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PhD Thesis

THE CHALLENGING DIAGNOSIS OF BONE DISEASE: A REAPPRAISAL OF PATHOLOGICAL BONE MARKERS BASED ON THE CAL MILANO CEMETERY SKELETAL COLLECTION

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Bibliography

List of publications realized during the course of this PhD.

Book chapters

Cattaneo C. and **Biehler-Gomez L** (2019). Il profilo di sant'Ambrogio. In C. Faccendini and C. Capponi, eds. *Apparuit Thesaurus Ambrosius. Le reliquie di sant'Ambrogio e dei martiri Gervaso e Protaso tra storia, scienza e fede*. Silvana Editore.

Biehler-Gomez L, Maderna E, Cattaneo C. (2020). The challenging diagnosis of cardiovascular disease in skeletal remains: distinction and recognition of atherosclerotic calcifications from known cases. In M. Binder, C. Roberts, D. Antoine, eds. *The Bioarchaeology of Cardiovascular Disease*. Cambridge University Press. In press.

Porta D, **Biehler-Gomez L**, Martino F, Cattaneo C. (2020). Studio antropologico e paleopatologico di Santa Maria Maddalena di Romano di Lombardia e San Lazzaro di Covo. In G. Medolago, ed. *Bartolomeo Colleoni e le reliquie della Maddalena e di Lazzaro da Senigallia a Covo e Romano*. Coglia Edizioni. In press.

Peer-reviewed articles

Biehler-Gomez L, Tritella S, Martino F, Campobasso CP, Franchi A, Spairani R, Sardanelli F, Cattaneo C. (2019). The synergy between radiographic and macroscopic observation of skeletal lesions on dry bone. *International Journal of Legal Medicine* 1-18.

Biehler-Gomez L, Giordano G, Cattaneo C. (2019). The appearance of breast cancer metastases on dry bone: implications for forensic anthropology. *Journal of Forensic and Legal Medicine* 61:5-12.

Biehler-Gomez L, Maderna E, Brescia G, Caruso V, Rizzi A, Cattaneo C. (2019). Distinguishing atherosclerotic calcifications in dry bone: implications for forensic identification. *Journal of Forensic Sciences* 64(3):839-844.

Biehler-Gomez L, Giordano G, Cattaneo C. (2019). The overlooked primary: bladder cancer metastases on dry bone. A study of the 20th century CAL Milano Cemetery Skeletal Collection. *International Journal of Paleopathology* 24:130-140.

Biehler-Gomez L, Giordano G, Cattaneo C. (2019). Multiple myeloma bone lesions in skeletal remains: report of two known cases from the 20th century CAL Milano Cemetery Skeletal Collection. *International Journal of Osteoarchaeology* 29:101-107.

Biehler-Gomez L, Maderna E, Brescia G, Cappella A, Rizzi A, Cattaneo C. (2019). "Aged" autopsy gallstones simulating dry bone context: a morphological, histological and SEM-EDS analysis. *International Journal of Paleopathology* 24:60-65.

Biehler-Gomez L, Castoldi E, Baldini E, Cappella A, Cattaneo C. (2019). Diabetic bone lesions: a study on 38 known modern skeletons and the implications for forensic scenarios. *International Journal of Legal Medicine* 133(4):1225-1239.

Biehler-Gomez L, Cabrini A, De Angelis D, Cattaneo C. (2018). How do skeletons with HIV present? A study on the identified CAL Milano Cemetery Skeletal Collection. *Legal Medicine* 33:11–6.

Cattaneo C, Mazzarelli D, Cappella A, Castoldi E, Mattia M, Poppa P, De Angelis D, Vitello A, **Biehler-Gomez L**. (2018). A Modern and Documented Italian Identified Skeletal Collection of 2127 Skeletons: the CAL Milano Cemetery Skeletal Collection. *Forensic Science International* 287:219.e1-219.e5.

Biehler-Gomez L, Cattaneo, C. (2018). The Diagnostic Implications of Two Cases of Known Rheumatoid Arthritis from the CAL Milano Cemetery Skeletal Collection. *Journal of Forensic Sciences* 63(6):1880-1887.

Biehler-Gomez L, Cappella A, Castoldi E, Martrille L, Cattaneo, C. (2018). Survival of Atherosclerotic Calcifications in Skeletonized Material: Forensic and Pathological Implications. *Journal of Forensic Sciences* 63(2):386-94.

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Abstract

The correct identification of diseases is fundamental for the construction of the biological profile of forensic and archaeological cases. The diagnosis of bone diseases consists in the comparison of the location and morphological characteristics of bone changes with the clinical literature and previous published cases for the identification of the causative agent. While the clinical literature may not be adequate to understand the morphology and distribution of dry bone lesions, the current paleopathological literature is mostly based on archaeological skeletons, on which very little is known, and specimens from pathology museums, with extreme manifestations of diseases often unrepresentative of classic cases.
This thesis aims to investigate the macroscopic diagnosis of pathological conditions based on skeletons with antemortem clinical diagnoses from a reference osteological collection: the CAL Milano Cemetery Skeletal Collection, and in particular atherosclerosis, rheumatoid arthritis, diabetes mellitus, HIV/AIDS, multiple myeloma and solid metastatic cancer. In addition to this main objective, several other research lines were explored including the histological analysis of non-skeletal calcified manifestations of diseases, the comparison between macroscopic and radiographic analysis of bone lesions and the description of bone lesions for pathological analysis on dry bones, through the examination of skeletal material from cemeterial, forensic and archaeological cases as well as samples extracted from well-preserved cadavers during autopsies.
This research not only implemented the scientific literature on bone diseases but also provided specific documentation to diagnose conditions previously unexamined and raised some important issues on the diagnosis of skeletal conditions on dry bone.

La corretta identificazione delle malattie è fondamentale per la costruzione del profilo biologico dei casi forensi e archeologici. La diagnosi di patologie ossee consiste nel confronto della posizione e delle caratteristiche morfologiche di lesioni ossee comparando la letteratura clinica e i precedenti casi pubblicati per l'identificazione dell'agente patogeno. Da un lato, la letteratura clinica non è adeguata a comprendere le manifestazioni morfologiche e la distribuzione delle lesioni ossee sull'osso secco; dall'altro, la letteratura paleopatologica si basa principalmente su scheletri archeologici, dei quali si sa molto poco e su esemplari di musei di anatomia patologica, con manifestazioni ossee estreme di malattie.
Questa tesi mira a studiare la diagnosi macroscopica di condizioni patologiche basate su scheletri con anamnesi cliniche antemortem utilizzando una collezione osteologica di riferimento: la *CAL Milano Cemetery Skeletal Collection*, focalizzandosi sull'aterosclerosi, l'artrite reumatoide, il diabete mellito, l'HIV/AIDS, il mieloma multiplo e il carcinoma solido metastatico. Oltre a questo obiettivo principale, sono state esplorate diverse linee di ricerca tra cui l'analisi istologica di manifestazioni calcificate non scheletriche di malattie, il confronto tra analisi macroscopica e radiografica delle lesioni ossee e la descrizione di lesioni ossee per le analisi patologiche attraverso l'esame di materiale scheletrico da casi cimiteriali, forensi e archeologici, nonché campioni estratti da cadaveri ben conservati durante autopsie.
Questa ricerca non solo ha implementato la letteratura scientifica sulle malattie delle ossa, ma ha anche fornito i mezzi per diagnosticare condizioni precedentemente non esaminate e ha sollevato alcuni problemi importanti sulla diagnosi delle condizioni scheletriche sull'osso secco.

Foreword

I would like to write a small note on the research performed in this thesis to clarify beforehand a few ethical considerations.

Despite the fact that skeletal remains have been used as a resource for research in the present PhD thesis, in accordance with the Italian law and ethical standards, it is important to specify that skeletons do no constitute one material among others for research purposes.

Skeletons are the remaining structures of breathing individuals, the last biological trace of their life to leave the Earth and the testimonies of past civilizations and ways of life. By studying skeletons, we do not only remember their life but we celebrate it. Skeletal remains are the most valuable resource of anthropologists; they allow us to reconstruct the life history of a person, understand the past, solve crimes, identify the dead (whether it be in mass catastrophes or in single cases), support the justice system and safeguard human rights. When we study skeletal remains, we do so consciously knowing that we are faced with a person. By opposition to the medical disciplines, this person is immune to pain and no harm can be inflicted; however, the notion of respect is of the utmost importance in our work and our treatment of the dead reflects it.

While the study of pathological conditions may seem ghastly for the layman, is it quite the opposite: bone lesions are not indicators of pain and suffering, but the scars of the survivors for only those who lived long enough may manifest bone changes. Their study helps us reconstruct the life of the deceased, understand the burden caused by pathological conditions, perform better diagnoses and even improve medical knowledge.

Anthropology is not a science of the dead but one of the living. It uses skeletons, cadavers and artifacts of past populations to reconstruct and understand life as it was. Hopefully, this note will help people better understand the meaning of this incredible discipline and my humble contribution to it.

Chapter 1. Introduction

"To the living we owe respect, but to the dead we owe only the truth."
Voltaire

1.1. The tales that dead men tell

While skeletons may represent death or the end of life in the collective mind, they are not, in fact, bland articulated structures devoid of identity. Quite the opposite, much of the history and identity of a person may be read on bare bones.

Biologically, the skeleton acts as a framework for attachment of the muscles and ligaments and thus supports the body. It protects vital organs by shielding the brain in the cranium, the spinal cord within the vertebrae and the heart, lungs and major blood vessels with the sternum, rib cage and spine; and it allows movement of the body by providing articulations. The skeleton is also the center for hematopoiesis (blood cells production in the bone marrow) and functions as a storage for calcium and iron in the body. But more than that, bones grow with us, they endure trauma, adapt to our activities and ways of life and suffer from our diseases. They are highly vascularized and in constant remodeling and so they are sponges full of information absorbed during the life of the individual.

When confronted to skeletonized human remains, the role of the anthropologist, whether in a forensic or archaeological context, is to reconstruct the life of the past individual through the construction of the "biological profile". The biological profile corresponds to a set of criteria determinable from skeletal remains after death but describing the individual during life. These criteria include the estimations of sex, age-at-death, ancestry and stature, the recording of anatomical variants and the analysis of traumatic and/or pathological lesions. Differentiating between male and female skeletons is based on sexual dimorphism, that is, phenotypic differences expressed through morphological variations in size and shape, as well as parturition (related to childbirth) (Phenice, 1969; Acsádi and Nemeskéri, 1970; Buikstra and Ubelaker, 1994; Walker, 2005, 2008; Spradley and Jantz, 2011; Klales, Ousley and Vollner, 2012). Age-at-death estimation in human remains relies mainly on degrees of skeletal maturation (dental development, bone ossification, growth and epiphyseal union) while the skeleton is growing (referred to as "subadults" in anthropology) (Scheuer and Black, 2004; Cunningham, Scheuer and Black, 2016) and the stage of skeletal degeneration in

fully grown skeletons (or "adults", different from legal majority) (Lovejoy *et al.*, 1985; Meindl and Lovejoy, 1985; Iscan and Loth, 1986; Brooks and Suchey, 1990; Buckberry and Chamberlain, 2002; Rougé-Maillart *et al.*, 2009; Baccino *et al.*, 2014). Ancestral geographical origin or ancestry estimation rests on the interpretation of skeletal morphological differences based on geographically-patterned human phenotypic variation (Hefner, 2009; Ousley and Jantz, 2012; Navega *et al.*, 2015). Stature estimation consists in the estimation of the individual's height from the application of equations based on bone lengths (Trotter and Gleser, 1952, 1958). Skeletal anatomical variants may be congenital, developmental or related to activity and can provide information on the individual's lifestyle, and potentially its genetic proximity with other skeletons sharing the same trait(s) within the same site (Hauser *et al.*, 1989; White and Folkens A., 2005; Christensen, Passalacqua and Bartelink, 2014). The study of trauma aims to identify antemortem (before death), perimortem (around the time of death) and postmortem (after death) injuries and reconstruct traumatic events (Rodríguez-Martín, 2006; Wedel and Galloway, 2013). Finally, pathological analyses repose on the observation and interpretation of bone abnormalities, either bone forming (osteoblastic) or bone remodeling (osteolytic), to understand disease burden (Steinbock, 1976; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Waldron, 2008; Grauer, 2012).

While most of the information gathered from skeletal remains concerns the individual at the time of death, they can also provide details about his life. Sex and ancestry are biological and relatively immutable information; age-at-death and stature are descriptors of the individual obtained at a specific moment of his life and thus relative to the individual at the time of his death; but signs of trauma and pathological conditions can tell quantity of information on the individual's health and life history. Consequently, after death and decomposition, skeletons remain and if read correctly and attentively, they can tell us much about the identity and life history of the persons they used to be.

1.2. Importance and significance of dry bone diagnosis

While the diagnosis of disease is of paramount importance in paleopathology, its significance in forensic sciences is underestimated.

Human paleopathology consists in the study of disease in ancient populations through

the examination of human remains (Aufderheide and Rodríguez-Martín, 1998). Consequently, the diagnosis of pathological conditions in skeletal remains is the core of the discipline of paleopathology. The question of the significance of disease in archaeological samples remains difficult to answer, but notable advances have emphasized theoretical issues in paleopathology. In particular, Wood *et al.* (1992) outlined three "conceptual problems" in the interpretations of health status of past populations from skeletal samples: first, "demographic nonstationarity" points out that population size is not immutable through time but markedly sensitive to changes in fertility; second, "selective mortality" highlights the selectivity bias in considering the dead (subject of the analysis) representative of the living; third, "hidden heterogeneity" underlines the variation between individuals in their susceptibility to disease. Based on these problems, the authors assert that "it is impossible to obtain direct estimates of demographic or epidemiological rates from archaeological samples". Another theoretical issue lies in the general assumption that signs of disease (especially infectious) are evidence of poor health and that skeletal remains affected by disease would represent the weakest of the population. Nonetheless, this may not be necessarily true. Most skeletal lesions are the result of chronic affections, meaning that the individual must have not only survived the initial and acute phase of the disease (e.g. in case of infection) but also lived long enough for bone involvement to occur. Far from an example of frailty and sickness, this implies a competent immune response in a relatively healthy individual – although, as Ortner, suggests it, less competent and effective "as one that successfully rids the body of the infectious organisms during the early stages of the disease" (Ortner and Aufderheide, 1991, p. 10). By opposition, individuals without bone lesions may have died first and rapidly during the acute stage of infection, thus representing the weakest individuals, despite the absence of skeletal manifestations of disease.

Forensic anthropology is the application of skeletal analysis to legal cases, including both dead and living individuals (e.g. assessing age in unaccompanied minors). In the scenario of an unknown skeletonized individual, the aim of the forensic practitioner is to provide sufficient information for a personal identification of the individual, for both legal and humanitarian reasons. The main methods of personal identification, specifically DNA, dental and fingerprint comparisons, consist in the superimposition and matching of antemortem and postmortem records. However, these methods cannot always be used: postmortem data may be lacking or damaged (DNA is highly

susceptible to contamination, teeth may be partially or completely absent and fingerprints are decomposed in skeletonized individuals), and antemortem data may not be available (e.g. migrants retrieved in the Mediterranean Sea). In such cases, additional descriptors are necessary. The anthropological "biological profile" will narrow the pool of potential candidates in the search for the identity of the deceased. But even when sex, age, ancestry and stature can be estimated, they are not always sufficient for a positive identification (e.g. if people with the same basic biological profile died during the same catastrophic event). Pathological markers on bones, as skeletal features that distinguish one individual from another, can then act as factors of individualization (Cunha, 2006). They will supply additional and specific postmortem skeletal information which may be compared to antemortem records, and may significantly orient the search among missing persons lists by sorting the individuals who may be included or excluded from further consideration. In combination with other personal descriptors, pathological conditions can contribute to the personal identification of unknown deceased by considerably narrowing the pool of potential matches. Therefore, the diagnosis of disease presents undeniable potential and should not be overlooked in forensic anthropology.

1.3. Accuracy of diagnosis on dry bone

Modern clinical diagnoses are based on a check-list of criteria including clinical manifestations, symptoms and test results, which may later be adjusted by the patient's response to treatment protocols. Clinical medicine and the study of bone disease present substantial differences in aims, methods and evidence. Indeed, in biological anthropology, the objective is not to cure but to diagnose; the patient is silent and devout of complains helpful for a diagnosis, his history is unknown and the array of clues orienting the diagnosis is considerably reduced as only his bones remain. In this context, different frameworks may be used for the diagnosis of disease: lesion-based approach, direct measurement of a diagnostic parameter (e.g. bone mineral density, cortical thickness) or direct identification of the causative microorganism (for infectious disease, e.g. DNA and biomolecular markers such as mycolic acids) (Mays, 2018). In spite of the efficiency and reliability of biomolecular methods, the lesion-based approach remains the foundation of the study of bone disease. It consists in the analysis of the morphology, position and distribution of lesions by comparison with

the clinical literature and previous publications of similar cases. The morphological aspect of bone lesions, their anatomical position on the bone and their pattern of distribution on the skeleton are carefully recorded and interpreted by comparing them to clinical or previously published cases presenting the same evidence of disease. This intuitive approach working from the "known" to the "unknown" (Mays, 2018) assures a sound diagnosis of disease.

As the lesion-based method in dry bone diagnosis possesses remarkable strengths, it also presents undeniable and important limitations. First and foremost, not all diseases affect the skeleton, only a small number of conditions have the potential to involve the skeletal system, and even those that can do not systematically affect all the individuals who have the disease, restricting our possibility to read health in bones. Second, the skeleton can only react in two ways: with abnormal bone proliferation (osteoblastic activity) or with abnormal bone loss (osteolysis). This limited array of physiological response to insult leads to a considerable overlap of skeletal manifestations between bone disorders. Some patterns of lesions however are typical and even pathognomonic of certain diseases (e.g. Pott's disease in tuberculosis) but variation in skeletal lesions of a given disease complicates the matter as not all disorders affect the skeleton in the same way (spinal involvement occurs in less than 1% of all patients with tuberculosis (Rezai *et al.*, 1995)). Consequently, in most cases, a certain diagnosis of disease cannot be reached and only a broad list of disorders that could be responsible for the lesions observed, or "differential diagnosis", remains. The aim is therefore to construct a differential diagnosis as narrow and accurate as possible. In addition, diseases affecting humans, their pathogenesis and our biological responses to disease may have changed over time: some diseases may have become extinct (Meyer *et al.*, 2002) or appeared later in time, pathogenesis may have evolved and produced different lesions in past populations (Grauer, 2012, p. 5), and our biological response to these disorders may not be the same as it was hundreds of years ago (Ortner, 2011a). Moreover, bone lesions in a skeleton may not be the result of a single disease: the living individual may have had more than one condition at the same time. Comorbidity is an important bias that may alter the aspect and distribution of bone lesions, confusing the diagnosis. Finally, relying on clinical data for comparison and interpretation of skeletal markers of disease carries serious limitations that must be acknowledged. Clinical standards and diagnostic criteria may not be adapted to the diagnosis of disease on dry bones because they were developed in a different aim, the treatment of living patients. As the main

interest of medical practitioners lies on diagnosis, management and treatment, skeletal manifestations of disease are incomplete for many disorders (Ortner and Aufderheide, 1991, p. 6; Ortner, 2011a). Furthermore, clinical data on skeletal manifestations of disease (when available) is dependent upon the sensitivity of the medical imaging technique. In fact, a change of about 40% in bone density is required to be discernable on plain radiographs (Ortner and Aufderheide, 1991, p. 8). Thus, clinical imaging may misread the morphology of some lesions or miss them altogether, confusing our understanding of the skeletal manifestations of the disease. For instance, the clinical literature states that tuberculosis predilects vertebral bodies, but this may be due to the fact that the involvement of vertebral arches is rarely seen on radiographs (Ortner and Aufderheide, 1991, p. 6). New bone deposition on the medial diaphysis of tibiae is commonly observed by paleopathologists but rarely reported in clinical studies (Ortner and Aufderheide, 1991, p. 7). Another example is the periosteal reactions on the visceral surfaces of ribs undetected by radiography and routine autopsies (Mays, 2018) but clearly identifiable through naked eye observation and proved to be related to intrathoracic chronic infections and tuberculosis (Kelley and Micozzi, 1984; Roberts, Lucy and Manchester, 1994; Matos and Santos, 2006; Santos and Roberts, 2006). In fact, in a study of osteoarthritis on 24 knee joints, Rogers and colleagues (Rogers, Watt and Dieppe, 1990) report that abnormalities were detectable radiographically in only two specimens, whereas 16 showed evidence of macroscopic bone changes. Additionally, medical imaging is performed in response to a patient's complaint and limited to a particular anatomical area, suggesting that "the total pattern of skeletal involvement in orthopedic diseases may not be well known" (Ortner and Aufderheide, 1991, p. 8). Sense of position and distribution are essential in the macroscopic study of bone disease as the limited array of skeletal physiological response to insult may overlap between diseases; thus, position and pattern of distribution may be crucial in distinguishing them. In the end, the information at our disposal in the diagnosis of disease of the living individual represented by the skeleton consists in basic biological data concerning the individual (sex, age, ancestry), the morphology and position of the lesions, and the pattern of distribution of these abnormalities.

1.4. Potential and limitations of reference collections

The main issue of the lesion-based approach in the diagnosis of bone disease is that the best outcome often consists in a speculative differential diagnosis difficult to narrow to a single cause. In order to effectively improve dry bone diagnosis, biases inherent to the lesion-based approach must be addressed to tip the scale in favor of a more reliable diagnosis. Reference collections may constitute such an option.

Reference collections, including identified osteological collections and pathology museum collections, are collections formed of selected and recovered material (skeletal and other biological elements) for research purposes. These collections composed of documented material constitute an ideal support for the development and testing of anthropological estimation techniques (Martrille *et al.*, 2007; Marlow and Pastor, 2011; Cappella *et al.*, 2017), the development of population-specific methods (Garvin, Sholts and Mosca, 2014; Gocha *et al.*, 2015), the analysis of secular change (Jantz and Jantz, 1999, 2016; Spradley, Stull and Hefner, 2016; Manthey *et al.*, 2017) and many methods for sex (Phenice, 1969), age (Lovejoy *et al.*, 1985; Meindl and Lovejoy, 1985; Iscan and Loth, 1986; Brooks and Suchey, 1990; Rougé-Maillart *et al.*, 2009), stature (Trotter and Gleser, 1952; Simmons, Jantz and Bass, 1990) and ancestry (Navega *et al.*, 2015) estimations were developed based on reference collections. The assemblage of osteological reference collections started in the beginning of the 20th century with the Hamann-Todd (United States) (Rothschild and Rothschild, 1995a), Terry (United States) (Hunt and Albanese, 2005), Raymond A. Dart (South Africa) (Dayal *et al.*, 2009) and Huntington (United States) (Jantz and Jantz, 1999) collections constituted of cadaver-derived human skeletons and later developed around the globe (Ubelaker, 2014) with the 21st century Identified Skeletal Collection in Coimbra (Portugal) (Ferreira *et al.*, 2014), the Luís Lopes Collection in Lisbon (Portugal) (Cardoso, 2006), the Human Skeletal Identified Collection of Modern Colombian Population in Bogota (Colombia) (Sanabria-Medina *et al.*, 2016), the Athens Collection in Athens (Greece) (Eliopoulos, Lagia and Manolis, 2007), the Granada Osteological Collection of Identified Infants and Young Children in Granada (Spain) (Alemán *et al.*, 2012), the UAB Identified Skeletal Collection in Barcelona (Spain) (Rissech and Steadman, 2011), the Pretoria Bone Collection in Pretoria (South Africa) (L'Abbé, Loots and Meiring, 2005), the Galler Collection in Basel (Switzerland) (Rühli, Hotz and Böni, 2003), the Sassari Collection in Bologna (Italy) (Hens, Rastelli and Belcastro, 2008), the Tedeschi

Collection in Padua (Italy) (Carrara, Scaggion and Holland, 2018) and now the CAL Milano Cemetery Skeletal Collection in Milan (Italy) (Cattaneo *et al.*, 2018).

Documentation in reference collections can include sex, age, ancestry, occupation, pathological conditions and cause of death. The study of these documented skeletons with recorded diseases can provide useful information on lesion morphologies and patterns of skeletal involvement for the comparative diagnosis of ulterior forensic anthropology and paleopathology cases. In addition to clinical literature, these studies illustrate and document macroscopic skeletal involvement in specific pathological conditions. The main issue with archaeological specimens is that, as the history and much of the evidence helpful for a diagnosis is lost, a certain diagnosis is impossible and almost inevitably results in a large differential diagnosis; by opposition, specimens from reference collections constitute a direct model for macroscopic comparison, given that the diagnosis is ascertained and known. This concurs with Hershkovitz and colleagues' (1998) observation: "As the life history of most archeologically derived skeletons is not usually available, information derived from clinically diagnosed cases has the potential to transform speculation into sound, data-based diagnosis". Clinical literature is a fundamental support in dry bone diagnosis, but as explained earlier, it also presents important limits in its application for the diagnosis of disease in skeletonized remains and thus morphological aspects of bone lesions and their distribution on the skeleton may not be adequately answered by the discipline. Dry bone diagnoses can only be supported by basic demographic information regarding the individual (namely, age and sex), morphology and position of the lesions, and pattern of distribution. Reference osteological collections can provide direct comparison of lesion morphologies and distributions in skeletons with known age and sex. Thus, the diagnosis remains built working from the "known" (here, a skeleton in which the disease is reported and ascertained) to the "unknown" (a skeleton under study). In this idea, reference osteological collections appear as an ideal support for studying and diagnosing disease on skeletal remains.

Nevertheless, lesion-based diagnoses based on comparisons with reference collections also present biases and limitations (Mays, 2018). In spite of its strengths, the documentation associated to the individual regarding pathologies and causes of death should be considered with care and perspective when making assumptions about the cause of lesion. Indeed, the presence of a pathological condition may not systematically result in bone lesions and cause of death may not necessarily be the

same as cause of lesion. In addition, the reliability of this documentation for the comparative diagnosis of dry bone depends on the method of diagnosis of the individuals in the reference collections. As our understanding of etiologies and pathogenesis considerably increased over the past century, so did our recognition, classification and diagnosis of diseases. In fact, the 2018 World Health Organization (WHO) International Classification of Diseases (ICD-11) repertories around 55,000 injuries, diseases and causes of death (World Health Organization, 2018). Thus, details of diagnostic methods are of crucial importance and if the diagnosis was not assessed by modern medical standards, its reliability may be questioned, constituting an important bias for ulterior comparative diagnoses. However, even in the case of a reliable diagnosis assessed by modern medicine, this specific condition may not be responsible for the lesions in the skeleton: the disease may not have involved the skeletal system or the individual may have been suffering from multiple conditions during life, which may bias the interpretation of lesions. Comorbidity remains an important issue, as often only one condition is reported in the associated documentation of reference collections (Brickley and Ives, 2010). Similarly, the state of preservation and completeness of the skeletons in osteological collections may constitute an important factor affecting the reliability of comparative diagnoses as it may impact the recovery and recognition of pathological lesions as well as the understanding of skeletal involvement. This state of preservation of the skeletal remains results from the conditions of conservation of the skeletal assemblages in museums, laboratories or universities, the method of recovery of the skeletons and the potential effect of taphonomic alterations. Pathological museums present a particular situation among reference collections as their sample selection strategies consisted in collecting the most unusual pathological cases. As a result, their specimens tend to present more severe and extreme expressions of disease, often unrepresentative of archaeological or forensic anthropology cases (Mays, 2018).

Finally, there is the argument in paleopathology that skeletons of individuals who died after the introduction of the antibiotics are not suitable for comparative diagnoses. The reasoning behind this argument is that the individuals who died in post-antibiotic era may have received treatment and modern medical treatments may impact the presence, frequency and aspect of bone lesions. By opposition, archaeological specimens represent individuals who died in the pre-antibiotic era and were unaffected by modern medicine; the pathological involvement of the skeleton is

therefore seen as the authentic and natural consequence of bone disease unaltered by treatment. Thus, the consideration of skeletons of individuals who died in post-antibiotic era and were potentially affected by modern medicine constitutes a bias for the comparison and diagnosis of conditions in archaeological specimens, unaltered by treatments. This theory that pre-antibiotic specimens represent natural expressions of diseases, unaltered by medicine, presupposes one of two possibilities: either people in the past did not resort to the medical treatments of their time, or medicine in the past was perfectly ineffective on the etiology and aspect of skeletal lesions, in healing or worsening the lesions. While the former is not only unlikely but also impossible to prove, the literature has clearly demonstrated the latter to be untrue: mercury, widely used and recommended in the past as a treatment of syphilis (Zuckerman, 2016), is known to be responsible for abnormalities in the development of permanent teeth in infants (Hutchinson, 1887), as well as dental defects referred to as "mercurial teeth" (Ioannou *et al.*, 2016), and Ortner (2003, p. 279) reported that the treatment with mercury exacerbated the extent and severity bone lesions, exceeding classic manifestations of the disease. In fact, Ortner (2003, p. 279) concludes that "At the very least, the implication is that we need to be very careful before assuming that a pre-antibiotic anatomical case of any skeletal disease is typical of the natural, untreated expression of the disease. Throughout human history medical practitioners used various substances to treat their patients and some of these treatments could easily have had adverse effects on the patients that could affect the skeletal manifestations". Consequently, the fact that the individual died in the pre-antibiotic era does not guarantee that any bone disease he may have suffered from represents a natural expression of the disease, unaltered by treatments. While specimens of pathological interest from osteological collections may show signs of healing or milder lesions due to the introduction of the antibiotics, skeletal lesions in pre-antibiotic individuals may also be altered by the effects of past medical treatments, without the paleopathologist being aware of it. Thus, the limit attributed to pathological specimens of osteological reference collections may also be applied to archaeological human remains, as their medical history is unknown. In addition, what is perceived as a bias in a paleopathological context may be of crucial interest in the forensic field. Indeed, all forensic skeletonized cases died in the post-antibiotic area and the chance of them receiving treatment during their lifetime is very high. Therefore, modern examples of anatomical cases with bone disease might prove insightful as the manifestations of

disease evolve with treatment. For instance, several studies (Holloway *et al.*, 2013; Steyn *et al.*, 2013; Steyn and Buskes, 2016) illustrated the changes in distribution and healing of tuberculous bone lesions in post-antibiotic anatomical skeletons from reference osteological collections, providing fundamental information for the recognition and identification of tuberculosis in forensic cases.

In conclusion, reference collections are a resource of considerable potential for the study of bone disease and the comparative diagnosis of unknown cases. While they do present limitations that must be acknowledged, they can also significantly contribute to our understanding of bone disease and its evolution. As of today, they are a limited and sometimes depreciated resource in paleopathology and a promising but unexploited asset in forensic anthropology.

Chapter 2. Aims and objectives

"Research is to see what everybody else has seen,
and to think what nobody else has thought."
Albert Szent-Gyorgyi

The lesion-based approach in the diagnoses of diseases consists in the analysis and interpretation of the morphology, position and distribution of lesions in an unknown case by comparison with the clinical literature and previous publications of similar cases. However, the lesion-based approach presents several biases. As explained earlier, these biases include the capacity of the disease to affect bones and its variability in pattern of affection, the potential non-linearity of the pathogenesis and pathological lesions through time, the challenge of comorbidity, the limits of clinical literature and the preservation of the material available for analysis.

In this PhD project, bone diseases and their diagnosis were studied with a new approach: instead of using solely archaeological material on which no information is known, this research was performed based primarily on modern skeletons with clinical antemortem diagnoses of diseases from the CAL Milano Cemetery Skeletal Collection, a reference osteological collection in Milan, Italy. In addition, samples extracted during autopsies at the medico-legal institute of Milan, forensic cases as well as selected archaeological specimens were included for focused studies.

This project was realized in two main stages. First, a macroscopic study of skeletons with recorded pathological conditions in their associated documentation; and second, the use of complimentary analyses to compare and confront macroscopic results. The research was thus divided in several and consecutive objectives:

- The first research line aimed at the macroscopic pathological analysis of skeletons with specific conditions clinically diagnosed before death in order to confront our observations with paleopathological definitions of the diseases to either strengthen or improve diagnostic criteria, while considering the limitations and biases inherent to the macroscopic method and the modern skeletal material.
- The second research line consisted in performing histological analyses of non-skeletal calcified manifestations and products of diseases in selective cases to further our understanding of pathological bone lesions and enhance the awareness, recovery, recognition and diagnosis of these pathological conditions.

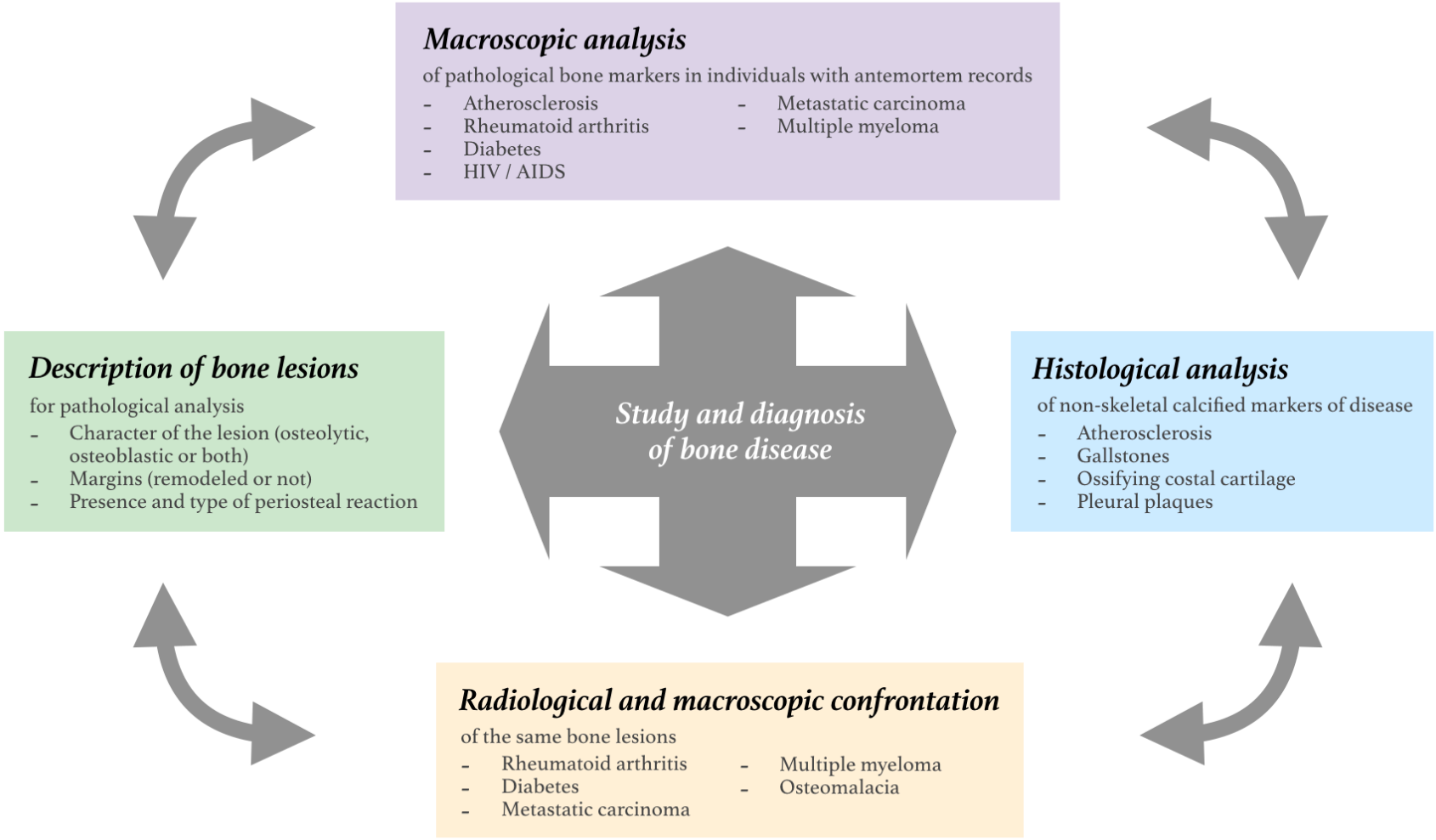
- The third research line focused on the comparison of macroscopic bone lesions with radiographic imaging of these same lesions. While clinical imaging has demonstrated its importance in the detection of bone lesions hidden from macroscopic observation, anthropologists have evidenced skeletal lesions in specific conditions unnoticed and unknown in the clinical literature, raising the questions: how do various types of macroscopic lesions appear radiologically? How much is seen and how much is invisible to radiographic imaging? Although we know the relevance of radiographic imaging for the detection and appreciation of the extent of bone lesions, are we really aware of the importance of a careful macroscopic description of bone lesions?
- Efforts in the field of paleopathology resulted in a standardized terminology for the description, communication and understanding of bone lesions. However, there is still considerable variation in the use of descriptive terms. The objective of the fourth research line of this project was to investigate the description of bone lesions for pathological analysis on dry bone.

With this intent, several pathological conditions were selected for this project based on their interest and relevance in the literature and scientific community, as well as the availability of the material in our collections. These include:

- Cardiovascular diseases, and in particular **atherosclerosis**;
- **Rheumatoid arthritis**, an erosive arthropathy;
- **Diabetes mellitus**, a metabolic condition characterized by chronic hyperglycemia and not directly involving the skeletal system;
- **Cholelithiasis,** a metabolic condition of the biliary tract;
- **Osteomalacia,** a metabolic condition characterized by impaired bone mineralization;
- **Human Immunodeficiency Virus** (HIV), a viral infection;
- **Multiple myeloma**, a neoplastic and hematological disorder;
- and **Metastatic cancer**, a neoplastic condition.

The main objective of this project was to study bone lesions in these particular conditions and discuss the morphological criteria for macroscopic or radiological diagnosis in comparison with the literature.

Balanced scorecard of this PhD project



Chapter 3. Literature review

"Study the past, if you would divine the future."
Confucius

Macroscopic diagnoses of diseases are based on the comparison of the position, distribution and aspect of lesions between what is observed in an unknown case and what is known and published in the literature. But how much do we actually know in the literature about skeletal diseases on dry bone?

3.1. Clinical literature

The study of bone disorders and trauma has been a subject of interest in medicine for centuries. As of today, there is a profusion of clinical literature on bone pathologies. As a matter of fact, orthopedics is the branch of medicine, and in particular surgical medicine, devoted to conditions involving the musculoskeletal system. Technologies have revolutionized the appraisal of musculoskeletal disorders, first with plain radiography and later with magnetic resonance imaging, computed tomography, nuclear medicine and ultrasound. But as discussed in the Introduction (Chapter 1), the plethora of clinical literature on bone disease is no warranty for a reliable diagnosis in the macroscopic analysis of dry bone lesions, which begs the question: how much do we really know about dry bone lesions?

3.2. Paleopathological literature

Manuals of paleopathology (Steinbock, 1976; Zimmerman and Kelley, 1982; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Mann and Hunt, 2005; Pinhasi and Mays, 2008; Waldron, 2008; Grauer, 2012) have accompanied and guided both novice and advanced researchers in the understanding and diagnosis of diseases on bare bones. These manuals are based to a greater (Zimmerman and Kelley, 1982) or lesser (Ortner, 2003) extent on established reference collections around the globe (Mays, 2018), and on the long experience of the authors with bone pathologies.
Most of the paleopathological literature is based on archaeological specimens and thus the use of this material for the comparative diagnosis of an unknown skeleton (either archaeological or forensic) poses the question of the reliability of the initial diagnosis

(when not confirmed by microbiology); which, in other words, corresponds to the limitations and biases inherent to the lesion-based approach in the diagnosis of disease. Consequently, the use of archaeological specimens as support of diagnosis of bone disease entails crucial limitations that may bias the diagnosis of disease in an unknown skeleton.

3.3. Reference collections

Studies based on reference collections allow a clear illustration and documentation of what to expect in specific pathological conditions. They bypass the unavoidable and large differential diagnosis of archaeological specimens in which a certain diagnosis is impossible and provide a direct model for macroscopic comparison. While epidemiology, etiology, pathogenesis and clinical manifestations will always be within the remit of clinical medicine, morphological aspect and distribution of lesions may not be adequately answered by the discipline. Macroscopic diagnoses on skeletal remains may only be supported by basic demographic information on the individual (age, sex, geographic origin), morphology and position of the lesions, and pattern of distribution. In this sense, reference collections constitute a strong medium for studying and diagnosing bone disease.

The reference collections most commonly used for research on bone diseases are the Hamann-Todd and Robert J. Terry collections in the United-States, the Coimbra and Lisbon collections in Portugal, the Galler collection in Switzerland, and the CAL Milano Cemetery Skeletal Collection in Italy. They have the common advantages of being identified and documented: demographic data (sex, age, date of birth, date of death and nationality), cause of death and diagnosed conditions (either autopsy-diagnosed or clinically diagnosed) are provided and associated to the skeletal remains.

- The Hamann-Todd Osteological Collection housed at the Cleveland Museum of Natural History is constituted of 3,300 unclaimed cadaver-derived skeletons from the beginning of the 20th century; the documentation for each individual includes demographic data, occupation, cause of death and autopsy report (Rothschild and Rothschild, 1995a; Ubelaker, 2014).
- The Robert J. Terry Anatomical Collection housed at the Smithsonian's National Museum of Natural History comprises 1,728 unclaimed defleshed cadavers who died in the first half of the 20th century; all the individuals of the collection have a

morgue record with demographic data and cause of death (Hunt and Albanese, 2005).

- The 21st Identified Skeletal Collection housed at the University of Coimbra includes 159 individuals of Portuguese descent, unclaimed and exhumed from the Coimbra cemetery with associated demographic data, cause of death and occupation; the individuals of the collection died between 1995 and 2008 (Ferreira *et al.*, 2014).
- The Luís Lopes Collection, also known as the Lisbon Collection, curated at the National Museum of Natural History of Lisbon, Portugal counts 1,692 skeletons who died between 1880-1975 and were buried in the cemeteries of Lisbon; documentation includes demographic data, cause of death and occupation (Cardoso, 2006).
- The Galler Collection is a pathology reference collection located at the National History Museum of Basel, Switzerland, and composed of 597 mostly macerated single bone specimens of all major pathological categories (autopsy-diagnosed) of the late 19th and early 20th centuries (Rühli, Hotz and Böni, 2003).

Focused studies have been published in the literature on particular conditions based on reference collections, and constituted the basis for selection of the pathological conditions reviewed below. For each condition, the pathogenesis, clinical manifestations and bone lesions, in particular based on reference collections will be reviewed from the literature. Dental diseases were not part of the review.

3.3.1. Infectious diseases

3.3.1.1. Non-specific infections

Certain infective bone lesions may present distinctive characteristics and thus may be ascribed to specific bacterial infections, in particular tuberculosis, treponemal disease and leprosy. However, pathological bone lesions wrought by other bacterial infections are relatively non-specific; that is, infective bone lesions are indistinguishable between different bacteria and the causative agent cannot be identified based solely on the macroscopic observation of skeletal remains (Roberts and Manchester, 2007, pp. 124–135). Bone involvement in bacterial infection will generally induce an inflammatory

response, which may affect the bone in three different sites: the inner or endosteal surface of bone, leading to osteomyelitis; the compact bone, causing osteitis; and the outer surface of bone, resulting in periostitis (Ortner, 2003, p. 181).

Staphylococcus aureus is the microorganism responsible for osteomyelitis in about 90% of cases. The radiological and paleopathological markers of osteomyelitis include the sequestrum, (devitalized fragments of avascular bone), the involucrum (envelope of new bone surrounding the necrotic sequestrum) and the cloaca (opening in the involucrum allowing drainage) (Gold, Hawkins and Katz, 1991; Lew and Waldvogel, 1997; Aufderheide and Rodríguez-Martín, 1998, pp. 178–181; Ortner, 2003, pp. 181–186). Osteitis appears radiographically as a cortical thickening with increased density (Chhem and Brothwell, 2008, pp. 93–99). Therefore, on dry bone, this abnormal thickening of the cortical bone can only be seen in transverse sections. Periostitis is an inflammation of the periosteum resulting in the deposition of new bone on the cortex; radiographically, periosteal reactions are separated in benign and aggressive types and can morphologically appear single-layered or multilayered, continuous, interrupted or complex (Ragsdale, Campbell and Kirkpatrick, 2018). Osteitis and periosteal reactions are not bone changes specific to bacterial infections and may be found in other conditions (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003); however, the problem of their non-specificity prevents a certain diagnosis from this bone abnormality. For instance, periosteal reactions appearing as plaque-like new bone formation with fine pitting and longitudinal striation were frequently observed on the cortical surface of the medial diaphysis of tibiae in archaeological skeletons but not noted in clinical context (Ortner and Aufderheide, 1991, p. 7). Several hypotheses were proposed to account for this bone change including stress indicator, recurrent minor trauma on this bone area close to the skin and ulceration induced from venous stasis in varicose veins (Roberts and Manchester, 2007, pp. 129–130), but the exact etiology of the lesion remains unknown.

3.3.1.2. Tuberculosis

Tuberculosis (TB) is a chronic and progressive infectious condition caused by the bacillus *Mycobacterium tuberculosis*. After the asymptomatic primary contact with the infectious agent, infection may become either latent or active. The immune system contains the bacilli preventing their replication in caseous and necrotic centers of

granulomas in the lungs, but the bacteria can survive in this material for years. The host's resistance against the microbial virulence will determine if the infection becomes latent (asymptomatic), also called persistent TB, or if it reactivates (with symptoms). Only 5-10% of healthy people infected develop an active disease. Factors weakening the immune system such as HIV infection, immunosuppressive drugs, malnutrition, aging and diabetes, may precipitate reactivation. At this point, the bacilli are revived and able to replicate in an uncontrolled manner, escape the granuloma, spread within the lungs and to other tissues (including bones and joints) via lymphatic route or bloodstream (Smith, 2003; Tierney and Nardell, 2018).

Clinical features of pulmonary tuberculosis include cough, chest pain, hemoptysis and respiratory failure. After physical examination, chest x-rays are usually performed and allow the observation of lung infiltrates with adenopathy and cavitation (Hopewell, 1994). Extrapulmonary TB, resulting from a failure to contain the bacilli, presents non-specific symptoms depending on the severity of the condition such as fever, weight loss and weakness, and may be concomitant with pulmonary TB, in particular in patients with HIV infection (Hopewell, 1994; Pigrau-Serrallach and Rodríguez-Pardo, 2013). Skeletal TB is slowly progressive; it causes pain, soft tissue swelling and limitation of joint movement. The spine and metaphyses of long bones (and by extension the joints) are most commonly involved due to the rich vascular supply of these areas. Spinal TB favors the thoracic and lumbar spine, causing paravertebral and epidural abscesses in about 50-80% of cases and lesions ranging from contiguous focal erosions on the anterior corners of the vertebral bodies to fragmentation of the vertebral bodies, disc space narrowing to intervertebral disk destruction and a progressive vertebral body collapse creating the characteristic gibbus deformation called "Pott's disease" (Jain, Sawhney and Berry, 1993; Hopewell, 1994; Shanley, 1995; Golden and Vikram, 2005). Neurological complications of spinal TB include spinal cord compression and in extreme cases, paraplegia or tetraplegia. Articular TB, much less common than spinal TB, typically consists in a slowly progressive monoarticular arthritis of the hip or knee (Golden and Vikram, 2005; Pigrau-Serrallach and Rodríguez-Pardo, 2013).

TB rarely affects the skeleton (Ortner, 2011b) but when it does, the lower spine is the most characteristic location involved in about 50% of cases, followed by the hip (Aufderheide and Rodríguez-Martín, 1998, pp. 118–141; Ortner, 2003, pp. 227–263, 2011b; Roberts and Manchester, 2007, pp. 135–142; Waldron, 2008, pp. 90–97). The disease creates lytic destruction in bone areas rich in hematopoietic marrow (essentially

trabecular bone) with virtually no new bone reaction. Lesions may heal without treatment and healing process in joint TB may terminate in the ankylosis of the articulation (Aufderheide and Rodríguez-Martín, 1998, pp. 118–141; Ortner, 2003, pp. 227–263, 2011b; Roberts and Manchester, 2007, pp. 135–142; Waldron, 2008, pp. 90–97). Although Pott's disease is a known sign of the infectious condition, Kelley and El-Najjar (1980) showed (based on skeletons with recorded TB from the Hamann-Todd collection) the importance of knowing the range of variation of the lesions in TB bone disease which may be circumferential (on the anterior and lateral sides of the bodies of the vertebrae), central (within the bodies) or paradiscal (on either side of intervertebral disc). In addition, while the neural arches have been described in the clinical literature as spared from the infection, Kelley and El-Najjar (1980) report three cases of neural arch involvement evidencing the issues of clinical diagnostic criteria (Ortner and Aufderheide, 1991, pp. 6–7). Mariotti *et al* (2015) furthered the research on TB bone lesions through the examination of erosions, cavities, enlarged foramina and periosteal reactions on the ribs of 244 skeletons (including 61 with known TB) from the Certosa cemetery in Bologna (Italy). Indeed, from the 1980s, studies suggested a positive (but not exclusive) correlation between TB and periosteal reactions on the visceral surfaces of ribs based on the Hamann-Todd (Kelley and Micozzi, 1984), Terry (Roberts, Lucy and Manchester, 1994), Coimbra (Santos and Roberts, 2001, 2006) and Lisbon (Matos and Santos, 2006) collections. Hershkovitz *et al.* (2002) investigated the relationship between the occurrence of *Serpens Endocrania Symmetrica* lesions and intrathoracic diseases, in particular TB (based on material from the Hamann-Todd collection), providing yet another skeletal marker suggestive of the condition. Ten years later, Pálfi and colleagues (2012) provided valuable illustration and details of the extreme osseous involvement in three juvenile skeletons from the Terry collection who died of TB and Holloway and her team (2013) examined 69 cases of skeletal TB from the Galler collection, before and after the introduction of pharmaceutical treatments to assess the impact of healing and treatment on the skeletal diagnosis of TB. Recent research (Steyn *et al.*, 2013; Steyn and Buskes, 2016) studied the evolution of TB bone changes in 205 skeletons who died of TB from South Africa reference collections (including the Pretoria bone collection (L'Abbé, Loots and Meiring, 2005) and the Raymond A. Dart Collection (Dayal *et al.*, 2009)), before, during and after the emergence of antibiotics and evidenced interesting changes. Not only did the number of lesions increased despite the introduction of antibiotics but the distribution of

lesions also varied: while costal and intracranial lesions increased, spinal manifestations, the most characteristic lesions of TB, decreased. These studies (Holloway *et al.*, 2013; Steyn *et al.*, 2013; Steyn and Buskes, 2016) illustrating the changes in TB lesions in post-antibiotic era may prove crucial in the pathological diagnosis of disease in forensic skeletonized cases.

A certain diagnosis is often difficult to assess on dry bones as many lesions due to TB are not specific to the infection (periosteal reaction on ribs, *serpens endocrania symmetrica*, erosions of the vertebral bodies, enlarged vertebral vascular foramina, lytic lesions). In these cases, biomolecular studies of ancient pathogens, such as mycobacterial DNA or mycolic acids, can allow the diagnosis of the disease in skeletal remains (Salo *et al.*, 1994; Nerlich *et al.*, 1997; Donoghue *et al.*, 2004; Redman *et al.*, 2009). Thus, archaeological specimens with a biomolecular confirmation of TB (Mays *et al.*, 2001) provide an ideal support for documentation of TB bone changes in the past, equal if not better than specimens from reference collections, as it removes the bias of the evolution of skeletal involvement through time in the comparative diagnosis of paleopathological specimens.

3.3.1.3. Treponemal disease

Treponematosis infection is divided into four types: yaws, bejel (endemic syphilis or Treponarid), venereal syphilis and pinta, but only the first three can affect the skeleton (Farnsworth and Rosen, 2006; Mitjà, Asiedu and Mabey, 2013; Marks, Solomon and Mabey, 2014). Venereal syphilis is caused by the spirochete *Treponema pallidum* subspecies *pallidum*. While this treponeme lacks metabolic activity, as well as oxygen and temperature adaptability, it possesses high invasive and attachment capabilities as well as a high motility allowing it to invade and survive in a wide variety of tissues for years. Even more challenging, *T. pallidum* cannot be cultured or genetically manipulated, hindering our understanding of its pathogenesis (Singh and Romanowski, 1999; LaFond and Lukehart, 2006; Peeling and Hook, 2006; Ho and Lukehart, 2011).

Syphilis develops in a multi-stage process and is responsible for protean clinical manifestations confusing the diagnosis, earning the alias of "great mimicker". Infection occurs by direct contact with active primary or secondary lesions. The replication of *T. pallidum* at the site of the inoculation induces a local inflammation resulting in a

painless chancre 3-6 weeks after infection and a moderate lymphadenopathy. This primary chancre spontaneously heals within 3-8 weeks, signaling the local clearance of the spirochete. However, at this point, *T. pallidum* has already spread systematically to deep and distant tissues and organs. About 3 months after the onset of the infection, secondary symptoms develop. The most common is a disseminated rash which may appear inconspicuous and can go undetected. Other, although less common, clinical signs include malaise, sore throat, muscle aches, weight loss, general lymphadenopathy, meningitis, ocular inflammation, inflammation of mucosal tissues and hepatitis (Singh and Romanowski, 1999; LaFond and Lukehart, 2006; Peeling and Hook, 2006; Ho and Lukehart, 2011). Symptoms of secondary syphilis usually resolve spontaneously within 3 months, when the host enters an asymptomatic latent stage leading, if kept untreated, to tertiary syphilis: *T. pallidum* evades immune detection and reactivates to cause symptoms in multiple invaded organs (LaFond and Lukehart, 2006; Ho and Lukehart, 2011). The Oslo Study (Gjestland, 1955) reported that about a third of patients with latent syphilis developed clinical signs of tertiary syphilis, often 20-40 years after primary contact. Gummatous lesions are localized tissue (usually skin) and bone destruction appearing as nodular ulcerative or necrotic lesions (15% of untreated syphilis); they rarely heal spontaneously but can resolve with appropriate antibiotic treatment. They are considered signs of "late benign syphilis" as they do not cause life-threatening complications unless they affect critical organs. Cardiovascular syphilis, another complication of tertiary syphilis, can manifest as aortic insufficiency, aortic aneurysms or coronary stenosis (10% of untreated syphilis). The primary invasion of the spirochetes may reach the central nervous system and left untreated or inadequately treated, the early neurosyphilis (6.5% of untreated syphilis) may spontaneously resolve or evolve either in asymptomatic meningitis or in acute syphilitic meningitis. In tertiary syphilis, the infection progresses to late neurosyphilis with general paresis and *tabes dorsalis* (Singh and Romanowski, 1999; LaFond and Lukehart, 2006; Peeling and Hook, 2006; Ho and Lukehart, 2011).

Primary yaws and bejel do not affect the skeletal system and lesions in both primary and secondary syphilis generally resolve spontaneously, implying that their diagnosis cannot be achieved on dry bones; however, if left untreated, the treponematoses can affect bones and thus may be recognized on skeletons. Nonetheless, the medical literature shows a considerable overlap in bone involvement of most cases of the three syndromes. Secondary yaws can cause bone changes including periostitis and osteitis,

in particular in the hands and feet (dactylitis) and in the long bones, creating the onion-layering aspect on x-rays. Tertiary yaws is rare but can cause gummatous lesions and bone deformities including the saddle-nose deformity, bowing of long bones or saber shin, destruction of the palate and maxilla (rhinopharyngitis obliterans or gangosa) and exostosis of the paranasal maxilla (goundou) (Farnsworth and Rosen, 2006; Mitjà, Asiedu and Mabey, 2013; Marks, Solomon and Mabey, 2014). Secondary bejel can be accompanied by osteitis and periostitis, and in its late form may evidence bowing of bones, bony gummata and osteolytic changes (Farnsworth and Rosen, 2006; Mitjà, Asiedu and Mabey, 2013; Marks, Solomon and Mabey, 2014). Periostitis and osteitis are also observed in secondary syphilis, but are transient and resolve spontaneously (Ortner, 2003, pp. 278–279). In the clinical literature, changes in tertiary syphilis include periostitis and gummatous lesions, particularly involving the cranial bones, tibia and clavicle, and tabes dorsalis resulting in Charcot's joints (Singh and Romanowski, 1999). Therefore, the argument in paleopathology is that a differential diagnosis between the three syndromes is likely to be difficult if not impossible on the basis of dry bone lesions.

After the analysis of 424 crania and calvariae and 250 long bones in 22 medical museums in Europe and based on his first-hand medical experience with yaws in Uganda, Hackett (1975, 1976) concluded that *caries sicca* (gummatous lesions on the cranium), first nominated by the anatomist Rudolf Virchow in 1896, and "nodes/expansions with superficial cavitation in long bones" (gummatous lesions on long bones) are pathognomonic traits of syphilis and of yaws and treponarid in relevant geographical areas. Other lesions suggestive of syphilis but not diagnostic, which Hackett (1975) referred to as "on trial", include cortical thickening of long bones or periosteal reaction on the cortical surface creating the appearance of enlarged bones with "larger than usual striae and pits". In addition, given the cardiovascular and neurological complications of syphilis, Hackett (1975) legitimately evokes sternal or vertebral erosions from aortic aneurysms and Charcot's joints from *tabes dorsalis* as potential lesions due to syphilis. Nonetheless, only about 8% of crania/calvariae and one long bone in Hackett's study had documentation supporting the diagnosis of syphilis beyond the morphological appearance of the bone. In spite of this, his work on treponematoses remains a reference for paleopathological analyses (Harper, Zuckerman and Armelagos, 2013; Mays and Vincent, 2013). Rothschild and Rothschild (1995b) later performed a more "quantitative" analysis on Bedouin and Guam

archaeological remains (bejel and yaws were the only treponemal diseases present in the Bedouin sites and in Guam respectively in that period) and 135 skeletons with diagnosed syphilis from the Hamann-Todd collection, determining the different skeletal distribution of the infectious conditions: syphilis and bejel appear to usually spare hands and feet, while yaws tends to affect more bones than the other treponemal conditions; however, all involve principally the tibiae (most commonly bilaterally), femora, fibulae and cranium. However, this research was met with criticism, particularly given the fact that the authors base their conclusions on a differentiation of the syndromes from undocumented cases (the Guam and Bedouin archaeological specimens) and refute the accepted consensus on the etiology of the disease (Heathcote *et al.*, 1998; Harper *et al.*, 2011). In addition, and contrary to tuberculosis, ancient DNA does not seem to be adequate for the study of the condition in dry bones as treponemal DNA is not preserved in human bone (Bouwman and Brown, 2005; Barnes and Thomas, 2006; von Hunnius *et al.*, 2007).

3.3.1.4. Leprosy

Leprosy is a chronic granulomatous infection caused by the obligate intracellular organism *Mycobacterium leprae*. The transmission of the disease is very low and poorly understood; risk factors include overcrowding and prolonged contacts. The bacillus enters the body via the nose and spreads through the circulation to the peripheral nerves and skin (Walker, 1983; Abulafia and Vignale, 1999; Eichelmann *et al.*, 2013). The clinical setting of the condition depends on the immune status of the host at and during the infection and is expressed in a spectrum with two opposite poles. At one pole, tuberculoid leprosy is stable, rarely contagious and characterized by high cell-mediated immunity, a limited number of bacilli and a few, limited lesions. These lesions are well-defined hypopigmented anesthetic macules or plaques with elevated borders, called "epithelioid granulomas", that may resolve spontaneously; however, the granulomatous inflammation of peripheral nerves will enlarge, causing an impairment of the peripheral nerves with sensory and motor loss and potential necrosis. At the opposite pole of the spectrum, lepromatous leprosy is defined as progressive and systemic with low cell-mediated immunity, high mycobacterial loads in skin and nerves, symmetrical and bilateral wide lesions as confluent papules and nodules named "lepromatous granulomas", greater nerve involvement and more

severe disability. Nonetheless, most patients exhibit manifestations intermediate to both polar forms, called borderline leprosy, where both granulomas may appear (Sehgal, 1987; Abulafia and Vignale, 1999; Walker and Lockwood, 2006; Gulia, Fried and Massone, 2010; Eichelmann *et al.*, 2013). Nerve involvement in leprosy leads to thickening of the peripheral nerves, pain, sensory and motor impairment causing disability and deformity. This impaired sense of touch can result in undetected trauma causing secondary infections and tissue damage, such as ulcers, osteomyelitis and neuropathic arthropathy. Other complications include osteoporosis, testicular damage and eye involvement (blindness occurring in 3.2% of patients) (Walker and Lockwood, 2006; Eichelmann *et al.*, 2013).

Nonetheless, this clinical classification cannot be achieved on dry bones as in living patients, although Andersen and Manchester (1994) suggest that symmetric lesions in the extremities may be attributed to lepromatous leprosy or borderline lepromatous leprosy. The small bones of the hands and feet are the preferred locations for bone lesions in leprosy, but the tibiae and fibulae may also be affected (Andersen, Manchester and Roberts, 1994; Ortner, 2003, pp. 278–289). These lesions are the result of ulcerations and infections secondary to long-standing nerve involvement and unperceived micro-trauma; they include periosteal reactions, osteomyelitis, destruction of the joints and ankylosis (Faget and Mayoral, 1944; Andersen, Manchester and Roberts, 1994). Two lesions in particular are characteristic of the infectious condition: severe erosion of the tubular bones of the hands and feet, particularly in the phalanges, and rhinomaxillary changes. The former is a late manifestation of leprosy, classically described as "concentric bone atrophy" of the phalanges (Faget and Mayoral, 1944; Møller-Christensen *et al.*, 1952). The latter corresponds to the clinical "Bergen syndrome", a facial disfigurement associated with a rhinomaxillary syndrome occurring in severe, advanced and long-standing lepromatous or near-lepromatous leprosy patients (Andersen and Manchester, 1992). Møller-Christensen (1952; 1953, 1961) described these rhinomaxillary changes osteologically based on his study of skeletal material from medieval Danish leper cemeteries and referred to them as "facies leprosa". In living patients, progressive changes may be observed: first, the infiltration of the mycobacteria in the nasal septum will induce a mucosal swelling; if the olfactory nerves are involved, anosmia may occur; later, the perforation of the nasal septum with sparing of the nasal bones will lead to "saddle-nose" deformity and the swollen mucosa may ulcerate resulting in its

atrophy; finally, late manifestations include perforation of the palate and loss of the central and lateral maxillary incisors (Andersen and Manchester, 1992). In skeletonized material, rhinomaxillary changes include resorption of the anterior nasal spine (present in about 80% of leprosy skeletons), bilateral symmetric resorption of the alveolar process of the maxilla (first extending from the antero-posteriorly and then progressing superiorly) leading to the loss of the central and lateral maxillary incisors, bilateral symmetric resorption of the margins of the nasal aperture, erosive changes on the nasal and oral surfaces of the palatine process of the maxilla leading to perforation of the hard palate, pitting on the nasal septum and resorption on the inferior nasal conchae (Møller-Christensen *et al.*, 1952; Andersen and Manchester, 1992).
However, all rhinomaxillary changes – except for the resorption of the anterior nasal spine caused directly by the bacilli – are signs of inflammatory reactions that may not be necessarily attributed to leprosy. For instance, a resorption of the alveolar process of the maxilla is not significant in the absence of upper incisors. Similarly, leprosy bone lesions in the post-cranial skeleton may be identical to lesions in osteomyelitis (from other causes), diabetic gangrene, gout, psoriatic arthritis, tuberculosis, dactylitis, sarcoidosis and *tabes dorsalis* in tertiary syphilis.

3.3.1.5. Fungal disease

Bone changes in fungal disease can occur via systemic hematological dissemination to the skeleton or via direct extension from soft tissue lesions (Ortner, 2003; Lupi, Tyring and McGinnis, 2005; Hay, 2006). The disease is rarely reported in the paleopathological record; in fact, Ortner (Ortner, 2003, pp. 329–332) presented only four cases from museum collections of archeological human remains in which a diagnosis of mycosis was plausible. Recently, however, Micarelli et al. (Micarelli *et al.*, 2019), reported a case from the 4th–6th centuries AD, Italy with skeletal lesions consistent with a mycosis (pitting with smooth edges, osteolytic lesions with sharp edges, blunt spiculae and remodelled areas with systemic distribution) and narrowed down the possible causative fungi to aspergillosis and cryptococcosis, as they are both native to Europe. Mycoses (in particular, blastomycosis, paracoccidioidomycosis, coccidioidomycosis, histoplasmosis, criptococcosis, candidiasis, aspergillosis, mucormycosis, sporotrichosis, maduromycosis) tend to present similar lesions, that is, osteolytic lesions with well-defined margins and punched-out appearance, and possible

periosteal reaction of variable extension, and a random distribution (Gehweiler, Capp and Chick, 1970; Moore and Green, 1982; Bradsher, 1992; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Lupi, Tyring and McGinnis, 2005; Hay, 2006). This random distribution of bone changes may allow their distinction from other infective diseases, whereas it renders the differential diagnosis among mycoses impossible (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003). Bone lesions in blastomycosis, coccidioidomycosis, paracoccidioidomycosis, and aspergillosis may simulate tuberculosis as well as primary and metastatic tumor, often causing misdiagnoses (Gehweiler, Capp and Chick, 1970; Moore and Green, 1982; Bradsher, 1992; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003). Similarly, nasomaxillary bone changes in mucormycosis may be confused with treponematosis and leprosy (Ortner, 2003). Hershkovitz and colleagues (1998) worked to answer this question by comparing a skeleton diagnosed in life with the fungal disease from the Terry collection with skeletons diagnosed with metastatic cancer and TB from the Hamann-Todd collection. They demonstrated that both the distribution and the aspect of lesions of the skeleton with blastomycosis are distinguishable from TB and metastatic cancer (for instance, both the cortical and trabecular bone are equally affected in blastomycosis, while this is not true in metastatic cancer and TB). However, this research was performed on only one skeleton with blastomycosis; it would therefore be interesting to see if the same analysis and distinguishing features are shared by other skeletons with the diagnosed fungal disease.

3.3.1.6. Parasitic infection

Parasitic infection in humans may be caused by endoparasites (including protozoa and helminth) or ectoparasites (flea, ticks, head and body lice). The first parasite find in archaeological material was found by Sir Armand Ruffer (Ruffer, 1910) in his recovery of well-preserved calcified eggs in the kidney tissue of Egyptian mummies dated circa 1,200 BC. Parasitic infection can thus be identified in skeletal remains through the recovery of hydatid cysts (in particular for Echinococcosis), ova and worms in (rehydrated) coprolites (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Roberts and Manchester, 2007; Reinhard *et al.*, 2013; Araújo, Reinhard and Ferreira, 2015) but also on dry bones such as in malaria.

Malaria is an infection caused by the parasites *Plasmodium falciparum* and to a lesser

extent *Plasmodium vivax* in endemic regions of the world. After a mosquito bite injects the parasites in the subcutaneous tissue, they migrate via the bloodstream and invade the liver where they bind to red blood cells. These merozoites destruct both parasitized and non-parasitized red blood cells, cause microvascular obstruction and induce systemic inflammation. Clinical presentation may be acute, with metabolic acidosis, severe anemia, cerebral malaria and respiratory distress or chronic, with a severe anemia in spite of a low parasitemia (Wickramasinghe and Abdalla, 2000; Miller *et al.*, 2002). Anemia is a common complication of both acute and chronic malaria (Ghosh and Ghosh, 2007). It may vary on a spectrum from mild, and thus benign, to severe and life-threatening. Hemolysis and impaired erythropoiesis, both direct and indirect consequences of the parasitic infection, are major factors in the etiology of anemia in malaria (Wickramasinghe and Abdalla, 2000). Nonetheless, comorbidities including iron, folate and vitamin B12 deficiencies, other nutritional deficiencies may cause anemia in malaria infection and anti-malarial therapy may aggravate it by hemolysis and bone marrow suppression (Ghosh and Ghosh, 2007).

Anemia is of special interest as it is a complication of malaria found to cause bone lesions. Indeed, while past studies have demonstrated the potential of biomolecular analyses to directly identify *Plasmodium falciparum* in ancient human remains (Nerlich *et al.*, 2008), Rabino Massa and colleagues (2000) performed immunological tests on Egyptian mummies dated 3200 BC and report porotic hyperostosis and *cribra orbitalia* (bone lesions commonly associated to anemia) in 92% of mummies tested positive for malaria. More recently, Smith-Gúzman (2015) furthered this question by performing a study on 98 individuals, including 27 with a reported cause-of-death of either malaria or anemia from the Golloway Osteological Collection, a documented collection of 592 unclaimed donated individuals from the Mulago hospital in Uganda (a region known to be holoendemic for malaria), and concludes that *cribra orbitalia*, spinal porosity, humeral cribra and femoral cribra are lesions indicative of malarial infection.

3.3.1.7. Viral infection

While measles and influenza do not affect the skeleton, other viral infections such as smallpox, rubella, poliomyelitis and the Human Immunodeficiency Virus (HIV) may lead to bone changes.

Smallpox is caused by the variola virus, specific to humans. The virus must pass from

one individual to the next in a continuing chain of transmission to sustain itself (Henderson, 1999; Moore, Seward and Lane, 2006). Thanks to a coordinated international effort initiated in 1966, the World Health Organization announced the worldwide eradication of smallpox in 1980 (Breman and Arita, 1980). The virus spreads by inhalation and infects the respiratory tract mucosa where it replicates, resulting in a primary viremia. During the immune response, the macrophages take on the virus which enters the reticuloendothelial system where it replicates, causing a second viremia and the onset of symptoms. General clinical manifestations of smallpox include fever, aching, chills, vomiting, raised pustular lesions and papular rash (Henderson, 1999; Moore, Seward and Lane, 2006). Skeletal complications may occur in up to 20% of young children (Davidson and Palmer, 1963) 1 to 6 weeks after the onset of smallpox (Cockshott and MacGregor, 1958) and manifest as "osteomyelitis variolosa". Initially, the lesions start as an arthritis with diffuse periarticular swelling, in particular in the elbows (about 80% of patients). The separation of the epiphysis at the epiphyseal junction is characteristic of osteomyelitis variolosa; severe lesions may provoke the detachment and destruction of the epiphysis causing disorganization of the joint. Marked periosteal reaction ensues and bone destruction may be observed in the small tubular bones of the hands and feet, also resulting in deformation of the joints. Lesions develop symmetrically, bilaterally and multifocally, primarily in the elbows but also in the wrist, knees and ankles, resulting in severe disability. Sequellae include ankylosis of the joints, reduction of the longitudinal growth with deformation and secondary osteoarthritis (Cockshott and MacGregor, 1958; Davidson and Palmer, 1963; Eeckels, Vincent and Seynhaeve, 1964; Jackes, 1983). Transverse bands of juxtametaphyseal osteoporosis can be seen on roentgenographic images (Eeckels, Vincent and Seynhaeve, 1964). Nonetheless, these lesions may appear similar to TB, syphilis, leprosy and sarcoidosis on dry bones (Lefort and Bennike, 2007) and a certain diagnosis based on the morphological appearance of the lesions may be impossible.

While the rubella virus (German measles) is harmless in children and adults, it may have serious consequences on fetuses. In adults, arthritis of the small joints of the hands and feet may be seen; in infants, rubella can provoke skeletal changes in the metaphysis of the long bones, manifesting as radiolucent bands due to poor mineralization, coarsening of the trabeculae and retardation of growth. In particular, "beaklike projections" may be observed in metaphyses during healing. If the infant survives the viral infection, bone changes may spontaneously resolve in a few months.

However, these changes may appear similar to early rickets and congenital syphilis and may be due to nutritional deficiencies (Aufderheide and Rodríguez-Martín, 1998, pp. 209–210; Ortner, 2003, pp. 336–337; Lewis, 2017, pp. 152–153).
Poliomyelitis (poliovirus) may lead to osteoporosis, pathologic fractures as well as underdevelopment and deformities in children including scoliosis, limb shortening (due to postpolio paralysis), talipes cavus and talipes equinovarus (high arched foot deformities) and coxa valga (increased femoral head to neck angle) (Aufderheide and Rodríguez-Martín, 1998, p. 212; Lewis, 2017, pp. 153–155). A possible case of poliomyelitis from the 15th century Portugal was reported by Umbelino and colleagues (Umbelino, Cunha and Silva, 1996) based on an asymmetry of the lower limbs with a shortening of the right leg and differential muscular activity, coxa valga, a scoliosis, a fracture on the right tibia and stress markers (Harris lines, *cribra orbitalia* and dental hypoplasia).

3.3.2. Metabolic diseases

3.3.2.1. Anemia

Anemia does not to a refer to a specific disease but to a symptom of an underlying condition. Anemia presents as a decline in the number of red blood cells (RBC), hematocrit or hemoglobin as a result of one of three basic mechanisms: blood loss, a deficient erythropoiesis (decreased production of RBC) or an excessive hemolysis (increased destruction of RBC) (Braunstein, 2017). Causes of anemia are numerous and may be classified in nutritional anemias (iron-deficiency anemia and megaloblastic anemia – due to vitamin B_{12}/folate deficiency), anemia of chronic disease (RA, kidney disease, cancer, HIV/AIDS, Crohn's disease as well as other chronic inflammatory conditions), aplastic anemia (consequent to infections, medicine, autoimmune diseases or exposure to toxic chemicals), hemolytic anemias (acquired or inherited, including the hemoglobinopathies with thalassemia and sickle cell anemia), malarial anemia and anemias due to other causes (Tefferi, 2003). Symptoms of anemia are consequent to hypoxia (as RBC function as oxygen carriers) and may include fatigue, weakness, pallor, drowsiness, dyspnea, angina, syncope, dizziness, headache and cold hands and feet (Braunstein, 2017).
Bone lesions in anemia result from marrow hyperplasia: in a hypoxic state, the kidneys release erythropoietin hormone to increase RBC production by the bone marrow in a

powerful stimulus. Marrow hyperplasia will then induce bone resorption leading to skeletal lesions including thickening of the cranial vault, "hair-on-end" appearance in the skull, metaphyseal widening, cortical thinning, trabecular coarsening and osteopenia (Aufderheide and Rodríguez-Martín, 1998, pp. 345–351; Ortner, 2003, pp. 363–376). Bone lesions of anemia occur in severe, chronic and long-standing cases and mild manifestations are not specific of the condition. Indeed, *cribra orbitalia* is a potential lesion of anemia but other causes include local inflammation, scurvy, trauma, osteoporosis and pseudopathology (Wapler, Crubézy and Schultz, 2004). While porotic hyperostosis is a possible manifestation of anemia, debate in the scientific community has questioned the validity of the iron-deficiency anemia hypothesis as its cause (Rothschild *et al.*, 2002; Walker *et al.*, 2009; Oxenham and Cavill, 2010; Rothschild, 2012; McIlvaine, 2015).

Hershkovitz *et al.* (1997) and Lagia *et al.* (2007) examined single cases of sickle cell anemia and thalassemia respectively from the Hamann-Todd collection and the Athens collection – constituted of individuals exhumed from cemeteries with known demographic data and causes of death (Eliopoulos, Lagia and Manolis, 2007). In the second study, however, the associated record only mentioned "anemia – heart failure" but a diagnosis of thalassemia was assessed by the authors from the analysis of bone lesions. After literature review and examination of their own cases, the authors suggest criteria allowing the distinction between the three main types of anemia: while all types of anemia may affect the skull, facial involvement and severe lesions are characteristic of thalassemia, long bone and spinal involvement (with H-shaped vertebrae on x-rays and enlarged basivertebral foramina) are typical of sickle cell and iron-deficiency anemia tends to cause generalized osteopenia (Hershkovitz *et al.*, 1997; Lagia, Eliopoulos and Manolis, 2007).

3.3.2.2. Vitamin D deficiency

Vitamin D holds a key role in the metabolic regulation of calcium and phosphorus and subsequently, in the mineralization of the osteoid. The most common causes of vitamin D deficiency include a lack of sunlight exposure associated with dietary insufficiencies, intestinal malabsorption as well as renal disease, and result in an insufficient mineralization of the newly formed bone (Berry, Davies and Mee, 2002; Brickley and Ives, 2010, chap. 5). In the pathological analysis of dry bones, the condition

is called "rickets" in infants, children and adolescents and "osteomalacia" in the grown skeleton (Mays and Brickley, 2018). The most severe manifestations of rickets occur in period of rapid growth, when poor mineralization of bones causes bending to weight-bearing bones and skeletal deformities: in crawling children the deformities affect the upper limbs while the lower limbs will be more commonly involved in walking children. In addition, rickets is associated with retarded growth, joint swelling, muscle weakness and delay in tooth eruption. In adolescents, both tetany and hypercalcemia may be observed. Moreover, delayed skeletal maturity may occur if the vitamin D deficiency is due to renal disease or intestinal malabsorption (Berry, Davies and Mee, 2002). The classical sign of osteomalacia is the pseudofracture or "Looser's zone", a small area of increased radiolucency on radiograph and corresponding to an accumulation of osteoid.

Considerable research has been devoted to the study of the skeletal complications of vitamin D deficiency over the past decades (Mankin, 1974; Mays and Brickley, 2018) based on pathology museum collections (Schamall *et al.*, 2003; Brickley, Mays and Ives, 2005) and refined with the study of archaeological specimens (Ortner and Mays, 1998; Mays, Brickley and Ives, 2006; Brickley, Mays and Ives, 2007, 2010; Ives and Brickley, 2014) allowing a confident diagnosis of the disease in skeletonized remains. Rickets is thus recognizable by the presence of cortical porosity in particular on the cranium but also on growth plates, flaring and thickening of rib ends and long bone metaphyses, medial bending of the tibia as well as distortion of the bones due to mechanical loading. Osteomalacia is characterized by pseudofractures and bending deformities. Pseudofractures appear macroscopically as small, usually bilateral, linear fractures with almost systematically evidence of repair forming an irregular callus. Their most frequent locations are on the ribs, spinous process and lateral border of the scapula, ilium, clavicle, distal ulna, pubic rami and femoral neck and shaft respectively. Bending deformities occur as a distortion of the bone under normal weight-bearing, due to the weakening and softening of the insufficiently mineralized bone and may affect the long bones, pelvis, sternum and cause kyphosis as well as scoliosis. Nonetheless, Ives and Brickley (2014) concluded in their large-scale study of 1181 skeletons that the manifestations of vitamin D deficiency observed in pathology museums (Brickley, Mays and Ives, 2005) were much more severe than what they found in archaeological excavations.

3.3.2.3. Vitamin C deficiency

Deficient vitamin C intake (ascorbic acid) causes severe impairment of collagen synthesis leading to the main feature of scurvy: sub-periosteal hemorrhages. These result primarily from a fragility of blood vessels susceptible to rupture and microfractures, and may occur throughout the skeleton. Vitamin C depletion also causes a depressed osteoblastic activity with a continuing or increased osteoclastic activity, resulting in a defective osteoid matrix formation. Radiographic changes thus include osteopenia, osteonecrosis, cortical thinning with periosteal new bone formation and demineralization with metaphyseal fractures in juveniles. However, reintroduction of vitamin C in the diet will completely resolve lesions after a few months (Fain, 2005; Brickley and Ives, 2006, 2010, pp. 41–74).

As a consequence of the inflammatory response to scorbutic chronic bleeding, diffuse abnormal bone porosity may be observed on the skull (and in particular the greater wings of the sphenoid, the roof and lateral margins of the orbit, the posterior maxilla, the interior surface of the zygomatic bone, the infraorbital foramen, the palate, the alveolar process of the maxilla and the coronoid process of the mandible), the scapula, and the metaphyseal ends of subadult bones. Other scorbutic bone lesions include new bone formation in the orbits and on the vault (due to the stripping of the periosteum from the underlying bone) and enlargement of the costochondral junction of the ribs ("scorbutic rosary"). In adults, affection of the gums may result in antemortem tooth loss (Aufderheide and Rodríguez-Martín, 1998, pp. 310–316; Ortner *et al.*, 2001; Ortner, 2003, pp. 383–392; Brickley and Ives, 2006, 2010, pp. 41–74). Documentations of dry bone lesions of scurvy were obtained from archaeological cases with probable diagnoses. As Ortner (Ortner *et al.*, 2001) points out, "confirmation of these findings with medically documented cases of scurvy would be very helpful" for future diagnoses.

3.3.2.4. Chronic kidney disease

Chronic Kidney Disease (CKD) results from the progressive and long-standing deterioration of renal function, with a clinical setting ranging from diminished renal capacity (renal insufficiency) to kidney failure, the end-stage renal disease (Malkina, 2018). The most common causes of CKD are diabetes mellitus and hypertension. Clinical manifestations include fatigue, anorexia, nausea, vomiting, taste disturbance,

nocturia and muscle cramps but many patients may remain asymptomatic (Webster *et al.*, 2017; Malkina, 2018). CKD is associated with an increased risk of cardiovascular morbidity and mortality (Moe and Chen, 2008). In fact, growing data indicates that individuals with CKD are more likely to die of cardiovascular disease than they are to reach kidney failure (Sarnak *et al.*, 2003; Moe *et al.*, 2007). CKD progression leads to increased osteoclastic activity and renal osteodystrophy: a complication of CKD materializing as osteitis fibrosa due to secondary hyperparathyroidism and osteomalacia (Hruska and Teitelbaum, 1995).

Kidney stones have been reported in ancient literature and found in preserved mummies dating back several thousands of years (Aufderheide and Rodríguez-Martín, 1998, pp. 284–286; Eknoyan, 2004). However, while kidney stones may be important risk factors for CKD, the chronic condition can also have a protective effect against kidney stone formation and so further studies are needed to better understand the relationship between kidney stones and CKD (Rule, Krambeck and Lieske, 2011).

Rothschild and colleagues (2002) investigated the possibility of diagnosing CKD on skeletal remains from the study of 94 individuals diagnosed during life with the condition from the Hamann-Todd collection: the authors reported the presence of several lesions including articular erosions, cysts and surface calcifications, bone porosity, osteochondritis dissecans, digital tufts alterations (referred to as "nicks"), periosteal reactions as well as osteopenia and osteomalacia on radiographs, and conclude that the lack of specificity of the lesions renders a certain diagnosis on skeletonized remains particularly difficult but that the presence of facial bone thickening, prognathism and an increased frequency of osteomas (or lesions resembling osteomas) should suggest further consideration for CKD.

3.3.3. Endocrine diseases

The endocrine system consists in a series of glands that produce and secrete hormones in the circulatory system that regulate metabolic processes in the body, including growth, development and maintenance of skeletal tissue. In particular, disorders of the pituitary, parathyroid and thyroid glands may affect the skeletal system.

Hyperpituitarism is defined by an excessive production of growth hormone by the pituitary gland and may result in gigantism and acromegaly, that is, excessive skeletal proportions. In addition, the association of increased size and weight with muscle

weakness may result in degenerative changes and skeletal distortions such as kyphosis and scoliosis. In hypopituitarism, the destruction of pituitary function leads to a form of dwarfism characterized by normally proportioned bones reduced in size and retarded skeletal maturation and dental eruption due to the loss of growth hormone effect (Aufderheide and Rodríguez-Martín, 1998, pp. 326–330; Ortner, 2003, pp. 422–425; Roberts and Manchester, 2007).

Hyperparathyroidism, characterized by an increased level of calcium in the blood plasma either primary or secondary to renal impairment, may affect the skeleton with diffuse osteopenia, subperiosteal porosity and osteitis fibrosa cystica. Low blood calcium level or hypoparathyroidism, is a rare condition which can lead to osteopenia and unspecific skeletal lesions (Aufderheide and Rodríguez-Martín, 1998, pp. 330–334; Marx, 2000; Ortner, 2003, pp. 429–430).

Bone changes in hyperthyroidism are generally mild, asymptomatic and unspecific, they include increased bone resorption leading to osteopenia, mildly increased stature and/or accelerated skeletal maturation (Aufderheide and Rodríguez-Martín, 1998, pp. 334–339; Ortner, 2003, pp. 426–428). Hypothyroidism is a clinical state resulting from an insufficient production of thyroid hormones. The most common cause today of hypothyroidism is iodine deficiency; in iodine-replete areas, the causes are congenital, due to chronic autoimmune disease (such as atrophic autoimmune thyroiditis or goitrous autoimmune thyroiditis), or iotrogenic, caused by destructive treatment for hyperthyroidism or thyroid cancer (Hall and Scanlon, 1979; Vanderpump and Tunbridge, 2002; Garber *et al.*, 2012). Any tissue may be affected in hypothyroidism. Clinical manifestations include fatigue, dry skin and hair, cold sensitivity, hoarseness of voice, constipation, muscle cramps, mental retardation and hearing loss (Hall and Scanlon, 1979; Gruber, 2012). Donald J. Ortner examined 12 skeletons with hypothyroidism from the Galler Collection and observed marked dwarfism in all 12 cases (with statures ranging from 105 to 145 cm), disproportionate bone development, delayed epiphyseal and apophyseal union as well as severe osteoarthrosis in particular in the hip and shoulder (Ortner and Hotz, 2005). It is however important to remember that specimens in pathology museums tend to present severe pathological manifestations (motivating their selection for entry in the museum) unrepresentative of archaeological cases (Mays, 2018; Mays and Brickley, 2018); therefore it would be interesting to compare Ortner's descriptions with additional material from other sources to improve diagnostic methods for the condition.

3.3.3.1. Paget's disease

Paget's disease of bone is a chronic condition of unknown etiology affecting the skeletal system in three progressive stages. Paget's disease may involve one bone (monostotic) or a few (polyostotic) generally affecting the axial skeleton, the end of the long bones and the clavicles. Initially, an osteolytic phase results in localized regions of sharply demarcated radiolucency known as "osteoporosis circumscripta". Lesions on the calvarium show marked reduction of the trabeculae in the diploe accompanied by thinning of the internal and external tables and may slowly coalesce. However, this type of lesion is not specific to the condition and may mimic tumor metastases. Subsequently, a mixed osteolytic-osteoblastic phase leads to and excessive production of poorly organized new bone in areas of the skeleton previously exhibiting a purely osteolytic character, terminating in a predominant sclerotic phase. On dry bones, this osteoblastic overstimulation is observable through enlargement of bones by periosteal new bone formation causing an increased circumference of the osseous element, and thickening of the cranial vault by endocranial and ectocranial bone formation. Nonetheless, pagetic lesions are circumscribed to the affected bones and do not spread to adjacent skeletal elements. Skeletal deformity and pathologic fractures are common features due to the mechanical weakness of the excessive abnormal bone apposition; they may occur in the skull, weight-bearing bones with bowing and vertebrae with vertebral compression and kyphosis (Aufderheide and Rodríguez-Martín, 1998, pp. 413–417; Ortner, 2003, pp. 435–442; Whyte, 2006; Singer, 2016).

Histology (light microscopy) is a particularly helpful method to confirm Paget's disease in dry bone material because of its characteristic "mosaic" pattern of woven and lamellar tissue, and pathognomonic "patchwork" architecture (De Boer, Van der Merwe and Maat, 2013); allowing for its diagnosis in archaeological material (Aaron, Rogers and Kanis, 1992; Roches *et al.*, 2002).

3.3.4. Neoplastic diseases

3.3.4.1. Benign tumors

Benign tumors tend to be solitary, slow growing and of limited size. The osteoma, and in particular, the "button" osteoma, is the most common tumor found in skeletons,

consisting of a smooth rounded mass of compact bone sharply demarcated, typically on the outer surface of the cranium, no larger than 20mm in diameter. By opposition, the osteoid osteoma occurs most often on a long bone of the legs in older children and young adults. The osteoblastoma is a rare tumor showing a slight radiolucency affecting the spine in adolescents and young adults. Chondromas are tumors of hyaline cartilage generally appearing in the bone (endochondroma); they predilect hands and feet and are found in all ages after 10 years. The osteochondroma is an osteocartilaginous exostosis appearing as a spongy mass near the growth plate on the metaphyseal surface of long bones; they tend to be solitary and to affect people younger than 21 years. Chondroblastomas are rare tumors with rounded, radiolucent and well-defined lesions occurring in the epiphyseal area of the long bones of immature individuals. Non-ossifying fibromas exhibit expanding lesions with a lobulated "soap bubble" appearance in the metaphyses of long bones (particularly the lower leg) of subadults. Giant cell tumors are locally destructive tumors favoring the knees of young adults. Osteolytic lesions in the epiphysis may extend to involve the metaphysis and the subchondral bone; however, giant cell tumors also retain some benign characteristics including a reluctance to perforate the bone cortex. Hemangiomas are solitary, small tumor of proliferating blood vessels. They may affect the vertebrae, reducing the trabeculae and increasing their diameter without altering the contour of the vertebral body, giving the aspect of a depleted trabecular structure with vertical striation on radiographs; or the cranium, where a "sunburst" appearance similar to osteosarcoma with circular margins may be observed (Aufderheide and Rodríguez-Martín, 1998, pp. 371–392; Ortner, 2003, pp. 504–523; Brothwell, 2008, 2012; Chhem and Brothwell, 2008).

3.3.4.2. Leukemia

Leukemia is a malignant condition of hematopoietic stem cells characterized by an excess of immature or abnormal leukocytes in the bone marrow, preventing the production of normal blood cells and leading to cytopenia. The type of leukemia may be classified as "myeloid" or "lymphoid" according to the lineage of precursor stem cell affected, as well as "acute" or "chronic" depending on the percentage of leukemia cells in the bone marrow or blood, resulting in four major categories of leukemia: acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia and

chronic lymphocytic leukemia (Emadi and Law, 2018). Infiltration of the malignant cells in the bone marrow leads to a diminution of RBC (leading to anemia), platelets (thrombocytopenia) and white blood cells (granulocytopenia). Clinical symptoms result from this impaired hematopoiesis: anemia induces fatigue, weakness, pallor, malaise, dyspnea, anorexia and tachycardia; thrombocytopenia causes mucosal bleeding, easy bruising, petechia and hemorrhages; and granulocytopenia leads to a severe vulnerability to bacterial, fungal or viral infection and fever. In addition, other organ infiltration can result in enlargement of the spleen or liver (splenomegaly and hepatomegaly), skin lesions (leukemia cutis), lymphadenopathy, bone and joint pain, as well as headache, stroke, visual and auditory involvement in meningeal infiltration (Lowenberg, Downing and Burnett, 1999; Sawyers, 1999; Emadi and Law, 2018).

Rothschild *et al.* (1997) examined in particular two skeletons from the Hamann-Todd collection who died of leukemia: a 3 year-old with acute lymphoblastic leukemia and a 60 year-old with acute myeloid leukemia. They describe superficial solitary and coalescing pits with smooth, minimally remodeled edges, minimal periosteal reaction and sparing the joints. This study helped some of the authors differentiate between leukemia and other conditions creating lytic lesions on the skeleton (Rothschild et al. 1998; Rothschild, Ruhli, et al. 2002). Nonetheless, this research was focused on only two skeletons; thus, additional cases of diagnosed leukemia could strengthen the criteria for diagnosis on dry bones.

3.3.4.3. Multiple Myeloma

Multiple Myeloma (MM) is a plasma cell malignancy characterized by monoclonal protein secretion, excessive osteoclastic activity and inhibition of osteoblastic bone formation, leading to progressive and destructive osteolytic bone disease (Bladé & Rosiñol 2007; Raab et al. 2009; Rajkumar 2009; Edwards et al. 2008; Kumar et al. 2017). Clinical manifestations include severe bone pain, pathologic fractures, osteoporosis, hypercalcemia, spinal cord compression (a rare complication), renal insufficiency (« myeloma kidney »), anemia and recurrent infections (Bladé and Rosiñol, 2007; Edwards, Zhuang and Mundy, 2008). MM can induce general immunodeficiency making infections a major cause of morbidity and mortality in the affected individuals (Bladé and Rosiñol, 2007).

Bone lesions in MM are described as exclusively lytic lesions, small and relatively

uniform in size, with a spheroid or "bubble-like" shape, sharp and well-circumscribed margins (or "punched-out"), and the absence of new bone reaction (Strouhal, 1991; Aufderheide and Rodríguez-Martín, 1998, pp. 351–354; Rothschild, Hershkovitz and Dutour, 1998; Ortner, 2003, pp. 376–382; Riccomi, Fornaciari and Giuffra, 2019). From reference collections, Rothschild *et al.* (1998) examined a skeleton from the Hamann-Todd collection and the pelvis, ribs and vertebrae of a second individual from the Mutter Museum in Philadelphia, both with an antemortem diagnosis of MM, and evidenced the different aspect of MM lesions by opposition to those of metastatic carcinoma and leukemia. However, MM remains the main differential diagnosis of metastatic bone disease and both conditions may be indistinguishable on dry bones (Ortner, 2003, p. 535).

3.3.4.4. Metastatic carcinoma

Carcinomas are solid tumors, they result from unregulated proliferation of cells and malignant transformation into cancerous cells occurring in epithelial tissues. After the malignant cells invade the adjoining local tissue, they can spread to distant sites or "metastasize" (Vassiliou, Chow and Kardamakis, 2013; Kirkpatrick, Campbell and Hunt, 2018). Symptoms vary depending on the primary organ of origin but general clinical manifestations of cancer may include pain, fatigue, weight loss, bleeding, swollen lymph nodes, headaches, dizziness, nausea and dyspnea. As Paget (1889) postulated in his "seed and soil" theory: metastatic growth (the seed) is dependent upon the properties and conditions of the microenvironment (the soil). The skeleton is a preferred site of metastases because bone microenvironment is particularly favorable to metastatic cells survival and development: first, bones are highly vascular which facilitates intravasation and extravasation of migrating malignant cells; second, the bone microenvironment is a reservoir of immobilized growth factors released during bone turnover that will attract new cancer cells and promote malignant cell proliferation (Roodman, 2004; Bussard, Gay and Mastro, 2008; Lipton and Vigorita, 2016). Cancer spread to bone may occur by different routes: through bloodstream, the lymphatic system, spinal fluid or by direct local contact of soft tissue with bone (Vassiliou, Chow and Kardamakis, 2013; Kirkpatrick, Campbell and Hunt, 2018). Bone metastases can lead to skeletal-related events, including severe bone pain, pathologic fractures, hypercalcemia, spinal cord compression as well as other nerve compression

syndromes, and are associated with an increased morbidity and mortality (Harvey and von Reyn Cream, 2007; Vassiliou, Chow and Kardamakis, 2013).

The most common malignancies to metastasize to bone are MM (75-95%), prostate and breast cancers (65-80%), followed by thyroid (60%), lung (30-40%) and kidney (20-25%) cancers. Malignant tumors of the bladder, rectum, pancreas, uterus, ovary and colon can also involve the skeleton with a frequency varying from 9 to 30%, while other gastrointestinal cancers rarely affect bones (Coleman, 1997, 2001; Roodman, 2004; Lipton and Vigorita, 2016). Bone metastases are located predominantly in hematopoietic areas of the skeleton, namely the vertebrae, pelvis, ribs, skull and ends of the long bones and are unusual distal to the knees and elbows (Aufderheide and Rodríguez-Martín, 1998, p. 388; Ortner, 2003, p. 534; Lipton and Vigorita, 2016). Lesions in metastatic carcinoma (MC) are typically classified in 3 types: osteolytic, osteoblastic and mixed metastases, but malignant tumors generally express both osteolytic and osteoblastic components on the skeleton and range from mostly osteoblastic to mostly osteolytic (Coleman, 1997, 2001; Brothwell, 2008). While Rothschild and Rothschild (1995a) demonstrated the importance of radiographic imaging of the skeleton for the observation of bone metastases, often hidden from macroscopic examination – in their study, 11 individuals were diagnosed with metastatic bone disease by visual examination compared to 33 with radiographs – they concede that some macroscopic lesions were not recognizable on x-rays (due to the 30-50% change in bone density required for observation on radiographs). Ragsdale *et al.* (2018) detailed the morphological bone changes in MC and advocate an organized approach for the diagnosis of metastatic bone lesions including distribution (solitary vs multifocal), character of margins, details of periosteal reactions, and remnants of mineralized matrix. In this idea, several studies based on reference collections (in particular the Terry, Hamann-Todd, Lisbon, Coimbra and the CAL Milano Cemetery Skeletal Collection collections) have examined bone lesions attributable to MC (Rothschild, Hershkovitz and Dutour, 1998; Marques *et al.*, 2018), and studies with knowledge of the primary organ of origin of the metastatic cancer from the associated documentation have documented the distribution and aspect of bone lesions in prostate carcinoma (Castoldi *et al.*, 2017), evidencing differences potentially useful for a distinction in an unknown case. The main issue in the diagnosis of MC is the differential diagnosis with MM and pseudopathology which may result challenging or even indistinguishable on dry bones (Ortner, 2003, p. 535; Kirkpatrick, Campbell and Hunt, 2018).

3.3.5. Diseases of the viscera: gallstones and urinary stones

Stones can be divided into two types: gallstones and urinary stones, which include stones from the bladder, kidneys and/or ureter. The pathogenesis of cholelithiasis and urolithiasis is still not fully understood and linked to genetic and environmental factors (I. S. Kim *et al.*, 2003; Knoll, 2010). These biological stones result from bacterial infections and alterations in the composition of the gallbladder bile (Carey, 1993; Johnston and Kaplan, 1993) or urine (Coe, Parks and Asplin, 1992; Knoll, 2010; Knoll *et al.*, 2011). Urinary stones and gallstones are often asymptomatic unless they cause obstruction and/or infection. In this case, the stone migration may cause biliary or renal colic associated with acute pain and unspecific symptoms such as nausea and vomiting (Preminger, 2016; Siddiqui, 2016).

The macroscopic study of these biological stones based on preserved collections of extracted stones (Womack, Zeppa and Irvin, 1963; Steinbock, 1990a, 1990b; I. S. Kim *et al.*, 2003) as well as their chemical analysis (Gault and Chafe, 2000; Ashok *et al.*, 2003; Chandran *et al.*, 2007; Abboud, 2008) and the reference differential diagnoses of soft tissue calcifications (Baud and Kramar, 1991; Komar and Buikstra, 2003) allowed their diagnosis in unknown dry bone context (Anderson, 2001; Giuffra *et al.*, 2008; Özdemir, Akyol and Erdal, 2015; Jaskowiec *et al.*, 2017).

3.3.6. Congenital diseases: skeletal dysplasias

Skeletal dysplasias are rare generalized skeletal abnormalities with a genetic basis often causing disproportionate short stature (Krakow and Rimoin, 2010). Over 60 years ago, a disproportionate dwarf was diagnosed as having either achondroplasia (if he had short limbs) or Morquio disease (if he had a short trunk) (Rimoin *et al.*, 2007). Today, the 2015 revision of the International Nosology and Classification of Genetic Skeletal Disorders lists 436 genetic disorders of the skeleton, classified on basis of clinical, radiologic and molecular criteria (Bonafe *et al.*, 2015). Clinical complications include cervical vertebra abnormalities such as atlantoaxial instability, fractures, spinal cord compression, joint pain and limitation, genu varum (bow legs) and genu valgum (knock-knees), eye disorders and hearing loss (Krakow and Rimoin, 2010).

In addition to paleopathology manuals (Aufderheide and Rodríguez-Martín, 1998, pp. 357–370; Ortner, 2003, pp. 481–501), several articles have documented cases of skeletal

dysplasias from pathological museums. Beighton *et al.* (1993, 1994) report infancy cases of osteogenesis imperfecta type II, thanatophoric dysplasia, achondroplasia and achondrogenesis, as well as adult cases of achondroplasia, Marfan syndrome, cleidocranial dysostosis, diaphyseal aclasia and severe rickets with dwarfism from the Museum of Pathological Anatomy in Vienna (counting about 44,000 specimens amassed over the last two centuries). Obviously, and as the understanding, terminology and diagnosis of these conditions evolved especially since the first publication of a nosology of skeletal dysplasias in 1970, these articles are based on post-mortem diagnoses performed by the authors. Similarly, Oostra *et al.* (1998) re-diagnosed all 360 congenital specimens from the teratological collection of the Museum Vrolik in Amsterdam and present cases of achondrogenesis type II, achondroplasia, Bloomstrand chondrodysplasia, Majewski syndrome, osteodysplastic primordial dwarfism, osteogenesis imperfecta type III and thanatophoric dysplasia, unspecified lethal chondrodysplasia and unspecified osteodysplasia. However, and as mentioned earlier, these specimens may represent severe cases of the conditions (as selected in pathological museums) which could prove unrepresentative of archaeological findings.

Macroscopic diagnoses of bone diseases are typically based on the morphology, position and distribution of lesions of an unknown case compared to the clinical and/or paleopathological literature. However, both the clinical and paleopathological literature present limitations. The sensitivity of medical imaging prevents the recognition of lesions otherwise observable macroscopically and the study of diseases in archaeological specimens, on which we know very little, impedes a certain diagnosis. Consequently, reference collections appear as a solid alternative: the skeletons are associated with a clinical (antemortem) or autopsy diagnosis based on modern medical standards, securing a certain diagnosis, and they may be used as a model for comparison with a documentation of both morphology and distribution of lesions. Nonetheless, biases remain, including the question of the cause of the lesion, selection strategies and state of preservation. While most categories of disease have been studied in reference collections – infectious, metabolic, endocrine, neoplastic, soft tissue and congenital diseases – few specimens have been examined and much confusion remains in dry bone diagnosis as manifestations of disease overlap.

3.4. Clinical and dry bone literature on joint disease

3.4.1. Osteoarthritis

Osteoarthritis (OA), also called osteoarthrosis, is a progressive degenerative joint disease and the most common form of arthritis (Hochberg, 2000; Johnson and Hunter, 2014). Although its pathogenesis remains incompletely understood, most authors agree on an enzymatic breakdown of the cartilage matrix. Whether the cause is pathomechanical or due to another etiology, fragments of the degraded matrix enter the synovium producing inflammation via inflammatory intermediaries. This leads to subchondral bone exposition, bone growth with sclerosis, bony cysts due to micro-fractures and osteophytes (Fitzgerald, Kaufer and Malkani, 2002; Berenbaum, 2013; Felson, 2013; Watts *et al.*, 2013).

About 15% of the population (Johnson and Hunter, 2014) and more than 58% of the individuals over 65 years old are affected by OA, with a higher rate for American Indians, Caucasian and African Americans compared to Hispanics, and Asian and Pacific Islanders (Dominick and Baker, 2004). There is a slight female predominance in the epidemiology of the disease and risk factors include age (especially after 50 years), excessive joint loading (high Body Mass Index), repetitive physical activity, previous trauma, and anatomic variants such as muscle strength and alignment (Hochberg, 2000; Fitzgerald, Kaufer and Malkani, 2002; Dominick and Baker, 2004; Weiss and Jurmain, 2007; Johnson and Hunter, 2014).

OA can affect any joint, but the most common locations are the hips, knees, spine, hands (especially the first carpometacarpal joint), feet (especially the first metatarsophalangeal (MTP) joint) and the acromioclavicular joint (Rogers *et al.*, 1987; Rogers, 2000; Fitzgerald, Kaufer and Malkani, 2002; Roberts and Manchester, 2007; Chew, 2012; Johnson and Hunter, 2014; Buikstra, 2019). The asymmetric loss of cartilage in the joint (due to differences in weight-bearing areas and/or mechanical impairment) produces bone-to-bone contact of the exposed bone surface resulting in focal abrasion with pitting, subchondral cysts due to microfractures with surrounding and underlying reactive new bone formation (sclerosis), highly polished areas from prolonged bone-to-bone friction known as eburnation, marginal bone remodeling related to joint repair (marginal osteophytes) and widening and flattening of the joint surface contour with the passage of time (Aufderheide and Rodríguez-Martín, 1998;

Hochberg, 2000; Rogers, 2000; Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Roberts and Manchester, 2007; Waldron, 2008; Braun and Gold, 2012; Vigorita and Ghelman, 2016). While the clinical diagnosis of OA relies on joint pain, swelling and stiffness, eburnation is the pathognomonic sign of the condition in dry bones (Rogers, 2000; Roberts and Manchester, 2007). In fact, it is important to remember that marginal osteophytes alone are not a sufficient indicator for diagnosis given that their formation can be related to the ageing process (Roberts and Manchester, 2007).

OA is the most common change observed in the skeleton (Rogers *et al.*, 1987). Easily identifiable with its typical features abundantly described in the literature (eburnation, marginal osteophytes, subchondral cysts and sclerosis), it can, however, co-exist with or develop secondary to several erosive arthropathies, including erosive osteoarthritis, rheumatoid arthritis and neuropathic arthropathy, potentially challenging its identification and diagnosis, especially if no pathognomonic signs for the co-existing conditions are present (Aufderheide and Rodríguez-Martín, 1998; Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Waldron, 2008; Vigorita and Ghelman, 2016).

3.4.2. Erosive osteoarthritis

Erosive osteoarthritis (EOA) is considered to be an aggressive subtype of OA typically affecting the hands of middle-aged women (Punzi, Ramonda and Sfriso, 2004; Mas and Rotés-Querol, 2007; Marshall *et al.*, 2013). Its etiology is unclear and tends to be more common among Caucasians and is present in about 2.8% of the general population and in 4-15% of patients with OA of the hands (Ehrlich, 2001; Punzi, Ramonda and Sfriso, 2004; Banks, 2010; Marshall *et al.*, 2013).

EOA produces both erosions and bone proliferation. The most characteristic lesion is the central erosion of the interphalangeal (IP) joints that differentiates it from the other arthropathies affecting the hands (Aufderheide and Rodríguez-Martín, 1998; Rogers, 2000; Punzi, Ramonda and Sfriso, 2004). This central erosion generates a collapse of the subchondral bone resulting in two typical erosive osteoarthritic deformities of IP joints: "gull-wing" and "saw-tooth" (Rogers, 2000; Punzi, Ramonda and Sfriso, 2004; Jacobson *et al.*, 2008; Waldron, 2008; Banks, 2010; Marshall *et al.*, 2013). Osteophytes and eburnation are a common feature of the condition and ankyloses of IP joints are described as being more common in EOA than in any other arthropathy (Aufderheide and Rodríguez-Martín, 1998; Punzi, Ramonda and Sfriso, 2004; Waldron, 2008; Banks,

2010). The X-ray lesions reflect the aforementioned macroscopic lesions as well as subchondral cysts and surrounding sclerosis (Jacobson *et al.*, 2008; Waldron, 2008; Banks, 2010). The lesions are found most commonly in the distal interphalangeal (DIP) and the proximal interphalangeal joints (PIP), more often symmetrical than asymmetrical. In decreasing frequency, EOA also affects respectively the first carpometacarpal joint, the metacarpophalangeal joints (MCP) and, lastly, the feet (Aufderheide and Rodríguez-Martín, 1998; Punzi, Ramonda and Sfriso, 2004; Banks, 2010).

The most reliable features of the condition are the central erosions and deformities of the IP joints. As a subset of OA, erosive osteoarthritic lesions can appear very similar, if not confusing (Vigorita and Ghelman, 2016). In addition, about 15% of all EOA patients will develop rheumatoid arthritis in their lifetimes, and since both diseases affect primarily the hands, a correct diagnosis may be challenging (Marshall *et al.*, 2013). Therefore, knowing the characteristic bone changes of these arthropathies on dry bones will help perform a correct and reliable diagnosis. Rogers and Waldron (1995) mention that only one case of EOA has been reported in the paleopathological literature (Rogers, Waldron and Watt, 1991); thus, documentation from clinically diagnosed dry bone cases could help the recognition of the condition in the future.

3.4.3. Septic arthritis

Septic arthritis may be caused by spreading of the infectious agent (most commonly, bacteria) to the joint cavity via hematogenous dissemination from a distant source, by direct inoculation (skin wound) or a contiguous infected bone. The infectious arthritis may involve one joint (classically, the hip or knee) or more. No changes can be noted on dry bones in acute septic arthritis. As the infection progresses, joint space narrowing, subchondral bone changes and complications such as subluxations or dislocations may manifest. Untreated septic arthritis is a potentially fatal condition which often terminates in bony ankylosis.

The main differential diagnosis of the condition is tuberculous arthritis: the latter tends to be more destructive and affects primarily children, whereas septic arthritis may affect all ages (Aufderheide and Rodríguez-Martín, 1998, pp. 106–107; Ortner, 2003, pp. 222–226; Roberts and Manchester, 2007; García-Arias, Balsa and Mola, 2011). Nonetheless, both conditions may be undistinguishable on dry bones. In addition, as

Roberts and Manchester (2007) explain: "septic arthritis may also be complicated by non-septic osteoarthritis or other joint diseases, so that, in any one individual, a number of joint diseases may present themselves but are impossible to differentiate from one another".

3.4.4. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic multifactorial disease with a pathological autoimmune response affecting primarily women (ratio of 2-3:1) around 30-50 years of age. With a prevalence between 0.8 and 2% in the general population, RA presents its highest rates of incidence among Native Americans (5-7%) and its lowest in Africa and South-East Asia, which suggests the presence of a genetic role in disease risk, mainly accounted for by genetic variation of the HLA DRB1 alleles (Lee and Weinblatt, 2001; Silman and Pearson, 2002; Alamanos and Drosos, 2005; Rindfleisch and Muller, 2005; Watts *et al.*, 2013; Smolen, Aletaha and McInnes, 2016). There is also evidence that "occurrence and severity of RA are related to genetic factors" according to Alamanos and Drosos (2005). Environmental factors include increasing age, obesity, family history, cigarette smoking and maybe also the influence of a protective effect of female hormones in RA. Smoking and infections might trigger the development of RA on genetically predisposed individuals and may influence the rate of development and severity of the pathology (Rindfleisch and Muller, 2005; Majithia and Geraci, 2007; Entezami *et al.*, 2011; Choy, 2012).

The disease consists of an inflammation of the synovium due to a combination of genetic, environmental and immunological factors. Consequently, a massive immune response takes place, producing increased angiogenesis and the formation of an aggressive front of tissue or "pannus" regulated by a complex cytokine and chemokine network producing the over-expression and over-production of TNF-α (Tumor Necrosis Factor). This then permits interactions between the infiltrated lymphocytes T and B, as well as fibroblasts and macrophages. The inflammation cascade results in an over-production of the pro-inflammatory cytokine interleukin 6 and the triggering of osteoclast activation, which in turn renders the pannus persistently inflammatory. As the latter extends and invades cartilage and bone, it leads to the destruction of local articular structures and the demineralization of subchondral bone (Firestein, 2000, 2003; Lee and Weinblatt, 2001; Scott, Wolfe and Huizinga, 2010; McInnes and Schett,

2011; Watts *et al.*, 2013; Smolen, Aletaha and McInnes, 2016).

In paleopathology, RA is described as mostly erosive with minimal new bone formation (Rogers and Waldron, 1995; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Waldron, 2008; Buikstra, 2019). In fact, Rothschild (2002) explains that unequivocal diagnosed cases of RA present with symmetrical erosions with marked periarticular osteopenia "but no new bone formation". The revised criteria of the American Rheumatism Association of 1987 for the classification of RA suggest seven clinical diagnostic criteria including the involvement of at least three joints, mainly PIP, MCP or wrist joints, in a symmetrical pattern with radiological erosions and/or periarticular osteopenia in the hand and/or wrist joints (Arnett *et al.*, 1988). Classically, RA is defined as polyarticular (usually affecting more than four joints) involving the hands and wrists (carpals, MCP and PIP) and is symmetrical (although not absolutely so in PIP and MCP) (Aufderheide and Rodríguez-Martín, 1998; Firestein, 2000; Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Roberts and Manchester, 2007; Waldron, 2008; Chew, 2012; Henrique da Mota *et al.*, 2013; Watts *et al.*, 2013; Vigorita and Ghelman, 2016). The erosions can be articular or para-articular and are typically U-shaped by opposition to the Ω(omega)-shaped erosions of psoriatic arthritis (Finzel *et al.*, 2011).

Possible lesions include "pencil-in-cup" deformities of the MCP and MTP joints, wrist ankylosis and ulnar deviation of the hand bones (Rogers, Watt and Dieppe, 1981; Aufderheide and Rodríguez-Martín, 1998; Brahee, Pierre-Jerome and Kettner, 2003; Ortner, 2003; Finzel *et al.*, 2011; Vigorita and Ghelman, 2016). Ankyloses of the carpals and tarsals are possible and not pathognomonic (Rothschild and Woods, 1991; Ortner, 2003) and they are considered to be a sign of extreme severity of the disease (Rogers *et al.*, 1987). The diagnostic potential of peripheral joint ankyloses in RA has divided the rheumatological community (Rothschild and Woods, 1991). On X-rays, subchondral cysts, osteopenia or more generally osteoporosis can also be observed. Although carpal bones, MCP and PIP joints are the most common locations, RA may spread with time and produce secondary changes in weight-bearing joints, including the elbows, shoulders, hips, knees, ankles, feet, cervical vertebrae as well as the radio-carpal, radio-ulnar and temporomandibular joints (Firestein, 2000; Brahee, Pierre-Jerome and Kettner, 2003; Erickson, Cannella and Mikuls, 2017). The involvement of the thoracic and lumbar spine is infrequent. The prevalence of involvement of the cervical spine (typically, the atlanto-axial joint) ranges between 17% and 88% (Kim *et al.*, 2015). It is a

late manifestation of long-standing RA associated with subluxation and reduced life expectancy (Jurik, 2011). Typically, DIP joints are not affected in RA (Rogers *et al.*, 1987; Ortner, 2003; Henrique da Mota *et al.*, 2013; Erickson, Cannella and Mikuls, 2017).
As a result, the presence of the bones of the hands and/or feet is essential to achieve a diagnosis of RA (Rogers *et al.*, 1987). Further studies on known cases of RA may thus provide additional information and help in the difficult diagnosis of the erosive arthropathy.

3.4.5. Ankylosing spondylitis

Ankylosing spondylitis (AS) is one of the four seronegative spondyloarthritides (SpAs) which also include enteropathic arthritis, psoriatic arthritis and reactive arthritis. The SpAs are inflammatory arthritides with poorly understood etiologies, clinically negative with regard to rheumatoid factors and associated with the variants of the HLA-B27 alleles. In terms of skeletal lesions, the SpAs tend to present sacroiliitis, spinal fusion, enthesitis and asymmetric peripheral oligoarthritis, mostly in the lower limbs (Arnett, 2000; Olivieri *et al.*, 2002; Waldron, 2008; Zochling and Smith, 2010; Watts *et al.*, 2013; Firestein *et al.*, 2017).
AS is the most common of the SpAs, affecting mainly males (ratio 2-9:1) and commencing at around 10-30 years of age. It is most common in Caucasians (0,1-0,9%) and least prevalent in African- Americans and Japanese, due to the distribution of the HLA-B27 gene in the population. As part of the SpAs, the pathogenesis of AS remains obscure; at present, it is known that it is an autoimmune and inflammatory arthritis and enthesitis with a clear genetic predisposition (Arnett, 2000; Olivieri *et al.*, 2002; Zochling and Smith, 2010; Watts *et al.*, 2013).
The pathognomonic bone signs for AS are bilateral symmetrical sacroiliitis and spondylitis (Braun and Sieper, 2007). Spine involvement in AS consists of syndesmophytes (vertically oriented osteophytes) and the consequent ascending ankylosis of the vertebrae creating a "bamboo spine" appearance, as well as osteopenia of the vertebral bodies resulting in the "squaring effect" of the vertebrae on x-rays (Rogers *et al.*, 1987; Aufderheide and Rodríguez-Martín, 1998; Sieper *et al.*, 2002; Ortner, 2003; Raychaudhuri and Deodhar, 2014; Buikstra, 2019). Entheseal and ligamentous (including apophyseal joints) ossification are particularly marked in AS and sacroiliitis is typically bilateral and symmetric (Rogers *et al.*, 1987; Aufderheide and Rodríguez-

Martín, 1998; Arnett, 2000; Ortner, 2003; Waldron, 2008; Zochling and Smith, 2010; Chew, 2012; Raychaudhuri and Deodhar, 2014). Peripheral joint involvement in AS is variable. It may be absent, or present as either a non-destructive mono/oligoarthritis of the large joints leading with time to their ankylosis or an asymmetric polyarthritis affecting mostly the hips, shoulders and knees (Rogers *et al.*, 1987; Aufderheide and Rodríguez-Martín, 1998; Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Braun and Sieper, 2007; Šlaus, Novak and Čavka, 2012; Watts *et al.*, 2013).

Although the axial involvement pattern is very distinctive and well-known in AS, peripheral manifestations are confusing. Indeed, the mono/oligoarthritis of large joints in AS resembles the peripheral involvement of enteropathic and reactive arthritis. Consequently, the axial involvement of AS is not only the most reliable feature for the diagnosis of the condition in dry bones, but also a necessary prerequisite.

3.4.6. Enteropathic arthritis

Enteropathic arthritis (EntA) is an inflammatory arthropathy present in 2 to 22% of Inflammatory Bowel Disease patients that includes Crohn's disease and ulcerative colitis, both idiopathic pathological conditions. EntA affects both sexes, with perhaps a slight female predominance, and shows geographical differences in disease risk due to immunological and genetic factors (notably the HLA-B27 gene distribution, more common in Caucasians) (Arnett, 2000; Olivieri *et al.*, 2002; Holden, Orchard and Wordsworth, 2003; Waldron, 2008; Voulgari, 2011; Resende *et al.*, 2013).

The exact mechanism responsible for the development of this arthritis is uncertain and consists of a reaction to an extra-articular infection, probably linked to abnormal bowel permeability combined with genetic and immunological influences (Holden, Orchard and Wordsworth, 2003; Voulgari, 2011). Axial involvement consists of bilateral symmetric (Waldron, 2008) or asymmetric (Ortner, 2003; Voulgari, 2011; Vigorita and Ghelman, 2016) sacroiliitis with spinal fusion. The symmetric sacroiliitis pattern and the spinal involvement can be either similar to AS or AS superimposed on EntA (Rogers *et al.*, 1987; Bruintjes and Panhuysen, 1992; Ortner, 2003; Waldron, 2008; Jurik, 2011; Voulgari, 2011; Jadon and McHugh, 2014). Entheseal changes are marked in EntA, mostly in the heels and knees (Rogers, 2000; Voulgari, 2011). The peripheral joint involvement can follow two different types. Type 1 is a non-erosive asymmetrical

arthritis of the large joints of the lower limbs affecting at most four articulations (Rogers, 2000; Holden, Orchard and Wordsworth, 2003; Ortner, 2003; Waldron, 2008; Zochling and Smith, 2010; Voulgari, 2011; Jadon and McHugh, 2014; Vigorita and Ghelman, 2016). Type 2, however, displays symmetrical involvement of at least five small joints consisting of erosive lesions (Holden, Orchard and Wordsworth, 2003; Zochling and Smith, 2010; Voulgari, 2011).

EntA is quite challenging to diagnose from skeletonized individuals without a medical history. The non-specificity of the lesions and their similarities with other arthropathies raises the problem of the identification of EntA in skeletal remains. Not only can the pathology co-exist with AS, but Type 1 peripheral arthritis with symmetric sacroiliitis is comparable to the lesions of AS and to reactive arthritis when the sacroiliitis is asymmetrical. Similarly, Type 2 joint lesions may be confused with psoriatic arthritis. In addition, as Waldron (2008, pp. 65–66) explains: "Although it is probable that both ulcerative colitis and Crohn's disease occurred in the past, it is not very likely that those who contracted either would have survived very long", thus precluding the possibility to affect bones. Waldron continues and concludes that in any case EntA could not be differentiated from classical AS or undifferentiated forms of SpAs.

3.4.7. Psoriatic arthritis

Psoriatic arthritis (PsA) is an inflammatory arthritis affecting 6 to 42% of patients with psoriasis. In over half of the cases, PsA is the result of long-standing psoriasis of about 7-12 years' duration (Dhir and Aggarwal, 2013). It affects both sexes with a slight female predominance in individuals around 40-50 years of age (Arnett, 2000; Fitzgerald, Kaufer and Malkani, 2002; Olivieri *et al.*, 2002; Myers, Gottlieb and Mease, 2006; Zochling and Smith, 2010).

Like the other SpAs, there is an idiopathic inflammation of entheses and the etiology responsible for the arthritis remains unclear: it consists of a combination of genetic predisposition (antigen HLA-B27) and environmental factors (an infection, for example) triggering an abnormal immune response. As in RA, T-cells, B-cells, macrophages and fibroblasts infiltrate the synovial tissue resulting in its hypertrophy. Cytokines, including TNF-α, stimulate both osteoclastic activity and the proliferation of inflammatory cells within the synovium, leading to its inflammation as well as to

bone erosion and new bone formation (Myers, Gottlieb and Mease, 2006; Jadon and McHugh, 2014).

Moll and Wright (1973) initially described four types of articular involvement that may accompany spondylitis and sacroiliitis: one consists of the extensive erosion of the DIP joints and represents less than 5% of cases; another, as uncommon as the first, is called *arthritis mutilans* and presents sacroiliitis and severe osteolysis in the IP, MCP and MTP joints; the third type and most common (about 70% of cases), is an asymmetrical oligoarthritis of the DIP, PIP, MCP and MTP joints; and the last type, present in about 15% of cases, is a symmetrical polyarthritis indistinguishable from RA. Recent research suggests that the asymmetrical oligoarthritic type gradually evolves into the symmetric polyarthritic type over time (Myers, Gottlieb and Mease, 2006; Dhir and Aggarwal, 2013).

More than the pattern of involvement, what differentiates PsA from the other arthropathies is its characteristic lesions. Acroosteolysis or the resorptions of the apical tufts in the hands and the "mouse ear" lesions of the IP joints are pathognomonic of the disease (Waldron, 2008). Whittling of the metacarpals and metatarsals or "pencil-in-cup" deformities of the MCP and MTP joints is also more typical of PsA (Aufderheide and Rodríguez-Martín, 1998; Rogers, 2000; Ortner, 2003; Roberts and Manchester, 2007; Waldron, 2008; Dhir and Aggarwal, 2013; Vigorita and Ghelman, 2016). Similarly to RA, PsA affects mostly the hands with marginal erosions as well as subchondral cysts and can present ankyloses of the IP, carpal and tarsal joints. Unlike RA, the lesions are Ω(omega)-shaped, both erosive and proliferative and can affect entire rays of the hands and feet; marginal erosions are poorly demarcated on x-rays; DIP joints are more affected than the PIP and MCP joints and representative of arthritis and the involvement of the TMJ joint is uncommon (Aufderheide and Rodríguez-Martín, 1998; Rogers, 2000; Ortner, 2003; Helliwell and Taylor, 2005; Waldron, 2008; Finzel *et al.*, 2011; Chew, 2012; Henrique da Mota *et al.*, 2013; Veale and Fearon, 2015; Vigorita and Ghelman, 2016).

Entheseal changes are common occurrences in PsA. Peripheral PsA can also be accompanied by sacroiliitis and spine involvement. Sacroiliitis can be similar to reactive arthritis (bilaterally asymmetric) (Aufderheide and Rodríguez-Martín, 1998; Rogers, 2000; Helliwell and Taylor, 2005; Waldron, 2008), or AS (bilateral symmetric) (Vigorita and Ghelman, 2016). Spine involvement in PsA may simulate that of reactive arthritis with asymmetrical large para-vertebral bridging and skip lesions

(Aufderheide and Rodríguez-Martín, 1998; Waldron, 2008; Baraliakos, Coates and Braun, 2015), or be indistinguishable from AS (Ortner, 2003; Helliwell and Taylor, 2005; Vigorita and Ghelman, 2016). Cervical spine involvement, in particular the atlanto-axial joint, is possible and may be similar to that of RA (Rogers, 2000; Laiho and Kauppi, 2002; Ortner, 2003; Helliwell and Taylor, 2005).

The diagnosis of PsA may result particularly challenging, especially given that the condition can co-exist with AS and may appear similar if not indistinguishable from RA (Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Waldron, 2008; Vigorita and Ghelman, 2016). Nonetheless, entheseal changes are typical of the SpAs and absent in RA, thus considerably helping in the differential diagnosis. In addition, the pattern of distribution of peripheral involvement and the characteristic lesions of PsA may also prove determinant in differentiating the condition from the other SpAs.

3.4.8. Reactive arthritis

Reactive arthritis (ReA), part of "Reiter's syndrome", develops in response to an infection in the gastrointestinal or urogenital tract. ReA affects predominantly young adults, from 20 to 40 years of age. It occurs in around 2-4% of patients infected and in about 30-40 cases per 100,000, involving mostly Caucasians due to the HLA-B27 gene distribution in the population (Kim, Klausmeier and Orr, 2009; Hannu, 2011; Selmi and Gershwin, 2014; Lahu *et al.*, 2015).

This inflammatory arthritis and spondyloarthropathy has a genetic predisposition and an onset as an over-stimulated autoimmune response to a triggering infection caused by a specific microorganism, including *Salmonella, Shigella, Yersinia, Campylobacter, Clostridium, Eschridia, Chlamydia, Ureaplasma* or *Mycoplasma* species of bacteria (Hannu, 2011; Selmi and Gershwin, 2014; Lahu *et al.*, 2015). However, it is "not clear how and in what form the bacterial components reach the joint and whether there are viable bacteria in the joint" (Kim, Klausmeier and Orr, 2009).

ReA (like the other SpAs) presents marked entheseal changes, typically in the heel as well as in the axial joints. Sacroiliitis is asymmetrical and spinal fusion generally occurs with skip lesions and para-vertebral bridging. As for the peripheral joints, they are involved in ReA in an asymmetrical mono- or oligoarthritis. The pathology involves the lower limbs most commonly, including the joints of the feet, knees, ankles and tarsals, but the hands can also be affected, although less often and only very mildly.

The lesions are erosive with reactive new bone and possible ankylosis of the tarsals (Aufderheide and Rodríguez-Martín, 1998; Rogers, 2000; Ortner, 2003; Waldron, 2008; Kim, Klausmeier and Orr, 2009; Hannu, 2011; Selmi and Gershwin, 2014; Cawley and Paine, 2015; Lahu *et al.*, 2015; Mays, Watt and Loe, 2016; Vigorita and Ghelman, 2016).
ReA, although very different from AS in its axial joint involvement, can also co-exist with the latter (Olivieri *et al.*, 2002), making its diagnosis in these cases dependent only on the peripheral bone lesions. However, the peripheral involvement is non-specific to the condition and can be very similar to those of EntA, PsA and AS, rendering the task quite difficult and adding to a problematic differential diagnosis. In this case, dry bone documentation of the skeletal manifestations of this condition from individuals clinically diagnosed before death could help the differential diagnosis of the arthropathy.

3.4.9. Diffuse Idiopathic Skeletal Hyperostosis (DISH)

Diffuse Idiopathic Skeletal Hyperostosis (DISH) is a skeletal disease of unknown etiology inducing entheseal calcification and ossification. In particular, it is characterized by the ossification of the anterior longitudinal ligament of the spine (especially the thoracic spine) with the production of flowing ossifications (limited to the right side of the vertebral bodies in the thoracic spine) of at least four contiguous vertebrae while relatively preserving the intervertebral disc space and the absence of changes associated with a degenerative disc disease or apophyseal joint ankylosis. DISH may also involve the heterotopic ossification of other locations, including peripheral joints and entheses, and is frequently associated with the ossification of the posterior longitudinal ligament and that of the ligamentum flavum (Resnick and Niwayama, 1976; Aufderheide and Rodríguez-Martín, 1998, pp. 97–99; Rogers and Waldron, 2001; Ortner, 2003, pp. 558–560; Sarzi-Puttini and Atzeni, 2004; Holgate and Steyn, 2016).
On dry bones, the earliest evidence of DISH dates back over 40,000 BP in the Shanidar 1 skeleton and is supported by flowing ossifications on L3 and L5, as well as diffuse ossifications of entheses (Crubézy and Trinkaus, 1992). The standardization of the diagnostic criteria of the condition by Resnick and Niwayama (Resnick and Niwayama, 1976) allowed the diagnosis of numerous cases (Aufderheide and Rodríguez-Martín, 1998, pp. 97–99; Ortner, 2003, pp. 558–560; Weisz *et al.*, 2011) and the re-evaluation of

previous diagnoses of ankylosing spondylitis. For instance, the radiographic reexamination of 13 royal Ancient Egyptian mummies (1492–1153 BC) permitted the exclusion of the diagnosis of ankylosing spondylitis based on the absence of sacroiliac joint erosions or fusion of the facet joints, including in two pharaohs initially diagnosed with the condition: Ramesses II, and his son Merenptah; and instead, allowed the diagnosis of DISH based on the ossification of the anterior longitudinal ligament along at least four contiguous vertebrae for four pharaohs: Amenhotep III, Ramesses II, his son Merenptah, and Ramesses III (Saleem and Hawass, 2014).

3.4.10. Gouty arthritis

Gout, also known as "the disease of kings", is a disorder of urate metabolism leading to an arthritis affecting mostly males (ratio 2-20:1) over 40-50 years of age. First identified in Egypt in 2640 BC, the spread of the pathology is coincident with western development through changes in dietary behavior and lifestyle. The higher rates observed in the Maori population in the development of gout compared to the rest of New Zealand might then be explained by a richer diet (K. Y. Kim *et al.*, 2003; Monu and Pope Jr, 2004; Nuki and Simkin, 2006; Ordi *et al.*, 2006; Zaka and Williams, 2006; Richette and Bardin, 2010). Gout possesses environmental (including hypertension, hyperlipidemia, obesity, insulin resistance, renal disease, low-level lead poisoning, uric acid calculi and endocrine disorders) and hereditary (family history in 80% of patients (Monu and Pope Jr, 2004)) risk factors, the latter suggesting a genetic susceptibility to the disease (K. Y. Kim *et al.*, 2003; Falasca, 2006; Zaka and Williams, 2006; Richette and Bardin, 2010).

Hyperuricemia, the result of either an over-production or under-excretion of uric acids, elicits deposits of monosodium urate crystals in and around the joints. Consequently, an immune response occurs characterized by a synovitis, promoting the phagocytosis of the crystals that causes the release of pro-inflammatory mediators. The interaction between the urate crystals and the inflammatory response ultimately results in nodules, called "tophi", containing the foreign bodies of crystals. The tophi deposited in sites of articulation finally lead to joint destruction through pressure and erosion (Aufderheide and Rodríguez-Martín, 1998; Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Monu and Pope Jr, 2004; Waldron, 2008; Richette and Bardin, 2010).

Gout arthritis is generally monoarticular and asymmetrical. Although it is renowned for its involvement of the first MTP joint called "podagra" (occurring in 85-90% of patients), it can affect any joint. Other commonly affected joints are the feet, ankles, knees, shoulders, elbows, wrists and hands. The erosion is typically a singular juxta-articular lytic lesion with a "punched-out" appearance surrounded by a sclerotic rim and exhibiting the pathognomonic "overhanging edge" or Martel's hook, particularly recognizable on x-rays (Aufderheide and Rodríguez-Martín, 1998; Rogers, 2000; Monu and Pope Jr, 2004; Waldron, 2008; Richette and Bardin, 2010).

Monoarthritic gout is relatively easy to diagnose, especially when the characteristic signs are visible. In this case, the differential diagnosis may be relatively straightforward: OA is rarely monoarticular and limited to a distal hand or foot joint, RA is symmetric and polyarticular and the SpAs manifest marked entheseal changes, spinal fusion and sacroiliitis.

3.4.11. Neuropathic arthropathy

Neuropathic arthropathy (NA), also referred to as Charcot's joint, is the arthropathic result of continued weight-bearing and undetected microfractures due to primary disturbances in the sensory and neurovascular innervation, produced in the absence of sensitivity to pain and altered proprioception. The primary causes of NA include syringomyelia, *tabes dorsalis,* diabetic neuropathy, neural leprosy, traumatic nerve damage, pernicious anemia and poliomyelitis. In syringomyelia, a rare pathology that produces a central lesion in the spinal cord, about 25% of patients develop NA, mostly in the large upper limb joints and unilaterally. Five to 10% of patients suffering from *tabes dorsalis*, a manifestation of tertiary neurosyphilis, present Charcot's joints, usually unilaterally in the large lower limb joints (often the knees). NA tends to affect feet more commonly the ankles in both neural leprosy and diabetes mellitus (Gupta, 1993; Canoso, 2000; Levy and Valabhji, 2008; Stanley and Collier, 2008; Wukich and Sung, 2009; Vigorita and Ghelman, 2016).

The neurotraumatic hypothesis, first developed by Virchow in 1886, postulates a mechanical cause to NA: peripheral neuropathy permits continued and unnoticed microtrauma because of the loss of proprioception associated with regular load-bearing, making the ligaments excessively relaxed and the unstable joint structures susceptible to microfractures, bone and cartilage fragmentation with debris formation,

demineralization of the affected bones, subluxations and deformities. The neurovascular hypothesis, introduced under the influence of Charcot's neurotrophic hypothesis, advances that the autonomic neuropathy causes vascular sympathetic dysfunction with a loss of thermoregulation. Reflex vasodilatation then leads to higher blood flow, which activates osteoclastic activity and resorption of the mineral matrix. This osteolysis and demineralization weakens the bone and predisposes it to minor trauma and, as a consequence, to NA (Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Trepman, Nihal and Pinzur, 2005; Chantelau and Onvlee, 2006; Giurato and Uccioli, 2006; Stanley and Collier, 2008; Wukich and Sung, 2009). However, these two hypotheses do not explain why the lesions in NA are unilateral when neuropathy is most commonly bilateral, or why NA is so uncommonly observed today compared to the high frequency of diabetic neuropathy. As a result, an inflammatory hypothesis arose and is now recognized. It presupposes a local inflammatory response and the release of pro-inflammatory cytokines in the joint as responsible for bone resorption (Molines, Darmon and Raccah, 2010).

Therefore, NA typically causes severe joint destruction and bone fragmentation with pathognomonic bone debris around the affected joint. Osteoporosis and/or sclerosis can be observed on x-rays, ankyloses are common, and osteophytes can be present (Aufderheide and Rodríguez-Martín, 1998; Fitzgerald, Kaufer and Malkani, 2002; Ortner, 2003; Trepman, Nihal and Pinzur, 2005; Stanley and Collier, 2008; Wukich and Sung, 2009; Vigorita and Ghelman, 2016).

The paleopathological diagnosis of NA is relatively straightforward if the lesion is unilateral, monoarticular, and surrounded by bone debris. However, it is important to note that lesions in NA may also appear similar to those of OA, post-traumatic OA, bone complications due to an infection, osteonecrosis, gout or even RA (Trepman, Nihal and Pinzur, 2005; Stanley and Collier, 2008); however, the condition is uncommon.

Consequently, despite the abundant literature on this subject, arthropathies remain particularly difficult to diagnose in skeletal remains. As demonstrated throughout this review, they can co-exist and may affect the skeletons similarly, thus confusing the diagnosis. Consequently, the paleopathological diagnosis may only be narrowed to a broad differential diagnosis with a reduced ability to discriminate specifically among them. To help paleopathologists in the differential diagnosis, we proposed a table

compiling the discriminating features of each arthropathy compared to the others. Further studies of known cases from documented sample populations may yet overcome these difficulties and provide insightful means to reach reliable diagnoses in spite of the limits imposed by skeletal analysis alone.

3.5. Metastatic bone disease: current notions and limitations

Cancer is the second leading cause of death worldwide, with an expected rise of new cases of about 70% over the next twenty years (WHO, 2017). It has been estimated that approximately 39,6% of men and women in the United States will be diagnosed with cancer at some point during their lifetime (National Cancer Institute, 2017). The high incidence and mortality of the disease has made it a subject of paramount interest for research over the past century, in particular in the search of effective treatments and cure (DeVita and Chu, 2008).

Although sarcomas may metastasize, it is a rare occurrence and metastatic carcinomas are much more common (Lipton and Vigorita, 2016). Any cancer has the potential to metastasize to bone (Joyce, 2012). Typically, bone metastasis happens late in the tumor progression (Talmadge and Fidler, 2010; Vassiliou, Chow and Kardamakis, 2013). Metastatic bone disease is associated with skeletal-related events, a higher morbidity and mortality (Vassiliou, Chow and Kardamakis, 2013), and usually, an incurability of the cancer (Roodman, 2004).

3.5.1. Antiquity of metastatic cancer

Metastatic carcinoma seems to be limited to vertebrates, as no evidence has ever been reported in invertebrates (Capasso, 2005). The oldest indication of metastatic cancer dates back to the Jurassic (200-700 million BP) and was suggested from an osteolytic lesion found in a dinosaur bone (Rothschild, Witzke and Hershkovitz, 1999). The earliest evidence of the disease in humans comes from osteolytic lesions on an Egyptian skull of 3000 BC that was diagnosed with nasopharyngeal carcinoma (Wells, 1963) but this diagnosis based on the sole evidence of a cranium is disputed (Binder *et al.*, 2014). Considering a full skeleton, the earliest diagnosis of metastatic cancer was assessed in a male of 25 to 35 years from ancient Nubia, circa 1200 BC (Binder *et al.*, 2014). Paleopathological evidence indicates a low frequency of the condition in

antiquity, even among the thousands of mummies analyzed to date (Capasso, 2005; David and Zimmerman, 2010); however, a comparative study between 905 skeletal remains from ancient Egypt (3200-500 BC) and 2457 skeletons from south Germany (1400-1800 AD) concludes that malignant tumors were not significantly fewer than expected given the changes in life expectancy (Nerlich *et al.*, 2006). Moreover, the Papyrus of Ebers (circa 1538 BC) and the Papyrus of Kahun (circa 1825 BC) include possible descriptions of uterus and breast carcinomas (David and Zimmerman, 2010). In ancient Greece, Hippocrates (5th century BC) used the word "carcinoma" as his diagnosis of a chest wall tumor and bleeding nipple of a woman from Abdera (Retief and Cilliers, 2011). He also described hard tumors in breast (Retief and Cilliers, 2011) and theorized that the cause of cancer was due to an excess of black bile; a theory that lasted until the Renaissance when Andrea Vesalius could not attest of the existence of black bile during anatomic dissections (Weinstein and Case, 2008). So why is the evidence of metastatic cancer so scarce in skeletal remains of antiquity? First, the shorter life span of ancient populations (about 40-50 years for the wealthy and 25-30 years for the non-elite (Preston, 1995; David and Zimmerman, 2010)) would reduce the risks of cell mutation, carcinogen exposure and development of bone metastases, associated with increasing age. However, there is growing evidence that age estimations in ancient skeletal remains may be underestimated, in particular for older individuals (Binder *et al.*, 2014). Second, the predominance of infectious conditions in antiquity (Marques, Santos and Cunha, 2013) limited the increase of the life span and a potential development of cancer. Third, the decreased quality of modern lifestyle compared to ancient populations may play a role in the high frequency of carcinomas observed today. Indeed, modern lifestyle is associated with smoking, lack of physical activity, unhealthy dietary habits, artificial environment as well as occupational and environmental carcinogens. Nonetheless, smoke exposure from open wood fires, actively used in the past, have carcinogenic properties similar to tobacco, especially when used indoors (Delgado *et al.*, 2005; Binder *et al.*, 2014). Fourth, the preservation of the integrity of the bone affected by metastases is essential for the diagnosis of the disease and highly dependent upon taphonomic conditions, which after centuries in the ground may have altered and destroyed the precious evidence of the condition. Nevertheless, the mummification process allows the preservation of tumorous lesions but the search of metastatic lesions in these collections has been largely unrewarding (David and Zimmerman, 2010). Finally, as metastatic lesions develop in vascularized

areas, it is the trabecular bone that is first and most commonly involved. Consequently, a macroscopic observation of the bone may not be enough for the identification of potential metastatic lesions and radiography proved to be an essential tool for the detection of cancer, but not systematically used in the anthropological analysis (Rothschild and Rothschild, 1995a; Binder *et al.*, 2014).

3.5.2. Why bone? The "seed and soil" framework

Tumor metastasis to the skeleton is the result of a cascade of events highly dependent on the interactions between the tumor cells and the bone microenvironment. The first step in this multistage process is the invasion by the primary tumor of the surrounding basement membrane. The neoplastic cells dissolve the extracellular matrix containing growth factors, stimulating their motility and develop angiogenesis, promoting their growth and supplying a route for migration through lymph or blood vessels. Hematogenous spread to the skeleton (in particular through Batson's venous plexus) is far more frequent than direct invasion and lymphatic spread to bone is rare (Rosenthal, 1997; Rybak and Rosenthal, 2001). Individual tumor cells detach from the primary tumor and enter the layered endothelial vascular wall (intravasation). They must then survive the velocity-induced shear forces of the blood flow, evade the "anoikis", the programmed cell death after detachment from the extracellular matrix and shield themselves from the immune system. The surviving tumor cells arrest in the circulation by adhering to the vessel endothelium of the target organ and exit the blood vessel by the same mechanism as intravasation (extravasation). The final step in the metastatic disease process is the adaptation of the neoplastic cells in the new hostile territory. In the bone, the tumor cell must survive the immune response, colonize and adapt to the new metastatic site and develop a vascular network to sustain itself. Indeed, angiogenesis will provide growth factors, oxygen, nutrients and metabolites to the metastatic lesion, allowing it to grow and proliferate. Finally, the neoplastic cells produce secondary metastases or "metastasis of metastases" that will repeat the same process and metastasize and colonize distant sites (Fidler, 1989; Gupta and Massagué, 2006; Steeg, 2006; Harvey and von Reyn Cream, 2007; Bussard, Gay and Mastro, 2008; Talmadge and Fidler, 2010; Faraji and Eissenberg, 2013; Randall, 2015; Lipton and Vigorita, 2016).

Why is bone such a preferential organ for metastasis? In 1889, Stephen Paget (Paget,

1889) addressed the question of favored metastatic sites and posed the principles of his "seed and soil" framework: "when a plant goes to seed, its seeds are carried in all directions, but they can only live and grow if they fall on congenial soil". Consequently, the development of the metastasis (seed) is dependent upon the conditions of the metastatic site (soil) (Mundy, 2002). The target site may either support the survival of the cancer cells, if the microenvironment possesses the necessary properties, or limit it (Talmadge and Fidler, 2010). Bone is a preferred site for metastasis because the bone microenvironment is highly favorable to the survival and proliferation of tumor cells. Indeed, bones present highly vascularized and metabolically active areas with a unique vascular structure, namely large diameter vascular sinusoids, inducing a slow blood flow and facilitating the intravasation and extravasation of the neoplastic cells (Mastro, Gay and Welch, 2003; Roodman, 2004; Bussard, Gay and Mastro, 2008; Vassiliou, Chow and Kardamakis, 2013; Lipton and Vigorita, 2016). Moreover, the bone microenvironment is a reservoir of immobilized growth factors released during bone remodeling that can both attract tumor cells and support them during the "vicious cycle" when in place (Roodman, 2004; Bussard, Gay and Mastro, 2008; Lipton and Vigorita, 2016). This "vicious cycle" is the result of the cross-talk between the migrated neoplastic cells and the bone microenvironment that feeds a disastrous loop of tumor growth and bone resorption (Guise *et al.*, 1996; Guise, 2002; Steeg, 2006; Gruber, 2012; Vassiliou, Chow and Kardamakis, 2013). The mechanisms responsible for bone formation orchestrated by the cancer cells are still unclear (Guise, 2002; Roodman, 2004; Theriault and Theriault, 2012) but they attest to a selective interaction of the tumor cells with the bone microenvironment (Guise, 2002; Guise, Yin and Mohammad, 2003). Metastatic cancer cells can manipulate the activity of osteoblasts (bone forming cells) and osteoclasts (bone remodeling cells) through the release of stimulators to incite the chemoattraction of cancer cells as well as support their survival (Guise, 2002; Guise *et al.*, 2006; Bussard, Gay and Mastro, 2008). Finally, the bone microenvironment presents some intrinsic properties favorable to the development and thriving of neoplastic cells, including low oxygen, low pH and high calcium and phosphate concentrations. While hypoxia will promote the proliferation and motility of cancer cells, acidosis, high calcium and phosphate will increase osteoclastic resorption (Vassiliou, Chow and Kardamakis, 2013) and thus contribute to the vicious cycle of tumor metastases.

3.5.3. Bone metastases

Metastatic cancers are far more prevalent than primary bone malignancies (Bussard, Gay and Mastro, 2008; O'Sullivan, Carty and Cronin, 2015). Bone is the third most common metastatic site in the body, after the lungs and liver (Mundy, 1997; Lipton and Vigorita, 2016) and the preferred site for metastasis in breast and prostate cancers (Coleman, 2001; Vassiliou, Chow and Kardamakis, 2013). Multiple myeloma (75-95%), prostate and breast cancers (65-80%) have the highest frequency of bone lesions, followed by thyroid (60%), lung (30-40%) and kidney (20-25%) cancers. Malignant neoplasms of the bladder, rectum, pancreas, uterus, ovary and colon can also involve bones with a frequency varying from 9 to 30%, while other gastrointestinal cancers seldom affect the skeleton (Coleman, 1997, 2001; Roodman, 2004; Lipton and Vigorita, 2016). Bone metastases are located predominantly in the vertebrae, pelvis, ribs, skull and ends of the long bones (Mundy, 1997; Chew, 2012; O'Sullivan, Carty and Cronin, 2015; Randall, 2015; Lipton and Vigorita, 2016). This pattern of distribution of bone metastases is mostly due to the direct route for spread of the Batson's venous plexus (Batson, 1940; Nathoo *et al.*, 2011). And metastases distal to the knees and elbows are unusual (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003).

The propensity of primary tumor cells to metastasize to bone demonstrates that bone metastases are a common clinical feature (Gruber, 2012). Typically, bone metastases are classified into three types: osteolytic, osteoblastic and mixed metastases (Coleman, 1997, 2001; Brothwell, 2008); however, they generally express both osteolytic and osteoblastic components and range from mostly blastic to mostly lytic (Mundy, 1997, 2002; Randall, 2015; Miler, Thomas and Shiozawa, 2017). Osteolytic metastases extend from osteoclastic resorption of cortical bone to complete focal bone destruction of the cortex, they can present as coalescing porosity, osteolytic foci or destruction of bone parts. Osteolytic foci are discrete, multiple, irregularly round osteolytic lesions of variable size with a denticulated or scalloped perimeter. On radiographs, the lesions manifest as geographic lucent areas (either well-defined or ill-defined, with or without sclerosis) or "moth-eaten" (multiple sites of osteoclasia) (Strouhal, 1991; Aufderheide and Rodríguez-Martín, 1998; Coleman, 2001; Ortner, 2003; Waldron, 2008; Theriault and Theriault, 2012; Ragsdale, Campbell and Kirkpatrick, 2018). Osteoblastic metastases can appear as new bone deposits coating the existing trabecula called "spongiosclerosis" (Ortner, 2003, p. 52), or as periosteal reactions, including woven

bone, lamellar bone (that is, remodeled woven bone), coral-like or even spiculated periosteal bone. The excess deposition of new bone is seen radiographically as an area of increased density and opacity and is defined as "osteosclerotic" (Vilar, Lezana and Pedrosa, 1979; Waldron, 1997, 2008; Aufderheide and Rodríguez-Martín, 1998; Smith, 2002; Ortner, 2003; Theriault and Theriault, 2012). Spiculated periosteal reactions may be "perpendicular to the cortex or slightly angulated, short or long, fine or coarse, few in numbers or forming a well-developed palisade" (Bloom *et al.*, 1987). "Hair-on-end" periosteal reactions are defined as parallel spiculated, whereas "sunburst" are divergent spiculated periosteal reactions (Ragsdale, Campbell and Kirkpatrick, 2018). While osteolytic metastases tend to be rapidly growing and aggressive, sclerotic metastases indicate a slower progression (Galasko, 1986; Rybak and Rosenthal, 2001; O'Sullivan, Carty and Cronin, 2015). Nonetheless, lamellated and spiculated periosteal reactions are aggressive types associated with malignancy (Bloom *et al.*, 1987; Miller, 2008). Mixed metastases present both osteolytic and osteoblastic criteria and manifest as a cortical osteolytic lesion surrounded by a sclerotic area on x-rays (Theriault and Theriault, 2012).

Most metastases to bone are osteolytic (75%), while 15% are osteoblastic and 10% are mixed; they are usually multifocal although solitary metastases may appear (Ortner, 2003). Certain solid tumors may show a predilection: the carcinomas of the kidney, lung and thyroid are predominantly osteolytic, prostate metastases are predominantly osteoblastic and breast cancer exhibits most commonly mixed metastases (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Brothwell, 2008; Waldron, 2008; Randall, 2015; Lipton and Vigorita, 2016). These mixed metastases in breast cancer may be the result of osteoblastic or sclerotic reaction as an attempt at bone repair surrounding osteolytic metastases in a predominantly lytic carcinoma (Mundy, 2002). The primary tumor mechanisms responsible for osteolytic and/or osteoblastic metastases are based on the specific expressions of tumor factors and their selective interaction with the osteoblastic and osteoclastic cells function and activity (Guise *et al.*, 1996, 2006; Guise, Yin and Mohammad, 2003). On the opposite, multiple myeloma is by definition purely osteolytic (Roodman, 2004; Randall, 2015) due to an impairment in osteoblastic cells function and an excessive osteoclastic activity resulting in severe bone loss (Bataille, Chappard and Klein, 1992; Giuliani, Rizzoli and Roodman, 2006; Theriault and Theriault, 2012).

Bone metastases can lead to skeletal-related events associated with an increased

clinical morbidity and life-threatening complications. It is estimated that an individual with metastatic carcinoma will experience a skeletal-related event every 3 to 6 months (Coleman, 2006). About two-thirds of patients with breast cancer will develop one or more skeletal-related events (Harvey and von Reyn Cream, 2007). These include severe bone pain, pathologic fractures, hypercalcemia, spinal cord compression and other nerve compression syndromes. Bone pain and impaired mobility are noted in 65-75% of patients with bone metastases (Vassiliou, Chow and Kardamakis, 2013). Pathologic fractures are most commonly observed in osteolytic metastases affecting the cortex of weight-bearing bones. Spinal cord compression is a medical emergency associated with a poor prognosis. Hypercalcemia is a life-threatening complication resulting from the release of calcium during bone destruction (Coleman, 2006; Harvey and von Reyn Cream, 2007; Vassiliou, Chow and Kardamakis, 2013; Randall, 2015). Although most of these skeletal-related events are clinical outcomes, pathologic fractures and vertebral collapse are possible to find on dry bones (Aufderheide and Rodríguez-Martín, 1998).

3.5.4. Diagnosis and differential diagnosis

The diagnosis of metastatic cancer depends on several factors: the age of the skeleton, the distribution of the lesions as well as their morphology, size and nature. Bone can only react in two ways: either with bone proliferation or with bone resorption. Consequently, osteolytic and osteoblastic lesions can be evocative of multiple pathological conditions. Therefore, other criteria have become necessary to distinguish them on dry bone, including the pattern of distribution of the lesions, their shape and characteristics, and the sex and age of the individual as some pathologies show a preference for certain sex and age categories. As described before, cancer metastases to bone can be osteolytic (coalescing porosity or round/oval osteolytic foci of various size), osteoblastic (spongiosclerosis or periosteal reaction) or mixed (with both osteolytic and osteoblastic components); they tend to be multifocal and located primarily in the axial skeleton, the skull and proximal ends of the femora and humeri with a sparing of the bones distal to the knees and elbows. Moreover, metastatic carcinoma has a peak onset in individuals over 40 years of age (Miller, 2008). This specific morphology, distribution and age category is suggestive of metastatic carcinoma (Anderson, Wakely and Carter, 1992; Duhig, Strouhal and Němečková, 1996; Šefčáková *et al.*, 2001; Marks and Hamilton, 2007; Luna *et al.*, 2008; Assis and Codinha,

2010; Wasterlain, Ascenso and Silva, 2011; Marques, Santos and Cunha, 2013; Binder *et al.*, 2014) but a differential diagnosis must be considered.

When the lesions are predominantly osteolytic, the most likely conditions responsible also include multiple myeloma, Langerhans cell histiocytosis, leukemia, secondary lesions to tuberculosis and taphonomic alterations (in particular osteophageous insects and roots). The ability of taphonomic bone alterations to mimic pathological lesions is the reasons why pseudopathology should always be considered in the differential diagnosis of osteolytic lesions. Weathering may cause cracking, flaking and in more advanced cases, removal of the external layers of the bone (Behrensmeyer, 1978). Carnivore alterations on skeletal elements includes grooves, scalloped edges, when teeth have penetrated the bone cortex, and puncture marks, described as shallow circular to oval lesions with small cortex fragments oriented perpendicularly to the bone surface and pressed into the interior (Milner and Smith, 1989). Insects, and in particular dermestid beetles and termites, may also present morphological similarities to osteolytic lesions. Dermestid beetles cause characteristic ovoid shaped pits of regular size and smooth delimited margins (Huchet *et al.*, 2011; Corron *et al.*, 2017). Derry (1911) first described the damage done to bones by termites making holes with "white, gnawed edges". Termites have been seen to favor skulls, creating pits, perforations and tunneling, associated with star-shaped marks, parallel striations and etched bone surface (Huchet *et al.*, 2011; Backwell *et al.*, 2012). Post-depositional damage caused by roots, termites or beetles can mimic the superficial appearance of osteolytic lesions due to cancer metastases. However, these lesions do not extend within the bone, they appear as regular demarcated tunnels and insects do not show a preference for particular skeletal districts (Strouhal, 1991; Binder *et al.*, 2014).

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*. The morphology of the skeletal lesions due to tuberculosis is not specific and primarily osteolytic in nature, and thus may overlap with osteolytic bone metastases. Nonetheless, tuberculosis is a disease of young people, with rare purely osteolytic lesions. Pott's disease (osteolytic destruction of a vertebral body causing a collapse of the superior vertebra and a sharp angulation of the spine due to the preservation of the neural arches of the vertebrae) is the most common form of skeletal involvement in bone tuberculosis. Lesions in the spine almost exclusively involve vertebral bodies, deformation of the joints and ankyloses may appear, affection of the bones of the hands and feet is frequent, and lesions on the cranium are rare in adults (Aufderheide

and Rodríguez-Martín, 1998; Ortner, 2003).

Langerhans cells histiocytosis (originally known as Histiocytosis X (Lichenstein, 1953)) represents three syndromes (Letterer-Siwe disease, Hand-Schtiller-Christian disease, and eosinophilic granuloma) of unknown etiology, characterized by an abnormal proliferation and mobility of histiocytic cells. This malignant pathology can produce sharply circumscribed osteolytic lesions of 10 to 20 mm with little to no reactive bone that may appear similar to bone metastases (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Marks and Hamilton, 2007). Nevertheless, the condition affects males twice as frequently as females with a frequency of 80% under 30 years and 50% under 10 years (Dorfman and Czerniak, 1998), and is thus less likely to be found in mature adults.

Leukemias are a group of malignant cancers of the myeloid and lymphoid hematopoietic cells of the bone marrow. Lesions present as numerous and diffuse superficial solitary pits (1 to 3 mm) with smooth minimally remodeled margins and/or as focal "fronts of resorption" in non-articular areas, thus leukemia can be distinguished macroscopically from metastatic carcinoma on dry bone (Rothschild *et al.*, 1997).

Multiple myeloma is a highly malignant plasma cell tumor emanating in the blood-forming bone marrow. Both multiple myeloma and metastatic carcinoma have a peak onset in individuals over 40 years, causing multifocal lesions with an outward progression in the bone, most commonly in the axial skeleton, thoracic cage, skull and proximal ends of femora and humeri (Strouhal, 1991). This considerable overlap explains why the distinction between these two diseases may be extremely challenging, if not impossible in some cases (Ortner, 2003). In spite of these difficulties, differences do exist. Multiple myeloma is purely osteolytic and affects males most commonly with a ratio of 2:1 contrasting with the female predominance and the possible remodeling of metastatic carcinoma. In the former, foci are numerous and scattered with a regularly spheroid or round geometry, sharp edges and smooth surrounding bone. By opposition, the latter exhibits less numerous and localized osteolytic foci, with an irregular morphology and scalloped margins. Moreover, lesions are thought to be of uniform small to medium size in multiple myeloma contrary to metastatic carcinoma, but this criterion is not sufficiently reliable alone (Rothschild, Hershkovitz and Dutour, 1998). On radiographs, lesions due to multiple myeloma are sharply demarcated and do not present an obscure, cloudy or "moth-eaten"

appearance characteristic of metastatic carcinoma (Strouhal, 1991, 1993; Rothschild, Hershkovitz and Dutour, 1998; Wakely *et al.*, 1998; Marks and Hamilton, 2007). Another element helping to distinguish multiple myeloma from metastatic carcinoma is the suggested restriction of myeloma lesions in the spine to the vertebral bodies with sparing of the posterior elements (Jacobson *et al.*, 1958).

Fungal disease, specifically blastomycosis, may also be differentiated from bone metastases in their involvement of cortical/trabecular bone: equal in the former, more extensive in the trabecular bone for the latter (Hershkovitz *et al.*, 1998).

The most common differential diagnosis on dry bone of proliferative lesions evocative of osteoblastic metastases includes Paget's disease, Ewing's sarcoma, osteosarcoma, and infectious conditions such as osteomyelitis and pulmonary tuberculosis.

Pulmonary tuberculosis is associated with a periosteal reaction on the ribs (Roberts, Lucy and Manchester, 1994) that may be confused with proliferative bone metastases, common in this skeletal area. However, rib periostitis in cases of known pulmonary tuberculosis is restricted to the visceral surface of the rib, mainly on the vertebral end and shows a layer of new bone that contrasts with the exuberant "coral-like" appearance of periosteal new bone observed in metastatic bone disease (Santos and Roberts, 2006). Osteomyelitis is a bone infection primarily involving the medullar cavity of long bones, characterized by a sequestrum (bone necrosis), surrounded by an involucrum (envelope of reactive new bone) and the presence of a draining cloaca (Ortner, 2003).

Primary bone malignancies, in particular Ewing's sarcoma and osteosarcoma, may appear similar to osteoblastic metastases. Sarcoma distant metastases are possible but considerably rarer than epithelial tumor metastases. Osteosarcoma is the most common primary bone tumor, presenting both osteolytic and osteoblastic lesions and often exhibiting the characteristic spiculated sunburst periosteal reaction. The malignancy is present in 80% of cases in individuals under 30 years of age, in males twice often as females, affecting primarily the knee but the proximal femur and humerus are other possible locations (Ortner, 2003; Brothwell, 2008; Miller, 2008; Waldron, 2008). Ewing's sarcoma is an uncommon primary tumor occurring in the femur, pelvis or tibia of adolescents and young adults (under 30 years), males more than females. The aggressive tumor may progressively involve more bones, with both osteolytic lesions and the typical "onion-skin" appearance of periosteal reaction (Biermann and Baker, 2000; Brothwell, 2008; Miller, 2008; Waldron, 2008).

Paget's disease is a chronic skeletal disease following three stages: (1) an osteolytic stage; (2) a mixed osteolytic and sclerotic stage with a predominant osteoblastic activity; and (3) a blastic stage with a declining osteoblastic activity (Ortner, 2003; Waldron, 2008; Merczi *et al.*, 2014). In the final stage, the bones become enlarged with cortical thickening due to periosteal bone deposition. The disorder typically affects the skull, lumbar spine, pelvis and proximal femur of individuals over 40 years. The best diagnostic criteria of Paget's disease is the pathognomonic mosaic pattern observable during histological examination (Ortner, 2003; Waldron, 2008; De Boer, Van der Merwe and Maat, 2013).

3.5.5. Identification of the primary organ of origin

The ultimate step in the analysis of bone metastases on dry bone is the tentative of identification of the organ of origin of the cancer. Of course, a definite diagnosis of the primary tumor on dry bones is too hazardous to assess. Nonetheless, distinctions in the pattern of bone involvement between primaries can sometimes allow the construction of a most probable case scenario.

As mentioned earlier, the carcinomas of the prostate, breast, thyroid and lung are the most common to metastasize to bone, with a frequency of 65 to 80% of cases for the first two primaries. Prostate cancer most commonly involves the vertebrae (in particular, the lumbar spine), pelvis, ribs, long bones (especially the femur), skull and sternum (Prates *et al.*, 2011; Lipton and Vigorita, 2016). Contrary to the majority of carcinomas, prostate bone metastases are predominantly osteoblastic with proliferative bone reaction and osteosclerosis. In fact, osteoblastic metastases are so typical in prostate cancer that "all patients with prostate carcinoma will develop osteoblastic bone metastases if they live long enough" (Mundy, 1997). However, an osteolytic component remains; mixed metastases may be observed and osteolytic ones are scarce (Lipton and Vigorita, 2016). Spiculated or "sunburst" periosteal reactions are a rare occurrence in metastatic carcinoma but known to prostatic cancer (Bloom *et al.*, 1987; Waldron, 1997, 2008; Castoldi *et al.*, 2017). Therefore, the high occurrence of prostatic bone metastases, male sex, the typical axial skeletal distribution and the specific morphology of the lesions with osteoblastic predominance makes the diagnosis of prostate cancer very likely (Tkocz and Bierring, 1984; Baraybar and Shimada, 1993; de la Rúa, Baraybar and Etxeberria, 1995; Wakely, Anderson and Carter,

1995; Mays *et al.*, 1996; Schultz *et al.*, 2007; Prates *et al.*, 2011; Merczi *et al.*, 2014).

Breast carcinoma is the most common cancer diagnosed in women, the leading cause of cancer-related deaths in female sex, and the first cause of death in women between 40 and 59 years (Randall, 2015; Siegel, Miller and Jemal, 2018). While breast cancer metastases to bone are predominantly osteolytic, most metastases are actually mixed (Rosenthal, 1997; Aufderheide and Rodríguez-Martín, 1998; Randall, 2015). The most common location affected are the vertebrae and pelvis, followed by the skull, ribs, femur and humerus (Allison *et al.*, 1980; Waldron, 2008; Randall, 2015; Lipton and Vigorita, 2016). Male breast cancer does occur but represents less than 1% of all breast cancer cases (Anderson *et al.*, 2010). Given the propensity of breast cancer metastases to bone, a mixed pattern of bone metastases primarily in the axial skeleton of a female individual over 40 years of age is highly suggestive of a breast metastatic carcinoma (Allison *et al.*, 1980; Strouhal, 1993; Smith, 2002; Melikian, 2006; Merczi *et al.*, 2014). Nonetheless, and despite this high likelihood, it does not exclude other carcinomas as the prime infection and a definite diagnosis cannot be achieved from the sole macroscopic observation of dry bone. Similarly, a case of osteolytic bone metastases distinct from multiple myeloma found on a male individual, especially if the ribs are particularly affected, may be suggestive of a case of malignant carcinoma of the lung (Grupe, 1988). But again, this combination of factors does not strictly exclude other carcinomas as possible primary organs of origin.

Consequently, the diagnosis of the primary site of infection on dry bone can only be narrowed down to the most probable case scenario, based on the sex and age of the individual, the skeletal distribution of the metastases and the morphology of the lesions; but in the end, "the final decision has to be made on probabilistic grounds" (Waldron, 2008).

The diagnosis of bone metastases is of significant value to both forensic sciences and the anthropological practice in archaeology. Nonetheless, the documentation of bone metastases remains scarce in the literature. Thus, more research based on established clinical diagnoses, such as studies on identified skeletal collections or radiological investigations, would increase the confidence in the anthropological diagnosis and help anthropologists in the differential diagnosis of unknown cases. In addition, further research on pathological biomarkers (Pérez-Martínez *et al.*, 2016) may allow a definitive diagnosis of the disease and could permit the identification of the primary organ of origin.

Chapter 4. Materials and Methods

"Life can only be lived forwards, but must be understood backwards."
Søren Kierkegaard

4.1. Materials

4.1.1. The CAL Milano Cemetery Skeletal Collection

The CAL Milano Cemetery Skeletal Collection is a part of the larger *Collezione Antropologica LABANOF* (CAL) collection under study at the LABANOF (*Laboratorio di Antropologia e Odontologia Forense*) and housed in the Department of Biomedical Sciences for Health, University of Milan, Italy (Cattaneo *et al.*, 2018). The CAL collection is composed of:

- the Archaeological Collection, comprising about 5,000 skeletons from over 50 Italian excavation sites representing over 2,000 years of history;
- the Histological Collection, constituted of about 2,000 slides for the microscopic interpretation of bone material, specifically age estimation, species diagnosis, interpretation of taphonomic and traumatic lesions, analysis of burnt osseous material and investigation of pathological lesions;
- the Forensic Osteological Series, containing bone calluses at different healing stages, traumatic lesions (gunshot injuries, sharp lesions, cuts, blunt force trauma, fractures), pathological lesions and elements for age estimations from individuals with recorded demographic data (pubic symphyses, 4th ribs and monoradicular teeth);
- the *Cranioteca* from the Psychiatric Hospital of *Mombello di Limbiate* (86 donated skulls from the early 1900 with cases of hydrocephaly, microcephaly and macrocephaly, among others);
- and the CAL Milano Cemetery Skeletal Collection.

The CAL Milano Cemetery Skeletal Collection is a modern and documented reference osteological collection of 2,127 unclaimed skeletal remains. Each of the individuals of the collection was first buried in a cemetery of Milan (*cimitero Maggiore*, *cimitero di Lambrate* and *cimitero di Baggio*) for a minimum of 10 years, before being exhumed by cemetery workers. In Italy, the article 43 of the Presidential Decree of the Italian

Republic (DPR) n.285 of September 10th, 1990 of the National Police Mortuary Regulation permits cemeteries to grant unclaimed skeletal remains to universities for education and research purposes.

The collection is continuously growing, but as of 2018 it was constituted of individuals from both sexes (51.5% female, 47.9% male and 0.6% of individuals for whom the information is lacking), mostly adults (91% of the collection) but with a valuable representation of subadults (9%, n = 189). The ages-at-death range from 0 to 104 years with years of birth extending from 1866 to 2000 and years of death ranging from 1910 to 2001. It is a modern osteological collection as about 80% of the individuals died after 1980 and 65% after 1990.

One of the main advantages of the collection is that the skeletal remains are associated with a documentation including demographic data (consisting of sex, age-at-death, date of birth and date of death) as well as the "ISTAT" (*Istituto Nazionale di Statistica*) death certificate. The completion of the "ISTAT" death certificates has become mandatory under the article 1 of the DPR of September 10, 1990 n.285 of the Italian Mortuary Police Regulation, and consists in the pronunciation of the death of the individual and the establishment of the cause of death for sanitary, epidemiological and statistical purposes. The medical doctor who pronounced the passing of the individual must indicate on the ISTAT certificate, in the event of a natural death, the morbid sequence that led to it as well as any relevant pathological condition, and in the circumstance of a violent death, the modality, description and complications that ensued. The recovery of the ISTAT certificates is still in progress and as of 2018, they were available for 1370 individuals of the collection (Cattaneo *et al.*, 2018).

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Forensic Anthropology Population Data

A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection

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ABSTRACT

The CAL Milano Cemetery Skeletal Collection is a modern and continuously growing identified osteological collection of 2127 skeletons under study in the *Laboratorio di Antropologia e Odontologia Forense* (LABANOF) in the Department of Biomedical Sciences for Health of the University of Milan (Italy), and part of the *Collezione Antropologica LABANOF* (CAL). The collection presents individuals of both sexes and of all age groups with a high representation of the elderly and an interesting sample of infants. Each individual is associated with a documentation that includes sex, age-at-death, dates of birth and death, and a death certificate that specifies the exact cause of death and the chain of events that led to it (related pathological conditions or traumatic events). It was also possible to recover for several individuals the autopsy reports and antemortem photographs.

This documented osteological collection is of crucial interest in physical and forensic anthropology: it provides unique teaching opportunities and more importantly considerable research possibilities to test and develop sex and age estimation methods, investigate key subjects of forensic relevance and discuss pathological markers, among others. The aim of this paper is to introduce the CAL Milano Cemetery Skeletal Collection as a new identified skeletal collection and present its research and teaching potential.

1. Introduction

In the past few decades, the need for identified skeletal collection has become clear and imperative. Bocquet-Appel and Masset demonstrated that simple regression methods resulted in a reflection of the age structure of the identified population studied [1]. Moreover, the morphology, efficiency and applicability of skeletal age indicators and sexually dimorphic traits vary across populations, calling for population-specific methods. Osteological collections are of paramount utility for the physical anthropologist, especially in a forensic context, where precise personal and medical details are crucial for the identification of unknown deceased or mass catastrophes victims; and in archeology, for a better reconstruction and understanding of the health and demography of past populations. Thus, identified skeletal collections constitute undeniable assets for research purposes. They allow for the development of new methods for age, sex, stature and ancestry estimations [2–9] and for testing and improving existing methods [10,11]. They provide data on specific populations, which in turn permits comparisons across populations and a higher scientific precision for the identification process in a forensic context. Indeed, the evident skeletal variations between different geographical populations call for population specific methods for at least sexing and aging skeletal remains [12–14]. Moreover, secular change due to environmental and genetic differences have been observed in both cranial and post-cranial features and may influence anthropological sex and ancestry estimations among others [15–20]. Identified skeletal collections are also a formidable and ideal tool for diagnosing disease and forensic studies given their documentation concerning known pathology and causes of death.

Although Usher specified that no such ideal exists, she described the criteria needed in an “ideal” human identified skeletal collection [21]: the age-at-death of the individuals should

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be known, by opposition to self-reported; the collection should represent the variation present in the population of interest including socioeconomic background, ethnicity and health; all ages and both sexes should be well represented; and the collection should be easily accessible.

The first identified skeletal collection started in the beginning of the 20th century with the Hamann-Todd (United States) [22], Terry (United States) [23], Raymond A. Dart (South Africa) [24] and Huntington (United States) [15] collections constituted of cadaver-derived human skeletons. Known collections are now developing around the globe [25] and becoming a source of substantial research and scientific progress, including the 21st century Identified Skeletal Collection in Coimbra (Portugal) [26], the Bocage Museum Identified Skeletal Collection in Lisbon (Portugal) [27], the Human Skeletal Identified Collection of Modern Colombian Population in Bogota (Colombia) [28], the Athens Collection in Athens (Greece) [29], the Granada Osteological Collection of Identified Infants and Young Children in Granada (Spain) [30], the UAB Identified Skeletal Collection in Barcelona (Spain) [31], the Pretoria Bone Collection in Pretoria (South Africa) [32] and the Sassari Collection in Bologna (Italy) [33].

Recently, contemporary repositories of skeletal data comprising various types of medical imaging have developed and added new possibilities to research and practice in forensic sciences. These virtual databases possess promising potential for scientific investigations; they may be based on any kind of medical image format ranging from radiological imaging to full 3D bone models compiled from computed tomography scans [34–36].

In this article, we present the CAL Milano Cemetery Skeletal Collection, a modern, known and documented collection of more than 2100 identified skeletons and growing, curated at the *Laboratorio di Antropologia e Odontologia Forense* (LABANOF) in the Department of Biomedical Sciences for Health of the University of Milan (Italy), and part of the *Collezione Antropologica LABANOF* (CAL). CAL was formally established by the University of Milan (*Università degli Studi di Milano*) as a collection of the University Museum System in December 2017 and acknowledged as a Museum Collection by the Lombardy Region and thus was inserted in the Lombardy Museum System in February 2018.

2. The CAL Milano Cemetery Skeletal Collection

2.1. Administrative and legal contexts

In Italy, the article 43 of the Presidential Decree of the Italian Republic (DPR) n.285 of September 10th, 1990 of the National Police Mortuary Regulation allows the cemeteries to grant unclaimed skeletal remains to universities for education and research purposes.

An agreement between the LABANOF, the city of Milan and the *Cimitero Maggiore*, the main cemetery of Milan, was concluded in 2008 for the acquisition and assemblage of a new and contemporary cemetery skeletal collection. Furthermore, and in collaboration with the *"ASL"* (*Azienda Sanitaria Locale*), it was possible to gather the documentation associated to each individual. This documentation includes demographic data (sex, age, date of birth, date of death) and the *"ISTAT"* (*Istituto Nazionale di Statistica*) death certificates (specifying the causes of death and the pathological conditions that led to it); when possible, autopsy reports were recovered at the Institute of Legal Medicine of the University of Milan.

2.2. Recovery and storage

Each individual is buried in a coffin and exhumed usually after 10 years. If the corpse is not skeletonized, the coffin is broken in order to let the soil enter and aid the skeletonization during the following 3–5 years. After this period of skeletonization, the body is exhumed again by cemetery workers and the skeletons are placed in metal boxes in an ossuary for 5 years. Finally, selected unclaimed skeletal remains can enter the collection, available for study at the *Laboratorio di Antropologia e Odontologia Forense* (LABANOF) housed in the Department of Biomedical Sciences for Health of the University of Milan (Italy).

The skeletons arrive in the laboratory in zinc boxes, equipped with an identifier plate where the name, date of birth, and date of death are reported. Each metal box holds a single individual and is associated with a cemetery form containing personal data and information regarding the burial and the exhumation. This documentation includes the modality of the burial, the exact date and place as well as the numbers of the grave and cemeterial area where the individual was buried. The demographic details regarding each individual, constituting sensitive data according to the law, are treated in agreement with the regulations and with prior authorization from the National Data Protection Supervisor. Each skeleton is stored and archived in the collection after the gathering of its associated data and made anonymous with a progressive number that will serve as an identifier.

3. Description of the collection

The collection of the skeletal remains began in January 2012 with the acquisition of the first 265 individuals. From there on, the collection saw a rapid numerical increase along with the abundant gathering of critical data, of both demographic and clinical/autopsy nature. Over the past three years, its expansion reached a total number of 2127 specimens coming from three cemeteries of Milan: *Cimitero Maggiore* (n = 1692, 80%), *Cimitero di Lambrate* (n = 294, 14%), and *Cimitero di Baggio* (n = 141, 6%), with a substantial number of complete or almost complete skeletons. It is important to note that the collection is in constant growth and so new individuals are continuously added.

3.1. Demographic composition

Currently, the cemetery collection is composed of 2127 skeletons of both sexes, with ages at death ranging from 0 to 104 years. Most of the collection is constituted of adult individuals (91%, n = 1938), but there is also a valuable representation of subadults (9%, n = 189). The years of birth range from 1866 to 2000 and the dates of death from 1910 to 2001. However, 80% of the individuals of the collection died after 1980 and 65% after 1990. Moreover, both sexes are equally represented. Indeed, the female sample constitutes 51.5% (n = 1096) of the collection, with ages-at-death ranging from 0 to 104 years old and the male sample presents 1019 individuals (47.9%), aged between 0 and 101 years old. The remaining 0.6% (n = 12) is constituted of unknown individuals (i.e., people for whom name or birth and death information were not available). A descriptive table of the collection is provided in Table 1.

Table 1
General descriptive table of the CAL Milano Cemetery Skeletal Collection.

	Numbers	Percentages
Males	1019	47.9%
Females	1096	51.5%
Adults	1938	91%
Subadults	189	9%
Including infants	97	4.5%
ISTAT medical data[a]	1370	64.4%
Recovered autopsies[a]	123	5.8%

[a] The recovery of the ISTAT death certificates and autopsy reports is still ongoing.

3.2. The associated documentation and the sanitary profile of the skeletal collection

The completion of the "ISTAT" death certificates has become mandatory under the Article 1 of the DPR of September 10, 1990 n.285 of the Italian Mortuary Police Regulation. In this article, the pronunciation of the death of an individual has to be communicated so that the cause of death can be irrevocably established. The intent of this regulation is to realize a national health survey for sanitary, epidemiological and statistical purposes. In case of natural death, the medical doctor who ascertains the death must indicate on the ISTAT certificate the morbid sequence that caused it as well as any other relevant disease that led to the terminal one; and in case of violent death, the modality, description and complications that occurred. So far it has been possible to retrieve the ISTAT certificates for 1370 individuals (64.4% of the entire collection), among which 1319 are adults (96%), and 51 subadults (4%). Half of the remaining individuals (n = 404, 19% of the collection) died before 1980 and thus the death certificates were not as complete. Similarly, we do not have the associated documentation for migrants and individuals who passed away in other districts than those of Milan (where the collection of the data was conducted).

The most common causes of death in the adults of the collection are cardiac arrest, often arising as the final consequence of concomitant pathologies, and cancer. The collection also presents a high percentage of traumatic causes of death including car accidents, falls, gunshot wounds and mechanical asphyxia. The association of the individuals with their death certificates allows us to obtain valuable and rare information such as the presence of relevant pathological conditions during life. As a result, neoplastic diseases are the pathological conditions most represented in the individuals of the cemetery collection. We also have numerous cases of diabetes, pneumonia and HIV as well as occasional cases of rheumatoid arthritis, tuberculosis and hepatitis.

The availability of autopsy reports, stored at the Institute of Legal Medicine of Milan, represents yet another precious source of information concerning the individuals of the collection. Of course, these reports are only available for individuals who were autopsied at the Institute of Legal Medicine of Milan. Most of the individuals concerned died of a violent death and/or in suspicious circumstances. In the autopsy reports, specific details about the individual can be found (including height, weight, health status and the date of the last time seen alive) as well as a detailed description of the external examination and the results of the analyses conducted on particular organs. Finally, the exact cause and manner of death are reported in addition to any injury involving soft tissues and skeletal elements. To this date, 123 autopsy reports have been collected.

Moreover, we were able to retrieve and add to the collection high quality antemortem photographs of 254 individuals. These individuals were specifically selected for the optimal state of preservation and completeness of their skulls, which is an excellent instrument for possible studies on face morphology and facial identification.

3.3. State of preservation of the skeletons

The skeletal preservation varies among the collection, ranging from almost complete skeletons, with just a few bones of the hands and feet absent, to skeletons with only few skeletal districts present. Generally, the bones of the lower limbs (femora and tibiae) are the best preserved of the skeleton. On the other hand, ribs, sacrum and pelvis are commonly the least well-preserved areas and hands are usually partially found (in 34% of cases they are completely absent). However, the loss of some skeletal districts was expected as the bodies have been buried in the soil for at least 10 years. This taphonomic condition could have caused the erosion and destruction of some bones, because of environmental elements and the decomposition process itself. In addition, the recovery of the skeletons has been carried out by cemetery workers by means of heavy vehicles, a step that was performed without any forensic anthropology attendance and could have caused the fortuitous loss of some skeletal elements. When collected, the individuals are completely skeletonized. No residual soft tissues were present in any case, except for remains of adipocere and decomposition fluids mixed with the soil, depending on the conditions of preservation of the coffin. Similarly, the aspect and integrity of the skeletal material can remarkably change from one individual to another, varying from well preserved skeletal remains to fragile, brittle and fragmented bones. Hair and nails are occasionally present.

The anthropological study of the collection is continuously ongoing. So far, over 385 individuals have been fully studied (including 324 adults and 61 subadults). These skeletons were selected in part randomly from the entire collection, and in part because of their known cause of death in order to perform focused research.

4. Potential and contribution of the collection

The CAL Milano Cemetery Skeletal Collection is a modern and documented skeletal collection that also has the advantage of being a representation of a modern European (Italian) population. Indeed, it presents individuals of both sexes and of all age groups associated with a unique and growing documentation constituted of death certificates, autopsy reports and sometimes photographs. No discriminating strategy of recovery of the individuals from the cemetery was defined and so they present a very diverse background in terms of pathological conditions and causes of death.

This representative ability of the collection allows a demographic and epidemiological reconstruction of the population of Milan for over 100 years. Also, this collection possesses an undeniable teaching asset. The various states of taphonomic completeness and preservation of the skeletons going from intact completeness to fragmentation allows for efficient and practical anatomy and anthropology lessons.

The availability of a European (Italian) modern skeletal collection is in fact a powerful tool for the development of the forensic anthropological practice, especially to expand and enhance its possibilities and efficiency in this specific geographical area. Indeed, known demographic information such as age, sex, ethnicity and date of death are critical data to validate anthropological methods or demonstrate their limitations in a particular geographic area or ethnic group. The contribution provided by the available antemortem data is also of great value in the study of subadults. In particular, to improve the accuracy of aging methods for living children. Furthermore, in general, trauma analysis on skeletons can improve the forensic expertise on living individuals. This aspect is of particular importance as it could be helpful in contexts such as child abuse and neglect.

The benefits of the collection are therefore not limited to demography, but are extended to other fields of interest of the discipline of anthropology such as the study of taphonomy and trauma. The prospect of this study is made possible by the presence of detailed autopsy reports (albeit just for a limited number of individuals) and death certificates. As a result, the collection material can allow a positive control in the traumatic analysis of a skeleton, which can lead to a better understanding of some of the trickiest aspects of the forensic (and archaeological) practice. For instance, the forensic analysis of traumatic lesions can become

very challenging when altered by the superimposition of taphonomic variables. Similarly, the diagnosis of a pathology can be misled by the confusing effect of taphonomy. Many studies have already been conducted in order to answer these questions [37–42] (among others). In this perspective, a context of trauma and taphonomy associated with the knowledge of the presence (or absence) and location of the lesions is one of a kind and could provide decisive data in this field of research. Moreover, the skeletons are often mixed with soil in the boxes. This soil is later collected and stored in a separate bag. In this context, the effect of the pH, composition and humidity of the soil on the preservation of the bone tissue could also be investigated.

The study of bare bones can also supply considerable information regarding the health of deceased individuals [43–45], whether in an archaeological or forensic context. The ISTAT death certificates retrieved in our skeletal collection offer an invaluable tool for the understanding of the aspect of these pathologies on bones given that we know that the individuals were clinically diagnosed with the diseases during life. Moreover, this knowledge can allow the testing of existing diagnostic methods and that of new ones, such as radiographic images, histological methods, isotopic analysis [46], biomolecular analysis [47], and the identification of pathogenic DNA [48–50]. The CAL Milano Cemetery Skeletal Collection has promoted extensive research and allowed the training of many students and future anthropologists. Indeed, it was the object of four PhD theses and of about twenty Master theses. The collection has already been used to study the challenging distinction between perimortem and postmortem fractures [51], to test aging methods on older individuals [52] and sex estimation methods [53] and to investigate pathological markers on bones [54,55].

The numerous studies conducted, still underway and planned testify the potential and contribution of the CAL Milano Cemetery Skeletal Collection to the research in physical anthropology.

Other skeletal series are also available for study at the LABANOF, including archaeological, histological, autopsy and skull collections. First, the CAL Historical Skeletal Collection is an archaeological assemblage of approximately 3000 individuals from 50 Italian (in particular Lombardy, Piedmont, Liguria, Veneto, Trentino Alto Adige, Lazio, and Puglia) and foreign (Switzerland) archaeological sites, ranging from the 9th century BC to the 17th century AD. Second, the Histological Collection counts more than 2000 slides mainly for the microscopic interpretation of bone material, including age estimation, species diagnosis, taphonomic and traumatic lesions interpretation, the analysis of burnt bones and the investigation of pathological lesions. Third, the Forensic Osteological Series consists of bone calluses (from different skeletal districts and at different healing stages), traumatic lesions (gunshot injuries, sharp lesions, cuts, blunt force trauma, fractures), pathological lesions and osteological elements for age estimation (pubic symphyses, 4th ribs and monoradicular teeth). Finally, the *cranioteca* from the Psychiatric Hospital of *Mombello di Limbiate* consists of 86 donated skulls from the early 1900 presenting some very interesting samples of hydrocephaly, microcephaly and macrocephaly among others.

5. Conclusion

The CAL Milano Cemetery Skeletal Collection is a continuously growing osteological collection of more than 2100 skeletons. Its unique aspect lies in two main advantages. First, it is a modern skeletal collection coming from three cemeteries of Milan without any discriminating sampling strategy, and thus it is representative of the modern Italian population. Second, each skeleton of the collection is associated with a documentation that includes demographic data (sex, age, date of birth and death) as well as a death certificate detailing the exact cause of death and the chain of events that led to it, whether pathological or traumatic.

Although the work on the skeletal collection has barely begun, it has already provided numerous and valuable studies in multiple fields of research of physical and forensic anthropology. The collection possesses a considerable potential for both teaching and research purposes, notably through the testing and elaboration of anthropological methods for sex and age estimation, the focus study of specific subjects of forensic relevance and the investigation of pathologies on bare bones.

The CAL Milano Cemetery Skeletal Collection has proven to be a solid base for research on both ancient and modern skeletal remains, as well as for a better interpretation and investigation of forensic cases.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

[1] J.-P. Bocquet-Appel, C. Masset, Farewell to paleodemography, J. Hum. Evol. 11 (4) (1982) 321–333.
[2] T.W. Phenice, A newly developed visual method of sexing the os pubis, Am. J. Phys. Anthropol. 30 (2) (1969) 297–301.
[3] C.O. Lovejoy, R.S. Meindl, T.R. Pryzbeck, R.P. Mensforth, Chronological metamorphosis of the auricular surface of the ilium: a new method for the determination of adult skeletal age at death, Am. J. Phys. Anthropol. 68 (1) (1985) 15–28.
[4] R.S. Meindl, C.O. Lovejoy, Ectocranial suture closure: a revised method for the determination of skeletal age at death based on the lateral-anterior sutures, Am. J. Phys. Anthropol. 68 (1) (1985) 57–66.
[5] M.Y. Iscan, S.R. Loth, Estimation of age and determination of sex from the sternal rib, Forensic Osteology, (1986) , pp. 68–89.
[6] S. Brooks, J.M. Suchey, Skeletal age determination based on the os pubis: a comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods, Hum. Evol. 5 (3) (1990) 227–238.
[7] C. Rougé-Maillart, B. Vielle, N. Jousset, D. Chappard, N. Telmon, E. Cunha, Development of a method to estimate skeletal age at death in adults using the acetabulum and the auricular surface on a Portuguese population, Forensic Sci. Int. 188 (1) (2009) 91–95.
[8] M. Trotter, G.C. Gleser, A re-evaluation of estimation of stature based on measurements of stature taken during life and of long bones after death, Am. J. Phys. Anthropol. 16 (1) (1958) 79–123.
[9] D. Navega, C. Coelho, R. Vicente, M.T. Ferreira, S. Wasterlain, E. Cunha, AncesTrees: ancestry estimation with randomized decision trees, Int. J. Leg. Med. 129 (5) (2015) 1145–1153.
[10] L. Martrille, D.H. Ubelaker, C. Cattaneo, F. Seguret, M. Tremblay, E. Baccino, Comparison of four skeletal methods for the estimation of age at death on white and black adults, J. Forensic Sci. 52 (2) (2007) 302–307.
[11] E.J. Marlow, R.F. Pastor, Sex determination using the second cervical vertebra—A test of the method, J. Forensic Sci. 56 (1) (2011) 165–169.
[12] T.P. Gocha, M.E. Ingvoldstad, A. Kolatorowicz, M.-T.J. Cosgriff-Hernandez, P.W. Sciulli, Testing the applicability of six macroscopic skeletal aging techniques on a modern Southeast Asian sample, Forensic Sci. Int. 249 (2015) 318.e1–318.e7.
[13] H.M. Garvin, S.B. Sholts, L.A. Mosca, Sexual dimorphism in human cranial trait scores: effects of population age, and body size, Am. J. Phys. Anthropol. 154 (2) (2014) 259–269.

[14] Y.M. Iscan, Progress in forensic anthropology: the 20th century, Forensic Sci. Int. 98 (1–2) (1998) 1–8.
[15] L.M. Jantz, R.L. Jantz, Secular change in long bone length and proportion in the United States, 1800–1970, Am. J. Phys. Anthropol. 110 (1) (1999) 57–67.
[16] R.L. Jantz, L.M. Jantz, The remarkable change in Euro-American cranial shape and size, Hum. Biol. 88 (1) (2016) 56–64.
[17] K. Spradley, K.E. Stull, J.T. Hefner, Craniofacial secular change in recent Mexican migrants, Hum. Biol. 88 (1) (2016) 15–29.
[18] K.E. Weisensee, R.L. Jantz, An examination of the differential effects of the modern epidemiological transition on cranial morphology in the United States and Portugal, Hum. Biol. 88 (1) (2016) 30–37.
[19] S. Cridlin, Piecewise regression analysis of secular change in the maximum femoral vertical head diameter of American white males and females, Hum. Biol. 88 (1) (2016) 47–55.
[20] L. Manthey, R.L. Jantz, M. Bohnert, K. Jellinghaus, Secular change of sexually dimorphic cranial variables in Euro-Americans and Germans, Int. J. Leg. Med. 131 (4) (2017) 1113–1118.
[21] B.M. Usher, Reference samples: the first step in linking biology and age in the human skeleton., in: R.D. Hoppa, J.W. Vaupel (Eds.), Paleodemography: Age Distributions from Skeletal Samples, Cambridge University Press, Cambridge, 2002, pp. 29–47.
[22] B.M. Rothschild, C. Rothschild, Comparison of radiologic and gross examination for detection of cancer in defleshed skeletons, Am. J. Phys. Anthropol. 96 (4) (1995) 357–363.
[23] D.R. Hunt, J. Albanese, History and demographic composition of the Robert J. Terry anatomical collection, Am. J. Phys. Anthropol. 127 (4) (2005) 406–417.
[24] M.R. Dayal, A.D.T. Kegley, G. Strkalj, M.A. Bidmos, K.L. Kuykendall, The history and composition of the Raymond A. Dart collection of human skeletons at the University of the Witwatersrand, Johannesburg, South Africa, Am. J. Phys. Anthropol. 140 (2) (2009) 324–335.
[25] D.H. Ubelaker, Osteology reference collections, in: C. Smith (Ed.), Encyclopedia of Global Archaeology, Springer, New York, 2014, pp. 5632–5641.
[26] M.T. Ferreira, R. Vicente, D. Navega, D. Gonçalves, F. Curate, E. Cunha, A new forensic collection housed at the University of Coimbra, Portugal: the 21st century identified skeletal collection, Forensic Sci. Int. 245 (Supplement C) (2014) 202.e1–202.e5.
[27] H.F.V. Cardoso, Brief communication: the collection of identified human skeletons housed at the Bocage Museum (National Museum of Natural History), Lisbon, Portugal, Am. J. Phys. Anthropol. 129 (2) (2006) 173–176.
[28] C. Sanabria-Medina, G. González-Colmenares, H.O. Restrepo, J.M.G. Rodríguez, A contemporary Colombian skeletal reference collection: a resource for the development of population specific standards, Forensic Sci. Int. 266 (Supplement C) (2016) 577.e1–577.e4.
[29] C. Eliopoulos, A. Lagia, S. Manolis, A modern: documented human skeletal collection from Greece, HOMO—J. Comp. Hum. Biol. 58 (3) (2007) 221–228.
[30] I. Alemán, J. Irurita, A.R. Valencia, A. Martínez, S. López-Lázaro, J. Viciano, et al., Brief communication: the Granada osteological collection of identified infants and young children, Am. J. Phys. Anthropol. 149 (4) (2012) 606–610.
[31] C. Rissech, D.W. Steadman, The demographic, socio-economic and temporal contextualisation of the Universitat Autònoma de Barcelona collection of identified human skeletons (UAB collection), Int. J. Osteoarchaeol. 21 (3) (2011) 313–322.
[32] E.N. L'Abbé, M. Loots, J.H. Meiring, The Pretoria Bone Collection: a modern South African skeletal sample, HOMO—J. Comp. Hum. Biol. 56 (2) (2005) 197–205.
[33] S.M. Hens, E. Rastelli, G. Belcastro, Age estimation from the human os coxa: a test on a documented Italian collection, J. Forensic Sci. 53 (5) (2008) 1040–1043.
[34] M. Kistler, S. Bonaretti, M. Pfahrer, R. Niklaus, P. Buchler, The virtual skeleton database: an open access repository for biomedical research and collaboration, J. Med. Internet Res. 15 (11) (2013) e245.
[35] F. Radetzki, T. Mendel, H. Noser, D. Stoevesandt, M. Rollinghoff, N. Gutteck, et al., Potentialities and limitations of a database constructing three-dimensional virtual bone models, Surg. Radiol. Anat. 35 (10) (2013) 963–968.
[36] E. Aghayev, L. Staub, R. Dirnhofer, T. Ambrose, C. Jackowski, K. Yen, et al., Virtopsy—the concept of a centralized database in forensic medicine for analysis and comparison of radiological and autopsy data, J. Forensic Leg. Med. 15 (3) (2008) 135–140.
[37] S.E. Calce, T.L. Rogers, Taphonomic changes to blunt force trauma: a preliminary study, J. Forensic Sci. 52 (3) (2007) 519–527.
[38] L. Corron, J.B. Huchet, F. Santos, O. Dutour, Using classifications to identify pathological and taphonomic modifications on ancient bones: do "taphognomonic" criteria exist? BMSAP 29 (1) (2017) 1–18.
[39] K. Moraitis, C. Spiliopoulou, Identification and differential diagnosis of perimortem blunt force trauma in tubular long bones, Forensic Sci. Med. Pathol. 2 (4) (2006) 221–229.
[40] J. Pokines, S.A. Symes, Manual of Forensic Taphonomy, CRC Press, 2013.
[41] K.J. Reinhard, T.M. Fink, Cremation in Southwestern North America: aspects of taphonomy that affect pathological analysis, J. Archaeol. Sci. 21 (5) (1994) 597–605.
[42] D.H. Ubelaker, B.J. Adams, Differentiation of perimortem and postmortem trauma using taphonomic indicators, J. Forensic Sci. 40 (May (3)) (1995) 509–512.
[43] D.J. Ortner, Identification of Pathological Conditions in Human Skeletal Remains, 3rd ed., Smithsonian Institution Press, New York, 2003 647 pp.
[44] T. Waldron, Palaeopathology, Cambridge University Press, Cambridge, United Kingdom, 2008 279 pp.
[45] A.C. Aufderheide, C. Rodríguez-Martín, The Cambridge Encyclopedia of Human Paleopathology, Cambridge University Press, Cambridge, United Kingdom, 1998 478 pp.
[46] M.A. Katzenberg, N.C. Lovell, Stable isotope variation in pathological bone, Int. J. Osteoarchaeol. 9 (5) (1999) 316–324.
[47] T.H. Schmidt-Schultz, M. Schultz, Bone protects proteins over thousands of years: extraction analysis, and interpretation of extracellular matrix proteins in archeological skeletal remains, Am. J. Phys. Anthropol. 123 (1) (2004) 30–39.
[48] W.L. Salo, A.C. Aufderheide, J. Buikstra, T.A. Holcomb, Identification of mycobacterium tuberculosis DNA in a pre-Columbian Peruvian mummy, Proc. Natl. Acad. Sci. U. S. A. 91 (6) (1994) 2091–2094.
[49] A.M. Gernaey, D.E. Minnikin, M.S. Copley, R.A. Dixon, J.C. Middleton, C.A. Roberts, Mycolic acids and ancient DNA confirm an osteological diagnosis of tuberculosis, Tuberculosis 81 (4) (2001) 259–265.
[50] S. Mays, E. Fysh, G.M. Taylor, Investigation of the link between visceral surface rib lesions and tuberculosis in a medieval skeletal series from England using ancient DNA, Am. J. Phys. Anthropol. 119 (1) (2002) 27–36.
[51] A. Cappella, A. Amadasi, E. Castoldi, D. Mazzarelli, D. Gaudio, C. Cattaneo, The difficult task of assessing perimortem and postmortem fractures on the skeleton: a blind text on 210 fractures of known origin, J. Forensic Sci. 59 (6) (2014) 1598–1601.
[52] A. Cappella, M. Cummaudo, E. Arrigoni, F. Collini, C. Cattaneo, The issue of age estimation in a modern skeletal population: are even the more modern current aging methods satisfactory for the elderly? J. Forensic Sci. 62 (1) (2017) 12–17.
[53] D. Gibelli, P. Poppa, M. Cummaudo, M. Mattia, A. Cappella, D. Mazzarelli, et al., Sex assessment from the volume of the first metatarsal bone: a comparison of linear and volume measurements, J. Forensic Sci. 62 (6) (2017) 1582–1585.
[54] L. Biehler-Gomez, A. Cappella, E. Castoldi, L. Martrille, C. Cattaneo, Survival of atherosclerotic calcifications in skeletonized material: forensic and pathological implications, J. Forensic. Sci. 63 (2) (2018) 386–394.
[55] E. Castoldi, A. Cappella, D. Gibelli, C. Sforza, C. Cattaneo, The difficult task of diagnosing prostate cancer metastases on dry bone, J. Forensic Sci. (2017).

For this project, skeletons were selected based on the content of the associated documentation of the individuals of the collection and in particular their recorded pathological conditions to perform focus studies on the skeletal markers of disease.

Consequently, a total of **134 skeletal remains** from the CAL Milano Cemetery Skeletal Collection were selected and examined for this project (Fig. 1) including:

- 12 individuals with a diagnosed vasculopathy;
- all three individuals with rheumatoid arthritis from the collection;
- all 38 individuals with diabetes from the collection;
- all nine individuals of the collection with HIV (including four with AIDS);
- one individual diagnosed post-mortem with osteomalacia;
- both individuals with multiple myeloma in the collection;
- all 14 individuals of the collection with breast cancer;
- all 13 individuals of the collection with bladder cancer;
- 45 individuals with recorded cancer for signs of metastatic bone disease;
- and 10 individuals as a control sample with various causes of death exclusive of those studied and previously mentioned.

In some cases, the individuals were diagnosed with more than one pathology of interest (Appendix 1).

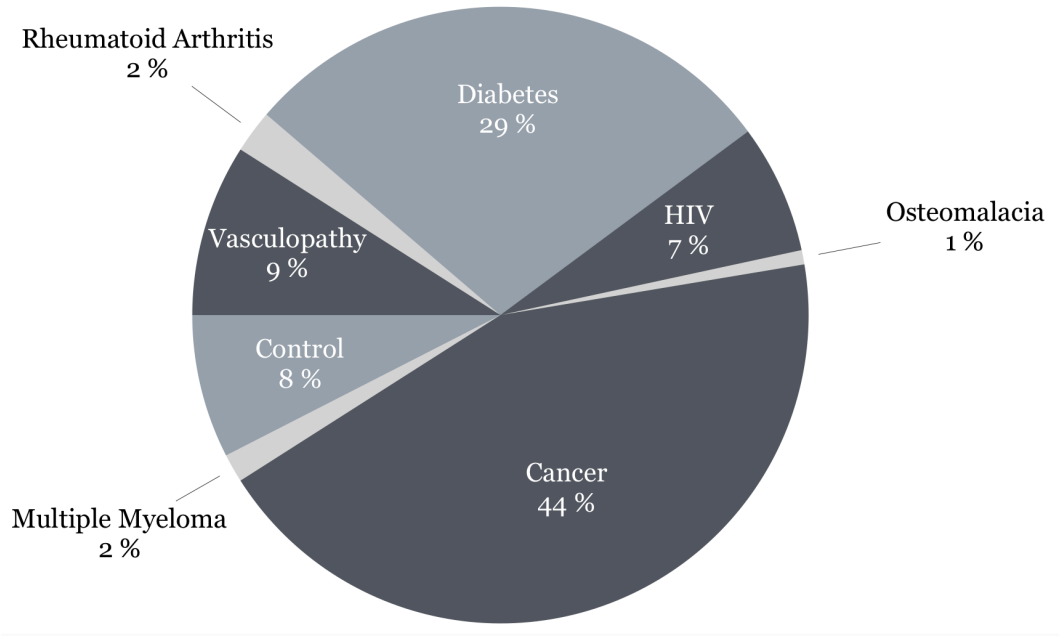

Figure 1: Distribution of the pathological conditions in the selected individuals

The distribution between sexes is almost even, with 69 females and 65 males (Fig. 2).

One hundred and seven of the 134 individuals selected (80% of the study sample) were comprised in the 60 to 89 years age groups, indicating an important representation of older individuals, although all adult age groups are represented in the study sample (Fig. 3).

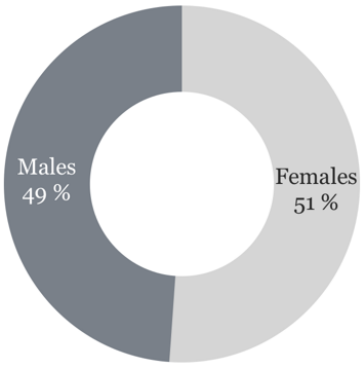

Figure 2: Sex distribution of the individuals selected

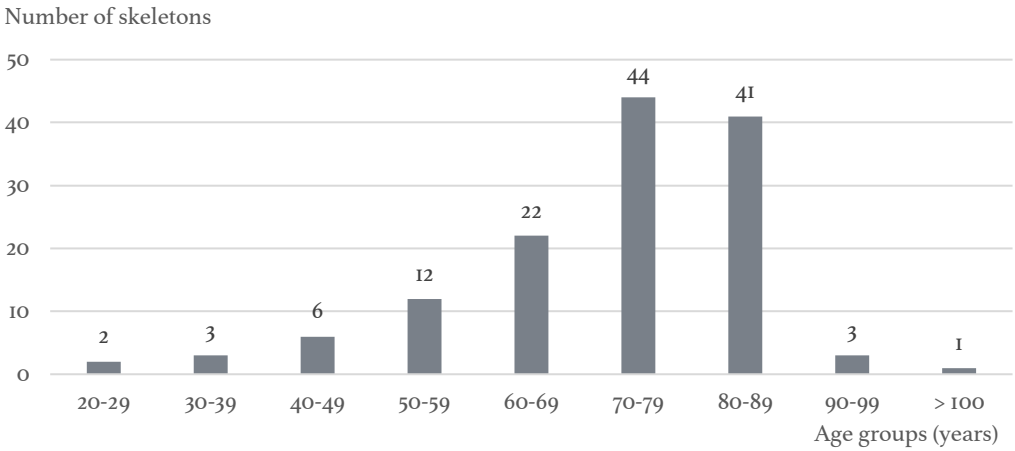

Figure 3: Age distribution of the skeletons selected

4.1.2. Archaeological specimens

Selected archaeological specimens were included for the particular lesions exhibited on bones. Specifically, four archaeological skeletons were used for this PhD project. Details on these individuals are reported below.

Tomb 3548 – Stratigraphic Unit 3707

Excavation site: Università Cattolica del sacro Cuore, Milan

Historical period: Roman imperial age (3rd-5th century AD)

Sex: Non-determinable **Age:** 3 to 5 years

Lesions: Diffuse woven periosteal new bone, metaphyseal flaring, unspecific dental defects, pathologic fracture on the right femur and ulna

Suspected diagnosis: Congenital syphilis (rickets less likely)

Stratigraphic Unit 4116

Excavation site: Università Cattolica del sacro Cuore, Milan

Historical period: Roman imperial age (3rd-5th century AD)

Sex: Non-determinable **Age:** 3 years ± 12 months

Lesions: Bowing of long bone diaphysis, metaphyseal flaring and porosity

Suspected diagnosis: Rickets

Tomb 185

Excavation site: Bolgare, Bergamo

Historical period: early Middle Ages (9th century AD circa)

Sex: Female **Age:** 23-39 years

Lesions: Osteolytic lesions with remodeled margins in hematopoietic-rich areas

Suspected diagnosis: Tuberculosis

Tomb 6

Excavation site: Università Cattolica del sacro Cuore, Milan

Historical period: Roman imperial age (3rd-5th century AD)

Sex: Female **Age:** Adult

Lesions: Large callus on the meta-diaphysis of the right tibia

Suspected diagnosis: Antemortem trauma

4.1.3. Autopsy samples

In addition to skeletal remains, samples from autopsies were collected for pathological analysis and consisted in three types of material: atherosclerotic calcifications, gallstones and pleural plaques.

Samples were extracted from well-preserved cadavers during autopsies at the Medico-Legal Institute of Milan between February 2014 and January 2017. They consisted in soft tissue extractions that were then submitted to maceration until the calcified elements were completely separated from the remaining soft tissues. Each sample was placed in a small, incompletely closed container and submerged in tap water. Every week, about 90% of the water was changed. The maceration lasted several weeks to several months, depending on the initial quantity of soft tissue attached to the calcified elements. After separation, the calcifications were retrieved and dried.

As a result, the calcified material collected during autopsies and macerated for this project includes:

- about **800 atherosclerotic calcifications** (including intact calcifications and fragments of larger calcifications) collected from 60 autopsies;
- **270 gallstones** extracted from 25 autopsies;
- and **two pleural plaques** from a single autopsy.

This material was later subjected to macroscopic analysis, histological observation and Scanning Electron Microscopy.

4.1.4. Forensic cases

Finally, forensic cases were also involved in this PhD project (Table 1). In particular, one sample of costal cartilage was taken from three forensic cases for a focused study. These forensic cases consist in cadavers autopsied at the medico-legal institute in Milan (Italy) and macerated in tap water for several months until the soft tissue could be completely removed from the bones. The skeletons were then dried and curated at the LABANOF.

Table 1: Description of the forensic cases examined for this project

Case	Sex	Age
Forensic case 1	Male	41 years
Forensic case 2	Male	78 years
Forensic case 3	Female	35-40 years

4.2. Methods

Each skeleton studied was first taken out of its storage container, placed on a table and separated from any associated material (e.g. clothing, jewelry, soil, botanical elements). They were then carefully washed with water and a toothbrush and let to dry. Taphonomically altered areas rendering the bone structure particularly fragile or with exposed trabecular bone were either completely spared or delicately cleaned with a paintbrush without water. The soil and dirt associated to the remains as well as the content of clothing or cranial vault (when applicable) were meticulously sieved with a 1.5 mm woven wire for the recovery of small bones (e.g. sesamoids or ear ossicles) and any calcified material.

Once cleaned and dried, the skeletons were laid down in anatomical position for anthropological analysis which includes the recording of the completion and preservation of the skeletal elements, the compilation of cranial and post-cranial measurements (Buikstra and Ubelaker, 1994; Bass, 2005), the documentation of anatomical variants (Hauser *et al.*, 1989; Buikstra and Ubelaker, 1994) and the

construction of the biological profile. The biological profile consists in sex (Phenice, 1969; Buikstra and Ubelaker, 1994), age-at-death (Lovejoy *et al.*, 1985; Meindl and Lovejoy, 1985; Iscan and Loth, 1986; Brooks and Suchey, 1990; Buckberry and Chamberlain, 2002; Scheuer and Black, 2004; Rougé-Maillart *et al.*, 2009; Cunningham, Scheuer and Black, 2016), ancestry (Hefner, 2009; Ousley and Jantz, 2012; Navega *et al.*, 2015) and stature estimations (Trotter and Gleser, 1958) as well as the analysis of traumatic (Rodríguez-Martín, 2006; Wedel and Galloway, 2013) and pathological lesions (Steinbock, 1976; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Waldron, 2008; Grauer, 2012).

In particular, several analyses were performed for the study of pathologies including macroscopic analysis, histological observation, Scanning Electron Microscopy and radiographic imaging.

4.2.1. Macroscopic analysis

Macroscopic analysis was performed on all the material studied as part of this project (skeletal remains and autopsy samples). Each skeletal element was attentively examined for any sign abnormal to the original bone structure. Taphonomic (post-mortem), traumatic and pathological lesions were distinguished based on morphological characteristics, including the location of the lesion, the aspect of the margins, the elasticity of the fractures, the changes in coloration, the effect of weathering on the cortical surface and the presence of bone remodeling (Behrensmeyer, 1978; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Rodríguez-Martín, 2006). Dubious pathological or pseudopathological lesions were examined under stereomicroscope for assessment of the above-mentioned criteria. Pathologic fractures were included in the analysis and diagnosis of bone lesions.

Pathological lesions were identified by comparison with the clinical and paleopathological literature (Steinbock, 1976; Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Waldron, 2008; Grauer, 2012) or positive controls from autopsy cases (golden standard). The exact location, distribution and number of the pathological lesions were recorded using anatomical references and their morphological characteristics (depending on the type of lesion: bone involvement, shape, margins, color and/or texture) were described according to standard paleopathological terminology (Buikstra and Ubelaker, 1994; Ortner, 2003; Buikstra,

Cook and Bolhofner, 2017). In addition, dimensions of lesions were measured with a Vernier caliper (sliding caliper). Finally, lesions were photographed for records.

4.2.2. Histological observation

Sixty samples, including 18 atherosclerotic calcifications, 18 gallstones, 18 samples of ossifying costal cartilage and six samples of pleural plaques, underwent histological analysis according to two protocols: undecalcified and decalcified.

Following the undecalcified protocol, the samples were ground so as to obtain thin sections using a grindstone Struers DAP-7 and progressive abrasive disks (Buehler micro cut disks, grains of 180, 320, 500, 1200, 2400, and 4000) applied progressively for a more optimal observation of the section under microscope (the first disks are used for grinding and the last ones for the polishing of the surface of the section). The thin sections obtained were embedded in Pertex (Pertex, mounting medium for light microscopy. Histolab: Gotebourg, Sweden) and kept at ambient temperature for 72h for solidification of the synthetic resin before observation with the optic microscope.

For the decalcified protocol, samples were first fixed in formalin (v/v, PH 7–7.6, ratio 20:1 v/v) for 24h and decalcified at room temperature in Decalc, 14% hydrochloric acid (Histo-Line Laboratories, Milan). Subsequently, each thin section was rinsed in tap water for 24 h, dehydrated in alcoholic scale, and embedded into paraffin. Finally, sections of 5 microns were cut from each block and stained with Hematoxylin and Eosin (H&E).

Of the 18 atherosclerotic calcifications that underwent histological analysis, 10 were subjected to the undecalcified protocol (three extracted from autopsies and seven collected from cemeterial cases) and eight followed the decalcified protocol (four were from well-preserved autopsy cases and four from cemeterial skeletal remains) (Fig. 4).

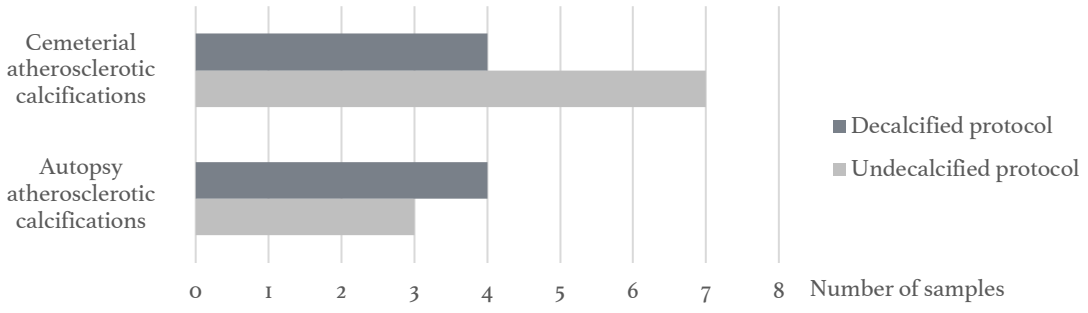

Figure 4: Histological analyses performed on the selected atherosclerotic samples

Fourteen gallstones used for thin sections were subjected to the undecalcified protocol. The structural integrity of the remaining four stones collapsed during the xylene passage before fixing the samples in paraffin preventing the completion of the decalcified protocol (Fig. 5).

Figure 5: Histological analyses performed on the selected gallstones

Of the 18 samples of costal cartilage, 10 samples underwent undecalcified histological analysis, including three from forensic cases and seven from skeletons of the CAL Milano Cemetery Skeletal Collection. In addition, eight samples underwent decalcified histological analysis: three from forensic cases and five from skeletons of the cemeterial collection (Fig. 6).

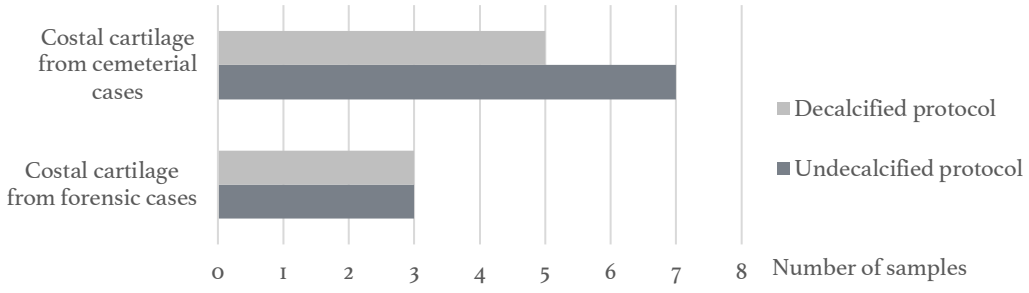

Figure 6: Histological analyses performed on the selected costal cartilage

Six samples from two large pleural plaques extracted during autopsy at the medico-legal institute of Milan from a well-preserved cadaver, were processed for histological analysis, including two following the decalcified protocol and four according to the undecalcified protocol (Fig. 7).

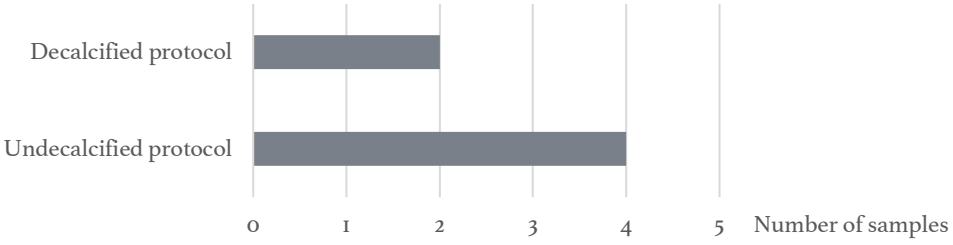

Figure 7: Histological analyses performed on the samples of pleural plaques

4.2.3. Scanning Electron Microscopy (SEM) and Energy Dispersive Spectrometry (EDS)

Scanning Electron Microscopy (SEM) was performed on five atherosclerotic calcifications from cemeterial (two sample) and autopsy cases (three samples) with a Cambridge Stereoscan 360 with electron gun, vacuum pump, and image acquisition software (Oxford, UK) for a better appreciation and understanding of these pathological markers of disease.

Nine gallstones underwent Scanning Electron Microscopy coupled with Energy Dispersive Spectrometry (SEM-EDS) with detector from 138 eV to 5.9 keV (Oxford Link Pentafet, Oxford, UK) to provide the elemental composition of the stones (Fig. 8).

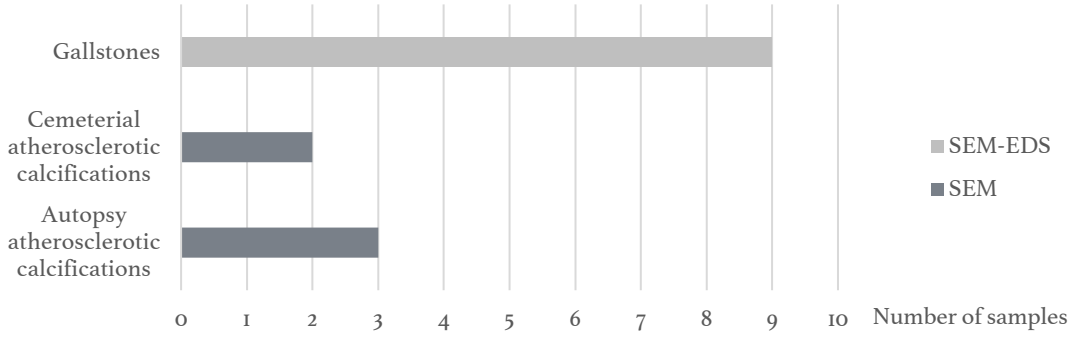

Figure 8: SEM and SEM-EDS analyses of the selected samples

4.2.4. Radiographic imaging

As one of the aims of this project was to compare the appearance and morphological characteristics of the same bone lesions macroscopically and radiographically, radiographs were carried out on single, multiple bones or almost entire skeletons depending on the distribution of bone lesions on the skeletons selected for the study. Radiographic imaging was realized in the service of radiology and medical imaging of the San Donato hospital center "IRCCS Policlinico San Donato" with a Siemens Luminos dRF Max1 with technical parameters ranging from 49.9 kVp to 80.9kVp and 0.2 mAs to 2 mAs.

Fourteen skeletons were selected from the collection for radiological analyses based on their associated clinical documentation as well as one skeleton with osteomalacia which was not diagnosed clinically but based on the lesions and bending deformities observed on the skeleton (Fig. 9).

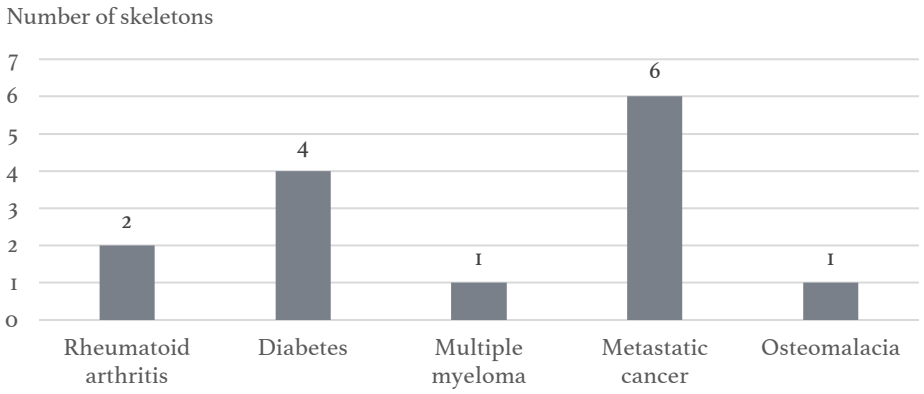

Figure 9: Distribution of the individuals selected for radiography per pathological condition

"Comparative sets" were realized of macroscopic pictures and plain x-rays of the same skeletal elements or bone lesions taken in the same position and assembled in a single figure side by side. The analysis of the complementary potential of radiographic and macroscopic observation of bone lesions was based on these comparative sets and performed per pathological condition.

4.3. Discussion of the Materials and Methods

4.3.1. Discussion of the materials

The selection of skeletons from a reference collection, the CAL Milano Cemetery Skeletal Collection, presents biases for the study and interpretation of pathological lesions that must be acknowledged. First, the skeletons present various stages of conservation including presence and preservation of the skeletal remains. Thus, the loss of osseous elements may result in the absence of crucial material for the interpretation of the distribution of bone lesions (in the case of multiple lesions) or in their identification (in the case of solitary lesions) and may bias the understanding of bone involvement when compared to skeletons with a better conservation. Similarly, taphonomic alterations may alter the morphological characteristics of pathologic lesions rendering them unrecognizable or even mimic the appearance of pathologic lesions (pseudopathology) complicating their diagnosis. Second, the associated documentation with clinical diagnoses does not constitute a golden standard. The diagnoses were established by medical doctors using modern medical standards, however, only a few conditions are reported in the ISTAT death certificate which does

not constitute an exhaustive medical history of the individual. Some individuals may have been suffering from pathological conditions that were not noted in the associated documentation, which may confuse the interpretation of the etiology of the lesions. Consequently, the finding of bone lesions in a skeleton with a diagnosed condition does not imply that the reported disease is responsible for the lesions found in the skeletal remains. Comorbidity is an important bias that must always be considered in the pathological analysis, especially given that the ISTAT certificates are not exhaustive recounts of the medical history of the individuals. In addition, misdiagnoses do occur in modern days and so this possibility remains. Third, even if the pathological condition were accurately diagnosed and reported in the associated documentation, it does not guarantee that the skeletons will evidence skeletal lesions. Indeed, not all diseases affect the skeletons and even those that do may not systematically cause bone lesions. Several factors may influence the involvement of the musculoskeletal system including idiosyncratic, environmental and genetic factors, the stage of development of the disease, the chronicity of the condition and the recourse to medication and/or surgery. Fourth, although occasional cases of surgical procedures are reported, the associated documentation does not generally provide information regarding medical treatment that the individual may have received, and as mentioned above, medical treatment is an important factor in the pathological involvement of the skeleton. Therefore, absence of evidence cannot be regarded as evidence of absence.

Samples extracted from well-preserved cadavers during routine autopsies at the medico-legal institute of Milan, namely atherosclerotic calcifications and gallstones, constituted another material selected for study in this PhD project. As they were collected *in situ* during autopsies, their diagnosis represents a golden standard. However, soft-tissue samples obtained from autopsies are not representative of forensic cases of skeletonized individuals or archaeological findings, which is why they were macerated for several weeks and dried to simulate a dry bone context.

Finally, four archaeological specimens from different excavations in Milan were included in this PhD project. Contrary to cemeterial skeletons, no reliable medical data was associated to these individuals. Nonetheless, this did not constitute a bias in our study because the archaeological material was not used as a basis for diagnosis of pathological conditions but in a research investigating the differences and discrepancies in the description of bone lesions. Therefore, archaeological specimens

were included for a study concerning the description of lesions using standard paleopathological terminology; references to suspected diagnoses and inferences on pathological diagnosis were not part of the research.

4.3.2. Discussion of the methods

The macroscopic analysis in the diagnosis of bone diseases consists in the naked eye observation, recognition, recording and diagnosis of bone lesions through their morphological characteristics and patterns of distribution. On the one hand, not all bone lesions are observable through macroscopic observation: many lesions are located in the trabecular structure of the bone, hidden from naked eye observation by the unaffected cortical shell of the bone, thus giving the fallacious appearance of a healthy bone, free from disease. Taphonomic alterations eroding the cortex of the bone and exposing the trabeculae can reveal the existence of skeletal lesions otherwise unperceivable by macroscopic analysis. However, the helpful effect of taphonomy is an element of luck that cannot be relied one for an exhaustive analysis of bone disease in a skeleton. On the other hand, naked eye observation of bone lesions is one of the most sensitive techniques for the observation of bone lesions involving the cortical layer, whether lytic or proliferative. One of the research lines was in fact to compare radiographic images with the macroscopic observation of different types of bone lesions to assess the strengths and weaknesses of each technique. In addition, macroscopic analysis is the first and most used tool of the anthropologist: radiography, computed tomography and scanner imaging are not routinely performed on skeletal material for logistic and financial reasons (Chhem and Brothwell, 2008, p. 15); therefore, naked eye observation is the initial step in the analysis of bone lesions and its results will determine the use of complimentary analyses. The first research line of this project consisted in the analysis of bone lesions in skeletons with reported conditions to provide supporting evidence in the ulterior pathological analysis of unknown skeletons in archaeological and forensic cases. Consequently, macroscopic analysis was the main method used in this project despite its pitfalls, which were always considered in the interpretation of the results obtained.

The preparation of thin sections for histological analysis is a destructive technique which allows the observation of the osseous material at the cellular level. Thus, this technique was used only on selective material, namely atherosclerotic calcifications

and gallstones, because of the availability of this material in our collections or because it was considered essential, as for ossifying costal cartilage and pleural plaques. Histological studies have demonstrated significant potential for a better understanding of the pathogenesis and etiology of the condition responsible but also to distinguish and identify diseases as specific histomorphometric characteristics are pathognomonic for specific diseases (e.g. Paget's disease) (De Boer, Van der Merwe and Maat, 2013).

Scanning Electron Microscopy and Scanning Electron Microscopy coupled with Energy Dispersive Spectrometry were performed solely on atherosclerotic calcifications and gallstones respectively for the same reason, the availability of the material, as they are also destructive analyses. Nevertheless, these techniques allow a specific topographical, morphological and content analysis of highly magnified structures.

All of the techniques used in this project aim in a precise observation of the morphological characteristics of bone lesions and an accurate recording of their pattern of distribution for their diagnosis. However, the lesion-based approach is not the only method for the diagnosis of diseases in skeletal remains: DNA and protein analysis, as microbiological techniques, permit a direct and unequivocal diagnosis of the conditions present on a skeleton (Nerlich *et al.*, 1997; Haas *et al.*, 2000; Donoghue *et al.*, 2004; Robbins *et al.*, 2009; Pérez-Martínez *et al.*, 2016). Paleomicrobiological techniques were not performed as part of this PhD project but are further perspectives planned for future research.

Chapter 5. Research lines

"Disease is very old, and nothing about it has changed.
It is we who change, as we learn to recognize what was formerly imperceptible"
Jean Martin Charcot

As detailed in Chapter 2, this PhD project was divided in several and consecutive objectives. In this chapter, the results of the four research lines investigated in this project will be presented.

5.1. Macroscopic study of skeletons with clinically diagnosed conditions

In this first research line, macroscopic analyses were performed on skeletons with specific pathological conditions reported in their associated documentation and clinically diagnosed before death, to analyze the morphological characteristics and distribution of bone lesions in these selected conditions (Appendix I).

Cardiovascular diseases

Twelve skeletal remains with a previous diagnosis of vasculopathy and 12 control individuals (with no record of vasculopathy in their associated medical documentation) were examined for evidence of cardiovascular diseases and in particular, atherosclerosis. Indeed, atherosclerotic calcifications act as markers of the cardiovascular disease and their recovery warrants the diagnosis of the condition. A total of 735 calcifications were found including 293 atherosclerotic calcifications. This research demonstrates that atherosclerosis, a cardiovascular disease, can be identified in skeletal remains.

Rheumatoid arthritis

Three individuals with rheumatoid arthritis were present in the collection: cases n°277, 1007 and 557. The first two skeletons manifested significant articular lesions, whereas case n°557 did not evidence any lesion suggestive of the condition and following the suggestion of a reviewer, was excluded from the article. Both shared common features consistent with the literature: symmetric erosions of bones in the hands, wrists and elbows, sparing of the distal interphalangeal joints, and the absence of sacroiliac and spinal fusion. The analysis of these two clinically diagnosed cases strengthens the

criteria for diagnosis of the condition in dry bone. This study is the first to examine skeletons with a clinical diagnosis of rheumatoid arthritis from a reference osteological collection.

Diabetes mellitus

All 38 individuals of the collection with diabetes were examined for possible lesions consistent with the condition and compared to 11 control individuals without a diagnosis of diabetes in their medical documentation. Although a diagnosis of diabetes cannot be asserted based solely on macroscopic observation, several lesions indicative of the condition could be identified and can suggest the condition in the differential diagnosis, including periosteal new bone formation, lysis of the distal tuft, lytic lesions, evidence of trauma, osteomyelitis, and osteochondritis dissecans. This is a pioneer research on the recognition of diabetic lesions on skeletons.

Human Immunodeficiency Virus (HIV)

Nine skeletons of the collection with a diagnosis of HIV, including four with Acquired Immune Deficiency Syndrome (AIDS) were subjected to a careful macroscopic examination for the study of HIV/AIDS induced lesions. Several lesions were observed including periosteal new bone formation, dental lesions, thickening of the frontal diploë, destructive localized porosity and evidence of trauma. None of the lesions reported can be directly linked to HIV because the virus does not directly affect bones in a macroscopic way. Nonetheless, the lesions found are consistent with HIV/AIDS-induced infections and inflammations and HIV-related risk factors. This is the first study to present skeletons with reported HIV and to perform an anthropological study on known HIV individuals.

Multiple myeloma

Both skeletons of the collection with an antemortem diagnosis of multiple myeloma were investigated for the presence, morphology and distribution of bone lesions. Both cases presented bone lesions with similar morphological traits and distribution: multifocal and round osteolytic lesions with sharply demarcated margins, relatively uniformly small in dimensions and specifically located in highly vascularized areas of the skeleton. The results strengthened the criteria for diagnosis of the condition and represent the third and fourth cases of skeletons with a documented diagnosis of the disease in the literature after the first two cases presented by Rothschild and colleagues

(1998) from the Terry Collection (Washington, D.C, United States) and the Mutter Museum (Philadelphia, PA, United States) (although the latter was undocumented in the article).

Breast metastatic carcinoma

All 14 individuals of the collection with a diagnosis of breast cancer in their associated medical documentation were examined for signs of metastatic disease. Forty-three percent of the study sample showed evidence of metastatic bone disease. The majority of the lesions were osteolytic, although osteoblastic and mixed metastases did also occur. The aim of this research was to analyze and illustrate the pattern of involvement and morphological characteristics of bone metastases in breast carcinoma to potentially differentiate the carcinoma in ulterior cases of metastatic bone disease. This is the first study to be performed in this sense on skeletons with recorded breast cancer.

Bladder metastatic carcinoma

Thirteen individuals with a diagnosis of bladder cancer were present in the collection. After a careful macroscopic examination, three skeletons manifested signs of metastatic bone disease. Metastases were mostly of a mixed nature (45%), although both osteoblastic (13%) and osteolytic (9%) were also found. The objectives of this research were the same as with bladder metastatic carcinoma: to illustrate and analyze the distribution, nature and morphological characteristics of bladder metastatic bone disease. Contrary to breast cancer, bladder carcinoma is rarely, if ever, considered in the differential diagnosis of the primary organ. This study is the first to document the macroscopic aspect of bone metastases in skeletons with recorded bladder carcinoma to help anthropologists recognize the condition.

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PAPER

ANTHROPOLOGY

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Survival of Atherosclerotic Calcifications in Skeletonized Material: Forensic and Pathological Implications

ABSTRACT: Atherosclerosis is a chronic inflammatory disease creating calcifying plaques in the arterial walls. Because its paleopathological diagnosis remains little studied on skeletal remains, its impact on forensic and archeological data is completely underestimated. Here, 24 skeletal remains from the *Milano Cemetery Skeletal Collection* have been studied to evaluate the chance of atherosclerotic calcification survival, retrieval, and identification. Through direct comparison with a known autopsy collection and literature, the identification and categorization of several types of calcifications were performed. Clothing elements such as tights or socks played a definitive role in the preservation of the calcifications; hence they are more likely to be found in forensic cases than in archeological ones. Therefore, vascular calcifications are possible to collect and identify in skeletal remains if sufficient care is given to their recovery. Consequently and as markers of the disease, such identification can provide valuable pathological information for forensic and archeological cases.

KEYWORDS: forensic science, forensic anthropology, physical anthropology, bone pathology, atherosclerosis, vascular calcifications, arteriosclerosis

Introduction

Markers of disease on skeletal remains are valuable tools in both forensic sciences and archeology. However, while pathologies that leave signs directly on bones are rather easy to detect, others do not. Some pathologies create calcifications and although they are products of the disease, they may be interpreted as nonrelevant in the anthropological analysis because they can be more insidious (especially when not *in situ*) and are less well known. Atherosclerosis is an example of these pathologies. Atherosclerotic plaques, as calcified material, are able to survive the decomposition and taphonomic processes. Their recovery in association with skeletal remains will bring additional and valuable information to the biological profile, and hence is of particular interest in forensic sciences. Indeed, this pathological information could be useful in cases of unknown individuals when confronted to ante-mortem data. For example, if an atherosclerotic calcification is recovered *in situ*, this information could be compared to ante-mortem medical imaging which could in turn provide a very strong element if not for personal identification, at least for a stronger match. When the most common methods of identification cannot be used (such as DNA, dental, and fingerprint comparison), any and all elements can be of great value if combined with other personal descriptors. Also, their systematic recovery and identification could lead to a reinterpretation of paleopathological data and therefore, to a better understanding of the disease itself. Regardless of the implications that can be extrapolated from fragments of artery calcifications, the first questions that come to mind are as follows: can such markers be recovered among skeletal remains despite taphonomic processes? And what do they look like? No study exists in this sense.

Atherosclerosis is a progressive chronic inflammatory and fibroproliferative disease creating plaques in the walls of any large and medium-sized artery (1). Atherogenesis is composed of five successive phases (2–5): first, the initiation of the lesion, characterized by the accumulation and oxidation of low-density lipoprotein (LDL) particles in a hemodynamic site of preference in the arterial wall, called a "fatty streak"; second, the inflammation, inducing the proliferation and differentiation of the monocytes conveyed in the intima (the inner layer of the arterial wall) by the immune response into macrophages, and their subsequent apoptosis; third, the macrophages take the oxydated LDL, forming a "foam cell" and undertake apoptosis creating a necrosis; fourth, the smooth muscle cells, which usually heal and repair the arterial wall after an injury, migrate from the media and accumulate around the necrotic core forming a fibrous plaque; finally, after a varying period of time, this necrotic core calcifies in the intima and advanced lesions can appear: atherosclerosis creating stenosis is not a fatal condition, but a ruptured plaque can cause a thrombosis and eventually death (4–7).

According to Towler (8), different types of vascular calcification have been distinguished, including atherosclerotic intimal calcification, aortic valve calcification, and medial artery

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calcification. Aortic valve calcifications are calcified nodules in the aortic valve leaflets, affecting the leaflet flexibility necessary for the systole and diastole mechanism. Medial artery calcification or Mönckeberg's arteriosclerosis, associated to type II diabetes mellitus and chronic kidney disease, takes place in the muscle layer or tunica media of the artery. Arteriosclerosis can impair any artery (most commonly the femoral, tibial, and uterine arteries) by increasing the arterial wall stiffness and reducing its elastance and is closely linked to lower extremity amputations in individuals with diabetes mellitus and cardiovascular morbidity and mortality (9,10). Scarce information is available in the literature concerning the macroscopic appearance of these calcifications and is reported here as follows: arteriosclerosis creates circumferential and contiguous nonluminal obstructive calcifications and atherosclerotic calcifications, by far the most common, are of tubular form (8,9,11–13).

The ectopic and dystrophic calcification mechanism (although still not well understood and unpredictable) can start from the early adult life and takes decades to develop (3,7,14–17). The atherosclerotic calcification process is not a passive accumulation of mineral deposition but an active and organized mechanism similar to bone formation (15–18). It has been hypothesized that arteries may contain hydroxyapatite, hematopoietic tissue and cells "phenotypically similar" to chondrocytes, osteoblasts, and osteoclasts; however, the cell type responsible for arterial calcification remains undetermined (15).

Some authors suggest that the presence of atherosclerotic plaques acts as a marker for the disease (15). As atherosclerotic lesions calcify in advanced stages, the presence of vascular calcifications indicates atherosclerosis, but the absence of calcified plaques does not prove the absence of the disease. Atherosclerotic calcifications have been divided into two major categories: microcalcifications (<2 mm) and macrocalcifications (>2 mm). Microcalcifications result from plaque rupture due to the inflammation and are responsible for thrombosis. When the response is anti-inflammatory, a regulated calcification and mineralization mechanism takes place, creating larger and stable calcifications (macrocalcifications) which "act as a barrier toward the spread of the inflammation" (7) as a physiologic defense to maintain the integrity of the arterial wall (14).

Clinical and epidemiological studies on recent populations demonstrated a correlation between increasing age and diffuse pattern of atherosclerotic calcification and showed that atherosclerosis seems to be prevalent in males under 60 and in females over 55–60 years. The main hypothesis accounting for this observation consists of a protective effect of female hormones, especially estrogen, in the atherogenic process (30,31).

Until recently, CVD was thought to be a modern disease and risk factors corresponded to our 20–21st century way of life, such as hypertension, diabetes, obesity, smoking, and physical inactivity (19). However, Czermak in 1852 and Ruffer in 1911 found atherosclerotic calcifications during the autopsies of Egyptian mummies (20–22). Challenging this assumption even further, the Horus study performed whole-body CT scanning on 137 mummies from four distinct populations dating from 3100 BCE to 1500 CE and found evidence of atherosclerosis in 34% of their sample (23). CT scanning was also used to find atherosclerotic calcifications on the frozen body of Ötzi the iceman (3300 BCE), discovered in 1991 in the Alps (24). Risk factors for ancient populations have been studied and reported the atherogenic effect of domestic smoke inhalation due to open fires in the habitation (similar to our modern passive smoke exposure), of past maternal infection and of systemic inflammation of chronic infections (22). In fact, several researches supported the idea of a strong association between chronic pro-inflammatory conditions (such as rheumatoid arthritis and periodontal disease) and atherosclerosis (25–27). One article even hypothesizes a link between atherosclerosis and osteoarthritis, as a subchondral bone ischemia (28). Other mentioned factors include the arterial degeneration over time and the genetic predisposition for CVD (22). Indeed, a recent research showed that Ötzi the iceman presented a number of single-nucleotide polymorphisms located on the 9p21 chromosome, hence indicating a strong association with CVD, and more specifically a predisposition for atherosclerosis, coronary heart disease, and stroke (29).

These results show a very high frequency of the diseaseof the disease. So high, in fact, that Ruffer (20) concluded his report by describing atherosclerosis to be as frequent 3000 years ago as today. However, mummies represent a very small proportion of the archeological data and so the diagnosis of CVD on this limited sample underestimates its actual frequency on past populations. Moreover, atherosclerosis is recognized in forensic pathology through characteristics such as luminal narrowing, thickening of an arterial wall and atheromatous findings, but is rarely diagnosed when confronted to skeletonized individuals (12). It is known that the medical diagnosis of atherosclerosis relies on the medical imaging of a plaque (17). It is also known that these plaques calcify in the atherogenic process over time. Evidence of the disease should, therefore, be possible to recover, even in an archeological context (11,13).

The investigation of atherosclerotic calcifications as markers for CVD becomes crucial not only in paleopathology but also for forensic purposes. Indeed, in case of skeletonized or advanced decomposed cadavers, a correct interpretation of recovered calcifications may provide valuable pathological data for the research of pathological conditions, the construction of an anthropological biological profile, and especially serve in creating a more thorough biological profile that may help toward the identification of unknown deceased when confronted to antemortem data. Nonetheless, and before stating a diagnosis, a careful and meticulous excavation/exhumation/recovery is necessary to collect any findings such as calcifications.

In this perspective, this study aimed to investigate the presence and detectability of calcifications in skeletal remains. In particular, it focuses on the macroscopic study and the identification of atherosclerotic and arteriosclerotic calcifications in forensic or paleopathological cases.

Methods

For this research, we selected a study sample consisting of 24 skeletons from the Milano Cemetery Skeletal Collection. This collection is a contemporary and documented skeletal collection of more than 2100 skeletons (with dates of death ranging from 1918 to 2001) available for study in accordance with the article 43 of the Italian National Police Mortuary Regulation (10th September 1990, n°285). This documentation includes demographic data, autopsy reports and *"ISTAT"* death certificates that specify the cause of death and any additional pathological conditions related to it. The individuals were buried in coffins, exhumed by cemetery workers (without any forensic anthropology expertise) twice in 15 years by means of heavy machinery such as backhoes and stored in zinc containers. In fact, the bodies are usually exhumed after 10 years and if they are not skeletonized, the coffins are broken so that the soil can enter and aid

the skeletonization for the following 5 years. They are finally placed in an ossuary for 5 years and then are able to enter our collection, available for study at the *Laboratorio di Antropologia e Odontologia Forense* (LABANOF).

The analyzed sample included 12 individuals whose associated documentation indicated vasculopathy or atherosclerosis/arteriosclerosis as a pathological condition, and a control group of 12 individuals (with no mention of vasculopathy in their ISTAT certificate) with a wider age range to test the potential range of application of the research (each subgroup being equally divided into females and males), as shown in Table 1 and Fig. 1. As a result, six of the 12 individuals of the control group are younger than their counterparts in the vasculopathy group. The inclusion of younger individuals for the vasculopathy group could not be achieved because it would require that the individual had died of a disease related to vasculopathy for it to be noted in the associated documentation, which rarely happens in younger individuals and this combination of criteria could not be found in our collection. However, the objective of this research was to assess the possibility of calcification survival, retrieval, and identification. Inferences regarding sex, age, and number of calcifications were noticed and so added as additional reported results, but were secondary to the aim of the study.

The exhumed skeletons were blindly studied without any knowledge of the associated documentation. The content of the box, of the cranial vault (when the state of preservation allowed elements to be kept in it) and even of clothing elements such as tights or socks (preserved despite high postmortem intervals), were thoroughly sieved with a 1.5 mm woven wire to separate calcified material (or suspected calcified material) from other elements. Indeed, many calcifications were found *in situ* (separated and preserved in the cranium, in socks or in tights) (Fig. 2) which facilitated their identification as cardiovascular calcifications. To strengthen our identification, we collected 10 atheromatous samples from autopsy cases and recovered the atherosclerotic calcifications after maceration and separation with the remaining soft tissues. They served as positive controls (golden standard) for comparison and identification of our cemeterial calcifications. These atherosclerotic calcifications are flat or convex–concave plaques, of various dimensions, thin but constituted of several layers, of irregular margins and of a light yellow color (Fig. 3). Through direct comparison with this known autopsy collection and images from the literature (8,11), it was possible to recognize distinctive criteria and identify atherosclerotic calcifications with certainty (Fig. 4).

An anthropological analysis was performed on each skeleton (sex, age, stature, as well as pathological and traumatic analysis), and possible bone manifestations related to circulatory disturbances were also investigated. To each skeleton presented in Table 1 was assigned a taphonomic appreciation to show the state of completeness of the skeleton: "almost complete" refers to over 90% of taphonomic recovery, "mostly complete" is between 60% and 90%, "fragmented" corresponds to 40–60%, and "badly preserved" means that less than 40% of the skeleton was recovered.

Finally, a macroscopic observation of the recovered calcified material was performed, consisting of a description based on several specific parameters: number, location (if found *in situ* the exact location was specified; for nonspecific location, such as the soil residues from the bottom of the box, "unknown" was noted), shape, color, range size, maximum length, and maximum thickness.

Results

Regardless of their typology, 735 calcifications (calcified material and definite non-bone elements) were recovered and studied in 20 of 24 skeletons (83% of the study sample), in both vasculopathy and control groups.

The macroscopic observations conducted on all the findings collected in the skeletons permitted to observe that the calcifications differ in morphology, color, and location. Based on these variabilities and by comparison with the literature (8,11) and the known autopsy collection, it was possible to achieve a categorization of four types of calcifications as a base for identification, as shown in Fig. 5. In this table, the description of ossified cartilage served as a distinctive type of calcified material to highlight the morphological differences with vascular calcifications; no ossified cartilage was collected in this study. Also, the non-identified calcifications are of unknown origin and morphologically discernible from the atherosclerotic and arteriosclerotic types.

The classification of all the findings into the different types of calcifications was made easier by the distinct characteristics of the cardiovascular categories, making them identifiable with certainty. Table 2 shows the distribution of the calcifications for each individual. As a result, 293 calcifications were identified as atherosclerotic, 205 as arteriosclerotic, five are suspected atherosclerotic calcifications, and 232 are non-identified (and so neither atherosclerotic nor arteriosclerotic). Atherosclerotic calcifications are present in 83% of the female sample (10 of 12 skeletons) and in 66% of the male sample (eight of 12). Of the 293 atherosclerotic calcifications recovered, a total number of 31 were found in males, while 262 were collected among female individuals. Also, all 205 arteriosclerotic calcifications were solely acquired in the female sample (Table 2, Figs 6 and 7).

No definitive calcification was found in the four individuals under 56 years (Fig. 8). Eighteen of the 20 remaining individuals presented atherosclerotic calcifications (eight males and all 10 females over 56 years). As shown in Table 2, intimal artery calcifications were collected in both vasculopathy (173 calcifications of 293, or 59%) and control groups (120 calcifications of 293, or 41%).

Discussion

The purpose of this research was to evaluate the possibility of finding calcified material associated with skeletal remains. Through the study of 24 exhumed skeletal remains, a total number of 735 calcified nonosseous components of the human body were collected, demonstrating that with the proper training to search for such material, it is possible to recover decisive pathological evidence. This procedure was a two-step method: first, the training in the identification of the vascular calcifications through comparison with an autopsy collection and literature (8,11) and the understanding of the distinctive criteria of these calcifications; and second, the sieving of the skeletal remains and associated elements to recover them.

It is important to note that the individuals were collected by cemetery workers by means of heavy vehicles such as backhoes, so no careful excavation of the bones or the associated soil was performed. Therefore, the number of calcifications present in the recovered material may be minimized compared to the actual number of calcifications in the individual during life. However, many calcifications were found inside clothing areas (tights or socks) or within the cranial vault, allowing their preservation.

TABLE 1—*Details of the study sample.*

Case n°	Sample	Sex	Age	Cause of Death	Related Pathological Conditions	Type of Calcifications Recovered	Skeletal Pathologies	State of Preservation of the Remains
1	Vasculopathy	F	76	Cardiac arrest	Chronic cerebral vasculopathy, Cachexia	Atherosclerotic + Non-identified	Osteoarthrosis + Dental abscesses	Almost complete
2	Vasculopathy	F	82	Cerebral coma	Cerebral vasculopathy	Atherosclerotic	Osteoarthrosis + Enthesopathy	Almost complete
3	Vasculopathy	F	84	Cardiac arrest	Atherosclerotic vasculopathy, Renal insufficiency	Atherosclerotic	Osteoarthrosis + Caries	Almost complete
4	Vasculopathy	F	85	Cardiac arrest	Cerebral vasculopathy, Senile dementia	Atherosclerotic	Osteoarthrosis	Mostly complete
5	Vasculopathy	F	87	Cardiac arrest	Cerebral vasculopathy, Atrial fibrillation, Coronaropathy	Atherosclerotic + Arteriosclerotic	Suspected rheumatoid arthritis + Osteoarthrosis	Almost complete
6	Vasculopathy	F	102	Cardiac arrest	Ischemic cardiopathy, Cerebral vasculopathy	Atherosclerotic	Osteoarthrosis	Mostly complete
7	Control	F	39	Nonspecified		–	Osteoarthrosis + Enthesopathy + Periodontitis & Caries	Mostly complete
8	Control	F	47	Cardiac arrest	Kidney failure in diabetic therapy, Pulmonary Edema	–	Osteoarthrosis + Periodontitis & Caries & Abscesses	Fragmented
9	Control	F	56	Cardiac arrest	Acute necrotic pancreatitis, Septic state, Metabolic coma	Atherosclerotic	Osteoarthrosis + Periodontitis & Caries	Mostly complete
10	Control	F	83	Cardiac arrest	Intestinal occlusion, Acute pancreatitis	Atherosclerotic	Osteoarthrosis + Periodontitis & Caries	Fragmented
11	Control	F	86	Cardiac arrest	Heart failure	Atherosclerotic	Osteoarthrosis + DISH + Enthesopathy	Badly preserved
12	Control	F	95	Cardiac arrest	Arterial hypertension, Parkinson's disease	Atherosclerotic + Non-identified	Osteoarthrosis + Deformation of right hip and shoulder related to trauma	Fragmented
13	Vasculopathy	M	69	Cardiac arrest	Acute cerebral vasculopathy	–	Osteoarthrosis + Suspected DISH + Chronic ear infection	Badly preserved
14	Vasculopathy	M	80	Cardiac arrest	Chronic encephalovasculopathy, Cerebral stroke	Suspected Atherosclerotic	Osteoarthrosis + Enthesopathy	Mostly complete
15	Vasculopathy	M	81	Heart failure	Atherosclerotic vasculopathy	Atherosclerotic + Suspected atherosclerotic	Osteoarthrosis + Osteomyelitis + DISH	Fragmented
16	Vasculopathy	M	83	Malignant hyperthermia	Cerebral vasculopathy, Cachexia	Atherosclerotic	Osteoarthrosis + DISH + Enthesopathy	Mostly complete
17	Vasculopathy	M	85	Cardiac Arrest	Vasculosclerosis, Senile deterioration	Suspected atherosclerotic	Osteoarthrosis + Enthesopathy	Almost Complete
18	Vasculopathy	M	87	Cardiac and respiratory arrest	Arterial hypertension, Arteriosclerosis, Cerebral stroke	Atherosclerotic + Suspected cartilage	Osteoarthrosis + Enthesopathy + Periodontitis	Almost Complete
19	Control	M	31	Hepatic failure	HIV infection, Pneumonia	–	Osteoarthrosis + Enthesopathy + Periodontitis & Caries	Almost Complete
20	Control	M	42	Respiratory failure	Chronic bronchopneumonia, Hepatic cirrhosis	Suspected atherosclerotic	Otseoarthrosis + Enthesopathy + Scoliosis + Caries	Almost Complete
21	Control	M	56	Cardiac arrest	Bladder cancer, Stroke, Cancer deterioration	Atherosclerotic	Osteoarthrosis + Enthesopathy + Metastases	Mostly complete
22	Control	M	81	Cardiac arrest	Heart failure	Non-identified	Osteoarthrosis + Enthesopathy + Suspected infection on right foot	Fragmented
23	Control	M	81	Cardiac arrest	Stroke, K-intestinal, Heart failure	Atherosclerotic	Osteoarthrosis + Enthesopathy	Fragmented
24	Control	M	85	Cardiac arrest	Cerebral hemorrhage	Atherosclerotic	Osteoarthrosis + Enthesopathy + Periodontitis & Caries + Periostitis on left lower limb	Badly preserved

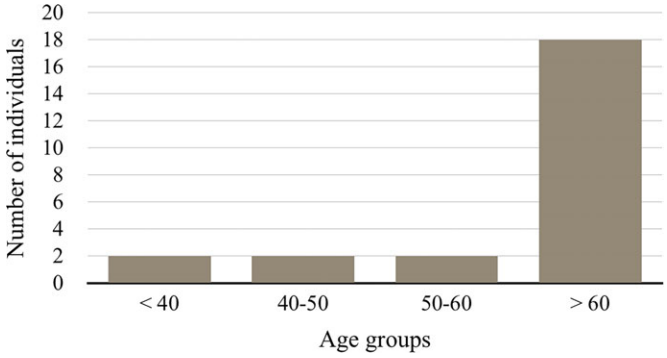

FIG. 1—*Distribution of the individuals of the study sample in age groups. [Color figure can be viewed at wileyonlinelibrary.com]*

FIG. 2—*Photograph of arteriosclerotic calcifications preserved inside tights. [Color figure can be viewed at wileyonlinelibrary.com]*

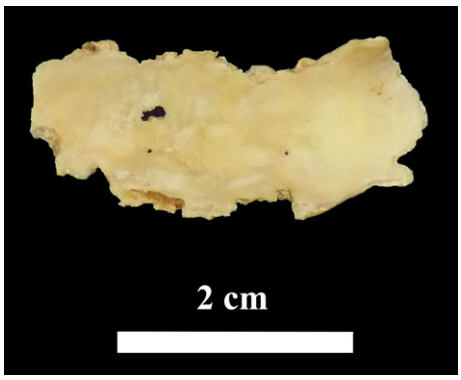

FIG. 3—*Photograph of an autopsy atherosclerotic calcification. [Color figure can be viewed at wileyonlinelibrary.com]*

All calcifications collected have been thoroughly studied through a macroscopic observation and then categorized in different types of calcifications. As reported in Fig. 5, atherosclerotic calcifications are of two different types: postcranial and cranial. They differ in shape, structure, and color. Postcranial calcifications are fragile convex–concave plaques, constituted of several layers with irregular margins, and colored with shades of light yellow to brown. The only difference with atherosclerotic plaques found in autopsies is the color (uniformly light yellow in the autopsy collection), which can be related to taphonomic processes. Cranial atherosclerotic calcifications (exclusively found *in situ*) are of a sinuous and semi-cylindrical shape, composed of an extremely fragile thin layer. They are uniformly light yellow and have irregular margins as well. Arteriosclerotic plaques, or medial artery calcifications (also always found *in situ*, as shown in Fig. 2), are long hollow cylinders formed by a uniformly brown and extremely fragile layer with irregular margins. Their long tubular shape is the most identifiable criterion, described in literature as "circumferential and contiguous" without obstruction of the lumen of the artery (8,9). Even though medial artery calcifications can affect any artery, they are more common in the femoral, tibial, and uterine arteries (10), which is why the cranial calcifications previously described can be identified as atherosclerotic (and not confused with arteriosclerotic findings), especially as cranial atherosclerotic calcifications are a common finding (30). The differentiation of atherosclerotic and arteriosclerotic calcifications was facilitated by their distinct characteristics (as seen in Fig. 5), making them efficiently identifiable even in a burial context after decomposition and skeletonization. Indeed, only five calcifications were too taphonomically altered to be identified with certainty as atherosclerotic and so were considered "suspected atherosclerotic" (about 0.7% of the total number of calcifications). Thus, if the calcifications were definitely differentiated from the atherosclerotic and arteriosclerotic categories, it was possible to affirm that they were due to another type of ossification/calcification mechanism. These nonvascular calcifications actually represent 232 non-identified materials (about 32% of the total number of findings). If it is possible to diagnose a disease such as CVD based on intimal artery calcifications, it would be interesting to apply the same reasoning to other diseases. In fact, these results show that there are many ectopic and dystrophic elements of unknown origin recovered in skeletal remains that could provide important evidence of other pathological conditions (for instance, myositis ossificans, gallstones, or calcified lymph nodes).

In terms of dimensions, the atherosclerotic calcifications found in the individuals have a maximum length ranging from 2.5 to 23 mm. As all fragments of atherosclerotic calcifications collected have a dimension superior to 2 mm, they can all be considered macrocalcifications, part of larger stabilizing plaques creating stenosis but physiologically defined to preserve the integrity of the arterial wall and diminish the risk of plaque rupture, and so of thrombosis. Also, some calcifications recovered were fragments of bigger calcifications; thence the dimensions of calcifications should be considered a minimal representation of the actual calcifications as well. Paradoxically and since these materials are fragments, the number of calcifications recovered is overestimated because it actually represents the number of pieces recovered and not of complete calcifications.

As no soft tissue elements (and so no arteries) are preserved in skeletal remains, the affirmation of the diagnosis of atherosclerosis on the base of sole calcifications is biased. Thus, the comparison with literature and a known autopsy collection corrects greatly this bias. Also, all cranial atherosclerotic calcifications and arteriosclerotic calcifications were recovered *in situ*. As a consequence, vascular calcifications found in skeletal remains and identified macroscopically can act as markers for the pathological diagnosis of CVD.

Despite previous studies demonstrating that females over 60 years are more subject to atherosclerosis than males (30,31), we observed little difference between the number of males and

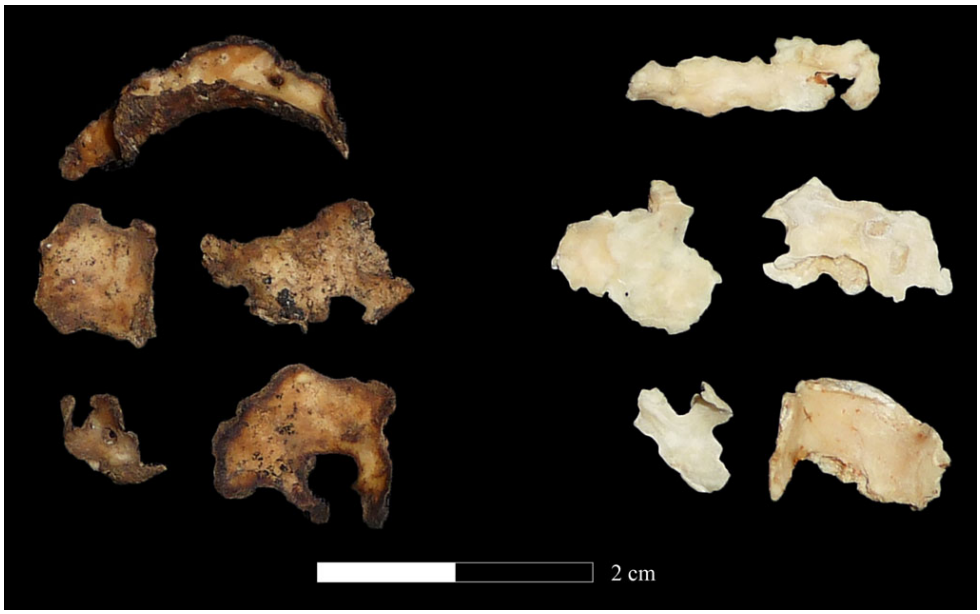

FIG. 4—*Morphological comparison between atherosclerotic calcifications recovered in this study (on the left) and autopsy atherosclerotic calcifications (on the right). [Color figure can be viewed at wileyonlinelibrary.com]*

Criteria	Atherosclerosis		Arteriosclerosis	Ossified cartilage	Non-identified
Location	postcranium	cranium	lower legs (in clothing elements)	thoracic area	unknown
Shape	convex–concave plaque	sinuous cylindrical	long linear hollow cylinder	superimposed pockets in a long cylindrical shape	superimposed pockets in an irregular shape
Structure	several layers superimposed	one thin layer	one thin layer	thick pockets	thick pockets
Color	shades of light yellow to brown	uniformly light yellow	uniformly brown	uniformly brown	uniformly brown to grey
Margins	sharp and irregular	thin and irregular	thin and irregular	thick and rounded	thick and rounded
Fragility	fragile	extremely fragile	extremely fragile	resistant	resistant
Image					

FIG. 5—*Categorization of the different types of calcifications recovered and compared to ossified cartilage. [Color figure can be viewed at wileyonlinelibrary.com]*

females affected by the disease (Fig. 6). Although the sexual difference in affliction is minor, results show an important difference in number of calcifications. Indeed, females have about 8.5 times more atherosclerotic calcifications than males (Fig. 7). Analogously, arteriosclerotic calcifications were only recovered in females. However, the literature does not provide any explanation concerning the sexual differences in numbers of atherosclerotic and arteriosclerotic calcifications.

Concerning age, no atherosclerotic calcifications were recovered in the four individuals under 56 years of age (Fig. 8). But these individuals may have had plaques in their arteries of an earlier stage of development and these soft-tissue plaques would have not survived the taphonomic and decomposition processes.

Literature also mentions an association between atherosclerosis and chronic pro-inflammatory infections such as rheumatoid arthritis and periodontitis (25–27). It was noticed in the anthropological analysis of the study sample (as documented in Table 1) that 57% of the individuals with periodontitis, 83% of the individuals with severe lytic arthrosis, the female with rheumatoid arthritis (individual n°5), and all three individuals with abnormally deep meningeal grooves on at least one parietal bone, had atherosclerotic calcifications. As a consequence, the information brought by the literature seems to coincide with the results of the study. However, it is, important to note that the observation of these pathologies is also related to age, as the individuals concerned are quite old. Moreover, bone signs like severe lytic arthrosis and the presence of deep meningeal impressions, two manifestations that could be linked to circulatory disturbances, appear connected to the presence of vascular atherosclerotic calcifications in the skeletal remains studied. These results cannot be considered evidence of atherogenic

factors, especially as they are observed on few and old individuals. Nevertheless, they are interesting observations that should be investigated in future studies.

The presence of a control group was essential in the investigation of the relation between the diagnosis of vasculopathy in life and the recovery of vascular calcifications. Indeed, its purpose in this study was triple: first, it permitted to include young individuals with different age intervals to study the significance of age; second, it served to verify the possibility of finding vascular calcifications in asymptomatic individuals (as supported by literature) by opposition to individuals with a diagnosed vasculopathy in which we expected to find them; and finally, it allowed us to compare the findings from both groups. As a result of the study, 59% of the calcifications were collected from the vasculopathy group and 41% from the control group. However and considering that the control group also included the four skeletal remains under 56 years in which no calcification was found, the difference in number of findings between both groups could be related to the age of the individuals knowing the existing correlation between age and recovery of vascular calcifications. Therefore, no relation can be assessed between the diagnosed vasculopathy mentioned in the death certificate and the presence nor the number of atherosclerotic calcifications. This result did not come as a surprise as clinical studies demonstrated a high presence of vascular calcifications in asymptomatic/randomly selected individuals (30,31). Lastly, only one individual from the vasculopathy group did not present any atherosclerotic calcification (individual n°13), but it should also be mentioned than its remains presented less than 40% of completeness, so the lack of evidence in this case could be due to the state of preservation. This finding demonstrates that vascular calcifications can be expected in individuals with a diagnosed

TABLE 2—*Distribution of the recovered calcifications.*

Case n°	Sample	Sex	Age	Atherosclerotic	Arteriosclerotic	Suspected Atherosclerotic	Non-identified
1	Vasculopathy	F	76	14	0	0	179
2	Vasculopathy	F	82	53	0	0	0
3	Vasculopathy	F	84	2	0	0	0
4	Vasculopathy	F	85	1	0	0	0
5	Vasculopathy	F	87	46	181	0	0
6	Vasculopathy	F	102	41	0	0	0
7	Control	F	39	0	0	0	0
8	Control	F	47	0	0	0	0
9	Control	F	56	20	0	0	0
10	Control	F	83	7	0	0	0
11	Control	F	86	1	0	0	0
12	Control	F	95	73	23	0	49
Total Females				258	204	0	228
13	Vasculopathy	M	69	0	0	0	0
14	Vasculopathy	M	80	1	0	1	0
15	Vasculopathy	M	81	8	0	1	0
16	Vasculopathy	M	83	1	0	0	0
17	Vasculopathy	M	85	1	0	0	0
18	Vasculopathy	M	87	1	0	0	0
19	Control	M	31	0	0	0	0
20	Control	M	42	0	0	3	0
21	Control	M	56	2	0	0	0
22	Control	M	81	0	0	0	4
23	Control	M	81	2	0	0	0
24	Control	M	85	15	0	0	0
Total Males				31	0	5	4
TOTAL				289	204	5	232

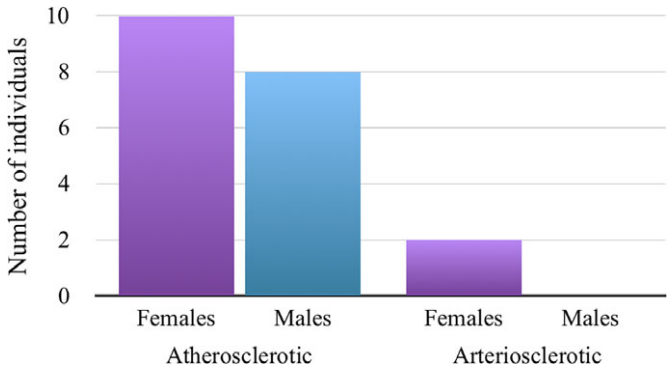

FIG. 6—*Distribution of the skeletal remains in which vascular calcifications were found according to sex. [Color figure can be viewed at wileyonlinelibrary.com]*

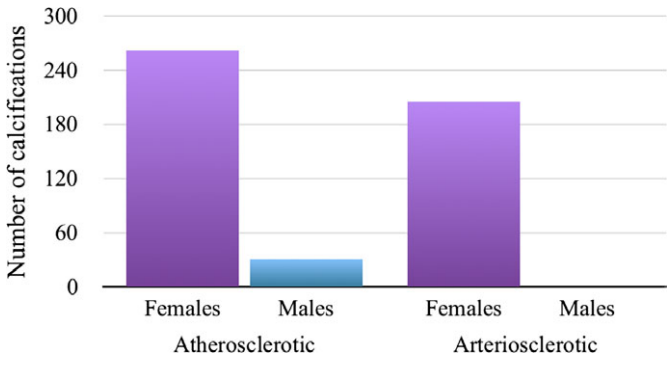

FIG. 7—*Distribution of the recovered vascular calcifications according to the sex of the individuals. [Color figure can be viewed at wileyonlinelibrary.com]*

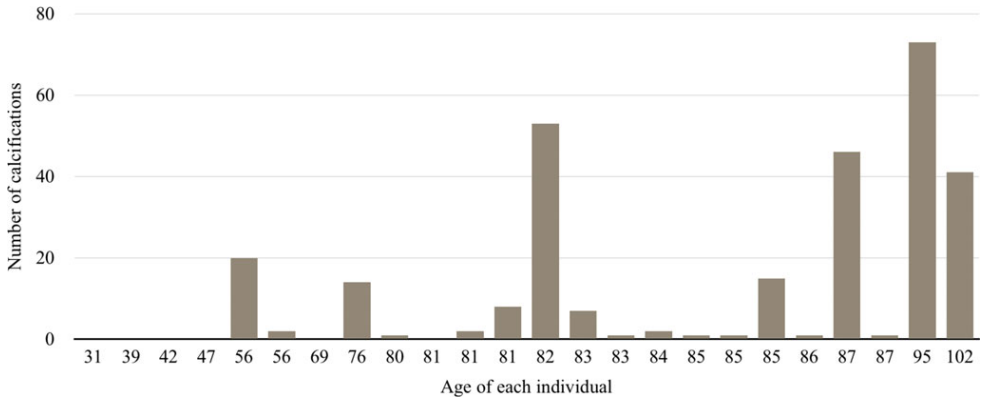

FIG. 8—*Distribution of the recovered atherosclerotic calcifications according to the age of each individual. [Color figure can be viewed at wileyonlinelibrary.com]*

vasculopathy, and adds a new argument in favor of their identification as markers for the disease.

Taphonomy plays a crucial role for recovery if the individual has very few calcified plaques in his arteries. However, it was not possible in this study to assess a correlation of cause to effect between the completeness of a collected skeleton and recovery of calcifications. Individuals 17 and 24, both aged 85 years, are a good example of this noncorrelation (Table 2). While only one calcification was recovered within an almost complete skeleton, 15 atherosclerotic calcifications were collected among the badly preserved skeletal remains of the second individual (Tables 1 and 2). On the opposite, the presence of socks or tights played a definitive role in the recovery of arteriosclerotic calcifications in particular (in both individuals 5 and 12). Considering their extremely fragile structure and the fact that they are common in the lower limbs (10), the preservation of clothing such as tights or socks appears as a taphonomic bias in favor of their conservation. Therefore, we expect vascular calcifications to be more likely to be found in forensic cases than in archeological ones.

The lack of evidence of the disease in skeletal remains in paleopathological and forensic literature cannot be seen as proof of its nonexistence. Atherosclerosis is both a past and modern CVD, with risk factors adequate to the time period, but still strongly related to age. The current study permitted to observe that the recovery and identification of vascular calcifications are possible in skeletal remains. Results show that vascular calcifications are present in higher numbers in females and are more correlated with age than with any other factor without being a direct consequence of the aging process. This difference in numbers of calcifications has never been studied before and could be of significant interest in the understanding of the disease. Nevertheless, this study sample was relatively limited in size and a generalization of the tendencies and interpretations brought out in this research regarding age, sex, and numbers of calcifications will require an increase of the size of the sample.

Given that atherosclerotic calcifications can act as markers of the disease, their identification can shed a new light on the representation of CVD in skeletonized cases and serve for forensic purposes, in providing specific and potentially identifiable pathological data. As proved possible in the present study in case of inhumation and high postmortem interval, it should be possible to find more evidence of CVD in forensic cases and archeological excavations with the appropriate training and methodology. In fact, if found in association with skeletal remains and since they act as markers of the disease, they could potentially lead to a new scientific re-interpretation of historical data.

Finally, as seen in this research, many calcifications remain unidentified; their study and the identification of the specific pathological mechanism responsible for their formation could provide new and valuable pathological markers, similar to atherosclerotic calcifications, for paleopathology and forensic cases.

References

1. Lam JYT. Atherosclerosis, the Merck Manual for health professionals, 2012; http://www.merckmanuals.com/professional/cardiovascular-disorders/arteriosclerosis/atherosclerosis (accessed April 6, 2016).
2. Lusis AJ. Atherosclerosis. Nature 2000;407(6801):233–41.
3. Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001;104(4):503–16.
4. Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol 2006;47(8 Suppl C):C7–12.
5. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011;473(7347):317–25.
6. Shah PK. Mechanisms of plaque vulnerability and rupture. J Am Coll Cardiol 2003;41(4 Suppl S):S15–22.
7. Pugliese G, Iacobini C, Fantauzzi CB, Menini S. The dark and bright side of atherosclerotic calcification. Atherosclerosis 2015;238(2):220–30.
8. Towler DA. Vascular calcification: a perspective on an imminent disease epidemic. IBMS BoneKEy 2008;5(2):41–58.
9. Lehto S, Niskanen L, Suhonen M, Rönnemaa T, Laakso M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1996;16(8):978–83.
10. Sinha S, Eddington H, Kalra PA. Vascular calcification: mechanisms and management. Br J Cardiol 2008;15(6):316–21.
11. Aufderheide AC, Rodríguez-Martín C. The Cambridge encyclopedia of human paleopathology. Cambridge, U.K.: Cambridge University Press, 1998.
12. Subirana-Domènech M, Borondo-Alcázar JC, Armentano-Oller N, Font Valsecchi G, Galtés Vicente I, Castellà-García J. Arteriosclerosis in paleopathology. Are macroscopic findings well known?. Int J Paleopathol 2012;2(4):246–8.
13. Binder M, Roberts CA. Calcified structures associated with human skeletal remains: possible atherosclerosis affecting the population buried at Amara West, Sudan (1300–800 BC). Int J Paleopathol 2014;6:20–9.
14. Frink R. Inflammatory Atherosclerosis: Characteristics of the Injurious Agent. Sacramento, CA: Heart Research Foundation, 2002.
15. Doherty TM, Asotra K, Fitzpatrick LA, Qiao J-H, Wilkin DJ, Detrano RC, et al. Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. Proc Natl Acad Sci U S A 2003;100(20):11201–6.

16. Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. Circulation 2008;117(22):2938–48.
17. Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, et al. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. Circulation 1996;94(5):1175–92.
18. Ruiz JL, Hutcheson JD, Aikawa E. Cardiovascular calcification: current controversies and novel concepts. Cardiovasc Pathol 2015;24(4):207–12.
19. WHO. World Health Organization statistics. Geneva, Switzerland: World Health Organization (WHO), 2015; http://www.who.int/mediacentre/factsheets/fs317/en/ (accessed March 6, 2016).
20. Ruffer MA. On arterial lesions found in Egyptian mummies (1580 B.C.-525 A.D.). J Pathol Bacteriol 1911;15(4):453–62.
21. Allam AH, Thompson RC, Wann LS, Miyamoto MI, Nur El-Din AE-H, El-Maksoud GA, et al. Atherosclerosis in ancient Egyptian mummies: the Horus study. JACC Cardiovasc Imaging 2011;4(4):315–27.
22. Thomas GS, Wann LS, Allam AH, Thompson RC, Michalik DE, Sutherland ML, et al. Why did ancient people have atherosclerosis?: From autopsies to computed tomography to potential causes. Glob Heart 2014;9(2):229–37.
23. Thompson RC, Allam AH, Lombardi GP, Wann LS, Sutherland ML, Sutherland JD, et al. Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet 2013;381(9873):1211–22.
24. Gostner P, Knapp R, Recheis W, Seidler H. The Iceman: discovery and imaging. Radiology 2003;226:614–29.
25. Scannapieco FA, Genco RJ. Association of periodontal infections with atherosclerotic and pulmonary diseases. J Periodontal Res 1999;34(7):340–5.
26. Libby P. Role of inflammation in atherosclerosis associated with rheumatoid arthritis. Am J Med 2008;121(10 Suppl 1):S21–31.
27. Tonetti MS, Van Dyke TE. Periodontitis and atherosclerotic cardiovascular disease: consensus report of the Joint EFP/AAP Workshop on Periodontitis and Systemic Diseases. J Clin Periodontol 2013;40(Suppl 14):S24–9.
28. Conaghan P, Vanharanta H, Dieppe P. Is progressive osteoarthritis an atheromatous vascular disease? Ann Rheum Dis 2005;64(11):1539–41.
29. Zink A, Wann LS, Thompson RC, Keller A, Maixner F, Allam AH, et al. Genomic correlates of atherosclerosis in ancient humans. Glob Heart 2014;9(2):203–9.
30. Flora GC, Baker AB, Loewenson RB, Klassen AC. A comparative study of cerebral atherosclerosis in males and females. Circulation 1968;38(5):859–69.
31. Allison MA, Criqui MH, Wright CM. Patterns and risk factors for systemic calcified atherosclerosis. Arterioscler Thromb Vasc Biol 2004;24(2):331–6.

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CASE REPORT

ANTHROPOLOGY

Lucie Biehler-Gomez,[1] *M.S.; and Cristina Cattaneo*,[1] *M.D., Ph.D.*

The Diagnostic Implications of Two Cases of Known Rheumatoid Arthritis from the CAL Milano Cemetery Skeletal Collection

ABSTRACT: Rheumatoid arthritis (RA) is the most common erosive arthropathy and is of particular interest to forensic sciences. However, its diagnosis on bones remains challenging. We examined two skeletons from the CAL Milano Cemetery Skeletal Collection diagnosed with the pathology during life, to test the validity of the diagnostic criteria on bones. The first showed typical lesions of RA described in the literature. The second exhibited secondary osteoarthrosis, suggesting long-standing RA. A differential diagnosis including all four seronegative spondyloarthropathies, erosive osteoarthritis, gout and neuropathic arthropathy was also considered. Both shared common features consistent with the literature: symmetric erosions of bones in the hands, wrists and elbows, sparing of the distal interphalangeal joints, and the absence of sacroiliac and spinal fusion. Given the paucity of studies on known RA skeletons, these results strengthen the criteria for diagnosis. This research is, to our knowledge, the first study on identified and known individuals with RA.

KEYWORDS: forensic science, forensic anthropology, rheumatoid arthritis, diagnosis, joint disease, erosive arthropathy

Rheumatoid arthritis (RA) has become a disease of crucial interest, especially because of the lack of a cure, its high prevalence, and the painful joint symptoms for which it is renowned for. In this article, we present and discuss two skeletal cases of diagnosed RA from the CAL Milano Cemetery Skeletal Collection. Osteological studies on identified collections are particularly valuable for the study of bone diseases and ultimately, for forensic purposes. The known demography but mostly the associated medical data makes the study on bone diseases completely different: although it is not a golden standard, because the presence of the disease does not necessarily imply the presence of bone lesions, it is an indicator and a rather reliable guide. In the forensic practice, the construction of a biological profile is of paramount importance. In the identification process, primary means of identification are not always available and, in these cases, any detail is of crucial interest. Although the diagnosis of a pathology such as RA will not allow a straightforward identification in a skeletonized forensic case, it will certainly help in the search for a possible match. For example, aged individuals who wander off and die in secluded areas are not a rarity in the forensic exercise, particularly with victims of Alzheimer: the authors have indeed seen several cases in the past; the remains may be found only years after, with no identification documents. However, anthropological estimates of age and sex might not be enough to narrow down the possible matches among a list of missing individuals. In this case, a correct interpretation of the pathological signs left on bones and a possible diagnosis could further restrict the search and thus provide crucial information to the forensic expertise.

Today, RA is present in 0.5–1% of the adult population worldwide (1–8), making it the most common erosive arthropathy (9) with a higher prevalence in North American tribes and a lower one in Africa and South-East Asia (2,4,5,10). It starts around 30–50 years (2,3,8,11,12), primarily in women with a ratio of 3:1 (2,8–10). Although the exact cause is unknown, RA is a multifactorial disease with genetic factors accounting for up to 50% of the risk of developing the condition and environmental factors such as smoking and infectious diseases that might trigger its development and influence its severity. Risk factors for this arthropathy thus include female sex, smoking, infectious conditions, and family history (1,2,4,5,7,10–14).

In terms of pathogenesis, RA is a progressive, systemic, chronic, and inflammatory arthropathy (1,15). The infiltration in the synovium of leukocytes induces an inflammation or «synovitis». The subsequent autoimmune response is massive and uncontrolled, leading to the creation of an aggressive inflammatory front, or « pannus ». The pannus is constituted of interacting B cells and T cells as well as macrophages that will induce the overproduction of chemokines and cytokines (including interleukin 1,6 and TNF-α) in a proinflammatory loop. These cytokines activate the osteoclastic activity; hence, the spreading of the pannus leads to cartilage and bone destruction in the joint. However, RA can also present extra-articular manifestations (consequences of long-standing vasculitis) and have systemic effects including cardiovascular diseases (atherosclerosis and its potential outcomes), lymphoma, anemia, insulin resistance, decreased bone mineral density, and a general heightened mortality rate (1,2,5,13,16,17).

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In the palaeopathological literature, RA is defined as erosive with minimal to absent new bone formation (9,18–20). The lesions are articular or para-articular, polyarticular, and symmetric (8,9,15,18,21–27). RA typically affects the small joints of the wrist and hands including carpal joints, carpometacarpal joints, metacarpophalangeal joints (MCP), metatarsophalangeal joints (MTP), proximal interphalangeal joints (PIP) and may spread to more distant weight-bearing joints (7,8,18,21–23,27,28). The arthropathy is also characterized by a relative sparing of distal interphalangeal joints (DIP) (9,15,28) and of sacroiliac as well as spinal fusion (12,19,29–33). RA can also cause erosions of the odontoid process leading to subluxations and dislocations (18,34,35), ulnar deviation of the bones of the hand (7,8,19,32,36), and possible « pencil-in-cup » deformities (31). Ankyloses are more common in the spondyloarthropathies (SpA) than in RA, although carpal and tarsal ankylosis are well known in RA and ankylosis of the PIP is possible (9,33,37,38). On radiographs, RA is characterized by osteoporosis and its precursor, osteopenia, and subchondral cysts (7,8,18,19,22,23,28,38). Evidence of the disease can be noted radiographically in 70% of cases in the 2 years following the diagnosis (2).

Despite the diversity of lesions for which RA is known for, its diagnosis on skeletal remains continues to be challenging for multiple reasons. First, the onset of the disease occurs generally between 30 and 50 years of age, which, in anthropology, implies that the individual must have lived long enough for the disease to develop but also mark the bones to be able to diagnose it. This simple requirement is, however, not obvious in the archeological context. Indeed, the life expectancy was estimated to be between 20 and 35 years in antiquity (39). The early age-at-death in ancient populations coupled with the low prevalence of the disease in younger individuals can therefore explain the rarity of RA findings in archeology (40). Second, a suspected RA diagnosis is always considered with a difficult differential diagnosis. For instance, the seronegative spondyloarthropathies can present similar peripheral lesions. Several criteria were used as discriminants in the literature for the differential diagnosis with RA. Compared to the SpA, the erosive and minimal new bone formation were always characteristics in favor of RA (41,42), particularly because the SpA present sacroiliitis and spinal fusion, contrary to RA (19,33,42,43). They also tend to be asymmetric in their peripheral joint involvement (30,33,36,42,44), and as previously mentioned, interphalangeal joint ankylosis is much more common. Erosive osteoarthritis, another lytic arthropathy affecting particularly the hands of middle-aged women (45) does not affect large joints (42). However, it does involve DIP joints just like psoriatic arthritis but unlike RA (19,46).

The objective of this study was to present the lesions observable on skeletons with known RA. Therefore, we will report the skeletal lesions observed on individuals with known RA, as proved by the associated medical documentation, and discuss the diagnosis of this arthropathy on skeletal remains based on our observations on these known RA skeletons. Moreover, we provide in this paper a table for the differential diagnosis of RA on dry bone that may be of use in the forensic analysis of skeletonized individuals. This study is, to our knowledge, the first on RA on known skeletal data.

Materials and Methods

The skeletons presented in this article are part of the CAL Milano Cemetery Skeletal Collection, a contemporary and documented anthropological collection of more than 2100 skeletons curated at the *Laboratorio di Antropologia e Odontologia Forense* (LABANOF) (47). In accordance with article 43 of the Italian National Police Mortuary Regulation (September 10, 1990, no 285), the individuals were exhumed after 2006, recovered by cemetery workers by means of heavy vehicles, placed in metal boxes, and then stored at the LABANOF. Each individual is associated with a documentation including autopsy reports (when performed) and *"ISTAT"* (*Istituto Nazionale di Statistica*) death certificates that specify the cause of death and any additional pathological conditions related to it.

For this study, we selected two individuals of the CAL Milano Cemetery Skeletal Collection who were medically diagnosed with RA while living as evident with the mention of « rheumatoid arthritis » in the associated medical documentation. A macroscopic osteological analysis was performed on each skeleton, and each lesion observed was recorded and described. The information provided on the ISTAT certificates is reported in the results for both cases. None of the individuals selected in our study were autopsied. It is important to note that no information regarding any treatment that might have influenced the course of the disease was provided in the associated documentation of any of the skeletons of the study.

The differential diagnosis included four SpA (ankylosing spondylitis, psoriatic arthritis, enteropathic arthritis, and reactive arthritis), erosive osteoarthritis, gout, and neuropathic arthropathy. The description of the nature, aspect, and pattern of involvement of the diseases of the differential diagnosis was established in Table 1.

Regarding terminology, several terms were used to describe the observed lesions and will be defined here. Pitting and porosity refer to a localized series of holes on the articular area of the bone. Marked pitting implies larger holes exposing more trabeculae. Eburnation is a focal polished area on the joint surface resulting from bone-to-bone contact. Marginal osteophytes are new bone apposition around the margin of the joint. Periosteal reaction indicates woven bone deposits on the cortical surface remodeling into lamellar bone over time.

Results

Case 1

The skeleton belonged to a female individual of 76 years, born in 1915 and died in 1991 of a cardiac arrest. The associated medical documentation also mentions that the individual presented a luxation of the cervical column. The vast majority of the bones were present (Fig. 1). For the feet, the fourth right distal phalanx, the third medial left phalanx, and the second to fifth left distal phalanges were absent. Regarding the hands, the trapezoid, metacarpal (MC) 1, MC4, and MC5 were present on the right side but the base and distal end of the MCs were taphonomically altered and so no lesion could be observed; all MCs, the first and third proximal phalanges, the third and fifth medial phalanges, and the fifth distal phalanx were present on the left side. Two calluses were found on the posterior parts of the right fifth and 12th ribs suggestive of healing antemortem trauma.

Eburnations were noted on the antero-superior part of the lateral femoral condyle, the postero-inferior side of the right patella, the superior part of the right glenoid fossa, and the costal facets of C1, C2, and L5. Porosity was present on the costal attachments of the sternum and on the manubriosternal synostosis and on the bodies of C6, C7, and L5. Large and marked pitting was noticed on the proximal ulnae, tibial plateaux, and the base of the phalanges of the left hand. Alteration of the normal shape of the bone with lytic resorption of the odontoid process (Fig. 2), the left

TABLE 1—*Criteria for differential diagnosis of rheumatoid arthritis.*

Other Pathologies Considered	Axial Involvement	Peripheral Joints Involvement	References
Rheumatoid arthritis	–	Erosive, polyarticular, symmetric, spreads to weight-bearing joints, affects hands and feet sparing DIP	(7, 15, 50, 55, 56)
Spondyloarthropathies			
Ankylosing spondylitis	Symmetric sacroiliitis, upward spine fusion, « bamboo » spine, syndesmophytes	Mono/oligoarthritis, mostly lower limbs	(56–58)
Psoriatic arthritis	Asymmetric sacroiliitis and spine fusion	Oligoarticular, DIP commonly affected, mostly hands, often asymmetric	(30, 56, 59)
Reactive arthritis	Asymmetric sacroiliitis and spine fusion	Asymmetric mono/oligoarthritis, large joints of lower limbs	(56, 60)
Enteropathic arthritis	Symmetric sacroiliitis and spine fusion	Nonerosive asymmetric oligoarthritis of the lower limbs	(24, 59, 61)
Erosive osteoarthritis	–	Asymmetric/symmetric, central erosion, DIP more affected than PIP, only hands, mostly females	(26, 45, 56)
Gout	–	Asymmetric « punched-out » lesion on MTP1 (podagra)	(56, 62, 63)
Neuropathic arthropathy	–	Asymmetric, severe destruction with bone debris	(50, 64)

DIP = distal interphalangeal joint; PIP = proximal interphalangeal joint; MTP1 = first metatarsophalageal joint.

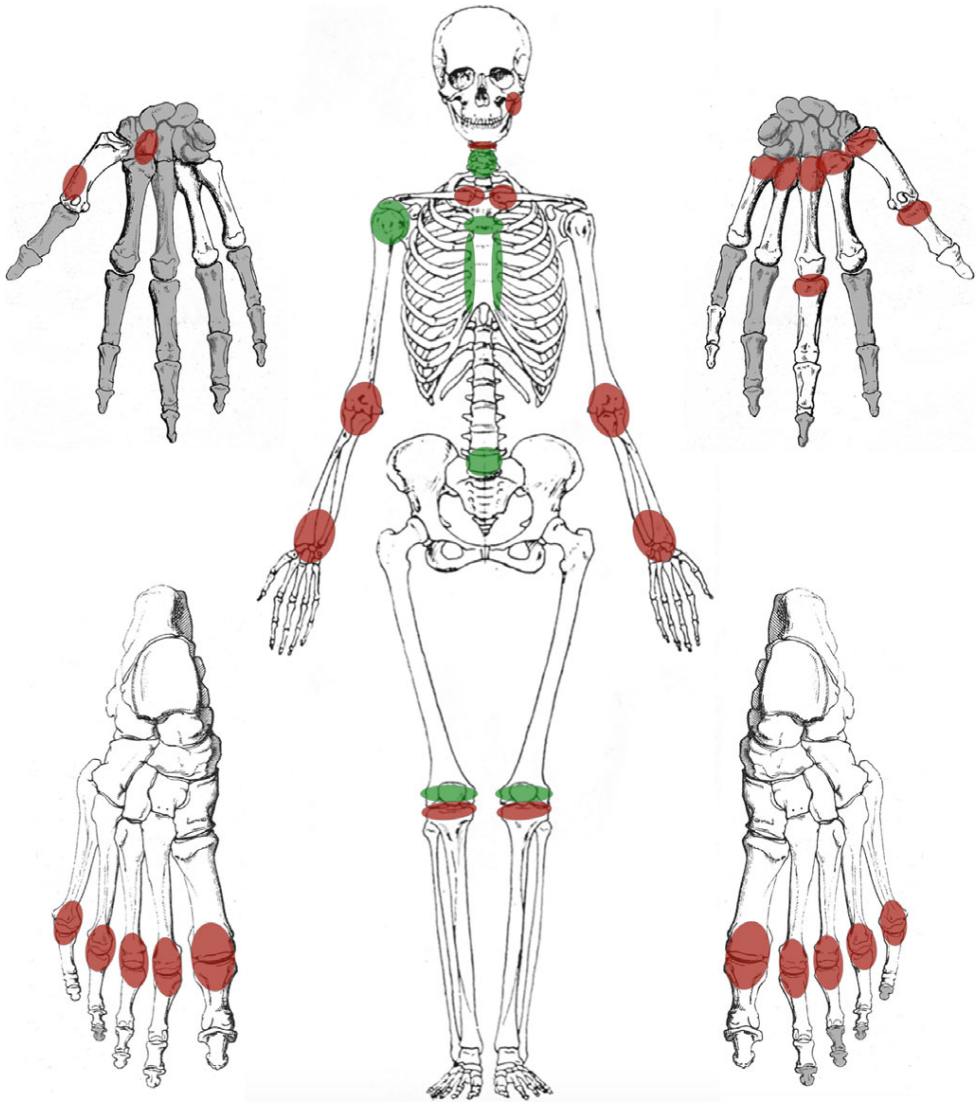

FIG. 1—*Schematic representation of the lesions noted in Case 1. Areas colored in green represent lesions associated to osteoarthrosis, whereas red lesions are associated with rheumatoid arthritis. Gray areas refer to missing bones. [Color figure can be viewed at wileyonlinelibrary.com]*

temporomandibular joint (TMJ) (Fig. 2), the sternal end of the clavicles, posterior right MC1 (Fig. 2), the base of left MC1-MC5 (Fig. 2), the heads of the metatarsals (MT) (Fig. 2), and the distal end of the ulnae was observed (Fig 2).

Case 2

The second skeleton of this research belonged to a woman of 88 years old, born in 1903 and died in 1991 of a cardiac arrest

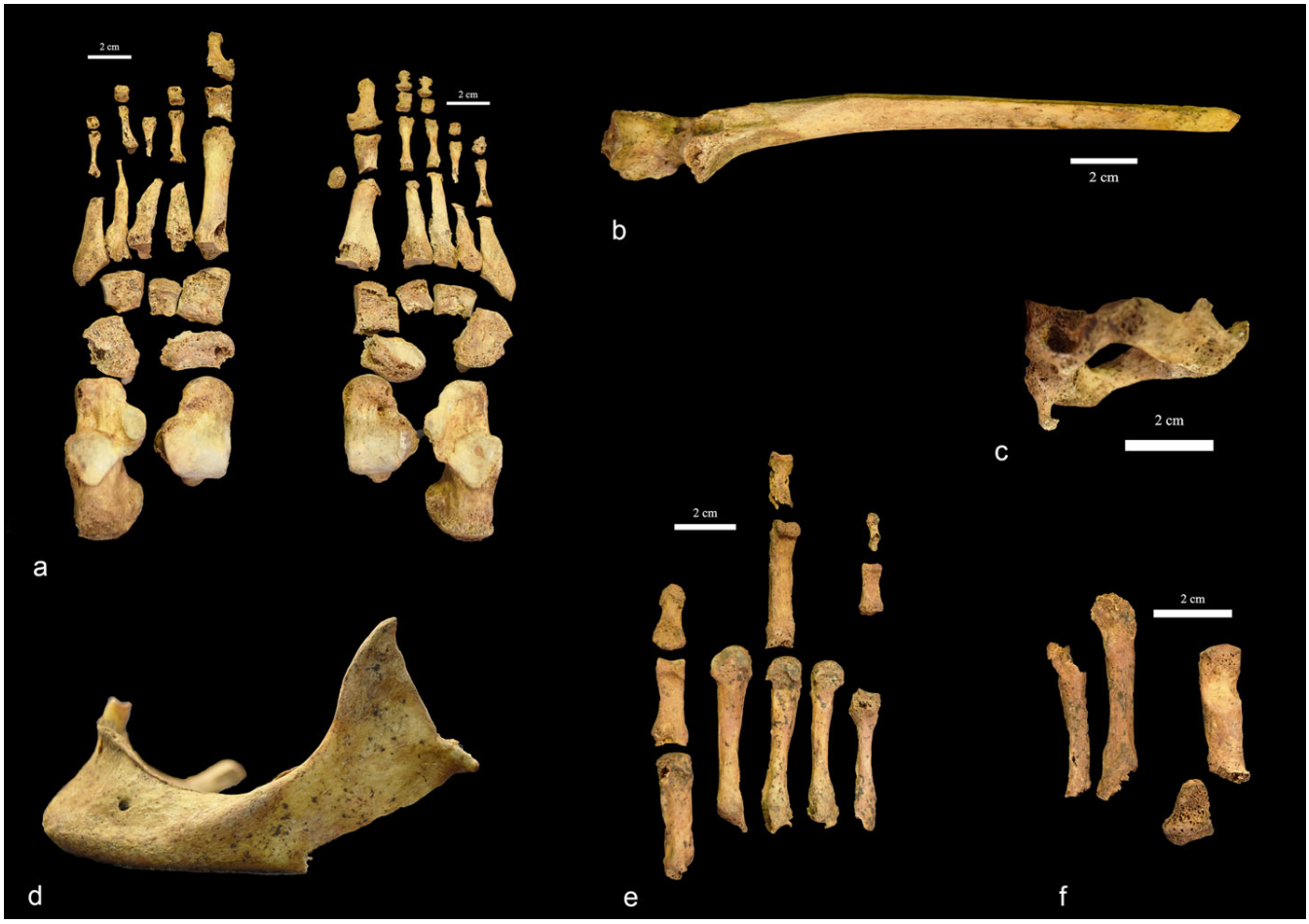

FIG. 2—*Details of some lesions noted in Case 1. (a) feet bones showing lytic resorption of the metatarsal heads deforming the metatarsophalangeal joints; (b) complete lytic resorption of the distal end of the left ulna; (c) lytic alteration of the odontoid process leading to a luxation of the cervical vertebrae; (d) lytic resorption of the left temporomandibular joint; (e) left hand bones with lytic deformation of the metacarpal bases; (f) right hand with lytic alteration of the trapezoid and the base of the first metacarpal. [Color figure can be viewed at wileyonlinelibrary.com]*

after a renal insufficiency and an irreversible pulmonary edema. Most of the bones of the skeleton were present except L4 and L5 (Fig. 3). For the hands, the right pisiform, capitate, hamate, and trapezoid as well as the second medial and distal phalanges and the fifth distal phalanx were absent. On the left hand, the pisiform, lunate, scaphoid, trapezium, trapezoid, and capitate were absent as well as the first proximal and distal phalanges. Three calluses were noted on the left ribs: two on the seventh and one on the eighth; given the advanced nature of their remodeling process, they were probably caused during the same traumatic event. Finally, enthesophytes were noted on the long bones of the upper limbs.

In the feet, marginal osteophytes were documented on both tarsometatarsal-1, first PIP, and first DIP joints, whereas more pronounced osteophytes altering the normal shape of the bones were present around the articular facets of the right navicular, talus, and calcaneus. Similarly, the bones of the ankles, knees, right wrist (only radius and ulna), elbows, and shoulders showed marginal osteophytes around the articulations (Fig. 4). The vertebrae presented marginal osteophytes on the bodies and articular facets (Fig. 4), Schmorl's nodes on the vertebral bodies of T7 to T10 and horizontal osteophytic bony bridges on the bodies of T8 to L3, fusing T8 and T9 together, as well as T11 with T12, and L1 with L2. On the hands, marginal bone apposition was observed on all types of joints: carpal, carpometacarpal, MCP, PIP, and DIP. Eburnation was reported on the head of the fifth right metatarsal, the left femoral head, and the lateral condyle of the right femur. Also, the left TMJ manifested bone apposition. Lytic activity was also noted in the skeleton: the periarticular margins of the ulnar heads, the articular base of both MC2, left MC3 and both MC4, as well as the base of the hamulus of the left hamate presented deep lytic lesions (Fig. 4).

Discussion

In this paper, we presented two different cases of known RA from the CAL Milano Cemetery Skeletal Collection. The main advantage of this collection consists in its documentation that includes diagnosed pathologies related to the cause of death. Therefore, we know that both individuals selected were medically diagnosed with RA during life. Given this valuable knowledge, it is interesting to see the lesions observed on the skeletons and how they relate to the disease. This study is the first to document RA skeletons from an identified skeletal collection and discuss its diagnosis for each case.

Case 1 presented eburnation as well as porosity on the articular attachments of the sternum and more marked porosity on

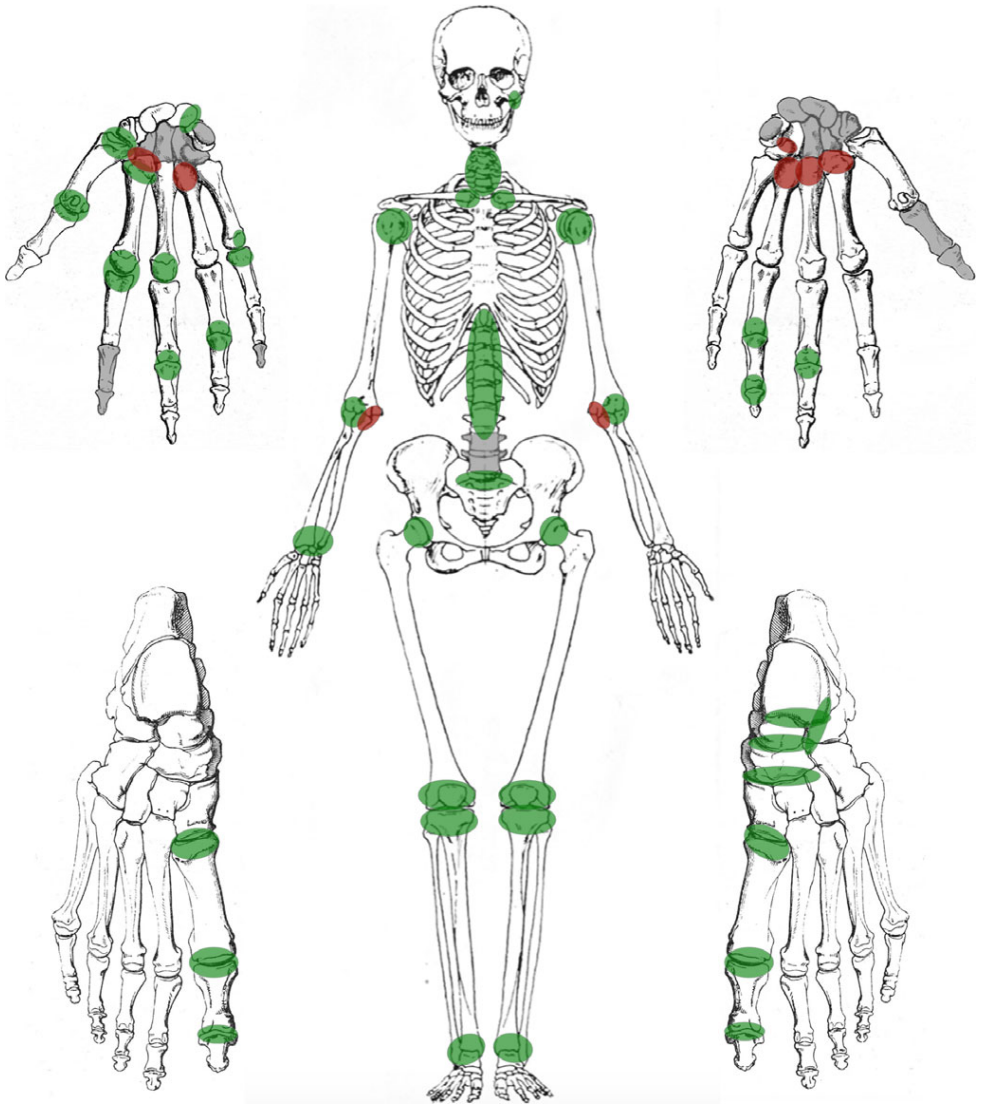

FIG. 3—*Schematic representation of the lesions noted in Case 2. Areas colored in green represent lesions associated to osteoarthrosis, whereas red lesions are associated with rheumatoid arthritis. Gray areas refer to missing bones. [Color figure can be viewed at wileyonlinelibrary.com]*

some vertebral bodies and the manubriosternal synostosis. Both of these lesions are typical of degenerative joint disease or osteoarthrosis (OA) (48,49), consistent with the age of the individual. The other peripheral lesions, more marked and represented in Figs. 1 and 2, are lytic and symmetric, when the taphonomic preservation allows bilateral comparisons. If we consider the literature, one can see that the differential diagnosis with erosive arthropathy, four SpA (ankylosing spondylitis, psoriatic arthritis, reactive arthritis, and enteropathic arthritis) gout, and neuropathic arthropathy should be included (Table 1). This individual in fact does not present any sacroiliac or spine fusion, in line with the exclusion of SpA. Moreover, it is clear that the lesions could not be confused with gout, erosive osteoarthritis, or neuropathic arthropathy because they are symmetric, polyarticular, and no DIP joint of the hands was affected. Hence, this known case strengthens the diagnostic bases for RA.

Case 2 also confirms the solidity of diagnostic standards. It showed marginal osteophytes on the bones of the ankles, knees, right wrist, elbows, shoulders, hands as well as on tarsals and vertebral bodies, Schmorl's nodes on vertebrae and eburnation on the fifth right metatarsal, and the articulations of the femora. These osteoarthritic lesions are quite common in older individuals (50,51) which is consistent with our skeleton of an 88-year-old woman. Lytic lesions were also reported in the hands and proximal end of ulnae. The lesions on the ulnae, MC2, and MC4 are symmetric. The bilateral counterpart of the left hamate was absent in the anthropological analysis and the right MC3 did not present any lytic lesion. The polyarticular distribution of the lytic lesions observed on the hands of the skeleton excludes classic gout and neuropathic arthropathy from the differential diagnosis (Table 1). Similarly, the erosive lesions were reported exclusively on the upper limbs, eliminating enteropathic arthritis and reactive arthritis from the differential diagnosis. No sacroiliac fusion was noted. Although enthesophytes were noted on the skeleton, they were located on the shafts of the long bones of the upper limbs. These sites refer to fibrous entheses and not to the fibrocartilaginous entheses characteristic of the SpA, such as in the hands, feet (especially the calcaneal attachments), TMJ, sternoclavicular, acromioclavicular, manubriosternal, and sacroiliac

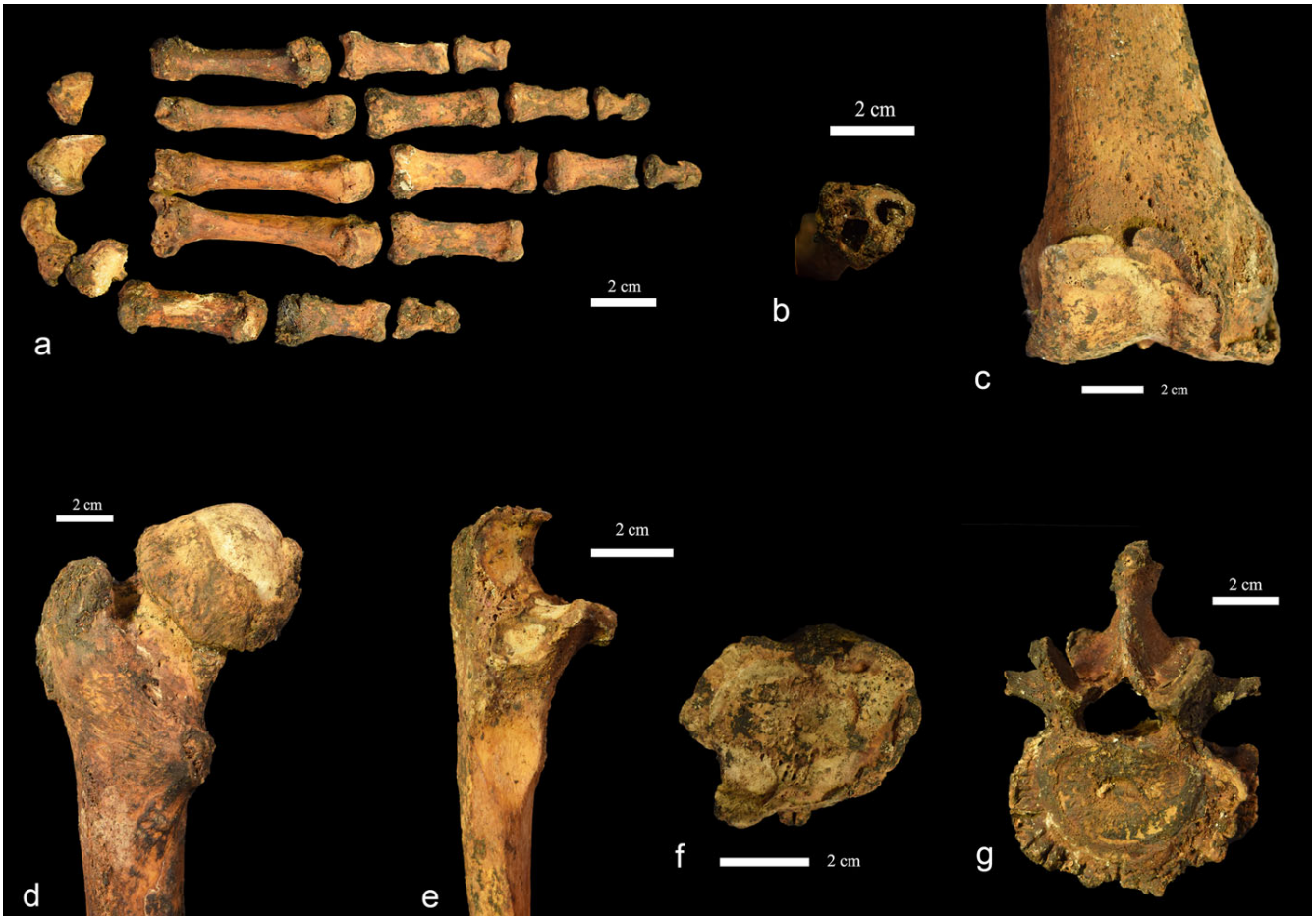

FIG. 4—*Details of some lesions noted in Case 2. (a) right hand showing proliferative alteration of the carpal, carpometacarpal, metacarpophalangeal, and interphalangeal joints; (b) proximal view of the second metacarpal of the left hand showing deep lytic lesions; (c) extensive marginal osteophytes on the distal part of the right femur; (d) marked marginal osteophytes around the head of the left femur (posterior view); (e) marginal osteophytes around the articular facets of the right proximal ulna, note the unusual deep pitting above the radial facet; (f) right patella showing extensive marginal osteophytes and localized pitting; (g) extensive marginal osteophytes on the fifth lumbar body. [Color figure can be viewed at wileyonlinelibrary.com]*

joints (52). Finally, none of seven distal phalanges showed lytic lesions, yet erosive osteoarthritis and psoriatic arthritis are known for their involvement of the DIP joints (Table 1). The proliferative and articular lesions located on the carpometacarpal joint and elbows may be related to a secondary event to RA, as secondary OA. The remaining lesions, comprising marginal osteophytes, alterations of the joint contour as well as eburnation may be associated with OA (48,49) and are congruent with the advanced age of the individual. OA and RA can coexist (7,9,18) and secondary OA is the result of long-standing RA (7). The presence of secondary OA lesions is thus very interesting because it can give us an idea of the duration of the pathology.

Hence, both of these known cases showed the typical traits described in the clinical literature: erosive polyarticular symmetric lesions affecting the hand and wrist bones as well as other more distant joints (here, the elbows). These traits are recognized characteristics of RA (2,7–9,15,18,19,21,23,24,27,28,38) and used in the differential diagnosis of previous studies (26,31,46,53,54). Moreover, they both lacked the same features that would have indicated other diseases included in the differential diagnosis: sacroiliac fusion, spinal fusion, asymmetric involvement, fibrocartilaginous entheses, oligoarthritis, DIP joints involvement, and bone debris.

Conclusion

In this paper, we presented the osteological analysis of two cases of known RA in a diagnostic perspective. To our knowledge, this research is the first to study RA on an identified skeletal collection. This type of study on known collections is of pivotal importance because it could contribute to the understanding of the etiology of the disease, its history, and especially its diagnosis on dry bone. RA is the most common erosive arthropathy today and dates back at least hundreds of years. As such, the study is of critical interest for forensic sciences, archeology, and even medicine. In the forensic anthropology practice, a correct interpretation of the bone markers for RA would allow a more precise biological profile and narrow the field of search within lists of missing persons for unidentified deceased. In this perspective, we reported the lesions observed in two known cases and discussed the diagnostic implications in a broad differential diagnosis.

The first case of this study strengthened the bases for the diagnosis of RA because it exhibited characteristic lesions of the disease described in both clinical and paleopathological literature, namely multiple symmetric marked lytic lesions located on the hands, wrists, and some more distant weight-bearing joints as well as the feet. The second case documented a comorbidity

with OA and displayed secondary OA through proliferative articular lesions. This particularity suggested a case of long-standing RA, providing additional information to the biological analysis of the skeleton. Symmetric lytic lesions typical of RA were observed on the hands, wrists, and elbows.

Consequently, common lesions due to RA could be easily identified in these two known cases: para-articular and articular erosions of multiple bones of the hands and wrists but also of more distant joints, in a symmetric pattern (as much as taphonomy allows it), sparing the DIP joints as well as sacroiliac and spinal fusion. These signs are consistent with the descriptions of the disease in the literature and given the paucity of studies on known dry bone cases, strengthen the criteria for diagnosis. We hope that this study will demonstrate the importance of research on identified collections especially for the study of bone pathology.

References

1. Choy E. Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. Rheumatology 2012;51(Suppl 5):v3–11.
2. Lee DM, Weinblatt ME. Rheumatoid arthritis. Lancet 2001;358 (9285):903–11.
3. Rindfleisch JA, Muller D. Diagnosis and management of rheumatoid arthritis. Am Fam Physician 2005;72(6):1037–47.
4. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res Ther 2002;4(3):S265.
5. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet 2016;388(10055):2023–38.
6. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423 (6937):356–61.
7. Vigorita VJ, Ghelman B. Arthritis. In: Vigorita VJ, Ghelman B, editors. Orthopaedic pathology. Philadelphia, PA: Lippincott Williams & Wilkins, 2016;710–68.
8. Wright V, Londino AV Jr. Inflammatory arthritis. In: Fitzgerald RH, Kaufer H, Malkani AL, editors. Orthopaedics. St Louis, MO: Mosby, 2002;819–25.
9. Ortner DJ. Erosive arthropathies, enthesopathies and miscellaneous pathological conditions of joints. In: Ortner DJ, editor. Identification of pathological conditions in human akeletal eemains. New York, NY: Smithsonian Institution Press, 2003;561–88.
10. Alamanos Y, Drosos AA. Epidemiology of adult rheumatoid arthritis. Autoimmun Rev 2005;4(3):130–6.
11. Entezami P, Fox DA, Clapham PJ, Chung KC. Historical perspective on the etiology of rheumatoid arthritis. Hand Clin 2011;27(1):1–10.
12. Majithia V, Geraci SA. Rheumatoid arthritis: diagnosis and management. Am J Med 2007;120(11):936–9.
13. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med 2011;365(23):2205–19.
14. Edwards CJ, Cooper C. Early environmental factors and rheumatoid arthritis. Clin Exp Immunol 2006;143(1):1–5.
15. Henrique da Mota LM, Afonso Cruz B, Viegas Brenol C, Alves Pereira I, Rezende-Fronza LS, Barros Bertolo M, et al. Guidelines for the diagnosis of rheumatoid arthritis. Rev Bras Reumatol Engl Ed) 2013;53 (2):141–57.
16. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular manifestations in rheumatoid arthritis. Mædica 2010;5(4):286–91.
17. Lopez-Mejias R, Castaneda S, Gonzalez-Juanatey C, Corrales A, Ferraz-Amaro I, Genre F, et al. Cardiovascular risk assessment in patients with rheumatoid arthritis: the relevance of clinical, genetic and serological markers. Autoimmun Rev 2016;15(11):1013–30.
18. Aufderheide AC, Rodríguez-Martín C, editors. Joint diseases. In: The Cambridge encyclopedia of human paleopathology. Cambridge, U.K.: Cambridge University Press, 1998;93–116.
19. Waldron T, editor. Diseases of joints, part 2. In: Paleopathology. Cambridge, U.K.: Cambridge University Press, 2008;46–71.
20. Rothschild BM. Not the Lucy, not the one. Clin Exp Rheumatol 2002;20 (6):741–3.
21. Chew FS, editor. Approach to joint disease. In: Skeletal radiology: the bare bones. Philadelphia, PA: Lippincott Williams & Wilkins, 2012;196–206.
22. Firestein GS. Rheumatoid arthritis. In: Humes HD, DuPont HL, Gardner LB, Griffin JW, Harris ED, Hazzard WR, et al., editors. Kelley's textbook of internal medicine. Philadelphia, PA: Lippincott Williams & Wilkins, 2000;1347–59.
23. Roberts CA, Manchester K, editors. Joint disease. In: The archaeology of disease. Ithaca, NY: Cornell University Press, 1995;99–123.
24. Rogers J, Waldron T, Dieppe P, Watt I. Arthropathies in palaeopathology: the basis of classification according to most probable cause. J Archaeol Sci 1987;14(2):179–93.
25. Rogers J, Watt I, Dieppe P. Medical history: arthritis in Saxon and mediaeval skeletons. Br Med J (Clin Res Ed) 1981;283(6307):1668–70.
26. Banks SE. Erosive osteoarthritis: a current review of a clinical challenge. Clin Rheumatol 2010;29(7):697–706.
27. Sommer OJ, Kladosek A, Weiler V, Czembirek H, Boeck M, Stiskal M. Rheumatoid arthritis: a practical guide to state-of-the-art imaging, image interpretation, and clinical implications. Radiographics 2005;25(2):381–98.
28. Erickson AR, Cannella AC, Mikuls TR. Clinical features of rheumatoid arthritis. In: Firestein GS, Budd R, Gabriel SE, McInnes IB, O'Dell JR, editors. Kelley and Firestein's textbook of rheumatology. Philadelphia, PA: Elsevier Health Sciences, 2017;1167–86.
29. Kacki S. Erosive polyarthropathy in a Late Roman skeleton from northern France: a new case of rheumatoid arthritis from the pre-Columbian Old Word? Int J Paleopathol 2013;3(1):59–63.
30. Veale DJ, Fearon U. What makes psoriatic and rheumatoid arthritis so different? RMD Open 2015;1(1):e000025.
31. Zias J, Mitchell P. Psoriatic arthritis in a fifth-century Judean Desert monastery. Am J Phys Anthropol 1996;101(4):491–502.
32. Middlemiss JH. Ankylosing spondylitis. J Fac Radiol 1956;7(3):155–66.
33. Rothschild BM, Woods RJ. Spondyloarthropathy: erosive arthritis in representative defleshed bones. Am J Phys Anthropol 1991;85(2):125–34.
34. Kim HJ, Nemani VM, Riew KD, Brasington R. Cervical spine disease in rheumatoid arthritis: incidence, manifestations, and therapy. Curr Rheumatol Rep 2015;17(2):9.
35. Jurik AG. Imaging the spine in arthritis – a pictorial review. Insights Imaging 2011;2(2):177–91.
36. Kim DK, Lee IS, Kim W, Lee JS, Koh BJ, Kim MJ, et al. Possible rheumatoid arthritis found in the human skeleton collected from the tomb of Joseon Dynasty, Korea, dating back to the 1700s AD. Int J Osteoarchaeol 2011;21(2):136–49.
37. Leden I, Forslind K, Svensson B. Ankylosis of wrist and small joints of the hand occurs in rheumatoid arthritis: diagnostic implication in paleopathology. Int J Paleopathol 2012;2(4):249–51.
38. Brahee DD, Pierre-Jerome C, Kettner NW. Clinical and radiological manifestations of the rheumatoid wrist. A comprehensive review. J Manipulative Physiol Ther 2003;26(5):323–9.
39. Preston SH. Human mortality throughout history and prehistory. In: Simon JL, editor. The state of humanity. Oxford, U.K.: Blackwell Publishers Ltd, 1995;30–6.
40. Thould AK, Thould BT. Arthritis in Roman Britain. Br Med J (Clin Res Ed) 1983;287(6409):1909–11.
41. Blondiaux J, Cotten A, Fontaine C, Hänni C, Bera A, Flipo R. Two Roman and medieval cases of symmetrical erosive polyarthropathy from Normandy: anatomico-pathological and radiological evidence for rheumatoid arthritis. Int J Osteoarchaeol 1997;7(5):451–66.
42. Hacking P, Allen T, Rogers J. Rheumatoid arthritis in a medieval skeleton. Int J Osteoarchaeol 1994;4(3):251–5.
43. Cawley WD, Paine RR. Skeletal indicators of reactive arthritis: a case study comparison to other skeletal conditions, such as rheumatoid arthritis, ankylosing spondylitis, ankylosing sero-negative SpA, and DISH. Int J Paleopathol 2015;11:70–4.
44. Rothschild BM, Turner KR, DeLuca MA. Symmetrical erosive peripheral polyarthritis in the Late Archaic Period of Alabama. Science 1988;241 (4872):1498–501.
45. Punzi L, Ramonda R, Sfriso P. Erosive osteoarthritis. Best Pract Res Clin Rheumatol 2004;18(5):739–58.
46. Hoffman GS. Polyarthritis: the differential diagnosis of rheumatoid arthritis. Semin Arthritis Rheum 1978;8(2):115–41.
47. Cattaneo C, Mazzarelli D, Cappella A, Castoldi E, Mattia M, Poppa P, et al. A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection. Forensic Sci Int. 2018.
48. Ortner DJ. Osteoarthritis and diffuse idiopathic skeletal hyperostosis. In: Ortner DJ, editor. Identification of pathological conditions in human skeletal remains. New York, NY: Smithsonian Institution Press, 2003;545–60.
49. Waldron T, editor. Diseases of joints, part 1. In: Paleopathology. Cambridge, U.K.: Cambridge University Press, 2008;24–45.
50. Ortner DJ. Identification of pathological conditions in human skeletal remains, 3rd edn. New York, NY: Smithsonian Institution Press, 2003.
51. Aufderheide AC, Rodríguez-Martín C. The Cambridge encyclopedia of human paleopathology. Cambridge, U.K.: Cambridge University Press, 1998.

52. Benjamin M, McGonagle D. The anatomical basis for disease localisation in seronegative spondyloarthropathy at entheses and related sites. J Anat 2001;199(5):503–26.
53. Šlaus M, Novak M, Čavka M. Four cases of ankylosing spondylitis in medieval skeletal series from Croatia. Rheumatol Int 2012;32(12):3985–92.
54. Mays S, Watt I, Loe L. An unusual erosive arthropathy from Medieval England. Int J Osteoarchaeol 2016;27(4):693–9.
55. Fitzgerald RH, Kaufer H, Malkani AL. Orthopaedics. St Louis, MO: Mosby, 2002.
56. Waldron T. Paleopathology. Cambridge, U.K.: Cambridge University Press, 2008.
57. Raychaudhuri SP, Deodhar A. The classification and diagnostic criteria of ankylosing spondylitis. J Autoimmun 2014;48–49:128–33.
58. Braun J, Sieper J. Ankylosing spondylitis. Lancet 2007;369(9570):1379–90.
59. Jadon DR, McHugh NJ. Other seronegative spondyloarthropathies. Medicine (Baltimore) 2014;42(5):257–61.
60. Lahu A, Backa T, Ismaili J, Lahu V, Saiti V. Modes of presentation of reactive arthritis based on the affected joints. Med Arch 2015;69(1):42–5.
61. Resende GG, Lanna CCD, Bortoluzzo AB, Gonçalves CR, Sampaio-Barros PD, da Silva JAB, et al. Enteropathic arthritis in Brazil: data from the Brazilian registry of spondyloarthritis. Rev Bras Reumatol Engl Ed 2013;53(6):452–9.
62. Richette P, Bardin T. Gout. Lancet 2010;375(9711):318–28.
63. Monu JUV, Pope TL Jr. Gout: a clinical and radiologic review. Radiol Clin North Am 2004;42(1):169–84.
64. Stanley JC, Collier AM. Charcot osteo-arthropathy. Curr Orthop 2008;22(6):428–33.

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ORIGINAL ARTICLE

Diabetic bone lesions: a study on 38 known modern skeletons and the implications for forensic scenarios

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Abstract
Diabetes mellitus is a condition with severe and life-threatening complications and epidemic proportions worldwide. The study of diabetes on bones can provide crucial information to the forensic practice, the archeological field and medical research. In this paper, the authors report and discuss the lesions observed on the skeletons of 38 individuals (plus 11 negative control samples) of the CAL Milano Cemetery Skeletal Collection with known diabetes. As a result, different types of lesions were highlighted in the feet: periosteal new bone formation, lysis of tuft, lytic lesions, evidence of trauma, osteomyelitis, and osteochondritis dissecans. In 50% of the skeletons of the study sample, lesions were located on bones of the first ray of the foot. Vascular calcifications were also collected and considered. None of these lesions is pathognomonic of diabetes and each implies a broad differential diagnosis that can be confronted with the upper and axial lesions. However, they are coherent with the disease development and complications. This study is the first to document skeletons with known diabetes from an identified collection and discuss their diagnostic potential.

Keywords Forensic anthropology · Bone pathology · Diabetes · Dry bone · Biological profile

Introduction

Diabetes mellitus (DM), one of the major threats to public health in the twenty-first century [1, 2], is predicted to be the seventh leading cause of death by 2030 [3]. Given its epidemic proportions, it has become essential to investigate the diagnosis of DM on bones for forensic, paleopathological, and medical purposes. Indeed, in the forensic practice, the correct interpretation of DM on bare skeletons would allow the construction of a more precise biological profile that could potentially lead to the identification of unknown deceased when compared to ante-mortem data. Primary means of identification are not always available in the identification process, and in these cases, any detail can be of crucial interest. Although the diagnosis of pathology such as diabetes will not allow a straightforward identification in a skeletonized forensic case, it will certainly help in the search for a possible match, providing additional descriptors. Indeed, the recognition of the pathology in an unidentified skeletonized forensic case could narrow the field of search among missing persons of a specific sex and age group. In a paleopathological perspective, the identification of diabetic cases could result in a new interpretation of archeological cases and past human behaviors, which would in turn provide substantial additional information on past and modern populations to the medical research for a better understanding of the disease. The study of modern osteological collections can aid in this objective if they have known pathologies [4, 5]. Based on the identified CAL Milano Cemetery Skeletal Collection [6], we present in this paper an observational study on 38 skeletons with known diabetes.

Diabetes affects about 20% of older adults [7] and 10% of the western population [8]. It is a metabolic disorder characterized by chronic hyperglycemia and presenting various etiologies, including two most common types [9]. The pathogenesis and genetic causes of the different etiologies remain incompletely understood. Type 1 diabetes mellitus (T1DM) represents less than 10% of all DM cases and can be either idiopathic (caused by another etiology) or autoimmune. For the latter, a combination of genetic predispositions and environmental factors will lead to the autoimmune destruction of

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pancreatic β-cells, definitely impairing the normal secretion of insulin and thus inducing hyperglycemia. By opposition, type 2 diabetes mellitus (T2DM) is responsible for about 90% of all cases of DM and for the epidemic scale of diabetes in both western and developing countries [1, 8–10]. T2DM pathogenetic process can result in a lack of insulin or in its insensitivity, ranging from "predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance" [9].

Complications of DM are not rare. They are generally separated into microvascular and macrovascular complications. The first includes retinopathy, nephropathy, and neuropathy. Depending on its severity and duration, hyperglycemia can generate diabetic neuropathy, a nerve ischemia which will damage nerve function [11, 12]. Neuropathy in DM can result in peripheral loss of the sense of touch, proprioception, temperature, and vibration (diabetic polyneuropathy), leaving the feet vulnerable to undetected trauma which can later cause foot ulcerations and infections as well as fractures, dislocations, destructions, and deformities of feet bones (such as Charcot's joints) [8–13]. Politi and colleagues report in a review that 50% of all diabetic patients will develop neuropathy in their lifetime [14]. Macrovascular complications in DM are becoming epidemic, especially in T2DM [15]. Diabetes amplifies the risk of cardiovascular morbidity and mortality. Indeed, because of its characteristic chronic hyperglycemia and insulin resistance, DM promotes atherosclerosis and plaque instability [11, 12, 15]. Atherosclerosis induces ischemia, which is not fatal per se, but a ruptured plaque can cause a thrombosis.

Bone mineral density (BMD) is also affected in diabetes: decreased in T1DM while normal or increased in T2DM; and associated to an increased fracture risk [7, 13, 16–19]. Undetected trauma can cause ulcerations and infections that can be observed on bones through infection markers such as osteomyelitis [20–23]. Neuropathic arthropathy (or Charcot's joints) appears in 0.2% of diabetic cases [24] and 25% of DM patients with leg complications [22], usually in individuals over 50 years with long-standing diabetes [19, 25]. It is a deformation and severe joint destruction with bone debris, classically in the feet (tarsometatarsal and metatarsophalangeal joints), secondary to a trauma and/or infection. Charcot's joint often occurs in diabetic patients with neuropathy and can lead to the amputation of the lower leg [8, 11, 19, 20, 22, 24–28]. Indeed, DM is the most common cause of amputation of the lower limbs in non-traumatic cases [24]. The lifetime risk for diabetic patients is about 20 to 40% for neuropathy, 50% for peripheral artery disease, and 15% for foot ulceration [24]. Neuropathy can also induce the "claw foot" deformity, characterized by a hyperextension of the metatarsophalangeal joints, hyperflexion of the interphalangeal joints and a cavus deformity of the foot arch. This posture puts a higher pressure on metatarsal heads which can create calluses over time [19, 24]. Although the feet are the most preferred site for lesions secondary to DM because of the undetected microfractures due to constant weight-bearing and impaired sensory innervations, hand lesions are also possible by means of etiologies similar to the microvascular mechanisms in diabetes. They include limited joint mobility, Dupuytren's disease, palmar flexor tenosynovitis, and the carpal tunnel syndrome [8, 13, 19, 25, 28]. Charcot's joint of the wrist is possible but extremely rare [13, 25, 28, 29]. However, Belcastro and colleagues explain that diabetic bone lesions are limited to the feet and do not involve the hands and facial skeleton [30]. They also describe diabetic osteopathy as an "ischemic osteolysis of the distal metatarsals and proximal phalanges of the foot, resulting in a distally tapered shape," or whittling [30]. The exact mechanism leading to diabetic osteolysis is not clearly understood and may not necessarily be associated with microvascular or neuropathic complications [19]. Diabetes shares common risk factors and complications with primary osteoarthritis, rheumatoid arthritis, cancer, diffuse idiopathic skeletal hyperostosis (DISH), and gout, though they do no present any link of causality [13, 17, 19, 25]. Patients with DISH are more likely to develop DM, especially given the common association with obesity [8, 13, 31, 32]; and diabetes "might play a role either directly or indirectly, through yet unknown mediators, in the pathogenesis" of DISH [31]. On the opposite, rheumatoid arthritis is not frequent in DM patients [8] and the association between diabetes and primary osteoarthritis remains uncertain [13]. According to Coughlin and colleagues, DM is a significant predictor of death for cancers of the liver, pancreas, bladder, and colon in men, and for those of the pancreas, breast, and colon in women [33]. The authors also mention the protective effect of DM on the development of prostate cancer.

The osteological literature gives little evidence of diagnosed diabetes. Indeed, we know that the disorder was identified based on CT observation of lower extremity arterial calcifications in Egyptian mummies [34]. In fact and to our knowledge, no definitive diagnosis of diabetes was ever performed on bare skeletons. The anthropological diagnosis of a pathology relies on bone lesions, either lytic or blastic, and any element associated to the skeleton, such as ectopic calcifications. In diabetes, bone affection is secondary to direct microvascular and macrovascular complications and unspecific to the condition. Indeed, Charcot's joints and osteomyelitis are possible (and not inevitable) ultimate results of neuropathic diabetes. Similarly, atherosclerosis, changes in the bone mineral density, fractures, and DISH are not pathognomonic indicators of the disease despite the existing associations. Dupras and colleagues mention in their study of bilateral amputations in ancient Egypt that "diagnosing diabetes from skeletal material alone is very difficult as no skeletal changes are specific to diabetes" [35]. This explains why the diagnosis of diabetes on bones cannot be defined.

In this article, we propose to investigate bone lesions and discuss their potential association to DM on individuals with known diabetes from an identified osteological collection. This will help recognize potential diabetes in forensic and archeological contexts and could influence the identification of missing individuals. Indeed, forensic cases of people who get lost, die, and decompose without identification documents on the scene are not rare. In these cases, sex and age estimations from the skeleton are not sufficient criteria to narrow down the search of the unknown deceased. The study of bone pathologies can provide crucial information for the construction of the biological profile, which is the main objective in the expertise of a skeletonized forensic case. The biological profile has no pretention to identify but only to narrow down the list of missing persons for identification or indicate possible descriptors, such as pathology, which may help in the search. If we correctly interpret the signs on bones, the recognition of a disease such as diabetes could further restrict the search within missing person lists, thus providing crucial information to the forensic expertise. As with previous publications [4, 5, 36, 37], studies on bone pathologies have a significant and unexplored potential in the forensic practice. Indeed, this is, to our knowledge, the first study to examine skeletons with clinically diagnosed diabetes.

Materials and methods

The study was conducted on 38 skeletons of known individuals suffering from diabetes mellitus and selected from the CAL Milano Cemetery Skeletal Collection [6]. The individuals of the collection were inhumed in coffins in the cemeteries of Milan, exhumed twice in 15 years (after complete skeletonization) by cemetery workers by means of heavy machinery and finally made available for research purposes in the LABANOF (Laboratorio di Antropologia e Odontologia Forense), in accordance with article 43 of the Italian National Police Mortuary Regulation (September 10, 1990; no. 285). The CAL Milano Cemetery Skeletal Collection has the double advantage of being contemporary and documented. This documentation includes demographic descriptions but also "ISTAT" (Istituto Nazionale di Statistica) death certificates that specify the cause of death and any pathological conditions related to it. As a result, it was possible to select individuals clinically diagnosed with DM, as evident by the mention of "diabetes mellitus" in their associated medical information.

We integrated in this study all the individuals of the CAL Milano Cemetery Skeletal Collection that matched our selection criterion: a mention of diagnosed diabetes in their medical history. Thirty-eight completed that criterion, including 20 males and 18 females with ages ranging from 52 to 88 years, a mean age of 74 years and a median age of 76 years (Table 1). The dates of birth of the selected individuals range from 1897 to 1938 and the dates of death range from 1984 to 1997. Given that in 31 of 38 cases the type of diabetes was not specified in the ante-mortem medical data, the effect of the different etiologies of DM (T1DM and T2DM) on bone lesions was not investigated.

After washing and drying, an anthropological analysis was performed on each skeleton, including biological, pathological, and traumatic analysis. During the study of the skeleton, we recorded any and every pathological lesion that could be observed as any sign "abnormal" to the original bone structure. Given the information obtained in the literature, the main focus of the analysis was oriented towards the lower legs; however, the lesions observed in the rest of the skeleton were also noted and considered. The location, type (lytic or blastic), and morphological aspect of the lesions were reported and the lesions were photographed.

Taphonomy and comorbidity are two potential biases in the study, which is why they were also considered. The skeletons were classified according to the state of preservation of the lower limbs, from most to least complete, and for more clarity, a graphic representation of the feet of each individual was provided in Table 1 (except for complete feet or when the feet were absent, which was specified). It appeared important to note any comorbidity mentioned in the death certificate as antemortem medical data because the etiology of these concomitant pathologies might interact and affect the type, severity, and morphology of the lesions that could be attributed to diabetes.

In particular, the evaluation criteria consisted in the macroscopic observation of the presence of: (1) periosteal reactive new bone; (2) lytic lesions; (3) lyses of tuft, later classified into "divot," "resorption," and "whittling" subcategories; (4) manifestations of osteomyelitis; (5) evidence of osteochondritis dissecans; and (6) vascular calcifications. The terminology "periosteal reactive new bone" refers to a mechanical or inflammatory response of the outer layer of the bone. It is a hypertrophic lesion, elevated from the normal cortical surface and with either fine porosity or striations due to longitudinal grooves in the reactive bone. "Lytic lesions" are localized tissue erosions characterized by a loss of cortical bone. A "lysis of tuft" was defined as a lytic lesion localized on the "distal phalangeal tubercle" or tuft [38] of the distal phalanges which includes different subcategories based on the descriptions by Rothschild and colleagues [39]: a "divot" corresponds to a focal defect located on the central extremity of the tuft; a "resorption" represents the loss of the tubercle as if it were resorbed; and the "whittling" indicates a distally concentric narrowing of the phalanx (Fig. 1). The small tubular bones of the feet are very fragile and subject to taphonomic alterations and so differentiating lytic lesions from taphonomic ones is sometimes very difficult. In these cases, we specified that the lesions were "suspected". Osteomyelitis was

Table 1 Details on the studied individuals and the observed lesions. Bones colored in gray were present and those left in white were absent from the anthropological study

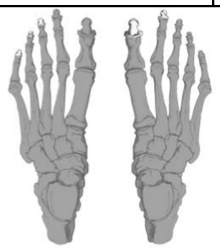
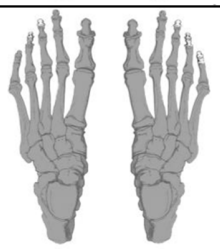
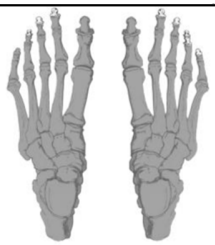
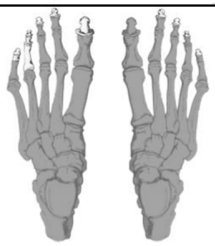
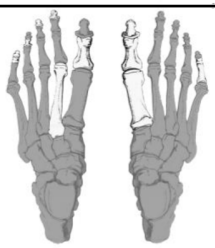
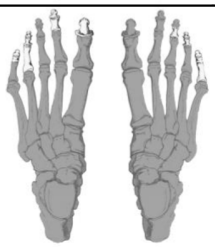
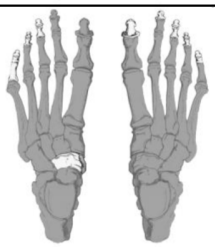
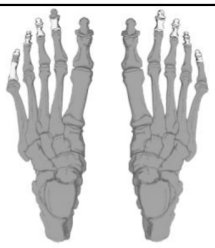
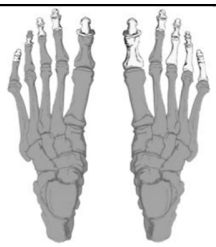
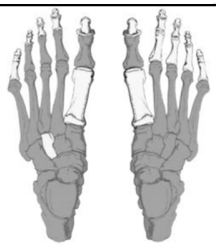
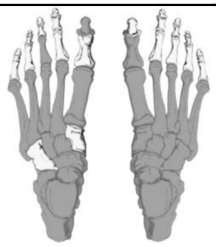
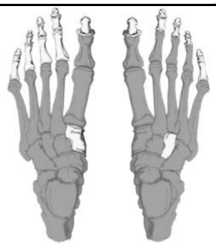
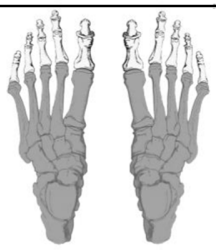
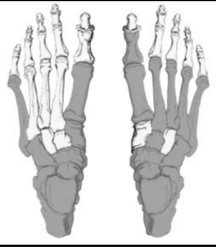
Case n°	Sex	Age	State of preservation of the lower limbs	Other antemortem medical data	Periosteal new bone formation	Lytic lesions	Lysis of tuft	Vascular calcifications	Osteomyelitis	Osteochondritis dissecans	Evidence of trauma	Comments on the upper and axial skeleton
1	M	68	Complete feet	Cirrhosis, hepatic neoplasia and terminal hepatic coma			x					Multiple large lytic metastases
2	F	80	Complete feet	Sclerohypertensive cardiopathy and chronic cardiac insufficiency	x							Remodeled fracture on the proximal metaphysis of the right humerus
3	M	70	Complete feet	Ischemic cardiopathy, chronic obstructive pulmonary disease, pulmonary edema, cardiac and respiratory insufficiencies	x	x	x				x	Antemortem callus on the left ulna
4	M	85	Complete feet	Anuria in prostatic cancer, cardiac insufficiency	x	x	x					Proliferative bone metastases on left ilium
5	F	68	Complete feet	Hypertension and myocardiac infarctus								
6	F	85	Complete feet	Renal insufficiency and previous acute myocardiac infarctus		x		x				Suspected rheumatoid arthritis
7	M	78	Complete feet	Chronic renal insufficiency, miocardiocoronarosclerosis and peptic ulcer	x		x					Multiple calluses on left and right ribs
8	F	82		Arrhytmic-hypertensive cardiopathy, bronchopulmonitis and acute heart insufficiency	x	x	x					
9	F	74		Dilated cardiomyopathy and cardiac insufficiency	x		x	x				

Table 1 (continued)

10	F	74		Comatose state after brain injury			x	x				
11	F	86		Arterial hypertension and chronic obstructive pulmonary disease		x	suspected			suspected		
12	M	88		Prostate cancer and chronic cardiopathy	x			x				Lytic and blastic bone metastases on vertebrae
13	M	77		Ictus right hemisphere, bilateral seizure, acute pulmonary edema, atrial fibrillation	x						x	
14	M	80		Renal insufficiency and brain natriuretic peptide (basal, right)	x		x	x				
15	F	68		Bronchopulmonitis and sepsis	x	x	suspected				x	

identified on bones as an enveloping periosteal bone deposition with a sequestrum [40]. Osteochondritis dissecans is a small and focal necrosis inducing the detachment of part of the bone [21]. Evidence of trauma, including calluses and fracture lines, were also reported because of the known association between DM and fracture risk. Lastly, vascular

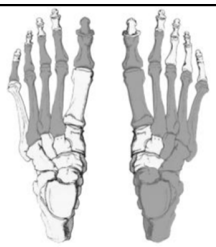
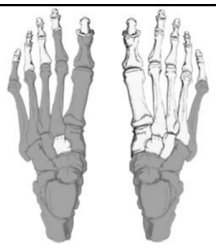
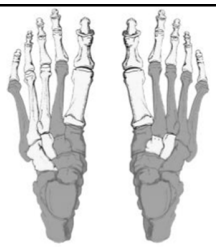
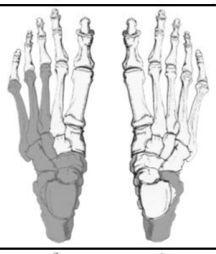
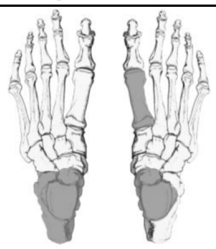
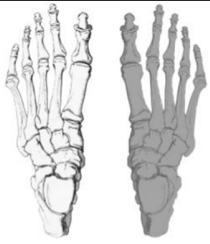
Table 1 (continued)

16	F	74		Intestinal occlusion and acute renal and cardiac insufficiencies							
17	M	52		Bilateral interstitial lung disease, severe respiratory insufficiency	x						Periosteal reaction on right lateral humerus and medio-distal ulna
18	F	77		Generalized vasculopathy and senile marasmus							
19	F	76		Cerebral ictus on right hemisphere and hypertension					x		Prosthesis on proximal right humerus
20	F	83		Encephalovasculopathy and cerebral ictus							Healed fracture on left distal radius
21	M	76		Cirrhotic cancer with bone metastases, metabolic compensation in diabetes mellitus, terminal collapsus syndrome	x	x		x			Multiple lytic metastases and callus on right distal ulna and right scapula

calcifications were also sought and collected in this research because they act as markers of cardiovascular disease [4] and could prove interesting given the known association between vascular diseases and diabetes.

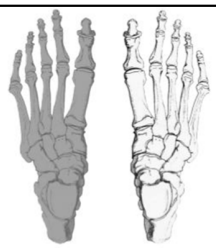
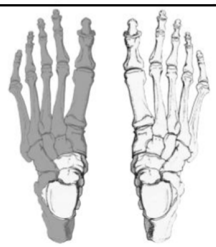
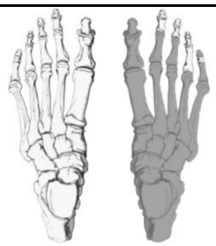
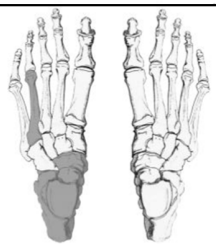
Moreover, we added a control sample of 30% of the initial study sample or 11 individuals without any mention of DM in their antemortem medical information, to compare the prevalence of the lesions found in the DM samples

Table 1 (continued)

22	M	54		Pulmonary tumor and respiratory insufficiency	x	x	x					
23	M	76		Cerebral ictus, cerebral vasculopathy and arterial hypertension								
24	F	83		Left pulmonary neoplasm								
25	F	87		Inferior limb gangrene, chronic arteriopathy and suspected pulmonary embolism								Substitutive prosthesis of left femoral head
26	M	75		Dilated cardiomyopathy post-ischemic, low tension, chronic renal insufficiency, acute pulmonary edema	x			x				
27	F	71		Chronic obstructive bronchopneumopathy and respiratory insufficiency	x							

with corresponding controls of both sexes and in the same age group. The control group is constituted of eight females and three males, with ages ranging from 54 to 85 years (median age of 75 years) who died as a consequence of malignant neoplasm, neoplastic cachexia, acute respiratory insufficiency, senile dementia, or cerebral coma

Table 1 (continued)

28	M	74		Ischemic cardiopathy and cardiac insufficiency	x		suspected	x				
29	M	58		Gastric neoplasm and hepatic metastases			suspected					
30	M	70		-	x	x						Remodeled fracture on the proximal right humerus
31	M	80		Ischemic cerebral vasculopathy, cerebral ictus with seizure, active duodenal gastric ulcer								
32	M	68	Only the femora present, no feet	Hypovolemic shock	x							Two rib calluses
33	M	61	No feet	Acute pancreatitis and sepsis								
34	F	79	No feet	Cerebral vasculopathy and hyperosmolar coma								Compression trauma on the left humeral head
35	M	77	No feet	Diabetic neoplasia and cardiac insufficiency								
36	F	66	Amputation left mid-femur, no feet	Severe diffuse arteriopathy and amputation left thigh	x							Multiple calluses on right ribs
37	M	70	No feet	Lymphoma and neoplastic cachexia								
38	F	78	No feet	Breast cancer and respiratory insufficiency								

without any mention of DM in their antemortem medical data. In terms of preservation of the lower legs, ten skeletons presented complete feet whereas the right distal phalanx I was absent from the last one.

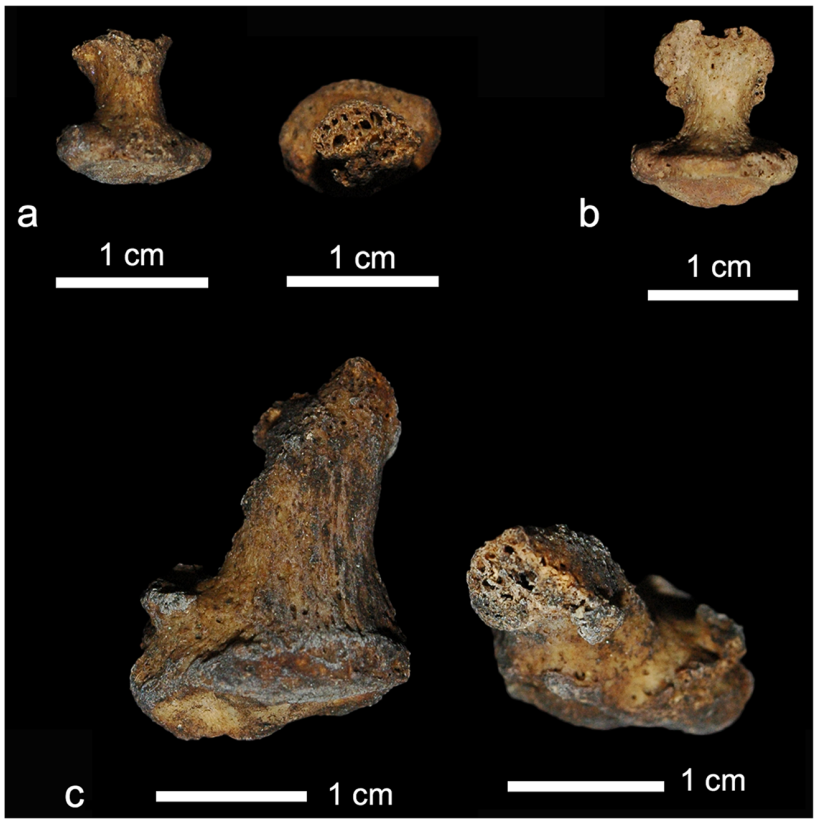


Fig. 1 Photographic details of the different types of lysis of distal tufts. **a** Superior and distal views of the fourth left distal phalanx of case no. 3, note the resorption of the tuft. **b** Superior view of the second right distal phalanx of case no. 4, note the central erosion of the tuft, called "divot." **c** Superior and distal views of the first right distal phalanx of case no. 3, note the concentric narrowing of the tuft, called "whittling"

Results

The classification concerning the state of preservation of the feet and the observed lesions on the individuals of this study are presented in Table 1. For clarity of reading, the details regarding the location and description of the lesions for each skeleton with diabetes will be reported here:

Case no. 1—male, 68; resorption of the left distal I-II-III tufts.
Case no. 2—female, 80; periosteal reaction on both distal phalanges I.
Case no. 3—male, 70; periosteal reaction on the body of both metatarsal (MT) 1, First proximal and distal phalanges I; suspected resorption of the distal part of the right fourth proximal phalanx; resorption of the left distal I, II, III, and IV tufts and whittling of the right distal I tuft; visible fracture line on the body of the left distal phalanx I; and marginal osteophytes, eburnation, and subchondral cysts on both first metatarsophalangeal (MTP) joints.
Case no. 4—male, 85; periosteal reaction on left distal I phalanx and resorption of the tuft; lytic lesion on the articular surface and medial side of right MT1; and resorption of the central tuft of the left distal II or "divot".
Case no. 5—female, 68; no lesion reported.
Case no. 6—female, 85; small lytic lesion on the medial base of right MT1.
Case no. 7—male, 78; periosteal reaction on femoral, tibial, and fibular shafts as well as on all MT, proximal phalanges, and distal phalanges I; resorption of right distal II tuft.
Case no. 8—female, 82; periosteal reaction on both MT1, first proximal and left distal phalanx I; lytic resorption of right MTP1 joint; lytic lesions on the head of left MT1 and suspected on right MT2; resorption of the right distal III, IV, and V and of the left distal I and V tufts.
Case no. 9—female, 74; periosteal reaction on the shafts of the tibiae and on the body of both MT1, first proximal phalanges and distal phalanges I; divot on left distal II tuft.
Case no. 10—female, 74; resorption of the left distal I and V tufts.
Case no. 11—female, 86; lytic lesions on the lateral heads of both MT1; suspected resorption of the right distal tuft I; osteochondritis dissecans on the distal calcaneal facet of the inferior left talus.
Case no. 12—male, 88; periosteal reaction on body of the distal phalanx I.
Case no. 13—male, 77; calluses on the medial side of the right calcaneus, talus, medial cuneiform, and MT1

evidencing a traumatic event on the medial side of the right foot; periosteal reaction on body of right MT1 and right distal phalanx I.
Case no. 14—male, 80; periosteal reaction on body of both MT1 and distal phalanges I; resorption of the left distal I tuft.
Case no. 15—female, 68; periosteal reaction on body of all MT, second-third-fourth left proximal phalanges and distal phalanges I; lytic lesions on the medial side of the head of left MT1; lytic erosion of the heads of both MT2 to MT5 and left proximal phalanges; misaligned remodeled trauma on 3rd right proximal phalanx; and suspected left distal I tuft resorption and flattening.
Case no. 16—female, 74; bones extremely fragmented, unreadable.
Case no. 17—male, 52; periosteal reaction on both femora, tibiae, and fibulae (shafts and both ends).
Case no. 18—female, 77; bones extremely fragmented, unreadable.
Case no. 19—female, 76; osteomyelitis with cloaca on the center shaft of right MT3.
Case no. 20—female, 83; no lesion reported.
Case no. 21—male, 76; lytic deformation of the head of MT5 and periosteal reaction on its body; suspected lytic metastases on the inferior left talus and left superior calcaneus with periosteal reaction.
Case no. 22—male, 54; lytic lesions on the lateral side of the head of right MT1; resorption of the left distal phalanx I tuft; periosteal reaction on the body of left distal phalanx I.
Case no. 23—male, 76; no lesion reported.
Case no. 24—female, 83; no lesion reported.
Case no. 25—female, 87; no lesion reported.
Case no. 26—male, 75; periosteal reaction on the body of right MT1.
Case no. 27—female, 71; periosteal reaction on body of right MT1, first proximal phalanx and distal phalanx I.
Case no. 28—male, 74; proliferation on MTP1, interphalangeal (IP) 1 and sesamoids; periosteal reaction on inferior MT1 and 1st proximal phalanx; and suspected whittling on left distal III phalanx.
Case no. 29—male, 58; suspected divot on left distal III tuft.
Case no. 30—male, 70; amputation left femur; lytic deformation of MTP5 joint; suspected lytic resorption of the head of the second and third proximal phalanges; and periosteal reaction on distal phalanx I, fifth proximal phalanx and MT5.
Case no. 31—male, 80; no lesion reported.
Case no. 32—male, 68; periosteal reaction on both femoral shafts (quite extended).
Case no. 33—male, 61; no lesion reported.
Case no. 34—female, 79; no lesion reported.
Case no. 35—male, 77; no lesion reported.
Case no. 36—female, 66; amputation left femur; periosteal reaction on left femoral shaft.
Case no. 37—male, 70; no lesion reported.
Case no. 38—female, 78; no lesion reported.

As a result, lesions in the lower limbs were noted in 25 diabetic individuals (66% of the diabetic sample), including 15 males (75% of the male group) and 10 females (55% of the female group). The individuals with recorded bone lesions were aged from 52 to 88 years with a mean age of 74 years. Six of 7 individuals with complete feet, 4 of 5 with only one foot, 13 of 19 with incomplete feet, and 2 of 7 without feet presented bone lesions in the lower limbs. The bones of two individuals (nos. 16 and 18) were too taphonomically altered to be readable for pathological analysis.

Regarding the observed lesions, two individuals had an amputation of the left leg (nos. 30 and 36), severed at the mid-femur (5% of the diabetic sample). Periosteal reactive new bone (Fig. 2) was noted on the lower legs of 19 individuals, corresponding to half of the diabetic sample and 76% of the individuals with recorded lesions on the inferior extremities. Thirteen individuals showed lysis of the distal tuft (34% of the diabetic sample and 52% of the individuals with lesions on the lower legs) which includes resorption, divot, and whittling (Fig. 1). Lytic lesions other than those located on the distal tuft were found on the bones of nine individuals—24% of the study sample or 36% of the individuals exhibiting lower leg lesions (Fig. 2). Evidence of trauma in the lower legs was observed in three cases (8% of the diabetic sample) whereas osteomyelitis and suspected osteochondritis dissecans were each observed on a single case (3% of the diabetic sample) (Fig. 3). Finally, vascular calcifications were recovered among the skeletal remains of eight individuals—21% of the study sample (Fig. 4).

It is interesting to note that of the 25 diabetic individuals with lesions on the lower legs, 19 presented lesions located on the first ray of the feet (76%): first metatarsal, first proximal phalanx, and first distal phalanx. Indeed, periosteal new bone on the first ray of the feet was recorded in 16 individuals of the total 20 (80%), including 6 individuals with reaction on each of the 3 bones of the first ray. Similarly, the distal phalanx of the first ray was involved in 9 of the 13 skeletons with observed lysis of tuft (69%) and lytic lesions located on the first ray bones were noticed in 6 of the 9 cases with lytic lesions (67%).

In the negative control group, 9 of the 11 skeletons did not exhibit any of the lesions cited above. Fifty-three vascular calcifications were collected in the skeletal remains of a woman of 82 years who was medically diagnosed with cerebral vasculopathy. The last skeleton, a male of 75 years, showed evidence of trauma with calluses on three bones, both first proximal phalanges and the left distal phalanx I, as well as periostitis on the body of that same distal phalanx.

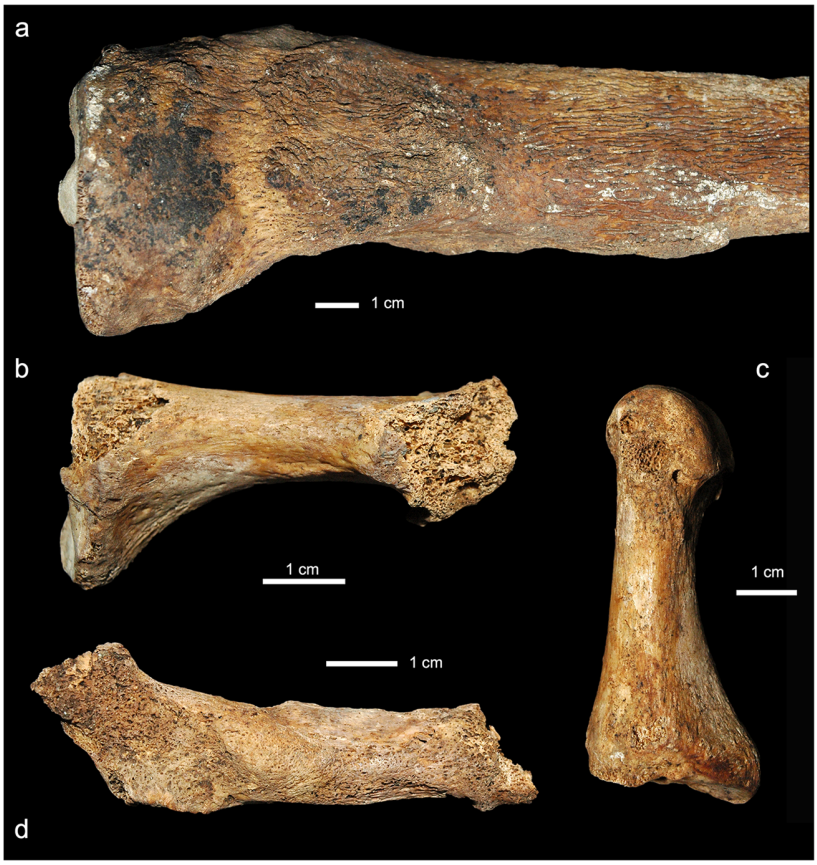


Fig. 2 Photographic details of periosteal new bone formation and lytic lesions. **a** Lateral view of the proximal right tibia of case no. 17, note the extensive periosteal reaction on the shaft. **b** Medial view of the first left metatarsal of case no. 8, note the periosteal reaction on the plantar part of the body (below) and the lytic lesion on the head (right). **c** Lateral view of the first left metatarsal of case no. 22, note the lytic lesion below the head (top). **d** Superior view of the fifth left metatarsal of case no. 21, note the lytic deformation of the head (right) and the periosteal reaction on the body

Discussion

The objective of this research was to investigate bone lesions potentially related to diabetes based on a study of 38 individuals with known diabetes. In this perspective, we reported every lesion that could be observed on the skeletons. These lesions could be classified into different types: periosteal reactive new bone, lysis of tuft, lytic lesion, evidence of trauma, osteomyelitis, and osteochondritis dissecans.

Periosteal new bone deposit is a nonspecific lesion that can be associated with many conditions including infections, tumors, trauma, altered circulation, and overlying soft tissue

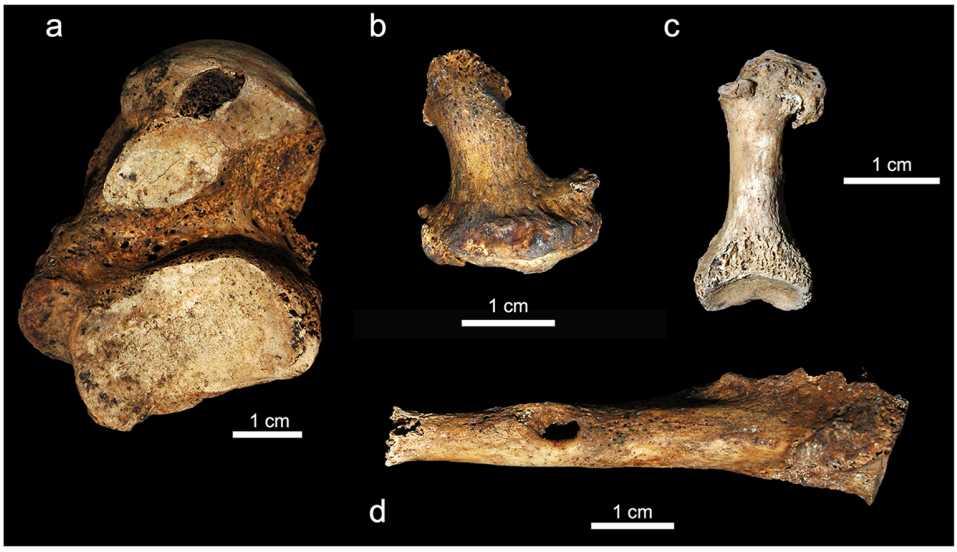


Fig. 3 Photographic details of specific lesions and trauma. **a** Inferior view of the left talus of case no. 11 with suspected osteochondritis dissecans. **b** Superior view of the first right distal phalanx of case no. 3 with visible fracture line. **c** Superior view of the third right proximal phalanx of case no. 15 with misaligned remodeled trauma. **d** Superior view of the third right metatarsal of case no. 19 with osteomyelitis and cloaca

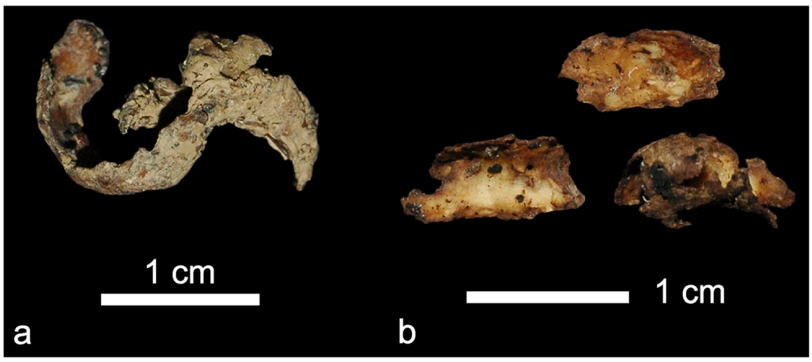


Fig. 4 Photographic details of recovered vascular calcifications found in the cranium of case nos. 26 (**a**) and 12 (**b**)

lesions [20]. These deposits of woven bone remodeling into lamellar bone over time can accompany the inflammatory response after trauma in an attempt to repair the bone [41]. In an anthropological context with diabetes, periosteal reactions can absolutely not be considered pathognomonic signs of the disease. First, the condition does not affect bone directly and so bone lesions are only secondary to the disease process. Second, and as previously mentioned, proliferative periosteal bone is an unspecific marker that can occur in many diseases, and these etiologies can also affect bone without systematically inducing periosteal bone deposits [42]. However, periosteal new bone is consistent with the pathogenesis of diabetes. The diabetic foot with impaired sensory innervations is susceptible to undetected microtrauma and the healing process of the bone would involve periosteal reaction on the surface of foot metatarsals and phalanges [41]. Similarly, proliferative periosteal bone can be a marker of the immune inflammation induced by a foot ulcer or gangrene, common in diabetes [43, 44]. This marker was found in the skeletons of half of the population (19 individuals of the diabetic sample) and in 79% of cases, the periosteal new bone was located on the first ray of the bones (15 individuals) (Fig. 2). However, no information on the relevance of the first ray involvement in DM could be found in the literature by us. Although not specific to any disease [42], this type of lesion, especially if located on the first ray bones of the feet, could prove an interesting possible indicator of DM if associated with other markers as it was observed in many skeletons of known diabetic individuals in the present study.

Lytic activity on the tuft of the distal phalanges is a destructive process due to impaired sensory innervations in diabetic neuropathy [45]. Rothschild and Benham reported three most common digital tuft alterations due to neuropathic diabetes: whittling, resorption and divot [39]. In this study, the analysis of the distal tufts was based on the descriptions by Rothschild and Benham and explained in the "Material and methods" section. As a result, we found lysis of the distal tuft in 13 individuals (34% of the diabetic sample): resorption of the tuft in ten skeletons, divot in three and whittling of the distal phalanges in two individuals (Fig. 1). It is interesting to note that in nine of ten individuals affected, resorptions of distal tufts were recorded on the distal phalanx of the first ray. But again, we could not find anything in the literature on first ray lesions to confront our results. In fact, literature is scarce regarding distal tuft lesions and thus conclusions from this unique sign are dangerous to draw. Indeed, Rothschild and Benham specify that lesions like resorption, divot, and whittling of the distal tufts are also common in individuals with spondyloarthropathies, leprosy, scleroderma, alcoholism, and syphilis [39]. None of these conditions were noted in the ante-mortem medical data or suggested from the anthropological analysis. The lysis of the distal tufts of the feet is difficult to observe in skeletons; first, because small tubular bones are rarely found in archeological cases, and second, because in forensic ones, the presence of tights or socks can damage their fragile structure. Nonetheless, they appear to be an interesting marker for the suggestion of DM in the differential diagnosis.

Lytic lesions reported in this research correspond to any bone erosion apart from those localized on the tufts of the distal phalanges. Small focal lytic lesion to more extensive lytic activity with deformity of the bone and joint were recorded in nine diabetic individuals (Fig. 2), including six individuals with alterations localized on the first rays of the feet. Although we do not have any information from the literature on the significance of these first ray lesions, both small and destructive lytic lesions are common on the feet of diabetic patients [44, 46]. Charcot's joints, typically cited in association with DM, were not observed in our study. Lytic lesions on the feet are not specific to diabetes and conditions such as gout, rheumatoid arthritis, secondary osteoarthritis, and traumatic events can also lead to lytic activity on the bones of the feet. Therefore, they should only be considered evocative of DM in a multifactorial approach.

Evidence of trauma including fracture lines and calluses were observed in the feet of 3 individuals (Fig. 3) and in the upper and axial skeleton of 11 individuals, for a total of 13 individuals with recorded trauma in the diabetic sample (Table 1). A higher rate of fractures in patients with DM was assessed in the literature [7, 13, 16–18] but the presence of fractures cannot be a hallmark of the condition, especially

considering the advanced age-at-death of the individuals of the study. However, the recording of fractures in individuals with known diabetes is compatible with the complications of the disease. Similarly, diabetes is a major cause of amputation of the lower legs and it is therefore not surprising to find amputation at the mid-femur in known diabetic individuals (case nos. 30 and 36).

Osteomyelitis is an infectious condition that can be subsequent to diabetic ulcer [20–23]. Osteochondritis dissecans is the result of a circulatory disturbance associated with trauma [40]. Both of these conditions are not specific to diabetes but they are possible results of complications of the disease. Only one case of each was found in the present diabetic population (Fig. 3).

Vascular calcifications act as markers of cardiovascular disease and in particular atherosclerosis [47]. Their recovery associated with skeletal remains allows the diagnosis of the pathology [4]. In this study, atherosclerotic calcifications were recovered among the skeletal remains of eight diabetic individuals (Fig. 4). Atherosclerosis is a macrovascular complication of diabetes but the condition is also present in 50% of individuals aged 40–49 years and in 80% of individuals aged 60–69 years [48], which concerns all the individuals of the study. Again, no conclusion can be drawn from a single indicator because of the broad differential diagnosis associated with an unspecific marker. However, vascular calcifications were found in clinical studies on diabetes [41, 46] and we report here atherosclerotic findings in 21% of the study sample on known diabetic individuals.

It should also be added that the diabetic individuals with observed bone lesions were aged from 52 to 88 years, implying an advanced age, and that we have no information regarding the duration of diabetes for each individual; therefore, it is possible that the lesions described in this paper are the result of long-standing diabetes and that they may not be apparent in recently diagnosed individuals. However, and despite the duration of the disease, they remain interesting markers.

The upper and axial lesions recorded in the skeletons can be divided into two categories: evidence of trauma and lesions due to concomitant pathologies namely rheumatoid arthritis and cancer. No lesion directly evocative of DM was found. Some lesions observed on the lower limbs could be related to upper and axial lesions or to concomitant pathologies. In case nos. 12 and 21, the periosteal new bone and lytic deformation recorded on the feet could be consequent to trauma on bones more prone to fracture because of DM; but the same reasoning can be applied to the existing cancer which can induce pathologic fractures [21]. In case no. 6, the small lytic lesion on the first metatarsal could be associated with rheumatoid arthritis suggested on the upper limbs and not be necessarily due to diabetes. However, it is also possible that the lytic lesions reported in the hands, radii, and ulnae can be due to diabetes. Indeed, radiographic lytic lesions in the bones of the hands in clinical cases of DM were reported long ago by Sakai and colleagues [46]. The ante-mortem medical information mentions a gangrene on the inferior limbs of case no. 32. This fact corroborates the diffuse periosteal new bone found on the femoral shafts that could have been caused by direct contact between the infected tissue and the periosteum of the femora. Similarly, the periosteal new bone noted on the left femur of case no. 36 could be subsequent to the amputation; either as a healing attempt or due to a gangrene on the left limb before it was amputated. The periosteal new bone formation and lytic lesions observed on the feet of case no. 15 can be due to trauma and/or ischemic osteolysis; none, either, or both correlated with the existing diabetes. Case no. 19 presented a reparative prosthesis on the proximal right femur. This surgical procedure could be consequent to a trauma that would have affected the right foot, inducing an infection and the observed osteomyelitis on the shaft of MT3. The fact that the individual was suffering from DM does not necessarily imply that he had bone lesions related to diabetes. Of course, the existing diabetes may have increased the risk of fracture which in turn increased the risk of skin ulcer, infection, and ultimately osteomyelitis.

However, lysis of the tuft, lytic lesions, osteochondritis dissecans, and osteomyelitis observed in individuals clinically diagnosed with DM during life where not found in the 11 controls. Indeed, ten of them did not show any lesion in the lower legs and the last one presented periostitis and a bone callus on the body of the left distal phalanx I as well as calluses on both first proximal phalanges. The periosteal reaction may be related to the traumatic event noted on three bones of the feet. As mentioned earlier, although in our results DM was associated with evidence of trauma, the presence of trauma in a skeleton cannot be pathognomonic for DM. There is thus no reason to think that the trauma and inflammation evidenced by the periosteal reaction were caused by DM. In one of the control cases, 53 vascular calcifications were collected. Vascular calcifications are correlated with increasing age and have been found in the skeletal remains of individuals over 56 years [4]; hence, they should not be considered an unusual finding in a woman of 82 with diagnosed cerebral vasculopathy. Even though periosteal reaction, evidence of trauma, and vascular calcifications were found in one of the control cases, they seem to remain interesting unspecific markers. Much more specific markers seem to be lysis of the tuft or lytic lesions in the feet given that they were absent in the controls and frequently reported in individuals with DM, contrary to osteochondritis dissecans or osteomyelitis who were only noted once. Consequently, diabetes may only be suggested in the differential diagnosis through a multifactorial approach considering different types of lesions, and where specific lesions such as lysis of the tuft and lytic lesions seem to have more weight.

Finally, 13 skeletons of the total 38 diabetic did not present any lesion observable on the bones of the lower legs. This

could be explained by the taphonomic incompleteness and bad preservation of the skeletons. Indeed, five of them did not have feet; one only had one foot which was composed of three carpals and the fourth metatarsal (case no. 31); six had incomplete feet including two too altered by taphonomy to be readable (nos. 16 and 18); and other two with less than 50% of the bones present and the first rays absent (nos. 24 and 25). But taphonomy does not explain everything, especially when case no. 5 had complete feet but no lesion was found. It is also possible that some individuals had a recent diabetes, or that they had not developed any diabetic complications at the time of death, or even that they had diabetic complications and long-standing diabetes but they had not affected the bones. Consequently, it is important to remember that the existence of diabetes does not necessarily imply the presence of bone lesions.

Conclusion

The study of the effect of diseases on the bone is of interest for forensic, archeological, paleopathological, and medical purposes. In this research, the skeletal remains of 38 individuals with known diabetes were studied, next to 11 negative controls, and all the observed lesions reported. Several types of lesions localized on the lower limbs were highlighted for their potential link to diabetes. Periosteal new bone formation, lysis of tuft, lytic lesions, evidence of trauma, osteomyelitis, osteochondritis dissecans, and vascular calcifications are markers unspecific to DM with broad differential diagnoses but they are consistent with the pathogenesis and complications of the disease. It was interesting to note that lesions were detected on the first ray bones of the feet in 76% of the individuals with lesions on the lower legs and in 50% of the total study sample of known diabetic individuals. Moreover, lysis of the tuft, lytic lesions, osteochondritis dissecans, and osteomyelitis were not found in the control group; only one case presented evidence of trauma and associated periosteal reaction; and vascular calcifications were found in a single case. These results strengthen the diagnostic value of the signs found on individuals with clinically diagnosed DM.

A definitive pathological diagnosis should never be made on a single bone marker but in a multifactorial approach. Although diabetes is very difficult to diagnose on bones because of the lack of pathognomonic traits, a combination of markers could suggest the consideration of the disease in the differential diagnosis. This is of paramount importance for the construction of a biological profile. In the forensic practice, precise information such as the possible presence of diabetes might not allow the direct identification of an unknown deceased individual but it will certainly help in the search for a better match among the missing by providing additional descriptors. This research is the first to document skeletons with known diabetes from an identified skeletal collection, and although the lesions found are not specific to the disease, they can support this hypothesis. We hope that this research based on skeletons with known diabetes will shed some light on the potential identification of this pathology on remains of forensic interest.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Zimmet P, Alberti KGMM, Shaw J (2001) Global and societal implications of the diabetes epidemic. Nature 414:782–787
2. Evans JMM, Newton RW, Ruta DA, MacDonald TM, Morris AD (2000) Socio-economic status, obesity and prevalence of type 1 and type 2 diabetes mellitus. Diabet Med 17:478–480. https://doi.org/10.1046/j.1464-5491.2000.00309.x
3. Mathers CD, Loncar D (2006) Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med 3:e442. https://doi.org/10.1371/journal.pmed.0030442
4. Biehler-Gomez L, Cappella A, Castoldi E, Martrille L, Cattaneo C (2018) Survival of atherosclerotic calcifications in skeletonized material: forensic and pathological implications. J Forensic Sci 63:386–394. https://doi.org/10.1111/1556-4029.13592
5. Castoldi E, Cappella A, Gibelli D, Sforza C, Cattaneo C (2017) The difficult task of diagnosing prostate cancer metastases on dry bone. J Forensic Sci 63:672–682. https://doi.org/10.1111/1556-4029.13617
6. Cattaneo C, Mazzarelli D, Cappella A, Castoldi E, Mattia M, Poppa P, de Angelis D, Vitello A, Biehler-Gomez L (2018) A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection. Forensic Sci Int 287:219.e1–219.e5. https://doi.org/10.1016/j.forsciint.2018.03.041
7. Schwartz AV (2003) Diabetes mellitus: does it affect bone? Calcif Tissue Int 73:515–519. https://doi.org/10.1007/s00223-003-0023-7
8. Arkkila PET, Gautier J-F (2003) Musculoskeletal disorders in diabetes mellitus: an update. Best Pract Res Clin Rheumatol 17:945–970. https://doi.org/10.1016/j.berh.2003.11.001
9. Alberti KGMM, Zimmet P (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med 15:539–553. https://doi.org/10.1002/(SICI)1096-9136(199807)15:7<539::AID-DIA668>3.0.CO;2-S
10. Brutsaert E (2017) Diabetes mellitus (DM), The Merck manual for health professionals [Internet]. Availablefrom: https://www.msdmanuals.com/professional/endocrine-and-metabolic-disorders/diabetes-mellitus-anddisorders-of-carbohydrate-metabolism/diabetes-mellitus-dm
11. Brutsaert E (2017) Complications of diabetes mellitus, The Merck manual for health professionals [Internet]. Available from: https://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/diabetesmellitus-and-disorders-of-carbohydrate-metabolism/complications-of-diabetes-mellitus
12. Fowler MJ (2008) Microvascular and macrovascular complications of diabetes. Clin Diabetes 26:77–82
13. Crispin JC, Alcocer-Varela J (2003) Rheumatologic manifestations of diabetes mellitus. Am J Med 114:753–757. https://doi.org/10.1016/S0002-9343(03)00140-2

14. Politi C, Ciccacci C, D'Amato C et al (2016) Recent advances in exploring the genetic susceptibility to diabetic neuropathy. Diabetes Res Clin Pract 120:198–208. https://doi.org/10.1016/j.diabres.2016.08.006
15. Beckman JA, Creager MA, Libby P (2002) Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 287:2570–2581. https://doi.org/10.1001/jama.287.19.2570
16. van Daele PL, Stolk RP, Burger H, Algra D, Grobbee DE, Hofman A, Birkenhäger JC, Pols HA (1995) Bone density in non-insulin-dependent diabetes mellitus. The Rotterdam study. Ann Intern Med 122:409–414
17. Burner TW, Rosenthal AK (2009) Diabetes and rheumatic diseases. Curr Opin Rheumatol 21:50–54. https://doi.org/10.1097/BOR.0b013e32831bc0c4
18. Vestergaard P (2007) Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis. Osteoporos Int 18:427–444. https://doi.org/10.1007/s00198-006-0253-4
19. Husni ME, Kroop SF, Simon LS (2005) Joint and bone manifestations of diabetes mellitus. In: Kahn CR, King GL, Moses AC et al (eds) Joslin's Diabetes Mellitus, 14th edn. Lippincott Williams & Wilkins, Boston
20. Waldron T (2008) Palaeopathology. Cambridge University Press, Cambridge
21. Aufderheide AC, Rodríguez-Martín C (1998) The Cambridge encyclopedia of human paleopathology. Cambridge University Press, Cambridge
22. Giurato L, Uccioli L (2006) The diabetic foot: Charcot joint and osteomyelitis. Nucl Med Commun 27:745–749. https://doi.org/10.1097/01.mnm.0000230066.23823.cc
23. Hartemann-Heurtier A, Senneville E (2008) Diabetic foot osteomyelitis. Diabetes Metab 34:87–95
24. Levy MJ, Valabhji J (2008) The diabetic foot. Surg 26:25–28. https://doi.org/10.1016/j.mpsur.2007.10.013
25. Gray RG, Gottlieb NL (1976) Rheumatic disorders associated with diabetes mellitus: literature review. Semin Arthritis Rheum 6:19–34. https://doi.org/10.1016/S0049-0172(76)80003-0
26. Martin MM (1952) Charcot joints in diabetes mellitus. Proc R Soc Med 45:503–506
27. Chantelau E, Onvlee GJ (2006) Charcot foot in diabetes: farewell to the neurotrophic theory. Horm Metab Res 38:361–367. https://doi.org/10.1055/s-2006-944525
28. Smith LL, Burnet SP, McNeil JD (2003) Musculoskeletal manifestations of diabetes mellitus. Br J Sports Med 37:30–35
29. Papanas N, Maltezos E (2010) The diabetic hand: a forgotten complication? J Diabetes Complicat 24:154–162. https://doi.org/10.1016/j.jdiacomp.2008.12.009
30. Belcastro MG, Mariotti V, Facchini F, Dutour O (2005) Leprosy in a skeleton from the 7th century necropolis of Vicenne-Campochiaro (Molise, Italy). Int J Osteoarchaeol 15:431–448. https://doi.org/10.1002/oa.799
31. Mader R, Lavi I (2009) Diabetes mellitus and hypertension as risk factors for early diffuse idiopathic skeletal hyperostosis (DISH). Osteoarthr Cartil 17:825–828. https://doi.org/10.1016/j.joca.2008.12.004
32. Sencan D, Elden H, Nacitarhan V, Sencan M, Kaptanoglu E (2005) The prevalence of diffuse idiopathic skeletal hyperostosis in patients with diabetes mellitus. Rheumatol Int 25:518–521. https://doi.org/10.1007/s00296-004-0474-9
33. Coughlin SS, Calle EE, Teras LR, Petrelli J, Thun MJ (2004) Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. Am J Epidemiol 159:1160–1167. https://doi.org/10.1093/aje/kwh161
34. Marx M, D'Auria SH (1986) CT examination of eleven Egyptian mummies. RadioGraphics 6:321–330. https://doi.org/10.1148/radiographics.6.2.3317542
35. Dupras TL, Williams LJ, de Meyer M et al (2010) Evidence of amputation as medical treatment in ancient Egypt. Int J Osteoarchaeol 20:405–423. https://doi.org/10.1002/oa.1061
36. Biehler-Gomez L, Cattaneo C (2018) The diagnostic implications of two cases of known rheumatoid arthritis from the CAL Milano Cemetery Skeletal Collection. J Forensic Sci. https://doi.org/10.1111/1556-4029.13799
37. Biehler-Gomez L, Cabrini A, De Angelis D, Cattaneo C (2018) How do skeletons with HIV present? A study on the identified CAL Milano Cemetery Skeletal Collection. Leg Med 33:11–16. https://doi.org/10.1016/j.legalmed.2018.04.007
38. White TD, Folkens AP (2005) The Human Bone Manual. Elesvier Academic Press, San Diego
39. Rothschild BM, Behnam S (2005) The often overlooked digital tuft: clues to diagnosis and pathophysiology of neuropathic disease and spondyloarthropathy. Ann Rheum Dis 64:286–290
40. Ortner DJ (2003) Identification of pathological conditions in human skeletal remains, 3rd edn. Smithsonian Institution Press, New York
41. Clouse ME, Gramm HF, Legg M, Flood T (1974) Diabetic Osteoarthropathy. Am J Roentgenol 121:22–34. https://doi.org/10.2214/ajr.121.1.22
42. Weston DA (2008) Investigating the specificity of periosteal reactions in pathology museum specimens. Am J Phys Anthr 137:48–59. https://doi.org/10.1002/ajpa.20839
43. Boel LWT, Ortner DJ (2013) Skeletal manifestations of skin ulcer in the lower leg. Int J Osteoarchaeol 23:303–309. https://doi.org/10.1002/oa.1248
44. Newman JH (1980) Non-infective disease of the diabetic foot. J Bone Joint Surg Br 63–B:593
45. Baer AN, Zahr ZA, Khan S, Polydefkis M (2012) Acroosteolysis in diabetes mellitus. J Rheumatol 39:2364–2365
46. Sakai M, Sakuma S, Ishigaki T (1986) Osseous lesions of the hand and foot in diabetes mellitus: correlation between magnification roentgenographic findings and clinical findings. Nagoya J Med Sci 48:55–65
47. Doherty TM, Asotra K, Fitzpatrick LA, Qiao JH, Wilkin DJ, Detrano RC, Dunstan CR, Shah PK, Rajavashisth TB (2003) Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. Proc Natl Acad Sci U S A 100:11201–11206. https://doi.org/10.1073/pnas.1932554100
48. Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, Rumberger J, Stanford W, White R, Taubert K (1996) Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing group. Circulation 94:1175–1192

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How do skeletons with HIV present? A study on the identified CAL Milano Cemetery Skeletal Collection

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ABSTRACT

With the Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS) pandemic, the study of HIV/AIDS on bones has become of pivotal interest for research in bone pathologies, forensic applications (especially in the matter of identification when confronted to antemortem data) and medical purposes. In this paper, we document and discuss the macroscopic lesions found on the skeletons of nine individuals with known HIV, including four with known AIDS, coming from the identified CAL Milano Cemetery Skeletal Collection. As a result, several types of lesions were observed on bones: periosteal new bone formation, dental lesions, thickening of the frontal diploë, destructive localized porosity and evidence of trauma. None of the lesions reported can be directly linked to HIV because the virus does not directly affect bones in a macroscopic way. However, HIV/AIDS-induced infections and inflammations and HIV-related risk factors may leave bone markers. The differential diagnosis of each of the lesions noted in this research and its potential link to HIV or AIDS was discussed. Although it is not possible to diagnose HIV on bare bones, this was not the focus of this study. To our knowledge, no anthropological study has ever been performed on known HIV individuals. With this paper, we present for the first time skeletons with known HIV.

1. Introduction

It is estimated that 36.7 million people are living with the Human Immunodeficiency Virus (HIV) infection worldwide and 1.8 million were newly infected in 2016 [1]. With the Acquired Immune Deficiency Syndrome (AIDS) pandemic, the chance of forensic cases with unknown skeletonized individuals infected by HIV becomes more important. Indeed and so far, HIV/AIDS is responsible for the death of 35 million people [1]. The investigation of HIV/AIDS-related bone lesions is interesting for paleopathology, forensic applications and medical purposes. Indeed and to our knowledge, no anthropological study has ever been performed on known HIV individuals. Moreover, the study of HIV/AIDS skeletal complications could lead to a better understanding of the disease process, which is of great consequence given that there is no cure.

Potentially, anyone can be infected by HIV. Viral transmission occurs through biological fluids such as blood, breast milk, semen and vaginal secretions. The mean time between HIV infection and clinical AIDS is estimated between 2 and 15 years. Africa is the area most affected with 25.6 million people living with HIV in 2016 and accounts for two thirds of the newly infected [1].

The pathogenic mechanisms of HIV/AIDS are particularly complex and multifactorial [2]. The virus primarily targets $CD4^+$ T cell membranes and inserts viral RNA into the host cell. The cell is then activated by exogenous stimuli and viral particles are expressed on its surface. Following the primary contact with the HIV infection, an outbreak of viral replication disseminates the virus through the bloodstream and lymphatics to lymphoid organs that will serve as a reservoir of viral replication. A strong immune response inhibits the viral process within weeks and establishes partial immunological control. Despite the immune containing mechanisms, the virus almost invariably escapes and induces a chronic persistent infection in the lymphoid tissue. Over time, the viral replication accelerates and outbalances the immune factors trying to contain or suppress it, leading to the destruction of the immune system which leaves the host inadequately equipped against opportunistic infections [3,4].

Known HIV complications on bones include low bone mineral density (BMD) and osteoporosis, osteonecrosis and more rarely, osteomalacia [5–7]. The implementation of antiretroviral therapy (ART) has seen a considerable increase in the life-expectancy of HIV infected

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individuals. However, it has been shown that long-standing ART is associated with a lower BMD in HIV individuals than in the general population [5,6,8–10], although other research found no evidence of the effect of ART on BMD [11, among others]. Osteoporosis deteriorates the microstructure of the bone, inducing a higher risk of fractures in the affected individuals. Most common in postmenopausal females, other risk factors include weight loss, physical inactivity, chronic inflammation, smoking, alcohol intake and corticosteroids, as well as AIDS-related muscle wasting, kidney disease, hypogonadism and vitamin D deficiency (common in HIV patients [6]) [5,6,8,10,12]. Osteomalacia is a rare disease defined by an impaired mineralization of the bone matrix, often due to a vitamin D deficiency and associated with low BMD and a high rate of fractures [10]. Moreover, HIV infection has a direct effect on the skeletal system. Targeted by HIV, T and B cells produce RANKL cytokines which differentiate and activate osteoclasts [13]. This stimulated osteoclastic activity will offset the homeostatic balance between bone production (osteoblastic activity) and bone remodeling (osteoclastic activity) and induce progressive bone loss.

Now, the general consensus is that low BMD in HIV infected individuals is the result of a multifactorial interaction between HIV infection, traditional osteoporosis risk factors, ART-related factors and HIV/AIDS-related conditions (such as muscle wasting, kidney disease, vitamin D deficiency and hypogonadism) [10,12–15]. In addition, long-standing HIV and ART use is associated with low BMD, osteopenia, osteoporosis, osteonecrosis, osteomalacia and a higher rate of fractures [5,9]. As a result, HIV infection is an independent risk factor for osteopenia, osteoporosis and osteonecrosis [7,12]. Osteonecrosis has been described in femoral heads, knees, hips and humeri of HIV patients [5] and ART is an independent risk factor [7]. ART exacerbates bone loss (2–6% loss in BMD) at the femora, lumbar spine and hips; all sites prone to fractures [5,9,12,13]. These multifactorial interactions between HIV-related risk factors and pathological mechanisms magnify the risk of fractures in HIV patients which is 2–4 times higher in the HIV infected population than in the general population [12].

Other complications of HIV infection affecting the bones have been described in the literature including septic arthritis, reactive arthritis, *Mycobacterium tuberculosis* infection, and osteomyelitis [4,7]. Finally, neuropathy has been mentioned as a possible complication of ART [4] which could potentially lead to bone lesions such as neuropathic arthropathy.

So how do skeletons with HIV present? None of the most common manuals define the disease on bones [16–18] and to our knowledge, no anthropological study has ever been performed on known HIV individuals. In the forensic practice, the identification of pathological bone markers is of crucial interest for the construction of a biological profile, which may in turn provide specific data that may narrow down the search of the unknown deceased among the missing. Knowing how known HIV skeletons present may allow to recognize the disease when faced with a skeletonized forensic case and may help look for a better match in the identification process. In this perspective, the aim of this paper is to document skeletons with known HIV and investigate potential HIV/AIDS-induced bone lesions based on a study sample of known HIV infected individuals from the identified skeletal collection of the CAL Milano Cemetery Skeletal Collection.

2. Materials and methods

The CAL Milano Cemetery Skeletal Collection is a contemporary and documented collection [27]. In accordance with article 43 of the Italian National Police Mortuary Regulation (September 10, 1990, n°285), human remains unclaimed by their relatives can be granted by cemeteries and ossuaries to universities for teaching and research purposes. The individuals of the collection were buried for 15–20 years until complete skeletonization and exhumed by cemetery workers by means of heavy machinery. The skeletons are then made available for research in the LABANOF (*Laboratorio di Antropologia e Odontologia Forense*) where they become part of the collection. Each skeleton is associated with a documentation that includes demographic data (sex, age, date of birth and date of death) and the *"ISTAT"* (*Istituto Nazionale di Statistica*) death certificate, that specify the cause of death and the pathological or traumatic chain of events that led to it.

For this research, we selected all the individuals of the CAL Milano Cemetery Skeletal Collection with a mention of HIV in their associated documentation. Nine individuals complete that single criterion: 2 females and 7 males. The age-at-death of the individuals selected for study range from 28 to 55 with a mean age of 39 years. The dates of birth range from 1941 to 1966 and the dates of death from 1991 to 1996. No information regarding the year of the diagnosis or any treatment that the individuals might have taken was found in the associated documentation.

Each skeleton was carefully washed and set to dry before the macroscopic anthropological analysis was performed, including biological, pathological and traumatic analysis.

3. Results

3.1. Case n°1

The first skeleton of this study belonged to a female of 41 years old. She was living with HIV for about ten years, had AIDS and had been suffering from a wasting syndrome for 4 months. The skeleton was very well preserved and almost complete except for some bones of the hands, thoracic and lumbar spine as well as rib cage. All of the teeth were lost antemortem. Given the advanced state of remodeling of the alveolar

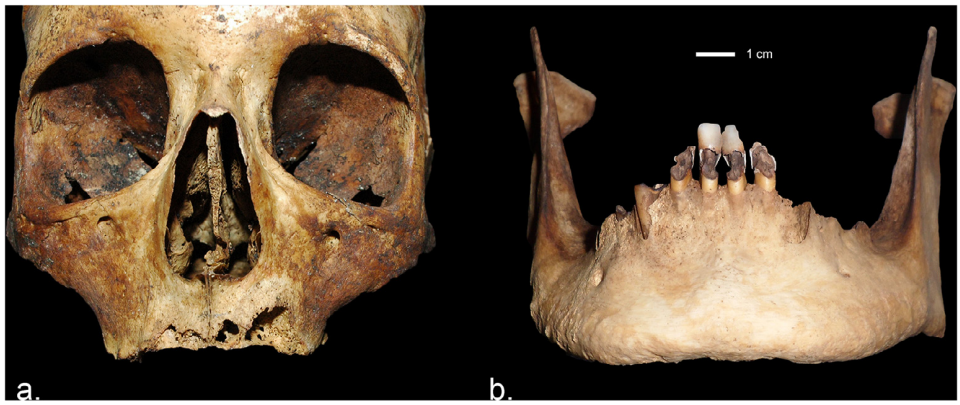

Fig. 1. Details of dental lesions. (a) Anterior view of the cranial bones of the face of case n°1, note the alveolar bone resorption in the area of teeth 22–25; (b) anterior view of the mandible of case n°5, note the tooth neck caries on 31, 32, 41 and 42 and the absence of crown on 43 and 44.

Fig. 2. Thickening of the diploë of the frontal bone. Inferior view of the cranial vault of case n°1, anterior is down.

bone, this edentulism was not recent. Maxillary bone resorption was noted on the area of teeth 21 and 22–25 (Fig. 1a). The cranial vault exhibited a thickening of the frontal diploë (Fig. 2). On the post-cranial skeleton, periosteal bone deposits were found on the proximo-lateral and distalo-medial parts of both tibiae (Fig. 3).

3.2. Case n°2

The second individual of this study was also a female of 41 years. She had a cerebral lymphoma and suffered from neoplastic cachexia and infective HIV complications for 6 months, including *Pseudomonas aeruginosa* and cytomegalovirus. The skeleton was severely altered by taphonomy, especially in the upper body. A majority of the hand bones, spine and thoracic cage elements were absent. The cranium was broken post-mortem and the facial bones were absent from the remains, like the mandible. A round surgical foramen was noted near Bregma, of about 1 cm of diameter. The left tibia presented periosteal new bone formation on its antero-medial shaft.

3.3. Case n°3

The third individual was a male of 28 years old who died of a cardiac arrest. HIV infection, pneumonitis and cirrhosis were also mentioned in the death certificate. The skeleton was very well preserved and almost complete; only the bones of the hands and feet were absent as well as some ribs and vertebrae, especially in the cervical spine. Regarding dental health, 31 and 33 only had the root remaining; 32 presented a tooth neck carious lesion and occlusal abrasion; and bone resorption was noted on the area of 14, 34, 42 and 44. An antemortem trauma was recorded on the distal epiphysis of the right radius with a

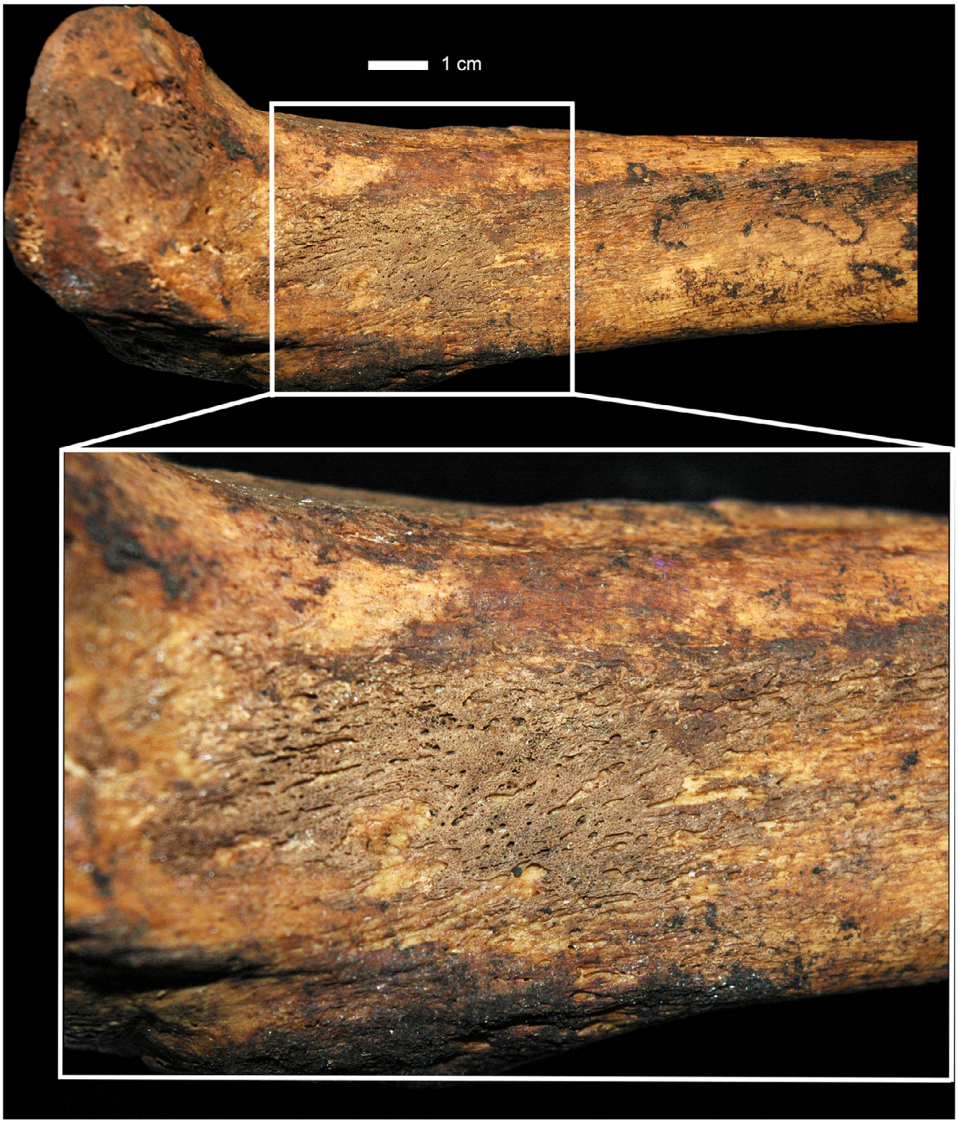

Fig. 3. Periosteal new bone. Lateral view of the proximal right tibia of case n°1 and zoomed area on the periosteal reaction.

Fig. 4. Evidence of trauma. Anterior view of the distal part of the right radius of case n°3 with a visible fracture line.

visible fracture line and remodeling suggesting a Colles' fracture (Fig. 4).

3.4. Case n°4

The fourth case was a male of 29 years old suffering from HIV infection for 10 years, cerebral toxoplasmosis for 13 months and cytomegalovirus that induced an encephalitis (2 months old) and chorioretinitis (8 months old). The skeleton exhibited an excellent preservation and apart from a few bones of the hands, some ribs and thoracic vertebrae, it was complete. Tooth neck caries were reported on 13, 27, 36 and 43, distal cavities on 26 and 44, an occlusal carious lesion on 46 and a destructive carious lesion on the crown of 35. Also, a postmortem crown fracture was noted on 16 and occlusal filling on 26 and 27. Finally, brownish stain was observed on all teeth present, probably due to a postmortem mechanism.

3.5. Case n°5

The fifth skeleton of this research belonged to a male individual of 31 years old. The death certificate details that he had HIV infection, pneumonia and hepatic failure. The bones of the skeleton were in excellent state of preservation. Regarding completeness, only a few vertebrae and some bones of the hands were missing from the box. Occlusal and tooth neck caries were reported on 31, 32, 41 and 42 (Fig. 1b). Teeth 43 and 44 only had the root remaining and the maxilla presented an old edentulism. Several antemortem traumata were noted on the post-cranium, including on the spinous processes of T4 and T7 as well as on the left nasal bone, underlying an extensive and localized periosteal new bone formation. Additional periosteal new bone was found on the internal surface of the posterior half of the 6th, 7th and 8th right ribs.

3.6. Case n°6

The sixth individual selected was a male of 35 years old. He had HIV infection, hepatic insufficiency, encephalitis for 5 years, hepatic, cerebral and respiratory complications for 1 year and AIDS. The skeleton was very well preserved and only a few bones were missing: C6, C7, 12th right rib and most of the bones of the hands. The maxilla showed alveolar resorption and edentulism. The mandibular dentition showed a bridge from 47 to 44 and a probable bridge from 37 to 34. Occlusal abrasion was noted on 31, 32, 33, 41, 42 and 43. Just like in case n°4, the teeth present exhibited brownish staining, probably post-mortem. Lastly, healed periosteal reaction was found on the posterior mid-femora and lateral tibial shafts.

3.7. Case n°7

The skeleton belonged to a 47-year-old male suffering from HIV infection, AIDS and a bronchogenic spinocellular carcinoma. The bones were in an excellent state of preservation. Some bones of the hands, spine and rib cage were missing and the feet were completely absent. All teeth were lost antemortem: the individual presented a complete and old edentulism with alveolar bone resorption. Localized destructive porosity was found on the left orbital surface of the greater wing of the sphenoid bone and to a lesser extent, in its right counterpart (Fig. 5).

3.8. Case n°8

The eighth individual presented in this paper was a male of 49 years old whose associated documentation mentions that he suffered from AIDS and bronchopneumonitis and died of cardiac arrest. The state of preservation of the bones was rather good; the sacrum was the most altered with a white discoloration and a particular fragility of the integrity of the bone. The completeness of case n°8 was similar to that of case n°7, with the absence of the feet, most of the bones of the hands as well as some ribs and spinal elements (C2, C3 and C4). The teeth presented generalized occlusal abrasion. Seventeen and 27 showed occlusal filling while 22 and 23 had a postmortem crown fracture. Finally, a suspected median cyst was recorded on the anterior left palatine.

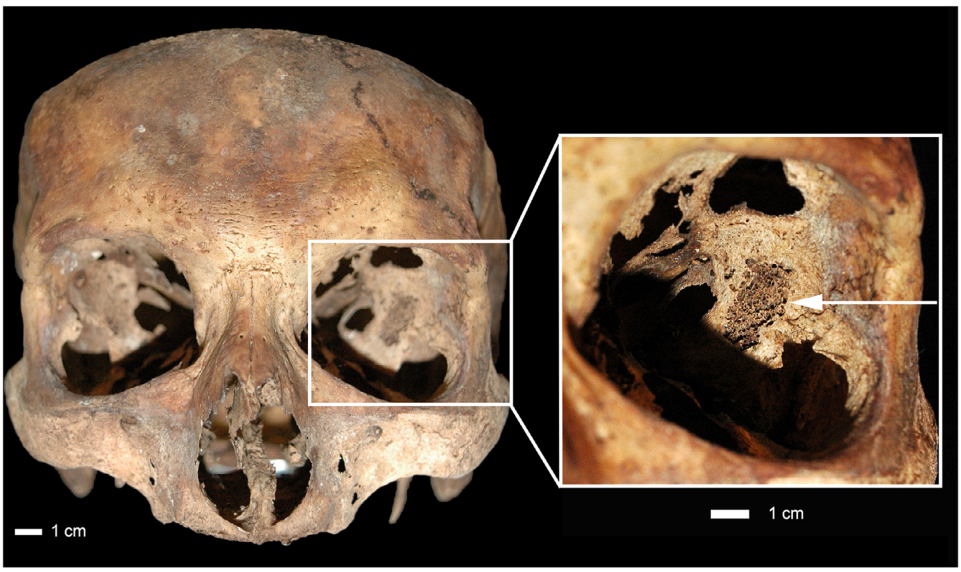

Fig. 5. Destructive porosity. Anterior view of the cranium of case n°7 and zoomed area on the porosity (white arrow) located on the orbital plate of the greater wing of the sphenoid (left orbit).

3.9. Case n°9

The last skeleton of this study belonged to a 55-year-old male who suffered from HIV infection for 8 years, Kaposi sarcoma, disseminated cytomegalovirus infection, atypical Mycobacterium for 1 year and interstitial pneumonitis for 9 days before death. The preservation of the bones was good but the skeleton was less complete than the previous ones. Most of the bones of the hands, some ribs and vertebrae, all the bones of the feet and the pelvic bones were absent. Dental plaque was noted on 31 and 41, occlusal abrasion on 22 and 45, and 24 only had the root remaining of the tooth. Brownish staining was observed on 16 and 17.

It is important to add that all the teeth examined in this study showed evidence of periodontal disease.

4. Discussion

The aim of this article was to report and discuss the lesions observed on known HIV cases from the identified skeletal collection of the CAL Milano Cemetery Skeletal Collection. As a result, several findings were reported on the studied individuals including dental lesions (antemortem loss of teeth, caries and cavities, periodontal disease, occlusal abrasion and alveolar bone resorption), periosteal new bone, thickening of the frontal diploë, marked porosity and evidence of trauma (fracture lines and calluses). In addition, a surgical foramen was found on the cranium of case n°2, but this lesion may be related to a medical invasive procedure as part of a treatment of the cerebral lymphoma she was suffering from.

Given that the individuals died between 1991 and 1996, they probably did not have access to any ART medication. Therefore, any long-term ART effect that can be observed today may be excluded from the analysis of the lesions in this study.

Of course, the reported lesions cannot be directly linked to HIV infection because HIV does not directly affect bone (except for a progressive diminution of BMD, which cannot be macroscopically asserted on bones). Nonetheless, HIV and later AIDS, make the individual susceptible to opportunistic infections that have the potential to mark the bones and hence be seen in the anthropological analysis. For instance, in case n°5, periosteal new bone was reported on the internal surface of the 6th, 7th and 8th right ribs which is consistent with the pneumonia the individual was suffering from. Periosteal new bone formation can be stimulated by “anything that breaks, tears, stretches, inflames or even touches the periosteum” [19]. A correct identification of the underlying cause of periosteal bone deposits is very challenging on bones and includes infection, trauma, vascular disease, tumor, metastases, some congenital and specific diseases and overlying soft tissue lesions [16,18–20]. Therefore, the observed periosteal reactions on the ribs are consistent with the inflammation of the lungs mentioned in the death certificate (as “pneumonia”). However, periosteal reaction is neither systematic, nor a diagnostic trait in itself [21]. In cases 1 (Fig. 3), 2, 5 and 6, the reported periosteal new bone (whether old or recent) could be due to an inflammatory immune response to an infection or to repeated microtrauma and an attempt at bone repair. Indeed, and as mentioned before, HIV infected individuals have a higher fracture risk and are more susceptible to opportunistic infections. In these cases, the exact mechanism responsible for the periosteal bone deposits is both difficult and hazardous to assess, but consistent with the associated medical documentation.

HIV and AIDS-induced conditions can mark the bones but other factors prior to the infection should also be considered. Indeed, drug users and drug addicts are populations at high risk for HIV infection [1,22]. The advanced dental health deterioration (Fig. 1) described in the individuals of this study could of course be attributed to bad dental hygiene. However, it is also possible that the individuals were drug users or even drug addicts which would cause and aggravate dental deterioration of the teeth, especially if associated with bad dental hygiene or chronic inflammatory conditions such as periodontal disease, observed on all recovered teeth. The condition of drug abuse was never reported in the clinical data available but this could be because it was unknown or unaccepted to family and physicians. Nonetheless, chronic drug use has the potential to result in the observed dental state but a long-standing bad dental hygiene could also achieve the same outcome. Moreover, not all drug users are HIV infected and so no strict correlation can be made from dental lesions to HIV infection.

The exact mechanism responsible for the thickened diploë of the calvaria observed in case n°1 (Fig. 2) is difficult to assess but several conditions can be explored. First and considering the sex and age of the individual (female over 40 years), hyperostosis frontalis interna (HFI) was suspected. Nevertheless, HFI consists in a thickening of the inner table of the bone [16,18] and the internal layer of the cranial vault did not show any hyperostosis, so HFI became less likely. Although Paget’s disease is predominantly a lytic condition, thickening of the cranial vault can be observed in advanced cases [16–18]; however, the lesion was limited to the cranium and no lytic lesions were found in the skeleton, hence the diagnosis of advanced Paget’s disease is unlikely. Thalassemia and Leontiasis Ossea (rare hypertrophic condition) can exhibit similar lesions to the one described [17,18] and so both are possible conditions although none of them was noted in the associated antemortem documentation. Therefore, the thickening of the frontal diploë was a unique finding in this study and may be due to an isolated condition.

Localized destructive porosity was noted on the orbital plates of the greater wings of the sphenoid in case n°7 (Fig. 5). This lesion may be the result of a chronic inflammation due to an eye infection which is not incompatible with an individual with a deficient immune system (HIV and AIDS) and susceptible to infections. No mention of a chronic eye infection was reported in the death certificate and so no exact identification of the condition responsible could be made.

The last type of lesion noted in this study is the evidence of trauma including antemortem bone calluses and fracture lines (Fig. 4). As explained before, it has been demonstrated that long-standing HIV infection is associated with a higher rate of fractures than in the general population. In this study, two individuals presented evidence of antemortem trauma (cases 3 and 5) which is compatible with the HIV complication. Nonetheless, it is not possible to directly link these traumata with any disease or virus infection because they could also be the result of occasional and unrelated traumatic events, potentially prior to the HIV infection.

This study of skeletons with known HIV may shed some light for forensic sciences. As already proven with other pathologies [23,24], studying how skeletons with known diseases present can give us an idea of what to expect when confronted to a skeletonized forensic case and may help find a better match towards the identification of the unknown deceased. Although it is not possible to diagnose HIV/AIDS based on the macroscopic analysis of bare bones, this paper showed what may be expected in skeletons with HIV infection. The presence of HIV biomolecular markers has been demonstrated in decomposed bloodstains [25] and in dry bone [26], thus this is the most reliable way to identify the disease in skeletal remains. However, macroscopic analysis still remains the main screening for diseases on dry bone and at times, biomolecular elements disappear and cannot be found, especially in very old bones.

The HIV/AIDS pandemic has made the study on the disease a subject of paramount interest, especially because the condition remains incurable. In this article, we performed the anthropological analysis of all known HIV individuals of the identified CAL Milano Cemetery Skeletal Collection. Despite the fact that it is not possible to diagnose HIV/AIDS on bones, several types of lesions were observed on this population of known HIV individuals: periosteal new bone formation, dental lesions, thickening of the frontal diploë, destructive localized porosity and evidence of trauma. Although the observed dental lesions may be the consequence of behaviors prior or independent of the viral infection (such as drug addiction), periosteal bone deposits and

destructive porosity can be correlated with AIDS-related infections and/or inflammations, noted in the associated antemortem documentation. This study is the first to describe how skeletons with known HIV present and may help in the differential diagnosis of similar cases.

5. Conflict of interest

The authors declare that they have no conflict of interest.

This article does not contain any studies with animals performed by any of the authors.

References

[1] WHO, HIV/AIDS World Health Organization Statistics, (2017). < http://www.who.int/mediacentre/factsheets/fs360/en/ > .

[2] A.S. Fauci, HIV and AIDS: 20 years of science, Nat. Med. 9 (2003) 839–843.

[3] A.S. Fauci, Host factors and the pathogenesis of HIV-induced disease, Nature 384 (1996) 529, http://dx.doi.org/10.1038/384529a0.

[4] S.B. Greenberg, C.J. Lahart, HIV/AIDS: clinical considerations, in: H.D. Humes, H.L. DuPont, L.B. Gardner, J.W. Griffin, E.D. Harris, W.R. Hazzard, T.E. King, D.L. Loriaux, E.G. Nabel, R.F. Todd, P.G. Traber (Eds.), Kelley's Textb. Intern. Med. fourth ed., Lippincott Williams & Wilkins, 2000, pp. 2320–2328.

[5] A.G. Cotter, W.G. Powderly, Endocrine complications of human immunodeficiency virus infection: hypogonadism, bone disease and tenofovir-related toxicity, Best Pract. Res. Clin. Endocrinol. Metab. 25 (2011) 501–515, http://dx.doi.org/10.1016/j.beem.2010.11.003.

[6] W.G. Powderly, Osteoporosis and bone health in HIV, Curr. HIV/AIDS Rep. 9 (2012) 218–222, http://dx.doi.org/10.1007/s11904-012-0119-7.

[7] V.J. Vigorita, B. Ghelman, D. Mintz, Osteomyelitis and septic arthritis, in: V.J. Vigorita, B. Ghelman, D. Mintz (Eds.), Orthop. Pathol. third ed., Lippincott Williams & Wilkins, Philadelphia, 2016.

[8] R. Guerri-Fernandez, J. Villar-Garcia, A. Diez-Perez, D. Prieto-Alhambra, HIV infection, bone metabolism, and fractures, Arq Bras Endocrinol. Metab. 58 (2014) 478–483.

[9] A.N. Ahmad, S.N. Ahmad, N. Ahmad, HIV infection and bone abnormalities, Open Orthop J. 11 (2017) 777–784, http://dx.doi.org/10.2174/1874325001711010777.

[10] G.A. McComsey, P. Tebas, E. Shane, M.T. Yin, E.T. Overton, J.S. Huang, G.M. Aldrovandi, S.W. Cardoso, J.L. Santana, T.T. Brown, Bone disease in HIV infection: a practical review and recommendations for HIV care providers, Clin. Infect. Dis. 51 (2010) 937–946, http://dx.doi.org/10.1086/656412.

[11] H. Knobel, A. Guelar, G. Vallecillo, X. Nogués, A. Díez, Osteopenia in HIV-infected patients: is it the disease or is it the treatment? AIDS 15 (2001) 807–808.

[12] I. Ofotokun, E. McIntosh, M.N. Weitzmann, HIV: inflammation and bone, Curr. HIV/AIDS Rep. 9 (2012) 16–25, http://dx.doi.org/10.1007/s11904-011-0099-z.

[13] I. Ofotokun, M.N. Weitzmann, HIV and bone metabolism, Discovery Med. 11 (2011) 385–393.

[14] C. Cazanave, M. Dupon, V. Lavignolle-Aurillac, N. Barthe, S. Lawson-Ayayi, N. Mehsen, P. Mercié, P. Morlat, R. Thiébaut, F. Dabis, for the G. d'Epidémiologie C. du S. en Aquitaine, Reduced bone mineral density in HIV-infected patients: prevalence and associated factors, AIDS 22 (2008) 395–402, http://dx.doi.org/10.1097/QAD.0b013e3282f423dd.

[15] B. Stone, D. Dockrell, C. Bowman, E. McCloskey, HIV and bone disease, Arch. Biochem. Biophys. 503 (2010) 66–77, http://dx.doi.org/10.1016/j.abb.2010.07.029.

[16] T. Waldron, Palaeopathology, Cambridge University Press, Cambridge, United Kingdom, 2008.

[17] A.C. Aufderheide, C. Rodríguez-Martín, The Cambridge Encyclopedia of Human Paleopathology, Cambridge University Press, Cambridge, United Kingdom, 1998.

[18] D.J. Ortner, Identification of Pathological Conditions in Human Skeletal Remains, third ed., Smithsonian Institution Press, New York, 2003.

[19] M. Richardson, Periosteal reaction, Approaches to Differ. Diagnosis Musculoskelet. Imaging, University of Washington, Department of Radiology, USA, 2001.

[20] J. Edeiken, P.J. Hodes, L.H. Caplan, New bone production and periosteal reaction, Am. J. Roentgenol. 97 (1966) 708–718, http://dx.doi.org/10.2214/ajr.97.3.708.

[21] D.A. Weston, Investigating the specificity of periosteal reactions in pathology museum specimens, Am. J. Phys. Anthropol. 137 (2008) 48–59, http://dx.doi.org/10.1002/ajpa.20839.

[22] B.M. Mathers, L. Degenhardt, B. Phillips, L. Wiessing, M. Hickman, S.A. Strathdee, A. Wodak, S. Panda, M. Tyndall, A. Toufik, R.P. Mattick, Global epidemiology of injecting drug use and HIV among people who inject drugs: a systematic review, Lancet 372 (2008) 1733–1745, http://dx.doi.org/10.1016/S0140-6736(08)61311-2.

[23] L. Biehler-Gomez, A. Cappella, E. Castoldi, L. Martrille, C. Cattaneo, Survival of atherosclerotic calcifications in skeletonized material: forensic and pathological implications, J. Forensic Sci. 63 (2018) 386–394, http://dx.doi.org/10.1111/1556-4029.13592.

[24] E. Castoldi, A. Cappella, D. Gibelli, C. Sforza, C. Cattaneo, The difficult task of diagnosing prostate cancer metastases on dry bone, J. Forensic Sci. (2017), http://dx.doi.org/10.1111/1556-4029.13617.

[25] C. Cattaneo, P.A. Nuttall, R.J. Sokol, Detection of HIV, hepatitis B and hepatitis C markers in discarded syringes and bloodstains, Sci. Justice 36 (1996) 271–274, http://dx.doi.org/10.1016/S1355-0306(96)72613-8.

[26] C. Perez-Martinez, G. Prieto-Bonete, M.D. Perez-Carceles, A. Luna, Usefulness of protein analysis for detecting pathologies in bone remains, Forensic Sci. Int. 258 (2016) 68–73, http://dx.doi.org/10.1016/j.forsciint.2015.11.009.

[27] C. Cattaneo, D. Mazzarelli, A. Cappella, E. Castoldi, M. Mattia, P. Poppa, D. De Angelis, A. Vitello, L. Biehler-Gomez, A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection, Forensic Sci. Int. (2018), http://dx.doi.org/10.1016/j.forsciint.2018.03.041.

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RESEARCH ARTICLE

WILEY

Multiple myeloma bone lesions in skeletal remains: Report of two known cases from the 20th century CAL Milano Cemetery Skeletal Collection

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Abstract

Multiple myeloma (MM) is of special interest in anthropology, in particular in the differential diagnosis with metastatic carcinoma. In this paper, we present two cases of known MM and discuss the criteria for diagnosis in comparison with the literature. Two skeletons from the identified CAL Milano Cemetery Skeletal Collection were selected for the antemortem clinical diagnosis of MM in their associated medical data. Each lesion observed during the anthropological analysis was reported and considered. Lesions were observed in both cases in the pelvis, sacrum, ribs, vertebrae, scapulae, proximal ends of femora and humeri as well as in the skull. Moreover, they presented similar morphological traits. Consequently, lesions in MM are multifocal, round, lytic, sharply demarcated, relatively uniformly small, and specifically located in highly vascularized areas of the skeleton. These results are consistent with data from the literature and strengthen the criteria for diagnosis of the condition.

KEYWORDS

bone metastases, bone pathology, multiple myeloma, neoplastic disease, paleo-oncology

1 | INTRODUCTION

Multiple myeloma (MM) is one of the major anthropological differential diagnosis of metastatic cancer, and both conditions may be indistinguishable on dry bones (Ortner, 2003). Consequently, studies on known individuals with the condition may provide new insight and additional information for the recognition of MM and its definitive diagnosis on skeletal remains. In this perspective, the aim of this paper is to present two cases of clinically diagnosed MM from the CAL Milano Cemetery Skeletal Collection, describe in detail the osseous lesions related to MM, confront both cases, and discuss the criteria for diagnosis in comparison with the literature.

Like other neoplastic diseases, MM is not only a modern occurrence: A possible diagnosis was suggested from the bones of a horned dinosaur, *Torosaurus latus*, from the late Cretaceous period (Capasso, 2005), and based on paleopathological records (Alt & Adler, 1992; Haidle, 1995; Morse, Dailey, & Bunn, 1974; Strouhal, 1991; Strouhal & Kritscher, 1990), the condition was "relatively common" in ancient human populations (Capasso, 2005).

MM represents 1.8% of all cancer cases and is the second most frequent malignancy of blood (after non-Hodgkin's lymphoma) in the Unites States. It affects men more commonly than women and Black Americans more than any other ethnicity with a rate twice as high than in White Americans (National Cancer Institute, 2017). The median age at diagnosis is 69 years with a range of 20 to 92 years, but only 2% of individuals are under 40 years of age. The first successful treatment for the malignancy was developed in the late 1960s and consisted in a combination of melphalan and prednisone. Today, the 5-year survival rate is of 49.6% by opposition to 29.8% in 1990; however, MM remains an incurable condition (Edwards, Zhuang, & Mundy, 2008; National Cancer Institute, 2017; Raab, Podar, Breitkreutz, Richardson, & Anderson, 2009).

The malignancy is most commonly characterised by an excess of bone marrow plasma cells and the secretion of monoclonal protein. The exact cause of the disease remains unknown, although genetic components seem to be an important factor. The development of MM is defined as a multistage process starting with the establishment of monoclonal gammopathy as a precursor lesion due to

genetic alterations. Genetic events transform the precursor disease state to myeloma, which may further progress to bone marrow-independent conditions such as extramedullary myeloma and plasma cell leukaemia (Kumar et al., 2017; Rajkumar, 2009). Interactions between MM cells and the bone marrow micro-environment generate a disastrous loop known as "vicious cycle" that will increase tumour growth, contribute to its survival and migration, and induce considerable bone damage (Kumar et al., 2017; Raab et al., 2009). The bone marrow micro-environment is constituted of haematopoietic cells, including B-cells, T-cells, and osteoclasts, as well as nonhaematopoietic cells, namely, bone marrow stromal cells, osteoblasts, and endothelial cells. Although the interactions between MM cells and the endothelial cells will contribute to tumour migration, the interplay between MM cells and bone marrow stromal cells, osteoblasts, T-cells, and B-cells will result in the proliferation of the malignant cells, resistance and inhibition of their apoptosis, neovascularization of the tumour, and substantial bone destruction due to an imbalance between excess of the osteoclastic activity and inhibition of the osteoblastic differentiation (Bataille, Chappard, & Klein, 1992; Edwards et al., 2008; Giuliani, Rizzoli, & Roodman, 2006; Kumar et al., 2017; Rajkumar, 2009). Moreover, MM cells express a programmed cell death ligand affecting T-cells, thus allowing immune evasion and leading to a general immunodeficiency in the individual (Kumar et al., 2017).

Consequently, infection is the major cause of morbidity in MM and frequently the cause of death. The risk of infection is estimated to be seven to 15 times higher in MM patients, due mostly to immunodeficiency. Another complication of MM is renal insufficiency caused by light chain tubular damage and leading to the commonly known "myeloma kidney." Renal insufficiency affects about 20% to 25% of patients with MM at the time of the diagnosis. Anaemia is a common complication of this malignancy and is associated with a poor prognosis (Bladé & Rosiñol, 2007). Finally, myeloma bone disease is the major cause of morbidity and mortality with a frequency of bone lesions of 70–100% (Coleman, 1997, 2001). Clinical manifestations include bone pain, spinal cord compression (in 10–20% of cases), nerve root compression, compression and pathologic fractures, and hypercalcaemia, the latter affecting 15–20% of patients (Bladé & Rosiñol, 2007).

MM remains a prevalent condition, making its study relevant for both modern (forensic cases) and past populations (paleopathological analyses). Studies based on known individuals with confirmed clinical diagnosis are of pivotal importance for the discipline of paleopathology. The information provided by the study of clinically diagnosed skeletons from identified skeletal collections will help in the realisation of a confident diagnosis when confronted to an archaeological skeleton where the clinical history is not available. The interpretation of pathological conditions on bones is a difficult undertaking with multiple inherent restrictions, but studies on individuals with clinically diagnosed diseases, have the potential "to transform speculation into sound, data-based diagnosis" (Hershkovitz, Rothschild, Dutour, & Greenwald, 1998). MM is one of the main differential diagnoses of metastatic carcinoma, making both diseases especially difficult to distinguish, and literature for MM on known dry bone collection is scarce. Thus, we present and discuss here the lesions found on two identified skeletons from the CAL Milano Cemetery Skeletal Collection (Cattaneo et al., 2018) and clinically diagnosed with MM during life.

2 | MATERIALS AND METHODS

This paper examines the skeletal remains of two individuals selected from the CAL Milano Cemetery Skeletal Collection for their antemortem medical data. This collection is a modern and documented skeletal collection of 2,127 skeletons, housed in the *Laboratorio di Antropologia e Odontologia Forense* (LABANOF), Department of Biomedical Sciences for Health, University of Milan, and made available for research purposes in accordance with Article 43 of the Italian National Police Mortuary Regulation (September 10, 1990, no. 285; Cattaneo et al., 2018). The collection is composed of unclaimed individuals buried in the cemeteries of Milan for a minimum of 10 years and exhumed by cemetery workers by means of heavy machinery. Each individual of the collection is associated with a documentation that includes sex, age, date of birth and death as well as a death certificate describing the cause of death and the pathological and/or traumatic chain of event that caused it. The individuals of the collection died between 1910 and 2001; they are evenly distributed among males and females and aged 0 to 104 years. Both individuals selected for the present study were clinically diagnosed with MM during life, as evident by the mention of "multiple myeloma" noted in the antemortem medical data associated to the skeletal remains.

A macroscopic analysis was performed on each skeleton and consisted in a careful observation of each bone to find bone lesions, and when such lesions were identified, they were recorded, measured with a Vernier calliper and photographed for records.

The interactions between MM cells and the bone micro-environment promote MM cell proliferation and migration, bone destruction, and inhibition of bone repair, which will be reflected in the macroscopic aspect and distribution of bone lesions in MM. Osseous lesions in MM are exclusively osteolytic (Randall, 2015; Roodman, 2004). MM creates geographic destruction in haematopoietic bone elements with well-defined or "punched-out" margins. These lesions have a hollow spheroid or "bubble-like" shape (Figure 1). They are multifocal, scattered, numerous, and small to medium sized (5 mm to 2 cm in diameter). The surrounding bone is smooth, and new bone reaction is absent (Aufderheide & Rodríguez-Martín, 1998, pp. 351–354; Ortner, 2003, pp. 376–382; Rothschild, Hershkovitz, & Dutour, 1998; Strouhal, 1991). Bone lesions in MM are most commonly found on the vertebrae, sacrum, pelvis, ribs, proximal femur and humerus, skull, and sternum (Ortner, 2003; Strouhal, 1991). A table compiling the morphological criteria described in the literature regarding MM lesions (Aufderheide & Rodríguez-Martín, 1998; Ortner, 2003; Rothschild et al., 1998; Strouhal, 1991, 1993; Waldron, 2008) and osteolytic lesions in metastatic carcinoma (Aufderheide & Rodríguez-Martín, 1998, pp. 388–390; Marks & Hamilton, 2007; Marques, Santos, & Cunha, 2013; Ortner, 2003, pp. 532–537; Ragsdale, Campbell, & Kirkpatrick, 2018; Rothschild et al., 1998; Strouhal, 1991; Waldron, 2008, p. 189) was realised for comparison (Table 1).

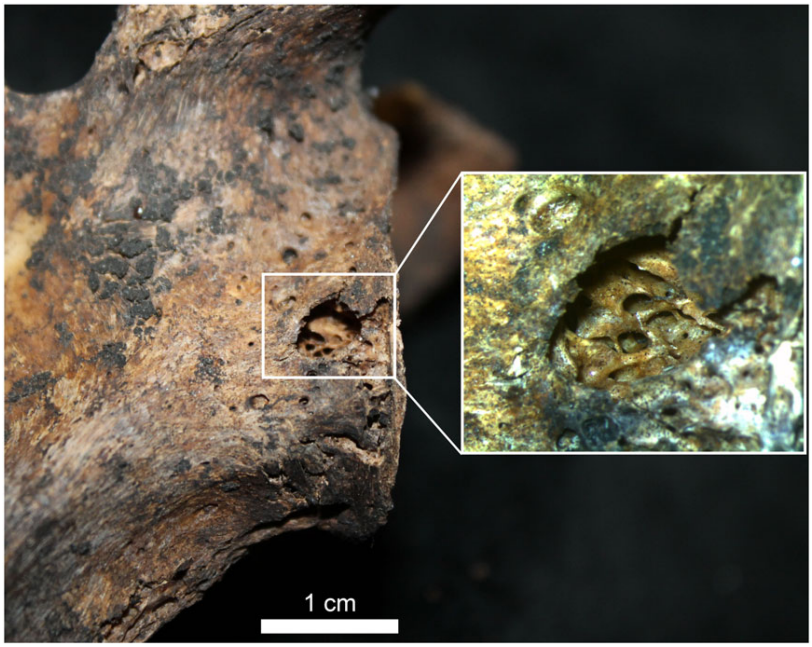

FIGURE 1 Detailed bone lesion in multiple myeloma. Close view of a small osteolytic lesion on the area adjacent to the glenoid fossa on the anterior left scapula. Note the spheroid lesion with well-defined margins and absence of new bone production (zoomed area) visible through the circular opening of the bone cortex [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Criteria of osteolytic lesions in multiple myeloma and metastatic carcinoma

	Distribution	Shape	Number	Size	Margins	Surrounding bone	New bone reaction
Multiple myeloma	Multifocal, scattered	Spheroid, "bubble-like"	Numerous, rarely solitary	Small to medium, 5 to 2 cm	Well-defined, "punched-out"	Smooth and unaffected	None
Metastatic carcinoma	Multifocal, discrete	Round or oval	Few, may be solitary	Medium to large (>1 cm)	Denticulated or scalloped	Often pitted	Common

3 | RESULTS

3.1 | Case 1

The first individual examined in this study was a female of 74 years, born in 1916 and died in 1991. The antemortem medical data mentions that she died of a cardiac arrest and suffered of MM as well as anaemia.

Each skeletal region was represented, but the skeleton was incomplete and fragmented in some areas (Figure 2). The skull was fragmented and severely altered by taphonomy. The scapulae and distal humeri were fragmented, and the bones distal to the elbows were absent. Most of the ribs were present, but few were complete. In the spine, only the cervical vertebrae and four fused thoracic vertebrae were present. The tibiae and fibulae were fragmented and impacted by taphonomic alterations, whereas the pelvic girdle and femora were complete and less damaged. Finally, the feet were present with only a few small bones absent.

Spheroid osteolytic lesions with well-defined margins were found scattered across the skeleton, located on the bones of the neurocranium, the mandibular rami, the sternal end of the clavicles, around the glenoid fossa and the spine of the scapulae, the proximal ends of the humeri and femora, the manubrium (Figure 3a), the ribs (Figure 3d,e), the bodies (Figure 3c) and vertebral arches (Figure 4) of the vertebrae, the sacrum (Figure 3b) and, the pelvic bones, in particular on the anterior and posterior ilia (Figure 5). These osteolytic lesions ranged from 1.29 to 9.31 mm in diameter. Discrete osteolytic lesions were rare and located near taphonomically damaged areas, thus they may not have been solitary before post-mortem bone breakage. Some lesions were coalescing in clusters (Figure 5). The fragmented state of the cranium allowed both endocranial and ectocranial observation of the osteolytic lesion, which showed that both outer and inner tables were involved with similar severity, whereas the greatest extent of the lesion was located on the diploë.

3.2 | Case 2

The second skeleton presented in this paper is a female of 61 years old, born in 1930, who died in 1991 of cardiac arrest. She was clinically diagnosed during life with MM as well as left bronchopulmonitis.

The remains were incomplete although all skeletal areas were represented (Figure 6). The neurocranium was fragmented in six parts, and the splanchnocranium was absent. The medial border of the left scapula was separated, the mandible was represented by its anterior portion, both radii and ulnae were fragmented distally, and the right femoral head was separated. In the thoracic cage, half of the ribs were complete, the remaining being either fragmented or absent. In the vertebral column, the posterior elements of three thoracic vertebrae were present. The sacrum was absent, but the coxal bones were rather well preserved. Finally, a few bones of the hands and feet were absent.

All lesions observed in this skeleton were spheroid osteolytic lesions with "punched-out" margins. The lesions were "bubble-shaped"

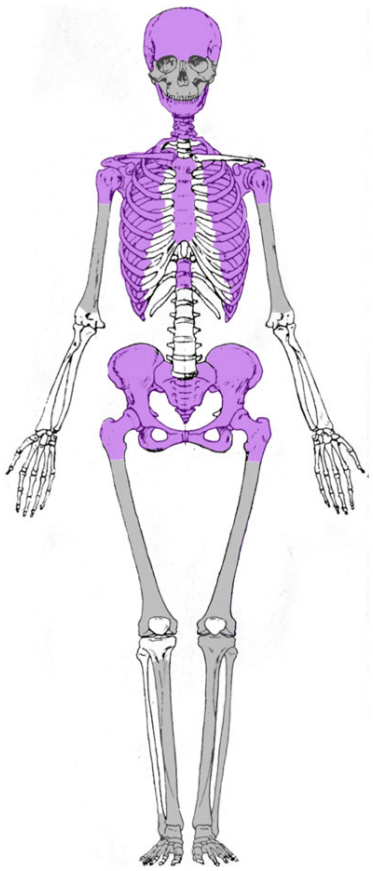

FIGURE 2 Schematic representation of Case 1. Purple colouring refers to areas of the bones affected with osteolytic lesions, and grey areas were present but unaffected [Colour figure can be viewed at wileyonlinelibrary.com]

and small in size, ranging from 1.58 to 4.32 mm in diameter. These osteolytic lesions were found on the cranium (in the internal portion of the right temporal bone), scapulae (in particular on the supraspinous fossa and adjacent to the glenoid fossa—Figures 1 and 7b), humeral and femoral heads (Figure 7a), lesser trochanters, vertebral ends of ribs (Figure 7c), posterior elements of thoracic vertebrae, and posterior iliac fossae. They appeared as focal clusters of osteolytic lesions localised in haematogenous areas scattered across the skeleton.

4 | DISCUSSION

The objective of this article was to present two dry bone cases of clinically diagnosed MM and discuss the criteria for diagnosis on skeletal remains. Both skeletons in this study presented bone lesions, which is not surprising given that the frequency at autopsy of bone lesions in MM was estimated to be close to 100% (Coleman, 1997, 2001).

All bone lesions observed in this study were exclusively osteolytic in nature; no new bone reaction or attempt at bone repair was found. This may be related to the biomolecular interactions between MM cells and the bone micro-environment resulting in increased osteoclastic differentiation and activation (hence, in bone destruction) and inhibition of the osteoblastic differentiation (preventing bone repair). The osseous lesions had a spheroid or "bubble-like" shape visible through the round opening of the bone cortex: they created geographic osteolytic destruction of the trabecular bone and perforated the bone cortex. The margins of the lesions were well defined, and the surrounding cortical bone was smooth and unaffected. The lesions were numerous, small and relatively uniform in size (ranging between 1.29 and 9.31 mm in diameter), sometimes coalescing in clusters, and scattered across the skeleton. These criteria are consistent with the traits described in the literature for MM (Aufderheide & Rodríguez-Martín, 1998, pp. 351–354; Ortner, 2003, pp. 376–382; Rothschild et al., 1998; Strouhal, 1991, 1993; Waldron, 2008, pp. 183–184) and assembled in Table 1.

These spheroid osteolytic lesions were located in the pelvis (in particular on the anterior and posterior ilium), sacrum, ribs (all areas were involved: head, body, and sternal end), vertebral bodies as well as vertebral arches, scapulae (specifically around the glenoid fossa

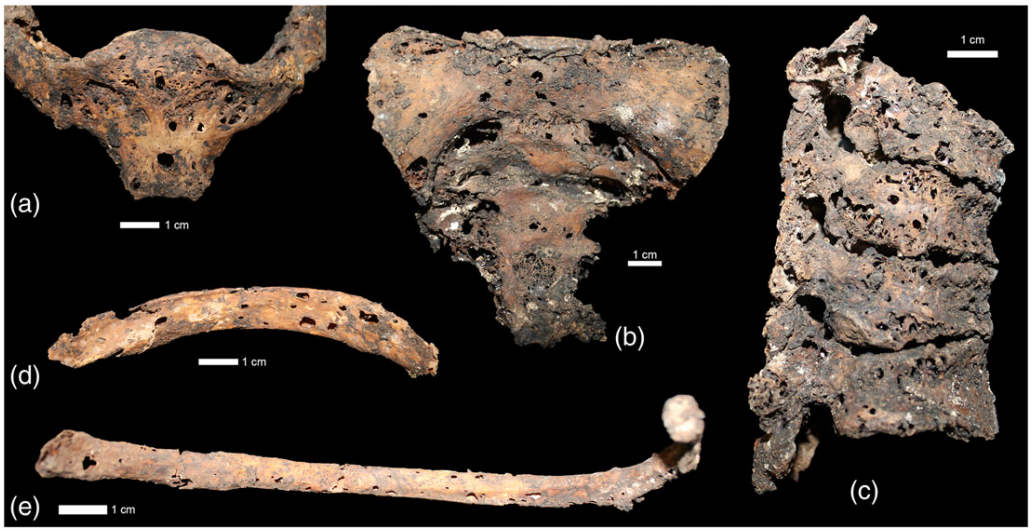

FIGURE 3 Detailed lesions of Case 1. (a) Anterior view of the manubrium, the cartilage of both first ribs is ossified; (b) anterior view of the sacrum; (c) right lateral view of four fused thoracic vertebrae; (d) internal view of a rib fragment; (e) internal view of a complete rib [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 4 Lesions on the posterior elements of the cervical vertebrae (Case 1). (a) Posterior view of the fourth cervical vertebra, superior is up; (b) inferior view of fifth cervical vertebra, posterior is up; (c) posterior view of the sixth cervical vertebra, superior is up; (d) posterior view of the seventh cervical vertebra, superior is up [Colour figure can be viewed at wileyonlinelibrary.com]

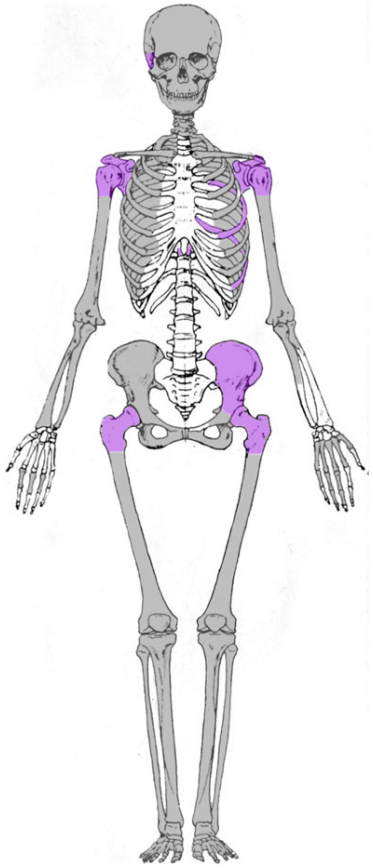

FIGURE 6 Schematic representation of Case 2. Purple colouring refers to areas of the bones affected with osteolytic lesions, and grey areas were present but unaffected [Colour figure can be viewed at wileyonlinelibrary.com]

and the scapular spine), proximal ends of humeri and femora (including the head, neck, and tubercles/trochanters), bones of the cranium, mandibular rami, and sternal end of clavicles (Figures 2 and 6). No lesion was found distal to the knees and elbows. The affected locations reflect a predilection for highly vascularized areas in the skeleton, consistent with the hematologic malignancy. Indeed, the fragmented state of the cranium of the first case allowed ectocranial and endocranial observation and showed that the diploë was affected to a much greater extent than both the inner and outer tables, through a more extensive destruction of the haematogenous bone compared

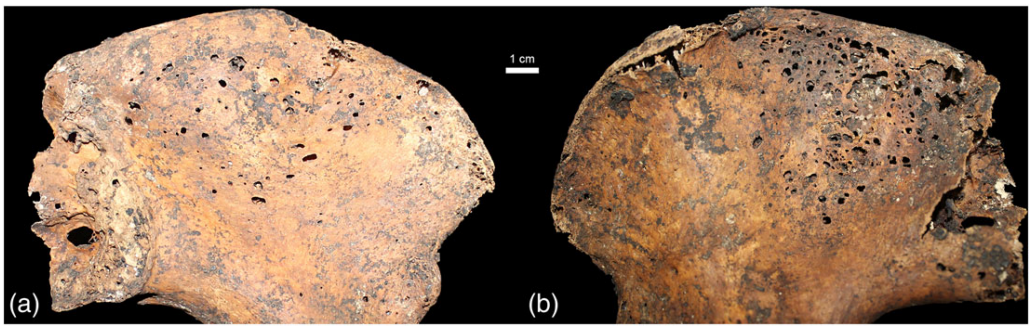

FIGURE 5 Lesions on left ilium (Case 1). (a) Anterior view, note the cluster of osteolytic lesions on the iliac fossa; (b) posterior view showing a coalescing cluster of osteolytic lesions [Colour figure can be viewed at wileyonlinelibrary.com]

FIGURE 7 Detailed lesions of Case 2. (a) Posterior view of the proximal left femur, note the small rounded lesions on the lesser trochanter; (b) superior view of the right subscapular fossa showing circular lytic lesions; (c) medio-posterior view of the vertebral end of the left eighth rib with multiple round osteolytic lesions [Colour figure can be viewed at wileyonlinelibrary.com]

with the cortical layers. Although Jacobson, Poppel, Shapiro, and Grossberger (1958) suggest that vertebral lesions in MM are restricted to the vertebral bodies by opposition to metastatic carcinoma, this was not observed in our study. In fact, both cases presented lytic lesions on the vertebral arches, as evidenced in Figure 4.

Both MM and metastatic carcinoma have a peak onset in individuals over 40 years and can cause multifocal osteolytic lesions affecting primarily bone areas rich in haematopoietic tissue, most commonly in the axial skeleton, thoracic cage, skull, and proximal ends of femora and humeri (Strouhal, 1991). This considerable overlap explains why the differential diagnosis between these two diseases may be extremely complicated on dry bones, if not impossible in some cases (Ortner, 2003, p. 537). Nonetheless, lesions in MM differ from osteolytic lesions in metastatic carcinoma by their size, shape, morphology, nature, and number (Rothschild et al., 1998; Strouhal, 1991; Table 1). Indeed, MM lesions are small, numerous, and scattered on the body (Figures 3, 4, 5, and 7), whereas lesions in metastatic carcinoma tend to be less numerous (they may even be solitary), medium-sized to large, and discrete. Osteolytic bone metastases are round to oval with denticulated margins and pitted surrounding bone (Aufderheide & Rodríguez-Martín, 1998, pp. 388–390; Marks & Hamilton, 2007; Marques et al., 2013; Ortner, 2003, pp. 532–537; Ragsdale et al., 2018; Rothschild et al., 1998; Strouhal, 1991; Waldron, 2008, p. 189). By opposition, MM lesions are spheroid with well-defined margins and smooth surrounding bone. Finally, an osteoblastic response is frequently observed in lesions of metastatic carcinoma, and MM lesions are exclusively osteolytic (Figure 1 and Table 1; Aufderheide & Rodríguez-Martín, 1998, pp. 351–354; Ortner, 2003, pp. 376–382; Rothschild et al., 1998; Strouhal, 1991, 1993; Waldron, 2008, pp. 183–184).

The state of preservation of both skeletons under study was rather similar: although the majority of bones were present, the skeletons were still incomplete and fragmented (Figures 1 and 2). This limiting factor did not prevent the morphological observation and recording of many MM lesions on the skeletons. Moreover, and despite the fact that we could not observe all the bones of both skeletons or all bones in their entirety, we were still able to understand the pattern of distribution of the lesions. Consequently, we do not believe taphonomic preservation to be a significant bias in this study. Although radiographic analyses have proven a useful tool in detecting bone metastases (Rothschild & Rothschild, 1995), they were not performed here because the point of the study was to document the macroscopic aspect of MM bone lesions in two cases of clinically diagnosed with MM during life.

Given that both cases were clinically diagnosed with MM during life, these results strengthen the criteria for diagnosis and illustrate the appearance of MM bone lesions.

5 | CONCLUSION

MM is a condition particularly considered in anthropology, especially in the differential diagnosis with metastatic carcinoma. In this article, we presented two cases of known MM from the CAL Milano Cemetery Skeletal Collection in order to document, discuss, and illustrate the morphological characteristics of MM bone disease.

Both cases presented similar aspects of bone lesions and patterns of distribution. No lesion was found distal to the knees and elbows. Consequently, bone lesions in MM are small spheroid osteolytic

lesions with well-defined margins scattered on the skeleton and located primarily in highly vascularized areas in the pelvis, sacrum, ribs, vertebrae, scapulae, proximal humeri, and femora and skull.

Because both cases presented in this paper were clinically diagnosed with MM during life, these results, consistent with the data from the literature, strengthen the criteria for diagnosis of MM on dry bone.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Alt, K. W., & Adler, C.-P. (1992). Multiple myeloma in an early medieval skeleton. *International Journal of Osteoarchaeology*, *2*, 205–209. https://doi.org/10.1002/oa.1390020304

Aufderheide, A. C., & Rodríguez-Martín, C. (1998). *The Cambridge encyclopedia of human paleopathology*. Cambridge, United Kingdom: Cambridge University Press.

Bataille, R., Chappard, D., & Klein, B. (1992). Mechanisms of bone lesions in multiple myeloma. *Hematology/Oncology Clinics of North America*, *6*, 285–295. https://doi.org/10.1016/S0889-8588(18)30345-9

Bladé, J., & Rosiñol, L. (2007). Complications of multiple myeloma. *Hematology/Oncology Clinics of North America*, *21*, 1231–1246. https://doi.org/10.1016/j.hoc.2007.08.006

Capasso, L. L. (2005). Antiquity of cancer. *International Journal of Cancer*, *113*, 2–13. https://doi.org/10.1002/ijc.20610

Cattaneo, C., Mazzarelli, D., Cappella, A., Castoldi, E., Mattia, M., Poppa, P., … Biehler-Gomez, L. (2018). A modern documented Italian identified skeletal collection of 2127 skeletons: The CAL Milano Cemetery Skeletal Collection. *Forensic Science International*, *287*, 219.e1–219.e5. https://doi.org/10.1016/j.forsciint.2018.03.041

Coleman, R. E. (1997). Skeletal complications of malignancy. *Cancer*, *80*, 1588–1594. https://doi.org/10.1002/(SICI)1097-0142(19971015)80:8+<1588::AID-CNCR9>3.0.CO;2-G

Coleman, R. E. (2001). Metastatic bone disease: Clinical features, pathophysiology and treatment strategies. *Cancer Treatment Reviews*, *27*, 165–176. https://doi.org/10.1053/ctrv.2000.0210

Edwards, C. M., Zhuang, J., & Mundy, G. R. (2008). The pathogenesis of the bone disease of multiple myeloma. *Bone*, *42*, 1007–1013. https://doi.org/10.1016/j.bone.2008.01.027

Giuliani, N., Rizzoli, V., & Roodman, G. D. (2006). Multiple myeloma bone disease: Pathophysiology of osteoblast inhibition. *Blood*, *108*, 3992–3996. https://doi.org/10.1182/blood-2006-05-026112

Haidle, M. N. (1995). A multiple myeloma of the late middle ages from unterregenbach, southwestern Germany. *International Journal of Osteoarchaeology*, *5*, 359–367. https://doi.org/10.1002/oa.1390050406

Hershkovitz, I., Rothschild, B. M., Dutour, O., & Greenwald, C. (1998). Clues to recognition of fungal origin of lytic skeletal lesions. *American Journal of Physical Anthropology*, *106*, 47–60. https://doi.org/10.1002/(SICI)1096-8644(199805)106:1<47::AID-AJPA4>3.0.CO;2-A

Jacobson, H. G., Poppel, M. H., Shapiro, J. H., & Grossberger, S. (1958). The vertebral pedicle sign: A roentgen finding to differentiate metastatic carcinoma from multiple myeloma. *The American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine*, *80*, 817–821.

Kumar, S. K., Rajkumar, V., Kyle, R. A., Van Duin, M., Sonneveld, P., Mateos, M. V., … Anderson, K. C. (2017). Multiple myeloma. *Nature Reviews Disease Primers*, *3*, 1–20. https://doi.org/10.1038/nrdp.2017.46

Marks, M. K., & Hamilton, M. D. (2007). Metastatic carcinoma: Palaeopathology and differential diagnosis. *International Journal of Osteoarchaeology*, *17*, 217–234. https://doi.org/10.1002/oa.874

Marques, C., Santos, A. L., & Cunha, E. (2013). Better a broader diagnosis than a misdiagnosis: The study of a neoplastic condition in a male individual who died in early 20th century (Coimbra, Portugal). *International Journal of Osteoarchaeology*, *23*, 664–675. https://doi.org/10.1002/oa.1294

Morse, D., Dailey, R. C., & Bunn, J. (1974). Prehistoric multiple myeloma. *Bulletin of the New York Academy of Medicine*, *50*, 447–458.

National Cancer Institute. 2017. SEER cancer stat facts: Myeloma [online] available from: https://seer.cancer.gov/statfacts/html/mulmy.html

Ortner, D. J. (2003). *Identification of pathological conditions in human skeletal remains* (3rd ed.). Smithsonian Institution Press: New York.

Raab, M. S., Podar, K., Breitkreutz, I., Richardson, P. G., & Anderson, K. C. (2009). Multiple myeloma. *Lancet*, *374*, 324–339. https://doi.org/10.1016/S0140-6736(09)60221-X

Ragsdale, B. D., Campbell, R. A., & Kirkpatrick, C. L. (2018). Neoplasm or not? General principles of morphologic analysis of dry bone specimens. *International Journal of Paleopathology*, *21*, 27–40. https://doi.org/10.1016/j.ijpp.2017.02.002

Rajkumar, V. S. (2009). Multiple myeloma. *Current Problems in Cancer*, *33*, 7–64. https://doi.org/10.1016/j.currproblcancer.2009.01.001

Randall, R. L. (2015). *Metastatic bone disease: An integrated approach to patient care*Springer.

Roodman, G. D. (2004). Mechanisms of bone metastasis. *New England Journal of Medicine*, *350*, 1655–1664. https://doi.org/10.1056/NEJMra030831

Rothschild, B. M., Hershkovitz, I., & Dutour, O. (1998). Clues potentially distinguishing lytic lesions of multiple myeloma from those of metastatic carcinoma. *American Journal of Physical Anthropology*, *105*, 241–250. https://doi.org/10.1002/(SICI)1096-8644(199802)105:2<241::AID-AJPA10>3.0.CO;2-0

Rothschild, B. M., & Rothschild, C. (1995). Comparison of radiologic and gross examination for detection of cancer in defleshed skeletons. *American Journal of Physical Anthropology*, *96*, 357–363. https://doi.org/10.1002/ajpa.1330960404

Strouhal, E. (1991). Myeloma multiplex versus osteolytic metastatic carcinoma: Differential diagnosis in dry bones. *International Journal of Osteoarchaeology*, *1*, 219–224. https://doi.org/10.1002/oa.1390010314

Strouhal, E. (1993). A case of metastatic carcinoma from Christian Sayala (Egyptian Nubia). *Anthropologischer Anzeiger; Bericht Uber Die Biologisch-Anthropologische Literatur*, *51*, 97–115.

Strouhal, E., & Kritscher, H. (1990). Neolithic case of a multiple myeloma from Mauer (Vienna, Austria). *Anthropologie (1962-)*, *28*, 79–87.

Waldron, T. (2008). *Palaeopathology*. Cambridge, United Kingdom: Cambridge University Press. DOI: 10.1017/CBO9780511812569

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Research Paper

The appearance of breast cancer metastases on dry bone: Implications for forensic anthropology

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ABSTRACT

Breast carcinoma is a major cause of morbidity and mortality in women. The study of bone pathologies presents considerable potential in anthropology, paleopathology, forensic science and medicine. In this paper, we present and discuss metastatic lesions found in the skeletons of known individuals from the CAL Milano Cemetery Skeletal Collection, clinically diagnosed with breast cancer during life. Fourteen skeletons from a contemporary and identified collection were macroscopically studied and metastases were identified by comparison with clinical literature. As a result, bone metastases were observed in 43% of the study sample. They were located most commonly on the ribs (28.1%), pelvic girdle (19.8%), vertebrae (15.6%), skull (15.6%), scapulae (10.2%) as well as proximal segment of the femora (8.4%) and humeri (2.4%) respectively, favoring sites of high vascularization. The majority of the lesions were osteolytic, although osteoblastic and mixed metastases did occur. Osteolytic metastases appear as coalescent porosity or round to oval perforating lesions on bones with denticulated margins and pitted surrounding bone, whereas osteoblastic metastases thickened the existing trabecula (spongiosclerosis). Mixed metastases were perforating lytic lesions exposing the osteoblastic activity in the underlying trabecular bone. These results, consistent with the data from the literature, strengthen the diagnostic criteria for metastases and illustrate the aspect of bone metastases in breast carcinoma.

1. Introduction

Breast carcinoma is the leading cause of death in women aged 40–59, the first cause of cancer-related deaths in female sex and the most common cancer diagnosed in women.[1,2] It has been estimated that the lifetime probability of developing invasive breast cancer in the United States of America is 1 in 8 women.[1] Although male breast cancer may occur, it is extremely rare and represents less than 1% of all breast cancer cases.[3] Breast and prostate cancers are the most common solid tumors to metastasize to bone with an incidence at autopsy of bone metastases ranging from 65 to 75%,[4–6] making it the first site of metastasis for these cancer primaries.[7,8]

In 1889, Stephen Paget postulated his “seed and soil” theory that presupposes that metastatic growth (seed) is dependent upon the favorable microenvironment provided by the bone matrix (soil).[9,10] The bone microenvironment is a storage of immobilized growth factors released during bone resorption that will attract tumor cells and stimulate their proliferation. This disastrous loop of tumor growth and bone resorption or “vicious cycle” is the mechanism responsible for osteolytic metastases. In short, breast tumor cells secrete osteoblastic and osteoclastic factors that will promote the phenotypic differentiation of bone cells to osteoblasts (bone forming cells) and osteoclasts (bone remodeling cells) and their activation. Bone forming cells synthetize growth factors kept within the bone matrix until the osteoclastic activity releases them, promoting the proliferation of tumor cells and attracting new ones.[7,11–18]

Bone metastases are classically divided into three types: osteolytic (when the osteoclastic activity predominates), osteoblastic (when bone formation overcomes bone resorption) or mixed (with both osteoclastic and osteoblastic activities). However, both components are generally expressed and bone metastases range from mostly lytic to mostly blastic. Breast carcinoma is predominantly osteolytic (80–90% of metastases) but osteoblastic metastases may occur (10–20%).[2,8,12,13] Through naked eye observation, osteolytic metastases materialize as coalescent porosity or perforations of the bone cortex. Perforating lesions are round to oval destructive bone lesions of various size with well-defined denticulated or scalloped margins, but the greatest extent of the lesion is located in the trabecular bone, often dissimulated from

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Table 1
Details on the study sample.

Case n°	Sex	Age	Antemortem medical data	Presence of bone metastases
1	F	70	Breast neoplasia, diffuse bone metastases, neoplastic cachexia	x
2	F	54	Left breast cancer, diffuse metastases, cardiac arrest	x
3	F	83	Right breast cancer, hepatic metastases, cardiac arrest	x
4	F	69	Right breast cancer, multiple metastases, neoplastic cachexia	x
5	F	91	Breast cancer and neoplastic cachexia	x
6	F	61	Breast cancer, bone metastases, neoplastic cachexia	x
7	F	86	Metastatic breast cancer, digestive hemorrhage, varicose ulcer on left inferior limb, cardiac arrest	–
8	F	75	Ovarian and right breast cancer, hepatic metastases, peritoneal carcinoma, neoplastic cachexia	–
9	F	78	Right ulcerated breast neoplasia, neoplastic cachexia, cardiac arrest	–
10	F	71	Cyrrhosis, rupture of esophageal varices, left mastectomy for malign tumor, cardiac arrest	–
11	F	78	Breast cancer and diabetes mellitus, respiratory insufficiency, cardiac arrest	–
12	F	90	Left breast cancer, diffuse metastases, neoplastic cachexia, aortic cardiopathy	–
13	F	89	Right breast cancer, diffuse metastases, massive tumoral abdominal mass, cardiac arrest	–
14	F	77	Operated left breast cancer, metastases, cachexia, cardiac arrest	–

macroscopic observation.[19–22] In addition, the surrounding bone may present pitting.[20] By opposition, coalescent porosity appears as a focal osteoclastic activity resulting in a confluence of superficial pits.[20] Osteoblastic metastases may manifest as deposits of new bone thickening the existing trabeculae or "spongiosclerosis"[23]; or as periosteal new bone deposited on the bone surface and either excrescent-coarse,[24] coral-like[25] or more rarely, spiculated.[26,27] Skeletal metastases tend to be multifocal rather than isolated.[2,11] Breast carcinoma most commonly metastasizes to the pelvis, vertebrae, ribs, skull, as well as the proximal ends of the humerus and femur.[2,5,28]

Bone metastases may lead to skeletal-related events associated with an increased clinical morbidity and life-threatening complications. About two-thirds of patients with breast cancer will develop one or more skeletal-related events.[29] The most common consequence to bone metastases is severe bone pain. Pathologic fractures occur when the disease is advanced, typically in osteolytic metastases involving the cortex of weight-bearing bones. Spinal cord compression may lead to paralysis and is associated with a poor prognosis. The effects of the tumor on the marrow may also lead to leukopenia. Hypercalcemia is a life-threatening complication resulting from bone destruction and noted in 30% of patients with bone metastases.[2,7,11,29,30] Most of these skeletal-related events are clinical complications; nonetheless, pathologic fractures and vertebral collapse may be observed on dry bones.[31] The most devastating consequence is that "once breast cancer has spread to bone, it is incurable".[32]

The study of bone pathology possesses considerable potential in several disciplines: in anthropology, for the understanding of a person's condition and way of life; in paleopathology, for the study of past diseases; in archaeology, for the reconstruction of past lifestyles; in forensic sciences, for the construction of a specific biological profile that will, for instance, help narrow down the search among a missing persons list and provide a possible match; and in the medical field, for a better understanding of the history and evolution of disease. Metastatic carcinoma is a very prevalent condition today and evidence of the disease has been diagnosed in ancient human remains,[23,28,31,33] making its study relevant for both modern and past populations. Previous research has demonstrated the relevance of the study of bone pathology to the forensic field.[34–36] A correct interpretation of bone signs and their diagnosis will provide specific information to the biological profile, which may in turn narrow down the search of a missing person and may help in the understanding of the cause of death. However, the forensic anthropologist cannot find what they are not trained to recognize. Consequently, we present in this paper a study on breast cancer metastases and their appearance on dry bone based on skeletons from a contemporary and identified skeletal collection.

2. Materials and methods

The present paper examines 14 skeletons of known individuals with breast cancer from the CAL Milano Cemetery Skeletal Collection.[37] This collection is a contemporary and identified skeletal collection of more than 2100 skeletons housed in LABANOF (*Laboratorio di Antropologia e Odontologia Forense*) and made available for research purposes, in accordance with article 43 of the Italian National Police Mortuary Regulation (September 10, 1990, n°285). The collection is constituted of unclaimed skeletal remains who were buried in the cemeteries of Milan for 10–15 years. The individuals of the collection died between 1910 and 2001, although over 80% of the individuals died after 1980. No selection strategy of the individuals of the collection was implemented and so all socio-economic groups are represented. The CAL Milano Cemetery Skeletal Collection is both contemporary and documented. This documentation includes demographic descriptions but also "*ISTAT*" (*Istituto Nazionale di Statistica*) death certificates, that specify the cause of death as well as the traumatic and/or pathological chain of events that led to it. Consequently, it was possible to select individuals with a mention of breast cancer in their medical data.

In this study, all of the individuals of the CAL Milano Cemetery Skeletal Collection with breast cancer recorded in their medical data were selected, which included 14 cases. All of the individuals were females of Italian descent with ages ranging from 54 to 91 years old and a mean age-at-death of 77 years. The dates of birth of the selected skeletons range from 1900 to 1937 and the dates of death from 1985 to 1997. All of the information obtained from the antemortem medical data was reported in Table 1.

The skeletons were carefully washed with water and a toothbrush, sparing taphonomically altered areas to minimize bone damage, and let to dry. Subsequently, a macroscopic anthropological analysis was performed on each of them, including biological, pathological and traumatic analyses. Based on the descriptions of bone metastases from the literature,[19–23,26,28,31,38] and our macroscopic observations of lesions on clinically diagnosed cases of breast carcinoma from an identified osteological collection, a table was created (Table 2) and compiles the macroscopic morphological criteria for the recognition on bone metastases. All of the bones were carefully examined for potential metastases. For each lesion identified as an osteolytic metastasis, the dimensions were taken with a Vernier caliper (sliding caliper) and reported. When multiple perforating osteolytic lesions were found communicating in the same area of a bone, they were counted as only one metastasis with multiple cortical openings. For osteoblastic metastases thickening the trabeculae or spongiosclerosis, the lesions were observed under a stereomicroscope to recognize the thickening of the trabecular bone or new bone within bone. Mixed metastases were identified when both osteolytic and osteoblastic components were found associated in the same lesion: an osteolytic lesion perforating the

Table 2
Macroscopic morphological criteria of bone metastases (based on the literature[19–23,26,28,31,38] and the macroscopic observations of bone lesions in the present study).

	Osteolytic		Osteoblastic		Mixed
Morphology	coalescent porosity	perforation of bone cortex	spongiosclerosis	periosteal reaction	perforation of bone cortex showing spongiosclerosis
Shape	cluster of small osteolytic lesions (superficial pits)	round/oval	bone-in-bone, thickening of trabecular bone	excrescent-coarse, coral-like or spiculated	irregular lytic lesion exposing a thickened trabecular bone
Margins	well-defined, denticulated or scalloped		–		well-defined, denticulated or scalloped
Surrounding bone	–	often pitted	–		often pitted
Location	cortical bone, but trabecular bone affected to a greater extent		trabecular bone	surface of cortical bone	cortical and associated trabecular bone
Observed in the present study	Cases 2 & 5	Cases 1, 2, 3 & 4	Case 6	–	Case 1

bone cortex (osteolytic component) and exposing a spongiosclerotic lesion (osteoblastic component). Finally, each bone metastasis was photographed for records.

3. Results

Of the 14 female individuals of our study sample clinically diagnosed with breast cancer during life, bone metastases were observed in six skeletons, constituting 43% of the study sample. The types and locations of the bone metastases found in the individuals affected were reported respectively in Tables 3 and 4. For clarity of reading, the cases in which bone metastases were identified will be described by individual.

3.1. Case 1

The skeleton was over 60% complete but badly preserved with many of the bones broken post-mortem or fragmented in several pieces due to taphonomic processes prior to the inclusion in the collection. In the axial skeleton, fourteen vertebrae were present as well as most of the ribs, the left pelvis and sacrum were preserved and the right coxal bone was fragmented in five parts. The limbs and scapular girdle were present except for a few bones of the hands and feet. The cranium was complete and the right ramus of the mandible was broken post-mortem and absent.

Regarding the distribution of the metastases:

- 27 were found in the pelvic bones,
- 22 in the cranium,
- 20 in the vertebrae,
- 18 in the ribs,
- 17 in the scapulae,
- 13 in the proximal part of the femora,
- three in the proximal part of the right humerus
- and one on the left condyle of the mandible.

While 114 metastases were osteolytic, seven were mixed with both osteoblastic and osteolytic components (Table 2): five mixed metastases were counted in the vertebrae (Fig. 1), one in the pelvis and one in the ribs. All lytic metastases observed in this case perforated the cortical bone (Table 2) and ranged in diameter from 0.97 mm to 71.78 mm.

Table 3
Types of metastases per individual affected.

Case n°	Sex	Age	Osteolytic	Osteoblastic	Mixed	Total (%)
1	F	70	114		7	**121 (72.4%)**
2	F	54	39			**39 (23.3%)**
3	F	83	1			**1 (0.6%)**
4	F	69	1			**1 (0.6%)**
5	F	91	1			**1 (0.6%)**
6	F	61		4		**4 (2.4%)**
Total			**156**	**4**	**7**	**167**

Table 4
Location of the metastases per type of lesion.

Location	Osteolytic	Osteoblastic	Mixed	Total	Total %
Skull	26			**26**	**15.6%**
Scapulae	17			**17**	**10.2%**
Humeri	4			**4**	**2.4%**
Vertebrae	20	1	5	**26**	**15.6%**
Ribs	45	1	1	**47**	**28.1%**
Pelvic girdle	30	2	1	**33**	**19.8%**
Femora	14			**14**	**8.4%**

Fig. 1. *Mixed metastasis.* Lateral view of the 9th thoracic vertebra (Case 1), note the lytic lesion on the lateral side of the vertebral body exposing a thickening of the underlying trabecular bone.

The pelvis was the area most dramatically affected by metastases. The coxal bones were riddled with metastases of varying size (from 6.44 to 70.20 mm), mainly around the iliac crests (Fig. 2), acetabulum (without involving the articular surface), ischiopubic and iliopubic rami. Although only one metastasis was counted in the sacrum, it involved over 25% of the bone and was located on the superior right area (Fig. 3a). In the vertebrae, 15 metastases were found in the posterior elements, while five were noted on the vertebral bodies. In the cranium, scapulae and the proximal end of the femora and right humerus, lytic metastases varied greatly in size (0.97–46.22 mm). The neurocranium presented 20 metastases, mostly in the parietal and temporal bones affecting primarily the diploë with an outward progression, as opposed to only two in the splanchnocranium (orbital plates of the sphenoid). In the scapulae, the inferior, medial and lateral borders as well as the spine were the most affected locations (Fig. 3b). The involvement of the right humerus and femora was limited to the head, surgical neck and areas of the tubercles or trochanters (Fig. 3c). In the thoracic cage, bone

Fig. 2. *Anterior view of the left ilium riddled with lytic metastases (Case 1),* especially around the iliac fossa and the acetabulum (fragmented).

metastases were mainly located on the superior or inferior borders of the ribs and around the heads (vertebral ends) (Fig. 3d and e).

3.2. Case 2

The skeletal remains were rather well preserved and most of the bones present. While the skull and lower limbs were complete and the majority of the ribs and vertebrae were present, the right humerus was fragmented and incomplete, and most of the bones of the hands as well as the right scapula, radius and ulna were absent.

A total of 39 bone metastases were reported in this skeleton, all osteolytic in nature with sizes ranging from 0.93 to 29.04 mm. Regarding skeletal distribution of the lesions:

- the ribs were the most affected with 28 metastases,
- four metastases were found in the pelvis,
- three in the vertebrae,
- two in the skull,
- and one in the proximal end of the femora and humeri (one metastasis each).

Metastases in the ribs were osteolytic perforating lesions on the superior and inferior borders of the bodies, as well as on the head, similar to those of case 1 (Fig. 3d and e). In the pelvis, the metastases were located around the iliac spine and were very destructive. All three metastases of the vertebrae were found in the neural arches of the thoracic spine (T2, T3 and T7) (Fig. 4a). In the skull, the bones affected were the occipital and the left parietal with one metastasis in each bone, and the greatest destruction focused on the diplöe. The metastasis observed on the femur was located on the greater trochanter whereas in the humerus, it was found below the head, posteriorly. Most of the metastases manifested as perforations of the bone cortex, with varying extent of destruction of the trabecular bone; however, the osteolytic metastasis in the humerus appeared as a localized confluence of superficial small osteolytic foci (of less than 1 mm in diameter) or "coalescent porosity" (Table 2).

3.3. Case 3

The skeleton was very well-preserved, except for a few fragmented thoracic and lumbar vertebrae and the right scapula. All bones of the right upper limb and some tubular bones of the left hand were missing but the remaining of the skeleton was present.

In this case, only one metastasis was found: a large lytic lesion of 17.67 mm on the right temporal bone (Fig. 5). Pitting of the bone was particularly marked with three smaller but relatively wide lytic lesions on the superior area of the main lesion.

3.4. Case 4

The skeletal remains of this individual were badly preserved by

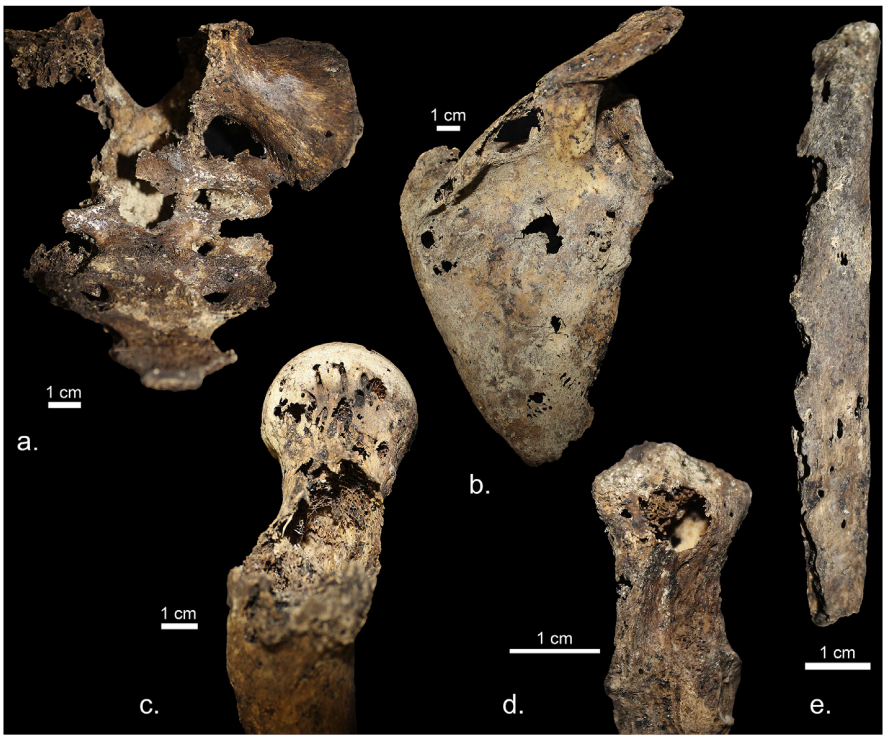

Fig. 3. *Osteolytic metastases (Case 1).* **a:** anterior view of the sacrum, note the destruction caused by the lytic metastasis; **b:** posterior view of the right scapula, note the lytic lesions with pitting along the lateral border, the superior part of the medial border, the scapular spine and around the glenoid articulation (both lesions located on the subscapular fossa are taphonomic); **c:** superomedial view of right femur, note the lytic metastases of various sizes on the femoral head and surgical neck; **d:** posterior view of the head of a lower left rib showing a lytic metastasis with well-defined denticulated margins; **e:** external view of a left rib fragmented (superior is on the right, anterior is up), note the lytic destruction-concentrated on the inferior border (left on the image) and porosity on the body.

Fig. 4. *Osteolytic metastases on posterior elements of vertebrae.* **a:** lateral right view of the 2nd thoracic of case 2; **b:** lateral right view of the 1st thoracic of case 5.

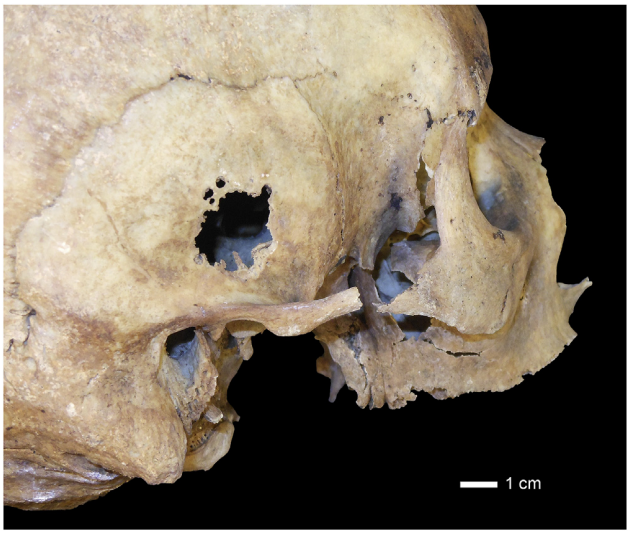

Fig. 5. *Osteolytic metastasis on the cranium (Case 3).* Right lateral view, note the irregular round shape of the lytic lesion with denticulated margins and surrounding pitted bone.

comparison with the previous cases of the study sample. Indeed, although the lower limbs were complete, most bones were fragmented with areas missing or separated. In particular, most of the rib fragments could not be identified and only two thoracic vertebrae were preserved in their original state.

One of this two vertebrae, belonging to the superior portion of the thoracic column, presented an oval and deep lytic lesion on its superior-anterior body of 5.4 mm (Fig. 6). The margins were denticulated and distinct from a perforation due to taphonomic processes.

3.5. Case 5

The skeleton was rather well preserved despite a high number of fragmented bones including the skull, scapulae, ribs, vertebrae, sacrum, coxal bones, fibulae and right tibia. The majority of the ribs and vertebrae were present but the sacrum was not readable.

An osteolytic metastasis was noted on the left part of the neural arch of the first thoracic vertebra consisting in coalescing superficial osteolytic lesions of less than 1 mm in diameter, or “coalescent porosity” similar to that of case 2 (Fig. 4b).

3.6. Case 6

This skeleton was largely incomplete. In the axial skeleton, only a few ribs and vertebrae were present but fragmented, as were the sacrum and the coxal bones. In fact, in the sacrum, only its superior portion was

Fig. 6. *Osteolytic metastasis on a vertebral body (Case 4).* Superior view of a superior thoracic vertebra (anterior is up).

present. The lower limbs and the skull were, however, well-preserved and complete. The large majority of the hand bones were missing and the right humerus was absent.

Four metastases were noted on this skeleton, all osteoblastic and thickening the existing trabeculae (“spongiosclerotic”). They were reported in an unidentified vertebral body (Fig. 7), head of an unidentified right rib, anterior-superior sacrum and posterior left coxal bone. For each of them, the bones were broken post-mortem allowing

Fig. 7. *Osteoblastic metastasis (Case 6).* View the trabecular bone of a fragmented vertebral body, note the thickening of the trabecular bone or “coating” as new bone is formed in the existing trabeculae.

the observation of the underlying trabeculae and the presence of osteoblastic metastases. There was no remaining evidence of overlying lytic lesions exposing the thickened trabeculae that would have indicated mixed metastases.

4. Discussion

Although Rothschild and Rothschild[39] rightfully showed that gross examination of bones was less sensitive than radiographic imaging in the identification of bone metastases, they also recognized that it is not possible to systematically perform radiographic screening of entire skeletons. Indeed, bone metastases involve primarily vascularized areas of bone, namely trabecular bone, generally hidden from macroscopic observation. Bone metastases may thus escape the notice of the anthropologist and the diagnosis of the disease, particularly important for the construction of the biological profile, may be lost. Consequently, the preferred method is an initial macroscopic analysis of the skeleton and when lesions suggestive of metastatic carcinoma are found, radiographic examination is performed to assess the extent and severity of the bone metastatic disease. In this study, 14 skeletons clinically diagnosed with breast cancer during life were macroscopically analyzed. In this perspective, we present, document and discuss bone metastases observed on known cases of breast cancer.

In our selected population, six individuals out of the total 14 exhibited macroscopic bone metastases. The remaining eight individuals (57% of the initial sample) did not show anything suggestive of metastatic cancer.

The presence or absence of bone metastases in the skeletons may be attributed to several factors which may be discussed. First, the state of preservation of the skeletal remains could have influenced the recovery of skeletal metastases. In a general point of view, we do not believe the state of preservation of the remains to be a pivotal factor. For instance, case 1 was heavily fragmented and incomplete and showed 121 metastases (72.4% of all the metastases observed in this study) while case 3 was particularly well preserved and almost fully complete but only one metastasis was found (0.6% of all metastases observed) (Table 3). Moreover, the state of preservation of the individuals without bone metastases was similar to that of the skeletons with metastases, ranging from very well-preserved to many bones absent or fragmented but with most skeletal areas represented. Nonetheless, and considering that in three out of six skeletons with metastatic bone disease only one metastasis was found, it is possible that few bones were affected in the individuals without identified bone metastases and that they may have been absent from the anthropological analysis or too fragmented to be readable. Second, the age of the individuals could impact the presence or absence of bone metastases. Metastatic bone disease has a peak onset in individuals over 40 years and a mean age-at-diagnosis of 62 years in breast cancer.[40] However, the affected sample of our population included both the youngest (54 years – case 2) and the oldest (91 years – case 5) individuals in our study sample. In addition, the individuals without bone metastases ranged from 71 to 90 years old, far older than the mean age-at-diagnosis in breast cancer patients. The duration, age-at-diagnosis or stage of development of the cancer was not provided in the antemortem medical data; it is therefore possible that the individuals without bone metastases were recently diagnosed or at an earlier stage of development of the cancer and that they did not present any skeletal metastasis while alive. Third, growing evidence shows that response to cancer varies between individuals and is influenced by their health-related quality of life, which may improve survival or be associated with increased morbidity and mortality[41,42]; thus inter-individual variations should also be considered. Fourth, although we have no information on the socio-economic background of the individuals of the study, epidemiological studies show variation in diagnostic intervals (and thus stage-at-diagnosis) depending on the presenting symptoms[43] and socio-economic groups.[44] Fifth, the influence of treatment. Apart from surgical interventions such as mastectomies (case 10 - Table 1), we did not have any information on potential treatments that the women of the study might have received; nonetheless, mastectomies, radiotherapy and chemotherapy, although not as developed and effective in the late 1980's to mid-1990's as they are today, could have reduced the risk of recurrence of the condition, the size of the tumor and the number of new bone metastases,[45,46] which does not alter our results on the morphological observation of bone metastases, only the interpretation of the total number of metastases. Finally, and as previously mentioned, macroscopic analysis is not the most sensitive and reliable type of examination to find bone metastases. Thus, the incidence at autopsy of bone metastases of 65–75% provided in the literature[4–6] may not be wrong in our population. Nevertheless, the point of this study was not to assess the incidence of bone metastases but rather to document and discuss the aspect of bone metastases on dry bone due to clinically diagnosed breast carcinoma.

It is difficult to draw interpretations from the number of metastases reported in each case because, as mentioned earlier, we have no information regarding the duration, treatment or stage of development of the disease; there may have been more metastases but located on osseous elements absent from the anthropological analysis or fragmented; and the total amount of skeletal metastases does not consider subcortical metastases inaccessible to macroscopic examination. Nonetheless, the high number of metastases in cases 1 and 2 with respectively 121 (72.4% of all the metastases observed in this study) and 39 (23.3% of all metastases) bone metastases (Table 3) is a strong argument in favor of an advanced stage of the disease in these two individuals. It may also be noted that in the two skeletons where metastases were absolutely expected because of their mention in the antemortem medical data (cases 1 and 6 – Table 1), skeletal metastases were indeed found during anthropological analysis.

Bone metastases were most commonly found in the ribs (28.1%), pelvic girdle (19.8%), vertebrae (15.6%), skull (15.6%), scapulae (10.2%) as well as proximal segment of the femora (8.4%) and humeri (2.4%) respectively (Table 4). These results concur with the data from the literature.[2,5,28] In the pelvic girdle, the sacrum as well as the areas around the iliac crest, acetabulum (excluding the articular surface), ischiopubic and iliopubic ramus were primarily involved (Figs. 2 and 3a) whereas in the skull, parietal and temporal bones were preferred (Fig. 5). In both locations, the perforation of the cortex revealed a more extensive lesion in the trabeculae/diploë, consistent with a preference of metastatic cells for highly vascularized areas. In the vertebral column, the literature mentions a predilection for the posterior elements in metastatic carcinoma, by opposition to multiple myeloma lesions exclusively located on the vertebral bodies.[47] In our study, the large majority of vertebral metastases were found on the posterior elements (Fig. 4), consistent with the data from the literature. Skeletal metastases in the thoracic cage were less rounded (Fig. 3e), making it difficult to differentiate them from taphonomic alterations. However, scalloped but defined margins allowed their identification. In addition, more extensive lesions did also occur in the ribs, focused on the head (Fig. 3d). In the scapulae, metastases were located on the different borders (inferior, medial and lateral) as well as on the spine (Fig. 3b) where they could show a deep involvement of the underlying trabecular bone. In the long bones, metastases were restricted to the head, surgical neck and trochanters/tubercles of the femora and humeri (Fig. 3c). They materialized as numerous lytic lesions of various size and depth. All osteoblastic and mixed metastases were found exclusively in the vertebral column (Figs. 1 and 7), pelvic girdle or ribs, the three areas most commonly affected by bone metastases in general (Table 4).

All three types of metastases were noted in the population studied: osteolytic, osteoblastic and mixed metastases. Osteolytic metastases were far more common (156 metastases – 93% of all metastases) than mixed (seven metastases – 4%) and osteoblastic metastases (four metastases – 2%). These findings confirm the data from the literature describing breast carcinoma as mostly lytic with 80–90% of metastases being osteolytic and possible, but rarer, osteoblastic metastases.[2,8,12,13]

In case 6, spongiosclerosis was observed because of the fragmented structure of the bones, which, after post-mortem alterations removing parts of cortical bone, left the underlying trabeculae apparent (Fig. 7). However, the bone was broken; thus, the possibility of an original mixed metastasis where the lytic activity would have been damaged by taphonomic alterations and only the osteoblastic one remained, cannot be excluded. Nonetheless, each osteolytic, osteoblastic and mixed metastasis identified presented recognizable criteria compiled in Table 2 from the descriptions of the literature and illustrated here. In this study, osteolytic metastases were found of two types: round or oval osteolytic lesions perforating the cortical bone, with denticulated margins, pitted surrounding bone and an outward progression (Fig. 5); or as coalescent porosity, a cluster of coalescing superficial pits (of less than 1 mm in diameter). Osteolytic metastases did not evidence any sign of healing or remodeling. Osteoblastic metastases were only reported in one individual (case 6) and manifested exclusively as a thickening of the existing trabecular bone, or spongiosclerosis (Fig. 7); no proliferative metastasis with periosteal reaction was observed here. Mixed metastases, also found in a single skeleton (case 1), appeared as a perforating osteolytic exposing the underlying abnormally thickened trabeculae (Fig. 1). Although several studies in the osteoarcheological literature have described lesions favoring a differential diagnosis of metastatic bone disease,[22,48–51] and even of breast metastatic carcinoma,[52–56] descriptions of known cases with a clinical antemortem diagnosis of the condition are rare. Because the individuals of the study sample were clinically diagnosed with breast malignancy during life, these results strengthen the criteria for the diagnosis of bone metastases and specifically illustrate the nature, aspect and pattern of distribution of skeletal metastases in breast cancer.

5. Conclusion

Malignant breast neoplasm is a major cause of mortality among women. A correct diagnosis of breast metastatic carcinoma on bones would provide crucial information for the construction of a biological profile in particular. In this study, we macroscopically examined 14 individuals clinically diagnosed with the breast malignancy during life and identified different types of metastases in six skeletons. The aim of this paper was to present and discuss the aspect of known bone metastases from breast cancer to document what to expect in an unknown skeletonized case. As a result, skeletal metastases from breast carcinoma affect most commonly the ribs, pelvic girdle, vertebrae, skull, scapulae, proximal segment of the femora and humeri, favoring sites of high vascularization. They are predominantly osteolytic, although mixed and osteoblastic metastases do occur. Osteolytic metastases appear as irregular oval perforating lesions on bones with denticulated margins and pitted surrounding bone, whereas osteoblastic metastases “coated” the existing trabeculae (bone-in-bone). Mixed metastases were perforating lytic lesions exposing the osteoblastic activity in the underlying trabecular bone.

The main scope of this article was to strengthen the bibliographic evidence of known metastatic bone lesions on dry bone as in the literature such evidence is scarce. In this manner, the proposal of a differential diagnosis within a biological profile may be more specific hence facilitating candidates among forensic cases.

Conflicts of interest

The authors declare that they have no conflict of interest.

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References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *Ca - Cancer J Clin.* 2018;68(1):7–30. https://doi.org/10.3322/caac.21442.
2. Randall RL. *Metastatic Bone Disease: An Integrated Approach to Patient Care.* Springer; 2015.
3. Anderson WF, Jatoi I, Tse J, Rosenberg PS. Male breast cancer: a population-based comparison with female breast cancer. *J Clin Oncol.* 2010;28(2):232–239. https://doi.org/10.1200/JCO.2009.23.8162.
4. Coleman RE. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. *Cancer Treat Rev.* 2001;27(3):165–176https://doi.org/10.1053/ctrv.2000.0210.
5. Lipton JF, Vigorita VJ. Metastatic bone disease. In: Vigorita VJ, Ghelman B, Mintz D, eds. *Orthopaedic Pathology.* third ed. Philadelphia: Lippincott Williams & Wilkin; 2016:594–619.
6. Roodman GD. Mechanisms of bone metastasis. *N Engl J Med.* 2004;350(16):1655–1664. https://doi.org/10.1056/NEJMra030831.
7. Vassiliou V, Chow E, Kardamakis D. *Bone Metastases: A Translational and Clinical Approach.* vol 21. Springer Science & Business Media; 2013.
8. Zhang Y, Ma B, Fan Q. Mechanisms of breast cancer bone metastasis. *Cancer Lett.* 2010;292(1):1–7. https://doi.org/10.1016/j.canlet.2009.11.003.
9. Paget S. The distribution of secondary growths in cancer of the breast. *Lancet.* 1889;133(3421):571–573. https://doi.org/10.1016/S0140-6736(00)49915-0.
10. Talmadge JE, Fidler IJ. AACR centennial series: the biology of cancer metastasis: historical perspective. *Cancer Res.* 2010;70(14):5649–5669. https://doi.org/10.1158/0008-5472.can-10-1040.
11. Guise TA, Kozlow WM, Heras-Herzig A, Padalecki S, Yin JJ, Chirgwin JM. Molecular mechanisms of breast cancer metastases to bone. *Clin Breast Canc.* 2005;5:S46–S53. https://doi.org/10.3816/CBC.2005.s.004.
12. Käkönen S-M, Mundy GR. Mechanisms of osteolytic bone metastases in breast carcinoma. *Cancer.* 2003;97(S3):834–839. https://doi.org/10.1002/cncr.11132.
13. Akhtari M, Mansuri J, Newman KA, Guise TM, Seth P. Biology of breast cancer bone metastasis. *Cancer Biol Ther.* 2008;7(1):3–9. https://doi.org/10.4161/cbt.7.1.5163.
14. Chen YC, Sosnoski DM, Mastro AM. Breast cancer metastasis to the bone: mechanisms of bone loss. *Breast Cancer Res.* 2010;12(6) https://doi.org/10.1186/bcr2781.
15. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. *Nat Med.* 2006;12:895. https://doi.org/10.1038/nm1469.
16. Guise TA, Mohammad KS, Clines G, et al. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. *Clin Canc Res.* 2006;12(20):6213–6216.
17. Guise TA, Yin JJ, Taylor SD, et al. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. *J Clin Invest.* 1996;98(7):1544–1549. https://doi.org/10.1172/JCI118947.
18. Mastro AM, Gay CV, Welch DR. The skeleton as a unique environment for breast cancer cells. *Clin Exp Metastasis.* 2003;20(3):275–284. https://doi.org/10.1023/A:1022995403081.
19. Ragsdale BD, Campbell RA, Kirkpatrick CL. Neoplasm or not? General principles of morphologic analysis of dry bone specimens. *Int J Paleopathol.* 2018;21:27–40https://doi.org/10.1016/j.ijpp.2017.02.002.
20. Marques C, Santos AL, Cunha E. Better a broader diagnosis than a misdiagnosis: the study of a neoplastic condition in a male individual who died in early 20th century (coimbra, Portugal). *Int J Osteoarchaeol.* 2013;23(6):664–675. https://doi.org/10.1002/oa.1294.
21. Rothschild BM, Hershkovitz I, Dutour O. Clues potentially distinguishing lytic lesions of multiple myeloma from those of metastatic carcinoma. *Am J Phys Anthropol.* 1998;105(2):241–250. https://doi.org/10.1002/(SICI)1096-8644(199802)105:2<241::AID-AJPA10>3.0.CO;2-0.
22. Strouhal E. Myeloma multiplex versus osteolytic metastatic carcinoma: differential diagnosis in dry bones. *Int J Osteoarchaeol.* 1991;1(3-4):219–224. https://doi.org/10.1002/oa.1390010314.
23. Ortner DJ. *Identification of Pathological Conditions in Human Skeletal Remains.* third ed. New York: Smithsonian Institution Press; 2003.
24. Anderson T, Wakely J, Carter A. Medieval example of metastatic carcinoma: a dry bone, radiological, and SEM study. *Am J Phys Anthropol.* 1992;89(3):309–323. https://doi.org/10.1002/ajpa.1330890305.
25. Santos AL, Roberts CA. Anatomy of a serial killer: differential diagnosis of tuberculosis based on rib lesions of adult individuals from the Coimbra identified skeletal collection, Portugal. *Am J Phys Anthropol.* 2006;130(1):38–49. https://doi.org/10.1002/ajpa.20160.
26. Bloom RA, Libson E, Husband JE, Stoker DJ. The periosteal sunburst reaction to bone

metastases. A literature review and report of 20 additional cases. *Skeletal Radiol.* 1987;16(8):629–634.
27. Vilar J, Lezana AH, Pedrosa CS. Spiculated periosteal reaction in metastatic lesions of bone. *Skeletal Radiol.* 1979;3(4):230–233. https://doi.org/10.1007/BF00360941.
28. Waldron T. *Palaeopathology*. Cambridge, United Kingdom: Cambridge University Press; 2008.
29. Harvey HA, von Reyn Cream L. Biology of bone metastases: causes and consequences. *Clin Breast Canc.* 2007;7(Supplement 1):S7–S13https://doi.org/10.3816/CBC.2007.s.001.
30. Coleman RE. Clinical features of metastatic bone disease and risk of skeletal morbidity. *Clin Canc Res.* 2006;12(20):6243.
31. Aufderheide AC, Rodríguez-Martín C. *The Cambridge Encyclopedia of Human Paleopathology*. Cambridge, United Kingdom: Cambridge University Press; 1998.
32. Guise TA. Breast cancer bone metastases: it's all about the neighborhood. *Cell.* 2013;154(5):957–958. https://doi.org/10.1016/j.cell.2013.08.020.
33. Capasso LL. Antiquity of cancer. *Int J Canc.* 2005;113(1):2–13. https://doi.org/10.1002/ijc.20610.
34. Biehler-Gomez L, Cappella A, Castoldi E, Martrille L, Cattaneo C. Survival of atherosclerotic calcifications in skeletonized material: forensic and pathological implications. *J Forensic Sci.* 2018;63(2):386–394. https://doi.org/10.1111/1556-4029.13592.
35. Biehler-Gomez L, Castoldi E, Baldini E, Cappella A, Cattaneo C. Diabetic bone lesions: a study on 38 known modern skeletons and the implications for forensic scenarios. *Int J Leg Med.* 2018:1–15. https://doi.org/10.1007/s00414-018-1870-0.
36. Biehler-Gomez L, Cabrini A, De Angelis D, Cattaneo C. How do skeletons with HIV present? A study on the identified CAL Milano Cemetery Skeletal Collection. *Leg Med.* 2018;33:11–16https://doi.org/10.1016/j.legalmed.2018.04.007.
37. Cattaneo C, Mazzarelli D, Cappella A, et al. A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection. *Forensic Sci Int.* 2018;287. https://doi.org/10.1016/j.forsciint.2018.03.041 219.e1-219.e5.
38. Marks MK, Hamilton MD. Metastatic carcinoma: palaeopathology and differential diagnosis. *Int J Osteoarchaeol.* 2007;17(3):217–234. https://doi.org/10.1002/oa.874.
39. Rothschild BM, Rothschild C. Comparison of radiologic and gross examination for detection of cancer in defleshed skeletons. *Am J Phys Anthropol.* 1995;96(4):357–363. https://doi.org/10.1002/ajpa.1330960404.
40. National Cancer Institute. SEER cancer stat facts: female breast cancer. https://seer.cancer.gov/statfacts/html/breast.html; 2017, Accessed date: 4 February 2018.
41. Spiegel D, Kraemer H, Bloom J, Gottheil E. Effect of psychosocial treatment on survival of patients with metastatic breast cancer. *Lancet.* 1989;334(8668):888–891https://doi.org/10.1016/S0140-6736(89)91551-1.
42. Sitlinger A, Zafar SY. Health-related quality of life: the impact on morbidity and mortality. *Surg Oncol Clin N Am.* 2018;27(4):675–684https://doi.org/10.1016/j.soc.2018.05.008.
43. Koo MM, von Wagner C, Abel GA, McPhail S, Rubin GP, Lyratzopoulos G. Typical and atypical presenting symptoms of breast cancer and their associations with diagnostic intervals: evidence from a national audit of cancer diagnosis. *Cancer Epidemiol.* 2017;48:140–146https://doi.org/10.1016/j.canep.2017.04.010.
44. Woods LM, Rachet B, Coleman MP. Origins of socio-economic inequalities in cancer survival: a review. *Ann Oncol.* 2006;17(1):5–19.
45. Diel IJ, Solomayer E-F, Costa SD, et al. Reduction in new metastases in breast cancer with adjuvant clodronate treatment. *N Engl J Med.* 1998;339(6):357–363. https://doi.org/10.1056/NEJM199808063390601.
46. DeVita VT, Chu E. A history of cancer chemotherapy. *Cancer Res.* 2008;68(21):8643.
47. Jacobson HG, Poppel MH, Shapiro JH, Grossberger S. The vertebral pedicle sign: a roentgen finding to differentiate metastatic carcinoma from multiple myeloma. *Am J Roentgenol Radium Ther Nucl Med.* 1958;80(5):817–821.
48. Assis S, Codinha S. Metastatic carcinoma in a 14th–19th century skeleton from Constância (Portugal). *Int J Osteoarchaeol.* 2010;20(5):603–620. https://doi.org/10.1002/oa.1084.
49. de la Rúa C, Baraybar JP, Etxeberria F. Neolithic case of metastasizing carcinoma: multiple approaches to differential diagnosis. *Int J Osteoarchaeol.* 1995;5(3):254–264. https://doi.org/10.1002/oa.1390050304.
50. Lieverse AR, Temple DH, Bazaliiskii VI. Paleopathological description and diagnosis of metastatic carcinoma in an early bronze age (4588 + 34 cal. BP) forager from the cis-baikal region of eastern siberia. *PLoS One.* 2014;9(12):e113919.
51. Wasterlain SN, Ascenso BF, Silva AM. Skeletal metastatic carcinoma: a case from 15th–20th century Coimbra, Portugal. *Int J Osteoarchaeol.* 2011;21(3):336–346. https://doi.org/10.1002/oa.1130.
52. Allison MJ, Gerszten E, Munizaga J, Santoro C. Metastatic tumor of bone in a Tiahuanaco female. *Bull N Y Acad Med.* 1980;56(6):581–587.
53. Smith MO. A probable case of metastatic carcinoma from the late prehistoric eastern Tennessee River Valley. *Int J Osteoarchaeol.* 2002;12(4):235–247. https://doi.org/10.1002/oa.618.
54. Merczi M, Marcsik A, Bernert Z, et al. Skeletal metastatic carcinomas from the roman period (1st to 5th century AD) in Hungary. *Pathobiology.* 2014;81(2):100–111.
55. Strouhal E. A case of metastatic carcinoma from christian sayala (Egyptian nubia). *Anthropol Anzeiger.* 1993;51(2):97–115.
56. Melikian M. A case of metastatic carcinoma from 18th century London. *Int J Osteoarchaeol.* 2006;16(2):138–144. https://doi.org/10.1002/oa.813.

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The overlooked primary: bladder cancer metastases on dry bone. A study of the 20th century CAL Milano Cemetery Skeletal Collection

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ABSTRACT

Objective: The aim of this article is to provide additional documentation of bone metastases to help anthropologists recognize the condition and potentially suggest the diagnosis of bladder carcinoma in differential diagnosis.

Materials and methods: Thirteen individuals clinically diagnosed with bladder carcinoma from the 20th century Milano Cemetery Skeletal Collection were macroscopically studied to document bone metastases in bladder cancer.

Results: Bone metastases were found through macroscopic observation in three individuals or 23% of the study sample. Metastases were mostly of a mixed nature (45%), although both osteoblastic (13%) and osteolytic (9%) also occurred. In particular, mixed and osteoblastic metastases exhibited different distribution patterns, even when affecting the same bones. The vertebrae (24.7%), skull (12.9%), ribs (11.7%), proximal humeri (7.8%), pelvis (5.2%), proximal femora (2.6%), sacrum (1.3%) and sternum (1.3%) were the most commonly affected. Osteolytic lesions included coalescing superficial pits or lesions perforating the bone cortex. Proliferative lesions manifested as spongiosclerosis or periosteal new bone. Mixed metastases were osteolytic lesions exposing a thickened trabecular bone or coalescent porosity with reactive new bone.

Conclusions: Bladder carcinoma metastases were mostly mixed, exhibiting periosteal reactions, perforations of bone cortex, spongiosclerosis and coalescing porosity.

Significance: Bladder carcinoma is rarely considered in the differential diagnosis of the primary organ. This study reports the macroscopic aspect of bone metastases in bladder carcinoma and may help anthropologists diagnose the condition in skeletons.

Limitations: Absence of evidence is not evidence of absence; some lesions may have been hidden from macroscopic observation and therefore missed.

Further research: Radiographic analysis and comparison with other neoplasms should provide additional details for the diagnosis of bladder cancer bone metastases.

1. Introduction

Urinary bladder cancer is the sixth most common cancer in the United States and the fourth most common malignant neoplasm diagnosed in men (American Cancer Society, 2018). Nonetheless, bladder carcinoma is rarely, if ever, considered in differential diagnosis of metastatic cancers observed archaeologically or forensically. The three most common carcinomas to metastasize to bone are prostate (65–80% frequency), breast (65–80%) and lung (30–40%), respectively (Coleman, 1997; Lipton and Vigorita, 2016). Prostate metastases are known to be most commonly proliferative, whereas breast and lung metastases to bone are predominantly osteolytic (Lipton and Vigorita, 2016; Randall, 2015; Waldron, 2008, pp. 186–188). Thus, in the paleopathological context, if the metastases are osteoblastic, a prostate origin of the cancer is the most likely diagnosis; if the metastases are predominantly osteolytic in a female, breast carcinoma is commonly proposed. In a male, pulmonary cancer is mostly frequently associated with osteolytic lesions.

To strengthen the diagnosis of metastatic carcinoma on skeletal remains, studies on identified skeletal collections provide information on known individuals (Marques et al., 2018; Rothschild and Rothschild, 1995). Some studies have focused on certain types of cancer, including leukemia (Rothschild et al., 1997) and prostate carcinoma (Castoldi et al., 2017). In this paper, we present dry bone metastatic lesions from

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identified skeletons clinically diagnosed with bladder cancer.

The exact causes of bladder cancers are unknown, but risk factors include male sex, age (above 55 years), white ancestry, chronic bladder infections and irritations, genetics and family history, prior chemotherapy or radiation therapy, smoking and chemical exposure. Bladder cancer most commonly affects older white males with a 4:1 male-to-female ratio, with an overall average age-at-diagnosis of 73 years. Indeed, 90% of individuals with bladder cancer are over 55 years old. Overall, the estimated chance of developing this type of cancer is one in 27 men and one in 89 women. Once the cancer has spread to distant parts of the body, the five-year survival rate is estimated at 5% (American Cancer Society, 2018).

The "seed and soil" theory developed by Stephen Paget in 1889 postulates that the development of the metastatic tumor (seed) is facilitated by the properties of the bone matrix (soil) (Paget, 1889; Talmadge and Fidler, 2010). Indeed, the distant target site may either encourage survival of tumor cells, if the microenvironment is favorable to metastatic growth, or limit it (Talmadge and Fidler, 2010). The bone microenvironment acts as a reservoir of immobilized growth factors. During bone turnover, these molecules are released and will both stimulate the proliferation of cancer cells and attract new ones. This "vicious cycle" is a continuous loop of tumor growth and bone resorption responsible for osteolytic metastases. In fact, osteoblastic (including endothelin-1 (ET-1) and platelet-derived growth factor (PDGF)) and osteoclastic factors (in particular, tumor-derived parathyroid hormone-related protein (PTHrP), necrosis-α (TNFα) and interleukins (IL-1, IL-8, IL-11, IL-15 and IL-17)) are secreted by tumor cells and promote the phenotypic differentiation of bone cells to osteoblasts (bone forming cells) and osteoclasts (bone remodeling cells) and their activation. An overexpression of osteoclastic factors (especially if associated with an inhibition of osteoblastic activation or the apoptosis of osteoblastic cells) will result in destructive bone lesions called osteolytic metastases. Alternatively, specific molecules stimulating the differentiation and activation of osteoblastic cells may result in excessive bone formation or osteoblastic metastases (Gruber, 2012; Guise, 2002; Guise et al., 2006, 2003, 1996; Steeg, 2006).

Consequently, skeletal metastases can be classified into three types: osteolytic (predominantly bone resorption), osteoblastic (predominantly bone formation) or mixed (both bone formation and resorption) (Coleman, 2001, 1997). Nonetheless, osteoclastic and osteoblastic components are generally both present, and skeletal metastases range from mostly osteolytic to mostly osteoblastic (Miler et al., 2017; Mundy, 2002; Randall, 2015). Prostate metastases are mainly osteoblastic and sclerotic while breast and lung secondaries are mainly osteolytic (Lipton and Vigorita, 2016; Randall, 2015; Waldron, 2008). Osteolytic lesions are irregularly round to oval and present a denticulated or scalloped perimeter. Moreover, the surrounding bone often exhibits pitting. Bone forming metastases or osteoblastic metastases may produce new bone deposits that thicken the trabeculae (Ortner, 2003, p. 535) or periosteal new bone, either excrescent-coarse (Anderson et al., 1992; Smith, 2002), coral-like (Anderson et al., 1992; Santos and Roberts, 2006; Smith, 2002) or more rarely, spiculated (Bloom et al., 1987; Vilar et al., 1979) fiber bone. Bone metastases tend to be multifocal rather than isolated (Guise et al., 2005; Randall, 2015), located on the vertebrae, pelvis, ribs, proximal ends of the humerus and femur and to a lesser degree, the skull (Lipton and Vigorita, 2016; Mundy, 1997; Randall, 2015; Waldron, 2008). Tumor metastases are most commonly disseminated through a hematogenous route and develop in osseous sites rich in hematopoietic marrow.

Skeletal metastases are associated with increased clinical morbidity and life-threatening complications. These include bone pain, pathologic fractures (typically in advanced disease with osteolytic metastases), spinal cord compression (with a risk of paralysis), leukopenia and hypercalcemia (observed in up to 30% of patients with bone metastases) (Coleman, 2006; Harvey and von Reyn Cream, 2007; Randall, 2015; Vassiliou et al., 2013). Although these are clinical complications that cannot be observed on dry bone, pathologic fractures and vertebral collapse are signs visible to the anthropologist (Aufderheide and Rodríguez-Martín, 1998, p. 388).

2. Materials and methods

In the present study, 13 skeletons of individuals with clinical diagnoses of bladder cancer from the CAL Milano Cemetery Skeletal Collection (Cattaneo et al., 2018) were examined macroscopically. This collection is a modern documented skeletal collection of 2127 skeletons housed in the LABANOF (*Laboratorio di Antropologia e Odontologia Forense*) made available for research purposes, in accordance with article 43 of the Italian National Police Mortuary Regulation (September 10, 1990, n°285). Documentation includes demographic descriptions as well as *"ISTAT"* (*Istituto Nazionale di Statistica*) death certificates. These death certificates specify the cause and the traumatic and/or pathological chain of events preceding death. As a result, it was possible to select individuals for whom bladder cancer diagnoses appeared in associated antemortem medical data.

The collection is composed of unclaimed individuals buried in Milan cemeteries for a minimum of 10 years and exhumed by cemetery workers by means of heavy machinery. Individuals presently in the CAL Milano Cemetery Skeletal Collection died between 1910 and 2001; they are evenly distributed among males and females and aged between 0 and 104 years at the time of death. Forty-eight percent have an age-at-death of between 70 and 90 years. Overall, 197 cases of cancer were reported as the cause of death (9% of the overall causes of death in the collection). In this research, all of the individuals of the CAL Milano Cemetery Skeletal Collection with bladder cancer reported in their associated medical data were selected, representing 13 cases (7% of cancer cases in the collection and 0.6% of the overall causes of death). The individuals include 10 males and three females with ages ranging

Table 1
Details on the studied individuals.

Case n°	Sex	Age	Antemortem medical data	Presence of bone metastases
CM 897	M	56	Bladder cancer and deterioration, stroke, cardiac arrest	yes
CM 422	F	59	Bladder cancer, multiple metastases, terminal cachexia	yes
CM 490	F	82	Bladder cancer, hepatic and pelvic metastases, neoplastic cachexia	yes
CM 39	M	76	Bladder cancer, neoplastic cachexia	–
CM 149	M	60	Bladder cancer, cardiac arrest	–
CM 164	M	77	Bladder cancer, myocardial infarctus	–
CM 310	M	58	Bladder cancer, hepatic and lymphatic metastases, cardiac arrest	–
CM 350	M	85	Bladder cancer, pulmonary metastases, cardiac arrest	–
CM 389	M	76	Bladder cancer, endoabdominal metastases, cardiac arrest	–
CM 407	F	86	Probable bladder cancer, secondary anemia, cardiac arrest	–
CM 456	M	71	Bladder cancer, hepatic metastases, cardiac arrest	–
CM 1039	M	60	Bladder cancer, hydroelectric imbalance, quadriplegia, cardiac insufficiency	–
CM 1141	M	78	Left bronchopulmonitis, respiratory insufficiency, bladder cancer, cardiac arrest	–

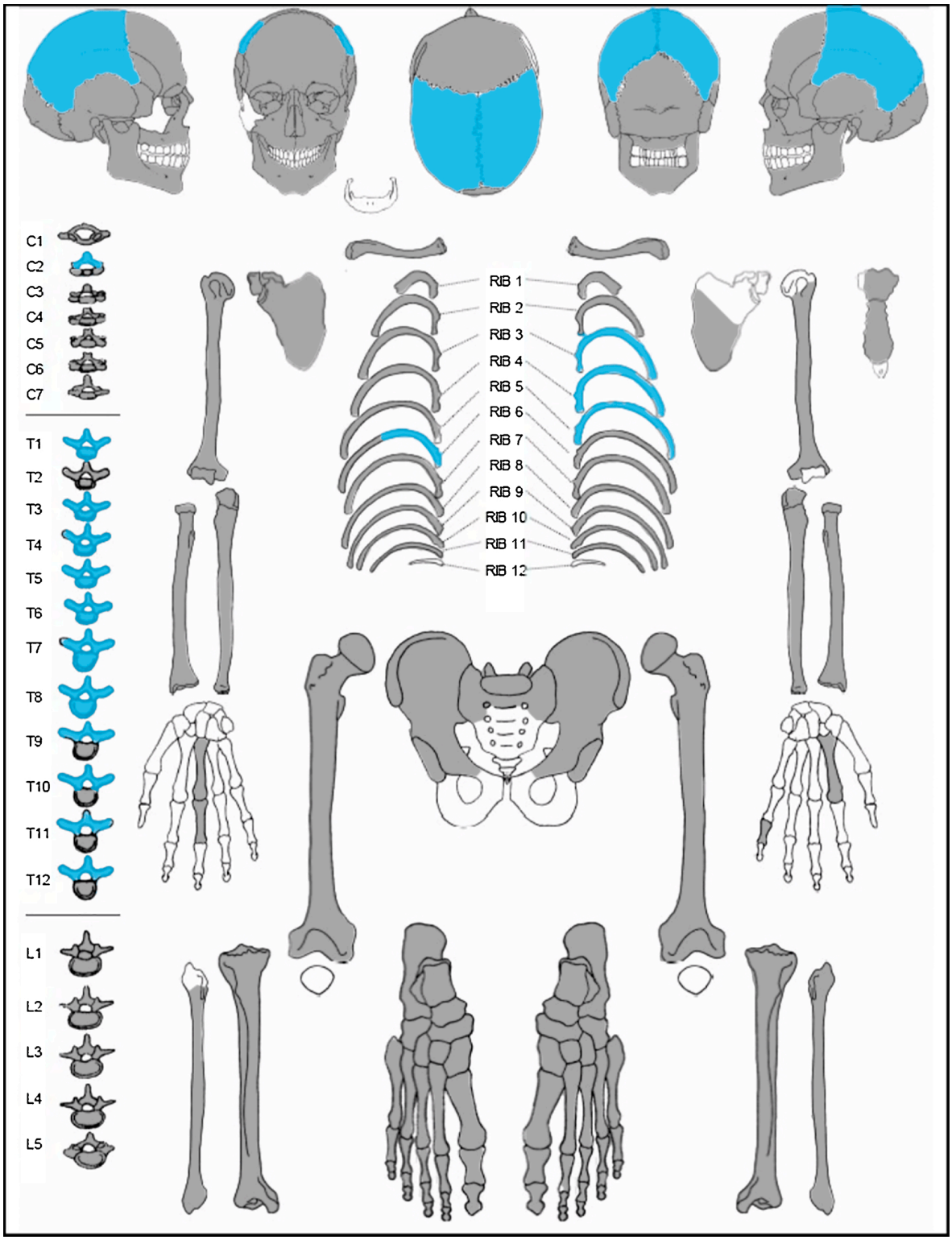

Fig. 1. Preservation of CM 897 and distribution of the pathological changes related bladder metastatic carcinoma. Affected areas are colored in blue and observable bones in grey (Image: INTERPOL DVI Form – Unidentified Human Remains). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

from 56 to 86 years and a mean age of 71 years (± 15 years). The dates of birth range from 1906 to 1940 and the dates of death from 1991 to 1997. All of the information available from the antemortem medical data is reported in Table 1.

Each of the skeletons was cleaned and put in anatomical position. A macroscopic anthropological analysis was then performed, including biological, pathological and traumatic analyses.

Metastatic bone lesions were identified by comparison with the

Fig. 2. *Periosteal reactions on the surface of ribs (CM 897).* a: excrescent-coarse aspect, thin layer of new bone; b: excrescent-coarse aspect with a thick layer of new bone; **c:** coral-like aspect; d: superior view showing the spicules of bones on the rib internal surface.

literature (Aufderheide and Rodríguez-Martín, 1998, pp. 388–390; Bloom et al., 1987; Marks and Hamilton, 2007; Marques et al., 2013; Ortner, 2003, pp. 532–537; Ragsdale et al., 2018; Rothschild et al., 1998; Strouhal, 1991; Waldron, 2008, p. 189). The descriptive macroscopic terminology for the lesions used in this paper is as follows.

- **Perforations of the bone cortex** are osteolytic foci destroying trabecular bone and to a lesser extent, its cortical envelope. They are typically round or oval osteolytic lesions with denticulated or scalloped margins. These geographic lesions are well-defined and the surrounding bone may present porosity. (Marks and Hamilton, 2007, Fig. 2; Marques et al., 2013, Fig. 2)
- **Coalescent porosity** is an osteolytic activity on the bone surface creating a coalescence and confluence of superficial pits. (Marques et al., 2013, Fig. 3)
- **Trabecular thickening or spongiosclerosis** (Ortner, 2003, p. 52) consists in a sub-cortical osteoblastic activity taking place within the space of the trabecular bone structure as “bone-in-bone”. The spongy bone visually appears abnormally thickened (Mays et al., 1996, Fig. 6; Ortner, 2003, p. 52; Tkocz and Bierring, 1984, Fig. 7).
- **Periosteal reactions** are deposition of new bone on the surface of the cortex. They may be described in several sub-types (active periosteal reaction, recognizable as thin layers of woven bone, were not observed in this study):
- *Excrescent-coarse:* solid thin to thick layer of fiber bone, rough to the touch (Anderson et al., 1992, Figs. 2, 3 and 4).
- *Coral-like:* thick new bone deposition with parallel spicules of fiber bone, resembling coral. Different from the divergent spiculated new bone deposition or “sunburst” reaction, not observed in this study (Santos and Roberts, 2006, Fig. 8).
- **Mixed lesions** are lesions with both osteolytic and osteoblastic changes observed. For instance, a lesion with cortex perforation (osteolytic component) exposing trabecular thickening or spongiosclerosis (osteoblastic component) would be considered mixed (Bloom et al., 1987, Fig. 1).

A careful examination was performed on all bones of each skeleton to find potential metastases. Dimensions of osteolytic lesions were taken with a Vernier caliper. Spongiosclerotic lesions were examined under stereomicroscope to assess trabecular thickening. Each bone lesion was then photographed for records and counted.

3. Results

Macroscopic bone lesions suggestive of metastatic carcinoma were recorded in three of the 13 individuals, representing 23% of the study sample. As this study focuses on bone metastases, only the skeletons in which bone metastases were found will be detailed here, one by one.

3.1. CM 897

The skeleton was very well-preserved and nearly complete (Fig. 1). Most hand bones and the 12th ribs were absent. The sacrum, right scapula and right humerus were fragmented, with the sacrum limited to its superior half, the scapula consisting of the medial and inferior borders, and the right humeral head being absent.

Our analysis recorded 22 bone lesions, including one osteolytic, four osteoblastic and 17 mixed (Table 2). The lesions were located on the parietal bones of the cranium (one lesion), the bodies and vertebral ends of unidentified ribs (nine lesions), and the neural arches and

Fig. 3. *Mixed metastasis on the 5th thoracic vertebra (CM 897).* Osteolytic lesion on the neural arch exposing the osteoblastic activity in the trabecular bone (left view).

lateral sides of the bodies of C2, T1 and T3 to T12 vertebrae (12 lesions) (Fig. 1). The single osteolytic lesion involved coalescent porosity found on the vertebral arch of the second cervical vertebra, whereas the osteoblastic lesions involved periosteal bone apposition located on the bodies of the ribs (Fig. 2). One mixed lesion consisted of an osteolytic lesion that exposed osteoblastic activity in the underlying trabecular bone (Fig. 3) located on the neural arch of T5. The remaining lesions on the cranium, vertebral ends of ribs and vertebrae manifested as coalescent porosity associated with reactive new bone. The diameter of the osteolytic and mixed lesions ranged from 1.39 to 15.51 mm.

3.2. CM 422

Overall, the skeletal remains were fragmented but almost complete (Fig. 4). All the vertebrae, apart from C5, were present. The cranium was fragmented in 23 pieces but all bones were observable except for the right maxillary. Both coxal bones and the posterior elements of the sacrum were present. Some bones of the hands and right foot were absent. The scapulae were fragmented. Both femoral heads and proximal ends of fibulae as well as the right humeral head were absent.

A total of 52 metastases were found in this skeleton. Six were osteolytic, perforating the bone cortex, 28 were spongiosclerotic and 18 were mixed lesions, of which 17 were perforating lesions exposing an abnormally thickened trabecular bone (spongiosclerosis). One consisted of coalescent porosity with reactive new bone on the left sphenoid (Table 2). Seven were found on the sphenoid and right parietal, two in the mandible (Fig. 5), one on the sternal body (Fig. 6a). Three lesions were located on each humerus (Fig. 6b), concentrated on the proximal epiphyses, four on vertebral arches (T2, T5 and L1) (Figs. 6c and 7), four on the ilia near the acetabulum and iliac crests, one on the superior part of the sacrum and one on the proximal end of each femur. The osteolytic metastases were found on the right sphenoid (three metastases) and proximal left humerus (three metastases), whereas the osteoblastic lesions were reported on the right sphenoid (one metastasis) and proximal femora (both metastases). The osteolytic and mixed lesions presented a diameter ranging from 2.58 mm to 12.78 mm. In addition, 25 lesions with spongiosclerosis were observed on unidentified broken fragments constituted of cortical and trabecular bone. In this specific situation, the fragmented state of the observable elements altered the understanding of the nature of the lesions. Indeed, these lesions may be the result of mixed lesions, with the osteolytic component (for instance, a cortex perforation) lost due to taphonomic processes and post-mortem breakage. As a strict osteoblastic nature of the lesion could not be confirmed, these 25 lesions were considered "dubious" (Table 2).

3.3. CM 490

This skeleton was fragmented and almost complete (Fig. 8). The cranium was broken in 29 pieces but only the occipital bone was missing from the analysis. The left radius, ulnae, right humerus, right scapula and fibulae were present but fragmented. Only fragments of the left ribs were present, whereas most of the right ribs were complete. The sacrum was represented by its superior half. A few bones of the feet and most bones of the hands were absent. The lower thoracic and lumbar spine was complete, but only a few more superior vertebrae were present. Both os coxae were present but the pubic bones were absent.

A total of three bone lesions were reported in this skeleton, all spongiosclerotic (Table 2) and all located on the vertebral column. They were located on the body of T8 (Fig. 9a), a fragment of an unidentified thoracic vertebral body (Fig. 9b), and the vertebral body of L1.

4. Discussion

The aim of this paper was to describe and discuss metastatic bone lesions from known bladder carcinoma. Three out of 13 skeletons clinically diagnosed with bladder cancer presented bone lesions evocative of metastatic carcinoma, corresponding to 23% of the study sample. This result is consistent with the 15–40% frequency at autopsy of bone metastases found in the literature (Coleman, 2001; Roodman, 2004).

Bone metastases were most commonly found in the vertebrae (24.7% of all bone metastases found), skull (12.9%), ribs (11.7%), proximal humeri (7.8%), pelvis (5.2%), proximal femora (2.6%), sacrum (1.3%) and sternum (1.3%) respectively (Table 3). In the vertebrae (24.7% of lesions), lesions were located on the vertebral arches and the bodies. Bone lesions in the thoracic region (13% of lesions) were found on the vertebral ends of ribs, the surface of vertebral bodies, and the sternum. Bladder carcinoma affected both the cranium (parietals and sphenoid) and the mandibular rami (12.9% of lesions). In the pelvic girdle (6.5% of lesions), lesions were noted near the iliac crest and the acetabulum, as well as on the superior aspect of the sacrum. These findings are compatible with published data on other carcinomas (Lipton and Vigorita, 2016; Mundy, 1997; Randall, 2015; Waldron, 2008, pp. 186–188) and demonstrate a preference of bone metastatic lesions for areas containing hematopoietic marrow.

All three types of bone metastatic lesions were observed: osteolytic, osteoblastic and mixed. Osteolytic bone lesions, found in CM 897 and CM 422, perforated the bone cortex (Fig. 6b) or expressed as coalescent porosity. Osteoblastic bone lesions manifested as spongiosclerosis (Fig. 6a and 9), an abnormal thickening of the trabecular bone, or as periosteal reactions (Fig. 2). Mixed bone lesions appeared as either coalescent porosity associated with reactive new bone or osteolytic lesions perforating the cortical bone and exposing a thickened trabecular bone (spongiosclerosis) (Figs. 3, 5, 6c and 7). Osteolytic lesions from either mixed or osteolytic metastases had irregular denticulated

Table 2
Types of metastases per individual affected.

Case n°	Osteolytic	Osteoblastic	Mixed	Dubious*	Total
CM 897	1	4	17		**22**
CM 422	6	3	18	**25**	**52**
CM 490		3			**3**
Total	**7**	**10**	**35**		**77**

* The "dubious" lesions refer to the 25 spongiosclerotic lesions found in bone fragments of unidentifiable origin of CM 422. Because of their fragmented state, the osteoblastic nature of the lesions could not be confirmed (they may be mixed lesion with the osteolytic component of the lesion destroyed by taphonomic alterations) and the lesions were therefore considered as "dubious".

(45% of lesions), 10 were proliferative (13% of lesions) and the last seven were osteolytic (9% of lesions). Rosenthal (1997) reports an elevated frequency of osteoblastic metastases in bladder carcinoma. Our study suggests that bladder carcinoma tends to produce mixed lesions, although both proliferative and osteolytic lesions also occurred. However, patterns vary by location. Mixed lesions were located on the vertebral arches or on the lateral sides of the bodies (Figs. 3, 6c and 7), whereas osteoblastic lesions were always observed within the spongy bone of the bodies of the vertebrae (Fig. 9). Similarly, mixed rib lesions were found near the vertebral ends and osteoblastic lesions on the internal surface of the bodies (extending to a lesser degree to the external surface) (Fig. 2). While femoral bone lesions were osteoblastic, those of the pelvic girdle were exclusively mixed (Table 3). In the vertebral column, thorax and the skull, mixed lesions were more common than proliferative or osteolytic lesions (Table 3). Of course, it is difficult to draw interpretative results from only three cases, but given that we know that these individuals were clinically diagnosed during life with bladder carcinoma, it is possible to say that bone metastases can indeed be found in bladder cancer, most commonly mixed, with osteoblastic and osteolytic metastases also possible.

Nonetheless, 10 individuals did not present any macroscopic pathological signs of metastatic bone disease. Observations of bone metastases may be influenced by several factors. Firstly, the state of preservation of the remains, both in completeness and conservation, influences the observation of bone metastases. Overall, we do not believe that taphonomy was a factor in our study because the preservation of the skeletal remains was similar across the series. The skeletons were almost complete, with a few bones absent (in particular in the hands and feet) and/or broken (Figs. 1, 4 and 8). All key areas where metastases were observed (namely, skull, vertebrae, ribs, proximal humeri and femora, pelvis and sacrum) could be observed in all 13 cases. Secondly, metastatic bone disease occurs during an advanced stage of the condition, with a peak onset over 40 years of age (Miller, 2008). In addition, the average age-at-diagnosis of bladder carcinoma has been estimated at 73 years in the United States (American Cancer Society, 2018; National Cancer Institute, 2017). Therefore, death at a relatively young age could bias the pathological analysis, because the individual could have had the disease, but the cancer would not have progressed sufficiently for bone metastases to have developed. In this study, the skeletal remains in which bone metastases were reported died at ages 56, 59 and 82 years. Two were far younger than the average published age-at-diagnosis of bladder cancer, whereas the unaffected individuals ranged from 58 to 86 years old. However, it is possible that some of these individuals were recently diagnosed and died before the cancer spread to the skeleton. Alternatively, bone metastases may have been present but localized on spongy bone, unobservable macroscopically. Ultimately, considering published incidence-at-autopsy for this carcinoma (Coleman, 2001; Roodman, 2004), we did not expect to find bone metastases in more than 2–5 skeletons of our study sample (15–40% incidence-at-autopsy of bone metastases in bladder carcinoma x 13 individuals in our study = expected 2–5 individuals with bone metastases), which is consistent with our result.

Radiographs have proven effective in finding bone metastases (Rothschild and Rothschild, 1995) but x-rays were not used in the present study. Indeed, skeletal remains from archaeological contexts are not usually systematically x-rayed. In this study, the absence of radiographic imaging implies that our results represent a minimum number of metastases. While 22 bone lesions were found in the first case (CM 897), 52 lesions were observed in the second (CM 422) and only three were noted in the third (CM 490). Although definite conclusions are dangerous to draw, especially given the limit of the macroscopic method for the identification of bone metastases, it may be inferred that the first two cases were more advanced in their metastatic progression than the third. The point of the present research was not to assess the incidence of bone metastases for bladder carcinoma but to provide additional information and documentation to help forensic anthropologists, paleopathologists and anthropologists recognize bone metastases in the dry bone analysis of skeletal remains and potentially consider the diagnosis of a bladder origin of the cancer.

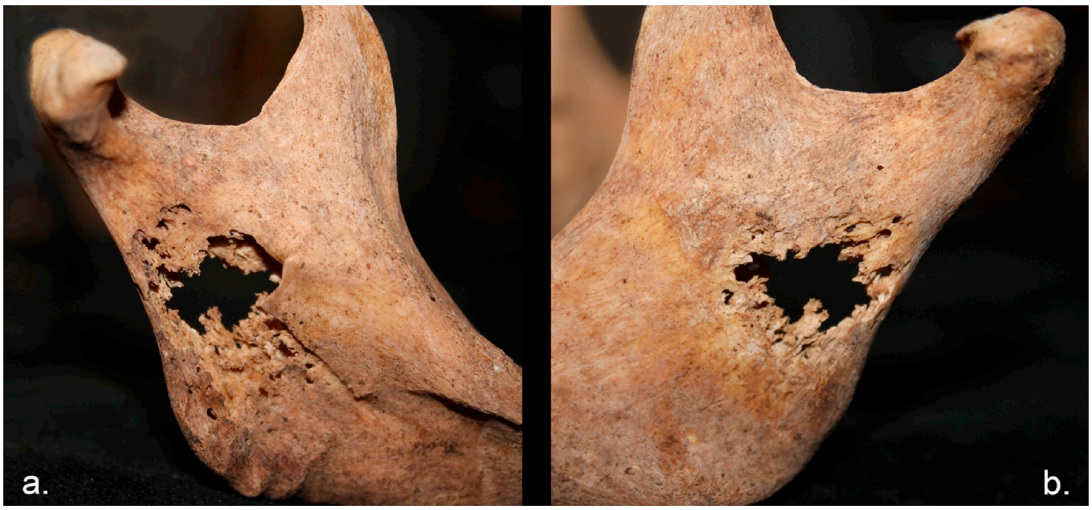

Fig. 5. *Mixed metastasis on the left ramus of the mandible (CM 422).* a: medial view; b: lateral view. Note the large oval osteolytic lesion with denticulated margins and pitted surrounding bone. The trabecular bone is affected to a greater extent by the osteolytic lesion than the cortical bone, implying an outward progression of the lesion. The metastasis is mixed but the proliferative activity is not observable on the picture.

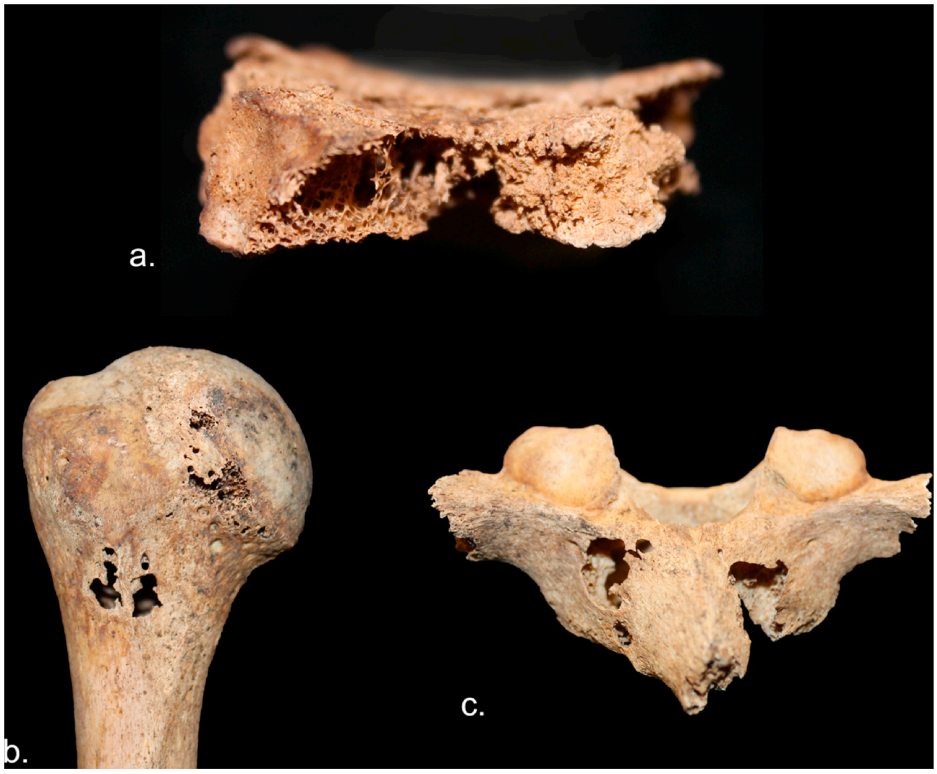

Fig. 6. *Detailed lesions of CM 422.* a: superior view of the sternal body, anterior is down, note the thickened trabecular bone on the right (osteoblastic metastasis - spongiosclerosis) compared to normal trabecular bone on the left; b: anterior view of the proximal left humerus showing an osteolytic metastasis; c: posterior view of an unidentified high thoracic vertebra with osteolytic lesions on both sides of the spinous process (mixed metastases, osteoblastic activity not observable on the picture).

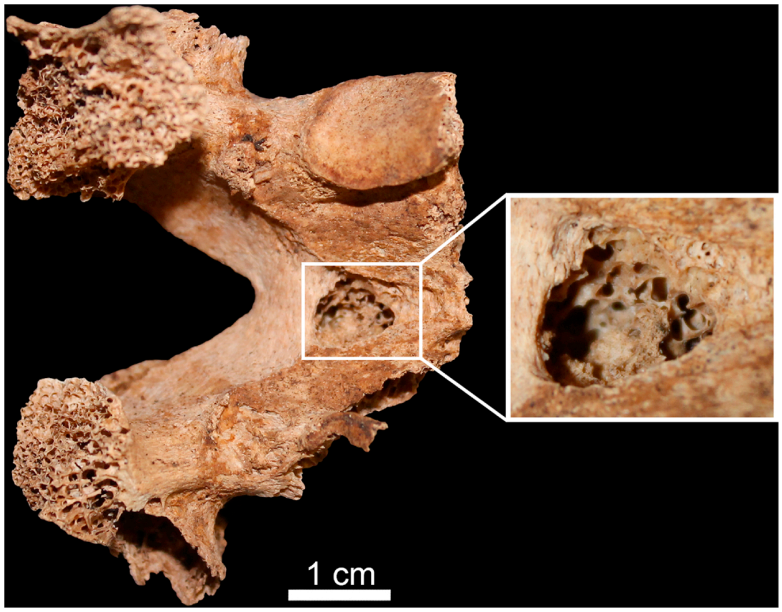

Fig. 7. *Lumbar vertebra with a mixed metastasis (CM 422).* Inferior view of an unidentified lumbar vertebra with an osteolytic lesion and osteoblastic activity (zoomed area).

5. Conclusion

The low incidence-at-autopsy and the paucity of documentation on bladder cancer metastases are responsible for the extremely rare consideration of this carcinoma in the diagnosis of metastatic cancer in skeletonized remains from archaeological or forensic contexts. In this paper, we performed a study on 13 cases clinically diagnosed with bladder carcinoma during life. As a result, three individuals showed bone lesions, representing 23% of the study sample. All three types of bone metastatic lesions were observed, including osteolytic, osteoblastic and mixed lesions. Osteolytic lesions were coalescing superficial pits or irregular round to oval lesions perforating the bone cortex and ranging from 1.39 to 15.51 mm in diameter. Proliferative lesions manifested as spongiosclerosis or periosteal new bone, either ex-crescent-coarse or coral-like. Mixed metastases were osteolytic lesions exposing a thickened trabecular bone (spongiosclerosis) or coalescent porosity with reactive new bone.

In the present study, bladder carcinoma metastases were in large

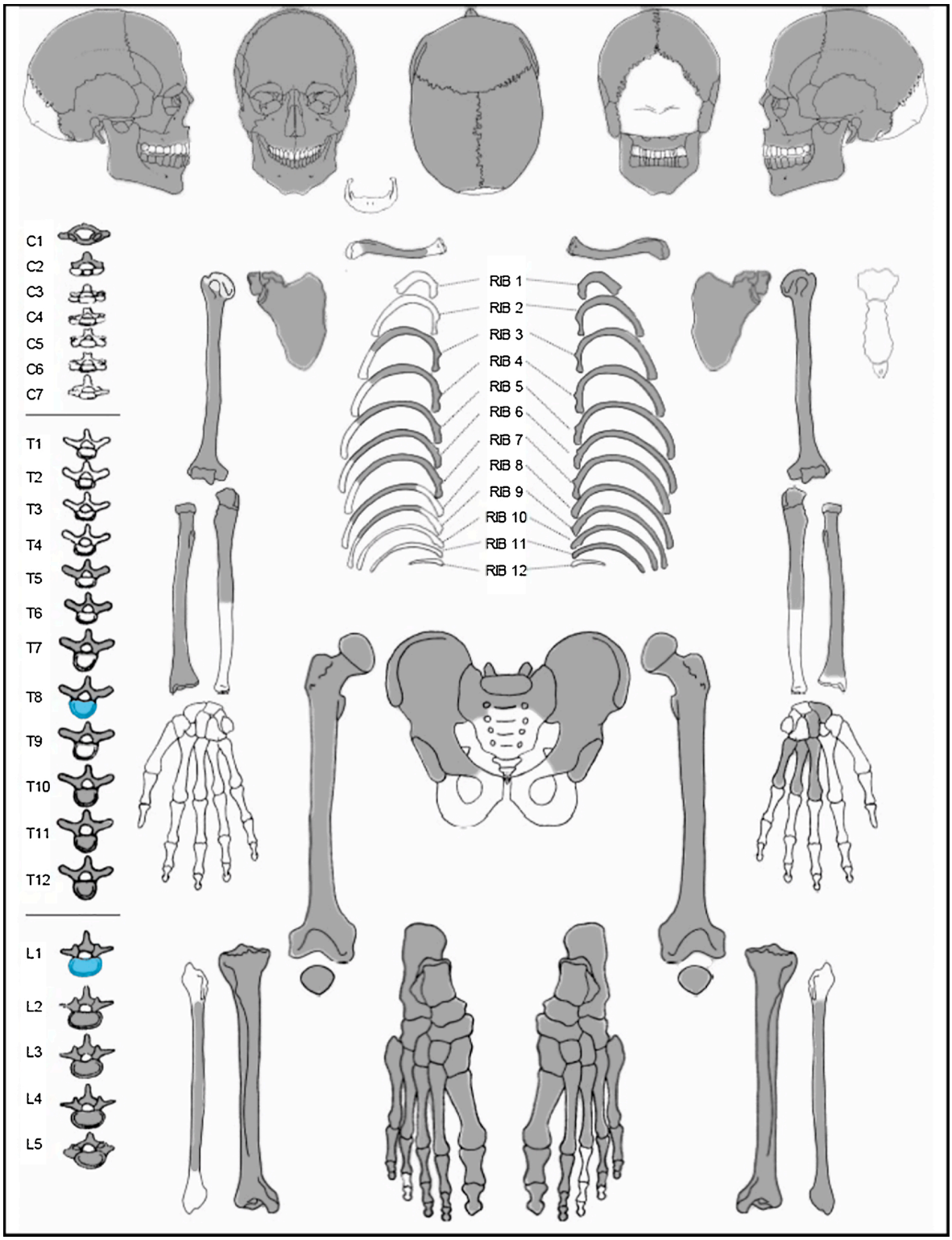

Fig. 8. *Preservation of CM 490 and distribution of the pathological changes related bladder metastatic carcinoma.* Affected areas are colored in blue and observable bones in grey (Image: INTERPOL DVI Form – Unidentified Human Remains). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

majority mixed (45% of lesions), although osteoblastic (13% of lesions) and osteolytic lesions (9% of lesions) also occurred. They were more commonly found in the vertebrae, skull, ribs, pelvic girdle, as well as proximal humeri and femora, favoring sites of high vascularization. However, bone metastases seemed to affect different areas of the same bones depending on their nature. Indeed, mixed lesions were found on or close to the vertebral arches, whereas osteoblastic lesions were systematically seen in the bodies of the vertebrae. In the ribs, mixed lesions

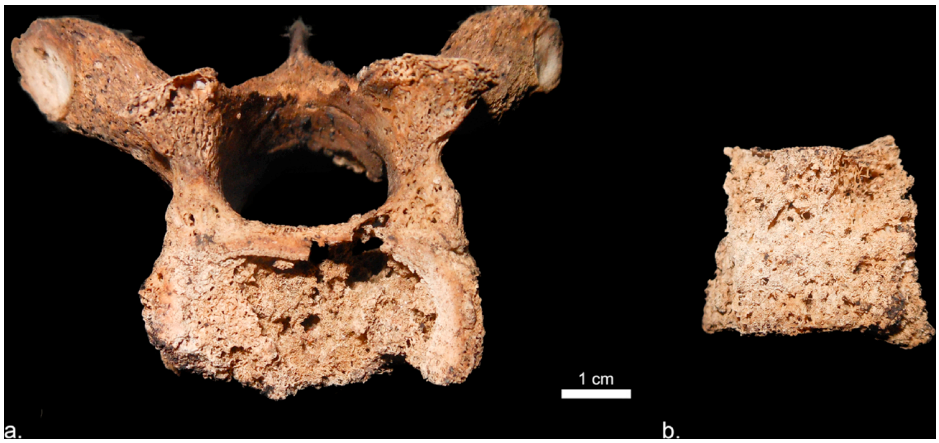

Fig. 9. *Detailed lesions of CM 490*. a: superior view of T8 (anterior is down) showing a proliferative metastasis on its body, note the new bone thickening the existing trabecular bone (spongiosclerosis); b: anterior view of a vertebral body with a spongiosclerotic lesion.

Table 3
Sites of bone metastases.

Location	Osteolytic	Osteoblastic	Mixed	Dubious	Total	Total %
Skull	3	1	6		**10**	**12.9%**
Humeri	3		3		**6**	**7.8%**
Sternum			1		**1**	**1.3%**
Ribs		4	5		**9**	**11.7%**
Vertebrae	1	3	15		**19**	**24.7%**
Pelvis			4		**4**	**5.2%**
Sacrum			1		**1**	**1.3%**
Femora		2			**2**	**2.6%**
Unidentified				**25**	**25**	**32.5%**

were located near the vertebral ends, while osteoblastic lesions were spread on the internal surface of the bodies.

This study is the first to document known bladder metastatic bone disease from an identified skeletal collection and may help recognize and identify the condition on dry bones.

Conflict of interest

The authors declare that they have no conflict of interest.

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References

American Cancer Society, 2018. Cancer Facts and Figures 2018. Atlanta. .
Anderson, T., Wakely, J., Carter, A., 1992. Medieval example of metastatic carcinoma: a dry bone, radiological, and SEM study. Am. J. Phys. Anthropol. 89, 309–323. https://doi.org/10.1002/ajpa.1330890305.
Aufderheide, A.C., Rodríguez-Martín, C., 1998. The Cambridge Encyclopedia of Human Paleopathology. Cambridge University Press, Cambridge, United Kingdom.
Bloom, R.A., Libson, E., Husband, J.E., Stoker, D.J., 1987. The periosteal sunburst reaction to bone metastases. A literature review and report of 20 additional cases. Skelet. Radiol. 16, 629–634.
Castoldi, E., Cappella, A., Gibelli, D., Sforza, C., Cattaneo, C., 2017. The difficult task of diagnosing prostate Cancer metastases on dry bone. J. Forensic Sci. https://doi.org/10.1111/1556-4029.13617.
Cattaneo, C., Mazzarelli, D., Cappella, A., Castoldi, E., Mattia, M., Poppa, P., De Angelis, D., Vitello, A., Biehler-Gomez, L., 2018. A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection. Forensic Sci. Int. 287, 219. https://doi.org/10.1016/j.forsciint.2018.03.041. e1-219.e5.
Coleman, R.E., 1997. Skeletal complications of malignancy. Cancer 80, 1588–1594. https://doi.org/10.1002/(SICI)1097-0142(19971015)80:8+<1588::AID-CNCR9>3.0.CO;2-G.
Coleman, R.E., 2001. Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat. Rev. 27, 165–176. https://doi.org/10.1053/ctrv.2000.0210.
Coleman, R.E., 2006. Clinical features of metastatic bone disease and risk of skeletal morbidity. Clin. Cancer Res. 12, 6243.
Gruber, R., 2012. Reader's digest of the pathophysiology of bone metastases. Wiener Med. Wochenschr. 162, 370–373. https://doi.org/10.1007/s10354-012-0110-3.
Guise, T.A., 2002. The vicious cycle of bone metastases. J. Musculoskelet. Neuronal Interact. 2, 570–572.
Guise, T.A., Yin, J.J., Taylor, S.D., Kumagai, Y., Dallas, M., Boyce, B.F., Yoneda, T., Mundy, G.R., 1996. Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis. J. Clin. Invest. 98, 1544–1549. https://doi.org/10.1172/JCI118947.
Guise, T.A., Yin, J.J., Mohammad, K.S., 2003. Role of endothelin-1 in osteoblastic bone metastases. Cancer 97, 779–784. https://doi.org/10.1002/cncr.11129.
Guise, T.A., Kozlow, W.M., Heras-Herzig, A., Padalecki, S., Yin, J.J., Chirgwin, J.M., 2005. Molecular mechanisms of breast Cancer metastases to bone. Clin. Breast Cancer 5, S46–S53. https://doi.org/10.3816/CBC.2005.s.004.
Guise, T.A., Mohammad, K.S., Clines, G., Stebbins, E.G., Wong, D.H., Higgins, L.S., Vessella, R.L., Corey, E., Padalecki, S., Suva, L., Chirgwin, J.M., 2006. Basic mechanisms responsible for osteolytic and osteoblastic bone metastases. Clin. Cancer Res. 12, 6213–6216.
Harvey, H.A., von Reyn Cream, L., 2007. Biology of bone metastases: causes and consequences. Clin. Breast Cancer 7, S7–S13. https://doi.org/10.3816/CBC.2007.s.001.
Lipton, J.F., Vigorita, V.J., 2016. Metastatic bone disease. In: Vigorita, V.J., Ghelman, B., Mintz, D. (Eds.), Orthopaedic Pathology. Lippincott Williams & Wilkin, Philadelphia, pp. 594–619.
Marks, M.K., Hamilton, M.D., 2007. Metastatic carcinoma: palaeopathology and differential diagnosis. Int. J. Osteoarchaeol. 17, 217–234. https://doi.org/10.1002/oa.874.
Marques, C., Santos, A.L., Cunha, E., 2013. Better a broader diagnosis than a misdiagnosis: the study of a neoplastic condition in a male individual who died in early 20th century (Coimbra, Portugal). Int. J. Osteoarchaeol. 23, 664–675. https://doi.org/10.1002/oa.1294.
Marques, C., Matos, V., Costa, T., Zink, A., Cunha, E., 2018. Absence of evidence or evidence of absence? A discussion on paleoepidemiology of neoplasms with contributions from two Portuguese human skeletal reference collections (19th–20th century). Int. J. Paleopathol. 21, 83–95. https://doi.org/10.1016/j.ijpp.2017.03.005.
Mays, S., Strouhal, E., Vyhnánek, L., Němečková, A., 1996. A case of metastatic carcinoma of medieval date from Wharram Percy, England. J. Paleopathol. 8, 33–42.
Miler, S.F., Thomas, C.Y., Shiozawa, Y., 2017. Molecular involvement of the bone marrow microenvironment in bone metastasis. Introduction to Cancer Metastasis. Academic Press, pp. 263–276. https://doi.org/10.1016/B978-0-12-804003-4.00014-1.
Miller, T.T., 2008. Bone tumors and tumorlike conditions: analysis with conventional radiography. Radiology 246, 662–674. https://doi.org/10.1148/radiol.2463061038.
Mundy, G.R., 1997. Mechanisms of bone metastasis. Cancer 80, 1546–1556. https://doi.org/10.1002/(SICI)1097-0142(19971015)80:8+<1546::AID-CNCR4>3.0.CO;2-I.

Mundy, G.R., 2002. Metastasis: metastasis to bone: causes, consequences and therapeutic opportunities. Nat. Rev. Cancer 2, 584–593.
National Cancer Institute, 2017. SEER Cancer Stat Facts: Bladder Cancer [WWW Document]. (Accessed 7.20.18). https://seer.cancer.gov/statfacts/html/urinb.html.
Ortner, D.J., 2003. Identification of Pathological Conditions in Human Skeletal Remains, 3rd ed. Smithsonian Institution Press, New York.
Paget, S., 1889. The distribution of secondary growths in cancer of the breast. Lancet 133, 571–573. https://doi.org/10.1016/S0140-6736(00)49915-0.
Ragsdale, B.D., Campbell, R.A., Kirkpatrick, C.L., 2018. Neoplasm or not? General principles of morphologic analysis of dry bone specimens. Int. J. Paleopathol. 21, 27–40. https://doi.org/10.1016/j.ijpp.2017.02.002.
Randall, R.L., 2015. Metastatic Bone Disease: an Integrated Approach to Patient Care. Springer.
Roodman, G.D., 2004. Mechanisms of bone metastasis. N. Engl. J. Med. 350, 1655–1664. https://doi.org/10.1056/NEJMra030831.
Rosenthal, D.I., 1997. Radiologic diagnosis of bone metastases. Cancer 80, 1595–1607. https://doi.org/10.1002/(SICI)1097-0142(19971015)80:8+<1595::AID-CNCR10>3.0.CO;2-V.
Rothschild, B.M., Rothschild, C., 1995. Comparison of radiologic and gross examination for detection of cancer in defleshed skeletons. Am. J. Phys. Anthropol. 96, 357–363. https://doi.org/10.1002/ajpa.1330960404.
Rothschild, B.M., Hershkovitz, I., Dutour, O., Latimer, B., Rothschild, C., Jellema, L.M., 1997. Recognition of leukemia in skeletal remains: report and comparison of two cases. Am. J. Phys. Anthropol. 102, 481–496. https://doi.org/10.1002/(SICI)1096-8644(199704)102:4<481::AID-AJPA5>3.0.CO;2-V.
Rothschild, B.M., Hershkovitz, I., Dutour, O., 1998. Clues potentially distinguishing lytic lesions of multiple myeloma from those of metastatic carcinoma. Am. J. Phys. Anthropol. 105, 241–250. https://doi.org/10.1002/(SICI)1096-8644(199802)105:2<241::AID-AJPA10>3.0.CO;2-0.
Santos, A.L., Roberts, C.A., 2006. Anatomy of a serial killer: differential diagnosis of tuberculosis based on rib lesions of adult individuals from the Coimbra identified skeletal collection, Portugal. Am. J. Phys. Anthropol. 130, 38–49. https://doi.org/10.1002/ajpa.20160.
Smith, M.O., 2002. A probable case of metastatic carcinoma from the late prehistoric eastern Tennessee River Valley. Int. J. Osteoarchaeol. 12, 235–247. https://doi.org/10.1002/oa.618.
Steeg, P.S., 2006. Tumor metastasis: mechanistic insights and clinical challenges. Nat. Med. 12, 895. https://doi.org/10.1038/nm1469.
Strouhal, E., 1991. Myeloma multiplex versus osteolytic metastatic carcinoma: differential diagnosis in dry bones. Int. J. Osteoarchaeol. 1, 219–224. https://doi.org/10.1002/oa.1390010314.
Talmadge, J.E., Fidler, I.J., 2010. AACR centennial series: the biology of Cancer metastasis: historical perspective. Cancer Res. 70, 5649–5669. https://doi.org/10.1158/0008-5472.can-10-1040.
Tkocz, I., Bierring, F., 1984. A medieval case of metastasizing carcinoma with multiple osteosclerotic bone lesions. Am. J. Phys. Anthr. 65, 373–380. https://doi.org/10.1002/ajpa.1330650406.
Vassiliou, V., Chow, E., Kardamakis, D., 2013. Bone metastases: a translational and clinical approach. Springer Science & Business Media.
Vilar, J., Lezana, A.H., Pedrosa, C.S., 1979. Spiculated periosteal reaction in metastatic lesions of bone. Skelet. Radiol. 3, 230–233. https://doi.org/10.1007/BF00360941.
Waldron, T., 2008. Palaeopathology. Cambridge University Press, Cambridge, United Kingdom.

5.2. Histological study of non-skeletal calcified markers of disease

The aim of this second research line was to provide additional information on specific calcified soft tissue material, that may easily be disregarded or misidentified mixed with the soil and dirt because they are not bone, to better recognize and identify the conditions in skeletal remains. These markers include atherosclerotic calcifications (atherosclerosis), gallstones (cholelithiasis), ossifying costal cartilage and pleural plaques.

Atherosclerotic calcifications

Eighteen atherosclerotic calcifications underwent histological analysis according to two protocols: undecalcified and decalcified; in addition, Scanning Electron Microscopy was performed on five atherosclerotic plaques. Each of the three analyses provided similar results: atherosclerotic calcifications are convex-concave plaques with a stratified structure, a pale-yellow coloration in autopsy cases and yellow to brown when recovered in dry bone. Histologically, undecalcified and decalcified sections showed a stratified aspect formed by superimposed layers, confirmed by the SEM analysis. The aim of this research was to document the morphological, histological, and SEM characteristics of atherosclerotic plaques to better recognize them among skeletal remains.

Gallstones

Two hundred seventy gallstones were macroscopically examined and 18 gallstones were subjected to histological analyses. Cross-sectional morphological analyses were also performed and nine gallstones underwent Scanning Electron Microscopy coupled with Energy Dispersive Spectrometry. As a result, gallstones vary in size, shape, color and texture. The cross-sectional surface correlates with chemical composition and is a valuable tool for classification into subcategories of stones, which can be confirmed by histological analysis. The elemental analysis yielded a higher frequency of carbon, calcium and phosphorus. The objective of this study was to provide additional information on the morphology and composition of gallstones based on the analysis of clinical samples "aged" by maceration to simulate a dry bone context.

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TECHNICAL NOTE

ANTHROPOLOGY

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Distinguishing Atherosclerotic Calcifications in Dry Bone: Implications for Forensic Identification

ABSTRACT: Atherosclerotic calcifications, as calcified atheromatous elements, are markers of cardiovascular disease. However, the literature gives little information regarding their morphological aspect, making their identification very rare in skeletonized cases. In this paper, we document the morphological, histological, and SEM aspects of atherosclerotic plaques collected from unclaimed cemeterial skeletal remains from an identified osteological collection and extracted from well-preserved cadavers autopsied at the medico-legal institute of Milan. Each of the three analyses provided similar results: atherosclerotic calcifications are convex-concave plaques with a stratified structure, a pale-yellow coloration in autopsy cases and yellow to brown when recovered in dry bone. Histologically, undecalcified and decalcified sections showed a stratified aspect formed by superimposed layers. Lastly, the SEM analysis showed a precise view of the stratified structure of the plaques in transverse section. As markers of disease, atherosclerotic calcifications can provide important antemortem information on the deceased that may be compared to antemortem data.

KEYWORDS: forensic science, forensic anthropology, forensic identification, calcified remains, atherosclerotic calcifications, vascular calcifications

Only some diseases leave direct signs on bone that can be interpreted by the anthropologist. Among them, there are some which induce the calcification over time of soft elements in the body. Consequently, these calcified materials become markers of disease. In both forensic anthropology and paleopathology, the recovery of these pathological markers is important because it can provide specific additional information on the deceased which may be compared to antemortem data; for instance, to narrow the search of a missing person in case of unknown deceased or even as factors of individualization of forensic cases. Although they are easy to identify in mummies and in fresh cadavers because of their preservation within soft tissue material and recovery *in situ*, their recognition in skeletonized cases, mixed with stones and soil and altered by taphonomy, becomes more challenging. Thus, it appears necessary in archeology and forensic sciences to know the typical features of these calcified materials, even microscopic.

Cardiovascular diseases, including atherosclerosis, are the first cause of death worldwide (1) and induce over time the calcification of soft tissue plaques in the arterial walls. Atherosclerotic calcifications are the result of a chronic and progressive disease in the intimal wall of any large- and medium-sized arteries (2). Consequent to a complex combination of genetic and environmental factors, low-density lipoproteins accumulate in lesions sites of the inner layer of the arterial layer which induces the migration of monocytes into the intima and their differentiation into macrophages. The foam cells constituted by the macrophages and lipoproteins then decay in a necrotic core. Consequently, smooth muscle cells migrate from the media layer of the artery to contain the lesion and form a fibrous plaque. With time, these fibrous plaques calcify and are able to survive decomposition and taphonomic processes (3–5). The exact mechanism regulating this ectopic and dystrophic calcification process remains unclear although similar to bone formation (6). It has been suggested that the calcification of arterial plaques could represent an attempt to preserve the integrity of the arterial wall by « strengthening weakened atherosclerotic plaques prone to rupture » (7,8).

In terms of epidemiology, there seems to be a protective effect of female hormones which explains why males tend to be more affected than females until 55-60 years of age when this ratio shift (9). Age is an important factor in the presence of calcified atherosclerotic plaques, although it is not a direct result of aging (8). Indeed, calcifications are present in 50% of individuals between 40 and 49 years and in 80% of individuals aged 60–69 according to Wexler et al. (7). Contrary to popular beliefs, atherosclerosis is not a modern disease and calcifications have even been found in the frozen body of Ötzi, the Iceman, dating about 3300 BC (10) and in Egyptian mummies (11). Vascular calcifications may be observed in antemortem radiographs and postmortem computed tomography (12,13).

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In this paper, we present the morphological, histological, and SEM aspects of known atherosclerotic plaques. The objective of this study is to provide additional and specific documentation on these insidious markers of disease so as to help forensic anthropologists and paleopathologists better recognize and identify them in the future.

Materials and Methods

This paper examines both autopsy and cemeterial (in other words, coming from dry known skeletal remains recovered from exhumations) atherosclerotic calcifications (Table 1). Atheromatous elements were extracted from sixty well-preserved cadavers from February 2014 to January 2017 during autopsies at the medico-legal institute in Milan (Italy) and "aged" by maceration to simulate a dry bone situation. The soft tissue extractions were put in ambient water in small containers incompletely closed. About 90% of the water was changed every week, until the calcified elements were completely separated from the remaining soft tissue. Depending on how much soft tissue was present, the maceration lasted several weeks to several months. In each soft tissue extraction, several atherosclerotic calcifications could be retrieved. Ultimately, the recovered calcified material was dried. These autopsy atherosclerotic plaques allowed the identification of cemeterial atherosclerotic calcifications found among the remains of 24 skeletons of the CAL Milano Cemetery Skeletal Collection buried for at least 10 years (14), as described in a previous study (15). The individuals autopsied and exhumed from the Milan cemeteries showing atherosclerotic calcifications were aged between 56 and 102 years old, with a mean age of 81 years old and a median age of 83 years old. Out of the 84 individuals concerned in this study (60 autopsied and 24 exhumed from cemeteries), 49 were male (58.3% of the study sample) and 35 were female (41.7% of the study sample). All of the individuals died of natural causes following cardiac or pulmonary complications, 12 individuals had a clinical diagnosis of vasculopathy, two individuals suffered from cancer (bladder and intestines malignant neoplasms) and single cases of hepatic cirrhosis, renal failure, pancreatitis, Parkinson's disease, and senile dementia were also present. The samples of both autopsy and cemeterial origins were subjected to the same types of analyses in order to confront their results and document the morphology of known atherosclerotic plaques.

In the morphological analysis, macroscopic criteria including shape, dimensions (measured with a vernier caliper) and color were recorded. Ten plaques were cut transversally with a scalpel and submitted to a horizontal grindstone (Struers DAP-7) and abrasive disks (Buehler micro cut disks, grains of 180, 320, 500, 1200, 2400, and 4000) used progressively for the observation of the cross-sectional surface.

Two protocols were used for histological analysis, undecalcified and decalcified. For the first protocol, ground sections of the samples (same process as in transverse section—horizontal grindstone Struers DAP-7 and abrasive disks) were processed in Pertex (Pertex, mounting medium for light microscopy. Histolab: Gotebourg, Sweden) and let to solidify for 72 hours in ambient temperature before observation with the optic microscope. For the second protocol, plaques were first fixed in formalin (v/v, PH 7-7.6, ratio 20:1 v/v) for 24 h, and decalcified at room temperature in Decalc, 14% hydrochloric acid (Histo-Line Laboratories, Milan). Subsequently, each section was rinsed in tap water for 24 h, dehydrated in alcoholic scale, and embedded into paraffin. Section of 5 microns was cut from each block stained with Hematoxylin and Eosin (H&E). Ten atherosclerotic calcifications underwent undecalcified histological analysis (three extracted from autopsies and seven from cemeterial skeletons) and eight were submitted to decalcified analysis (four extracted from autopsies and four from cemeterial skeletons), all coming from seven different individuals.

Lastly, Scanning Electron Microscopy (SEM) was performed with a Cambridge Stereoscan 360 with electron gun, vacuum pump, and image acquisition software (Oxford Link Pentafet, Oxford, UK) on four autopsy atherosclerotic calcifications from different individuals.

Results and Discussion

Fifty-one of the 60 atheromatous elements extracted during autopsies originated from the aorta (43 in the abdominal aorta, six in the thoracic aorta and two unspecified), five from the iliac arteries, two from the carotid arteries, one from the pulmonary artery, and the last one from the basilar

TABLE 1—*Distribution of the samples per type of analysis.*

			Histological Analysis		
Origin of the Material	No. of Individuals	Morphological Analysis	Undecalcified	Decalcified	Scanning Electron Microscopy
Autopsy cases	60	200	5	4	3
Cemeterial skeletons	24	293	3	4	2

TABLE 2—*Results per type of analysis.*

		Histological Analysis		
Origin of the Material	Morphological Analysis	Undecalcified	Decalcified	Scanning Electron Microscopy
Autopsy cases	Convex-concave plaques with a stratified structure and a pale-yellow coloration	Stratified layers of fibrous tissue	Stratified layers of fibrous tissue with "ghosts" of macrophages foam cells and lipid cholesterol cleft and in some cases, a calcification core	Multilayered structure
Cemeterial skeletons	Convex-concave plaques with a stratified structure and a yellow to brown coloration			

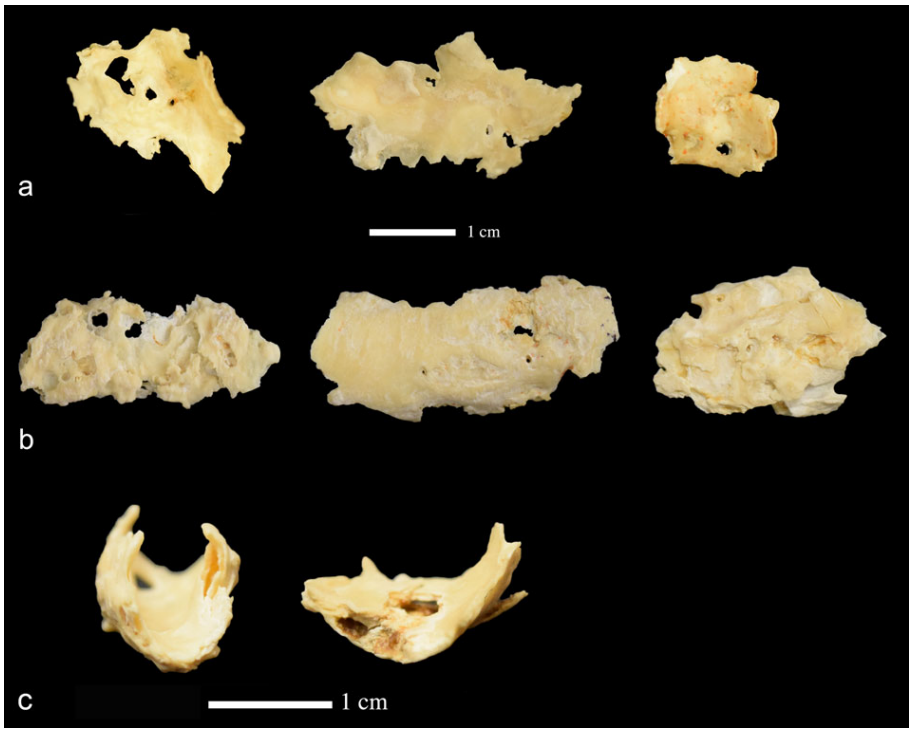

FIG. 1—*Morphology of autopsy atherosclerotic plaques. (a) luminal view, note the smooth internal surface for the lumen of the artery; (b) external view, note the superimposed layers of calcifications that give the stratified structure to the plaque; (c) transversal view, note the convex-concave shape of the plaques.*

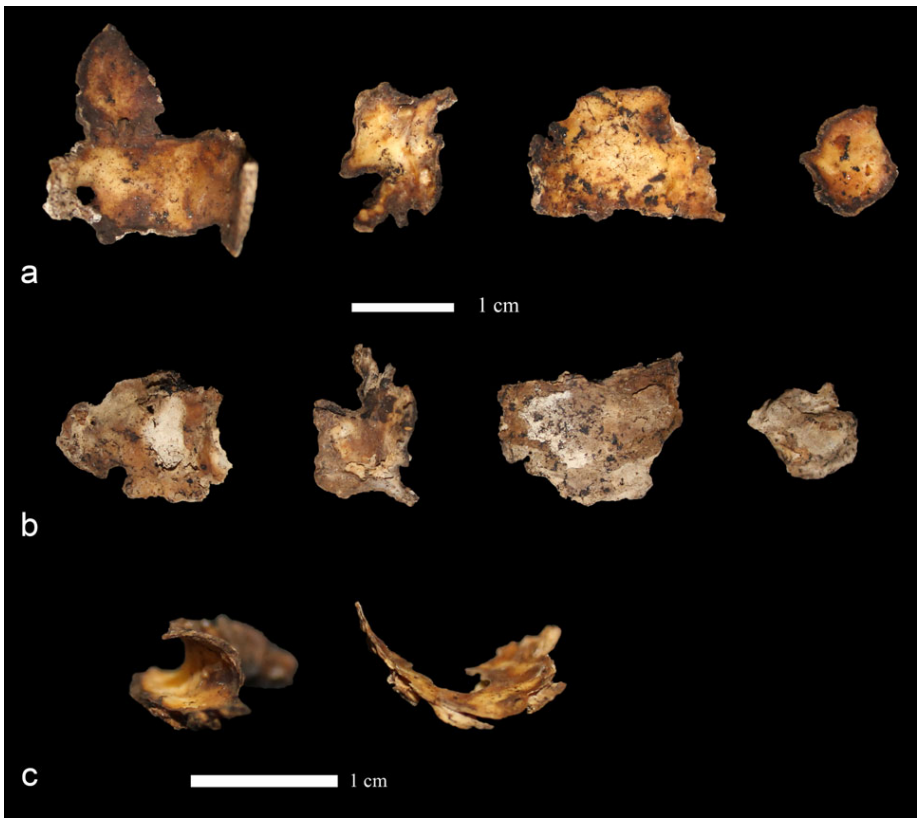

FIG. 2—*Morphology of cemeterial atherosclerotic plaques. (a) luminal view, note the smooth internal surface for the lumen of the artery in a bright yellow/orange color and the margins in dark brown; (b) external view, note the superimposed layers of calcifications that give the stratified structure to the plaque; c: transversal view, note the convex-concave shape of the plaques.*

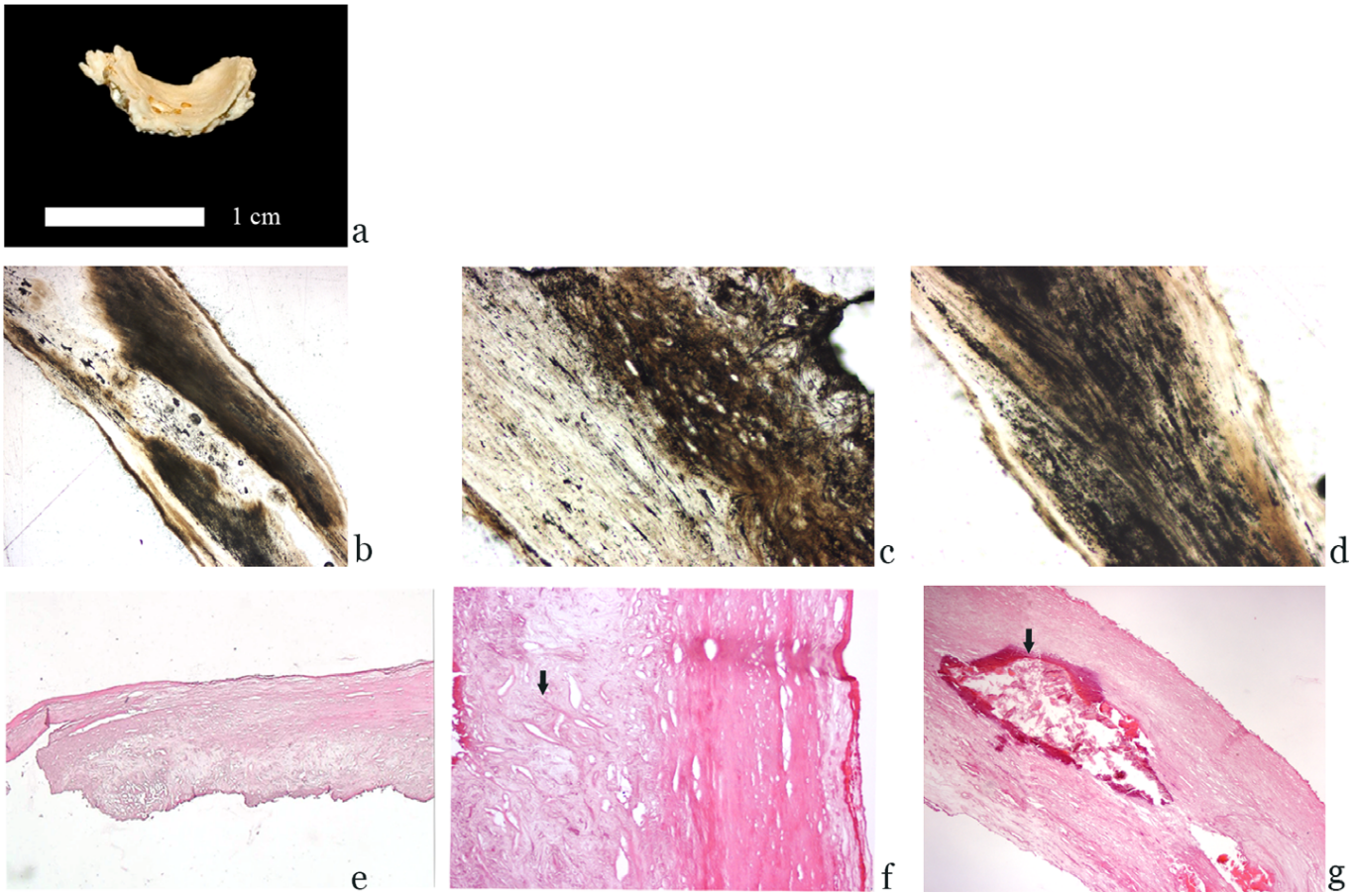

FIG. 3—*Histological comparison of decalcified and undecalcified protocols on an autopsy plaque. (a) macroscopic view; (b, c, and d) histological pictures of undecalcified sections illustrating the stratified structure of atherosclerotic plaques, original magnification x1(b), x4(c), x10(d); (d, e, and f) decalcified sections of the same autopsy sample, semi-thin sample stained in H&E with "ghosts" elements (f: black arrow) and calcification core (g: black arrow), original magnification x1(e), x4(f), x10(g).*

artery. The exact original location of the cemeterial atherosclerotic calcifications is unknown but some were found *in situ* (15), attached to dried soft tissue remains on the left side of thoracic or lumbar vertebrae, suggesting the abdominal aorta.

Each of the three analyses of plaques provided similar results (Table 2), regardless of their original location site in the body. In terms of morphology, autopsy atherosclerotic calcifications are pale yellow convex-concave plaques constituted of superimposed layers (Fig. 1). Cemeterial calcifications present the same characteristics, except for a change in coloration ranging from yellow to brown attributed to taphonomic processes (Fig. 2). In both cases, they exhibit a smooth texture on the luminal surface which may be attributed to the modeling of the continuous passage of the blood flow (Figs 1a and 2a) and a stratified structure viewed from the tunica media of the artery (Figs 1b and 2b). This stratified structure is caused by a successive calcification process over time, consistent with the pathogenesis of atherosclerotic calcifications, progressing in an outward direction (16). The formation of the atheromata and their calcification in the tunica intima of the artery gives them the convex-concave shape of vascular vessels as observed in transverse views of both forensic and cemeterial calcifications (Figs 1c and 2c). The calcifications present a thickness ranging from 1 to 4 mm and a length of 5 to 34 mm.

The histological analyses showed, for both decalcified and undecalcified samples, a stratified structure supporting the pathological etiology of atherosclerotic plaques and reflecting the literature description of soft tissue samples (17–20). In particular, the semi-thin decalcified samples stained in H&E showed a clear picture of the stratification in both autopsy and cemeterial samples (Fig. 3). Moreover, the H&E stained sections for both "aged" autopsy and cemeterial plaques showed the arterial layers surrounding a lipid-rich tissue, in some cases with calcified core preserved, and demonstrated the presence of a fibrous wall component suggesting a continuum between intima and atherosclerotic plaques wall. The lipid cholesterol cleft and foaming macrophages remained as ghost structures in the core of the transverse sections of the plaques after fixation and decalcification processing (Fig. 3). In undecalcified sections, the "ghost" elements could not be recognized and only the structure in superimposed layers could be seen (Fig. 4). This may be explained by the general higher thickness of the sections in undecalcified sections compared to decalcified ones. However, when the grinding process was increased to obtain thin undecalcified sections, the integrity of the samples became compromised and a majority of the sample was lost, rending the sections useless for observation. Consequently, while decalcified sections allowed the observations of lipid cores and foaming macrophages, the undecalcified process destroyed the cholesterol

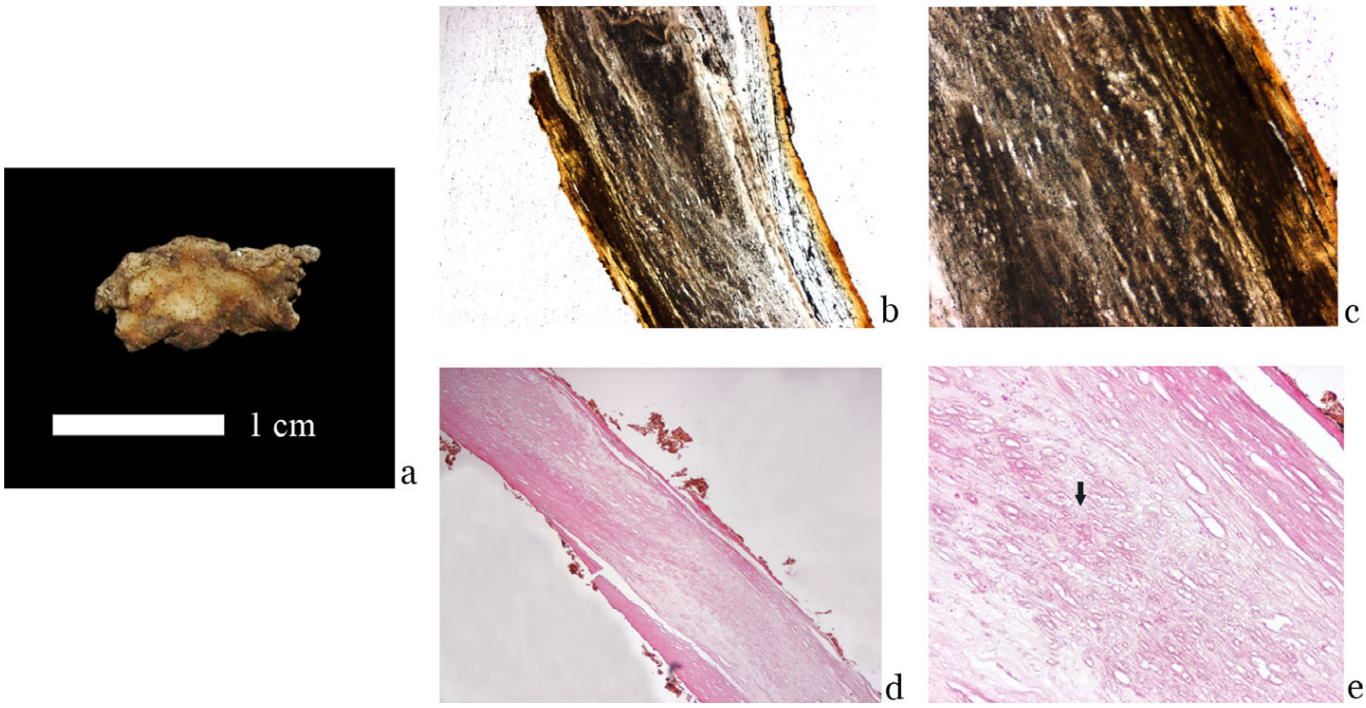

FIG. 4—*Histological comparison of decalcified and undecalcified protocols on a cemeterial plaque. (a) macroscopic view; (b and c) histological pictures of undecalcified sections, original magnification x1(b), x4(c).; (d and e) decalcified sections of the same autopsy sample stained in H&E with postmortem contaminations and "ghosts" elements (e: black arrow), original magnification x1(d), x4(e).*

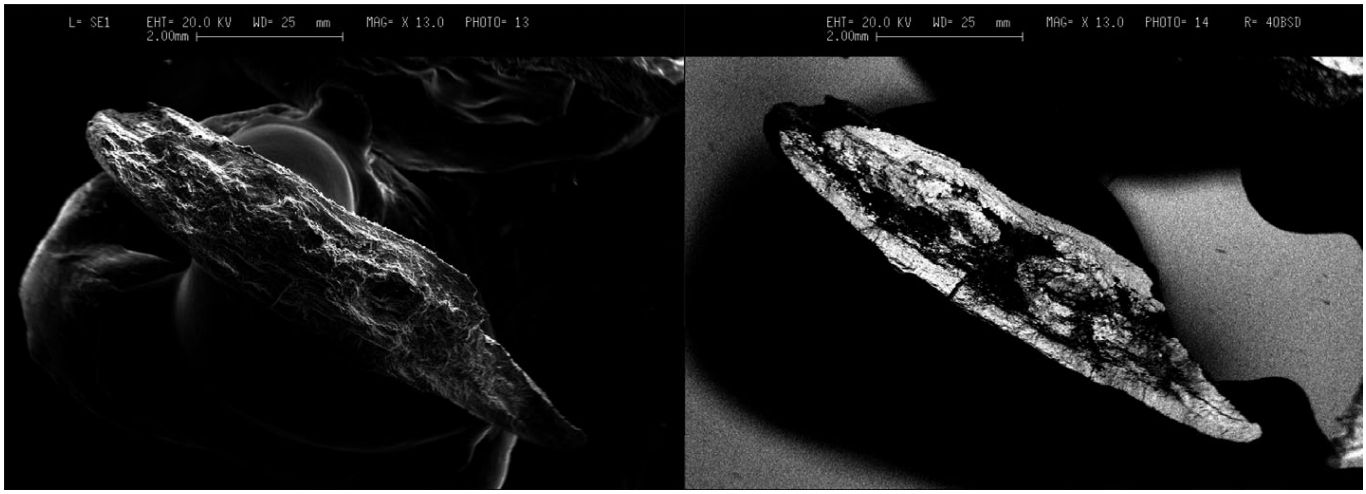

FIG. 5—*Scanning Electron Microscopy of an autopsy atherosclerotic plaque. (a) magnification x13; (b) Back-Scatter Detector (BSE) magnification x13.*

crystals and compromised the study of the plaques in thin sections. Although "ghost" elements could not be observed on undecalcified histological sections, their stratified aspect remained very clear and identifiable.

The SEM analysis also showed the stratified structure of the plaques (transverse view) in great details but no other structure could be identified (Fig. 5). Compared to classic microscopy, the SEM multilayered aspect is not specific to atherosclerotic calcifications, whereas histological sections provide very characteristic details with cores of extracellular lipids and "ghost" macrophages elements that may be related to the atherosclerotic process and may thus be useful in the identification of atherosclerotic plaques. SEM analyses of atherosclerotic plaques have been published in the literature (21,22) but not from dry bone specimens. Nonetheless, they describe a multilayered structure of the plaques, consistent with our findings.

This documentation of known atherosclerotic calcifications, both extracted from cadavers then "aged" by maceration and collected from cemeterial skeletal remains, has potential not only for their recognition among other calcified material but also to allow the diagnosis of the pathology responsible for their formation, namely atherosclerosis, a cardiovascular disease. Indeed, as atherosclerotic calcifications act as markers of the disease (6), their identification warrants the diagnosis of the cardiovascular condition, even after decomposition of the arteries, decades of burial and alterations due to mixing with the soil. Consequently,

their recognition holds considerable importance. This is why, in this paper, we decided to "age" atherosclerotic calcifications extracted from cadavers by maceration to simulate dry bone context and compare the results of three types of analyses, morphological, histological, and SEM, with identified atherosclerotic plaques from cemeterial skeletal remains (15) buried for at least 10 years, in order to provide documentation on known atherosclerotic calcifications that may be used for their identification in unknown dry bone context.

Atherosclerosis is very common in adults and even more in elderly. With time, atheromata calcify into atherosclerotic plaques and are able to survive decomposition and taphonomic processes. Therefore, they should be recovered in skeletonized cases, whether in an archeological or a forensic context. The lack of documentation on the morphological appearance of these vascular plaques, especially after a long postmortem interval and altered by decomposition and taphonomic processes, explains why they are not more commonly found. The retrieval of atherosclerotic calcifications associated with skeletal remains can provide valuable additional pathological information on the deceased which is especially not negligible in a forensic context. Indeed, they may be compared to antemortem medical information and help in the identification of unknown deceased. The objective of this paper was to document the aspect of known atherosclerotic plaques extracted from well-preserved cadavers during autopsy cases and "aged" by maceration to simulate a dry bone context as well as atherosclerotic calcifications collected from cemeterial skeletal remains of the CAL Milano Cemetery Skeletal Collection with three different approaches. Macroscopically, atherosclerotic plaques possess similar morphological characteristics regardless of their original location in the body or whether they come from a cemeterial or "aged" autopsy context. They are convex-concave plaques with a stratified structure and a coloration varying from uniform pale-yellow to yellow and brown depending on the alterations they were subjected to. Histologically, they show a concentric layered structure. In the H&E stained decalcified sections, it was possible to observe arterial layers surrounding a core of extracellular lipid and cholesterol crystals, some macrophages and foam cells that were identified as "ghost" elements. Finally, the SEM analysis highlighted in great details the stratified structure of the calcifications but did not provide specific information on the constitution of the plaques that could be used in their identification.

In conclusion, we presented here the morphological, histological, and SEM aspects of known atherosclerotic plaques to document what to expect when confronted to calcified material. The aim of this study was to give new insight to help forensic anthropologists and paleopathologists better recognize atherosclerotic plaques and diagnose the pathology in dry bone context.

References

1. WHO. Cardiovascular diseases (CVDs) – World Health Organization statistics, 2017; http://www.who.int/mediacentre/factsheets/fs317/en/(accessed October 9, 2018).
2. Lam JYT. Atherosclerosis, The Merck manual for health professionals, 2012; http://www.merckmanuals.com/professional/cardiovascular-disorders/arteriosclerosis/atherosclerosis (accessed October 9, 2018).
3. Lusis AJ. Atherosclerosis. Nature 2000;407(6801):233–41.
4. Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001;104 (4):503–16.
5. Falk E. Pathogenesis of atherosclerosis. J Am Coll Cardiol 2006;47(8 Suppl):C7–12.
6. Doherty TM, Asotra K, Fitzpatrick LA, Qiao J-H, Wilkin DJ, Detrano RC, et al. Calcification in atherosclerosis: bone biology and chronic inflammation at the arterial crossroads. Proc Natl Acad Sci U S A 2003;100(20):11201–6.
7. Wexler L, Brundage B, Crouse J, Detrano R, Fuster V, Maddahi J, et al. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical implications. A statement for health professionals from the American Heart Association. Writing Group. Circulation 1996;94(5):1175–92.
8. Frink RJ. Inflammatory atherosclerosis: characteristics of the injurious agent. Sacramento, CA: Heart Research Foundation, 2002;111.
9. Flora GC, Baker AB, Loewenson RB, Klassen AC. A comparative study of cerebral atherosclerosis in males and females. Circulation 1968;38 (5):859–69.
10. Gostner P, Knapp R, Recheis W, Seidler H. The iceman: discovery and imaging. Radiology 2003;226:614–29.
11. Thompson RC, Allam AH, Lombardi GP, Wann LS, Sutherland ML, Sutherland JD, et al. Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet 2013;381(9873): 1211–22.
12. Towler DA. Vascular calcification: a perspective on an imminent disease epidemic. IBMS Bonekey 2008;5(2):41–58.
13. Blau S, Ranson D, O'Donnell C. An atlas of skeletal trauma in medico-legal contexts. San Diego, USA: Academic Press, 2018;796.
14. Cattaneo C, Mazzarelli D, Cappella A, Castoldi E, Mattia M, Poppa P, et al. A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection. Forensic Sci Int 2018;287:219.e1–5.
15. Biehler-Gomez L, Cappella A, Castoldi E, Martrille L, Cattaneo C. Survival of atherosclerotic calcifications in skeletonized material: forensic and pathological implications. J Forensic Sci 2018;63(2):386–94.
16. Libby P. Vascular biology of atherosclerosis: overview and state of the art. Am J Cardiol 2003;91(3A):3A–6A.
17. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation 1995;92(5):1355–74.
18. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20(5):1262–75.
19. van Engelen A, Niessen WJ, Klein S, Groen HC, van Gaalen K, Verhagen HJ, et al. Automated segmentation of atherosclerotic histology based on pattern classification. J Pathol Inform 2013;4(Suppl):S3.
20. Ababneh B, Rejjal L, Pokharel Y, Nambi V, Wang X, Tung C-H, et al. Distribution of calcification in carotid endarterectomy tissues: comparison of micro-computed tomography imaging with histology. Vasc Med 2014;19(5):343–50.
21. Schembri L, Congiu T, Tozzi M, Guasti L, Cosentino M, Marino F. Scanning electron microscopy examination and elemental analysis of atherosclerotic calcifications in a human carotid plaque. Circulation 2008;117(23):e479–80.
22. Curtze SC, Kratz M, Steinert M, Vogt S. Step down vascular calcification analysis using state-of-the-art nanoanalysis techniques. Sci Rep 2016;6:23285.

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"Aged" autopsy gallstones simulating dry bone context: A morphological, histological and SEM-EDS analysis

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ABSTRACT

Objective: The aim of this paper is to provide information on the morphology and composition of gallstones based on clinical samples in order to assist paleopathologists and bioarchaeologists in recognizing their presence in archaeological contexts.
Materials and Methods: 270 gallstones were extracted and macerated from autopsies conducted at the *Istituto di Medicina Legale* in Milan (Italy) in order to simulate a dry bone recovered from archaeological contexts. Morphological, histological, and elemental variation was documented.
Results: Gallstones vary in size, shape, color and texture. The cross-sectional surface correlates with chemical composition and is a valuable tool for classification into subcategories of stones. Histological analysis can confirm the classification. Elemental analysis yielded a higher frequency of carbon, calcium and phosphorus.
Conclusions: Although identification of gallstones in archaeological contexts can be challenging, familiarity with morphological, histological, and elemental variation can assist researchers in the field and laboratory.
Significance: Identifying gallstones in archaeological populations will assist researchers in estimating their frequency in the past and the environmental, cultural, and biological conditions leading to their presence.
Limitations: Small sample size derived from a modern and limited autopsy population may minimize the types and degree of variation present in the past. Effects of climate, soil, and taphonomy were not evaluated.
Suggestions for Further Research: Examination of larger samples derived from diverse populations may reveal greater variation or more diagnostic aspects of stones.

1. Introduction

Gallstones (cholelithiasis) are quite common, present in about 10% of individuals over 40 years and in 30% over 70 years (Siddiqui, 2016; Waldron, 2008). It is estimated that 25% of patients with cholelithiasis or urolithiasis (urinary stones) develop a recurrent stone (Johnston and Kaplan, 1993; Knoll et al., 2011; Siddiqui, 2016). Stone formation is a multifactorial condition associated with risk factors, which include female sex, advancing age (gallstones are uncommon in infants and children), high body mass index, rapid weight loss, familial history of gallstones, increasing number of pregnancies, diabetes mellitus type 2 and diet and activity (Festi et al., 2008; Shaffer, 2006). The highest prevalence of cholelithiasis is seen in Native-Americans and in decreasing order in Euro-Americans, Europeans, African-Americans and black Africans (Shaffer, 2006). These results suggest a genetic predisposition to stone formation (an estimated 30% genetic component) (Sanders and Kingsnorth, 2007; Shaffer, 2006). Cholesterol stones represent more than 75% of gallstones in western countries (Johnston and Kaplan, 1993; Siddiqui, 2016) and are caused by an "imbalance in the chemical constituents of bile" (Sanders and Kingsnorth, 2007). Indeed, when the bile becomes oversaturated with cholesterol, this excess is precipitated as solid microcrystals that accumulate and grow into stones. Pigment stones appear to be present in older individuals than cholesterol stones and are mainly composed of calcium bilirubinate, inorganic calcium salts and fatty acids (Johnston and Kaplan, 1993; Siddiqui, 2016).

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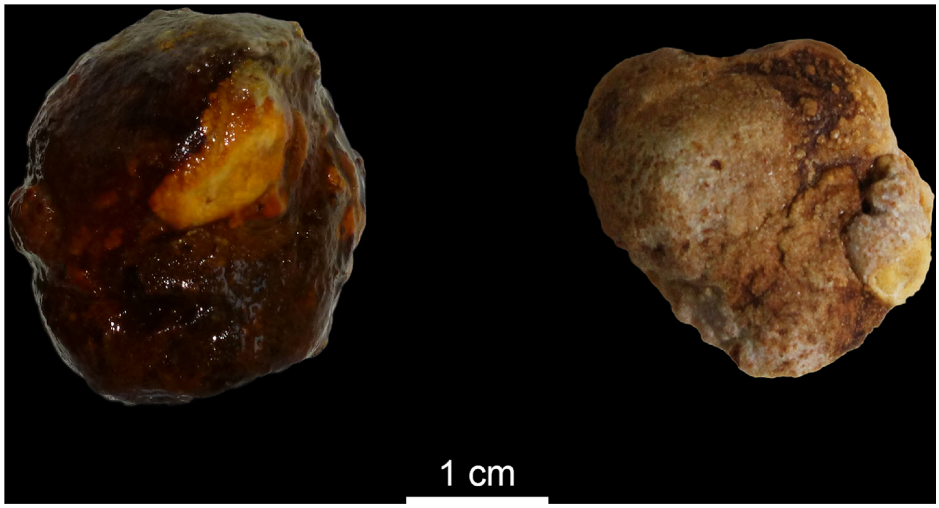

Fig. 1. Extracted gallstone from a fresh cadaver (left) and after maceration to mimic the effects of archaeological "aging" (right).

In spite of their common occurrence in modern populations, gallstones are less commonly found in the paleopathological record. Angel (1973) reported two brown faceted gallstones found between the ribs and iliac crests of a 45-55-year-old male in Mycenae, Greece, dated 1600–1500 BCE. Smith and Dawson (1924) note the presence of gallstones in a mummified priestess from Amen dated 1085–95 BCE, and Gray noted radio-opaque gallstones in a mummy of the late Dynastic period (525–343 BCE) (Gray, 1967). The mummified remains of Kha, the architect of Amhenotep III, who lived between 1460 and 1400 BCE, contained 14 gallstones (Cesarani et al., 2009). Similarly, the well-preserved body of a 50-year-old woman from the Han dynasty (*circa* 200 BCE), buried in an airtight coffin in the province of Hunan, China, revealed the presence of several gallstones (Wei, 1973). A study by Munizaga et al. (1978) showed that gallstones were recovered in 2 out of 75 mummies dated 100–300 CE from northern Chile. Detecting cholelithiasis in skeletal remains is more challenging, but gallstones have been found in a skeleton from Merovingian Germany (*circa* 750 CE), a 9th-century burial in Hérault, France, at least 6 burials in the Ohio Libben Woodland site dated 1000–1200 CE (Steinbock, 1990), and in a London grave dated 1000–1200 CE (White and Dyson, 1988). Clearly, without mummification of tissues or the recovery of stones *in situ* near the anatomical location of the gallbladder, detecting the presence of gallstones in archaeological contexts is difficult. Hence, the goal of this study was to provide researchers with an understanding of the wide morphological characteristics of gallstones in order to facilitate identification and successful recovery.

2. Materials and methods

From February 2014, to January 2017, 270 gallstones were extracted during 25 autopsies in the *Istituto di Medicina Legale* in Milan, Italy (Fig. 1). Each of the 25 autopsy extractions underwent maceration in tap water for several weeks until the calcified elements were completely separated from soft tissues and biological liquids.

The macroscopic analysis focused on several criteria, including the location where the calcified elements were found in the autopsied body, the general morphology (including shape, texture and color), and dimensions (measured with a Vernier caliper) of the stone. For the cross-sectional analysis, one stone from each autopsy extraction (a total of 25 stones) was cut transversally with either a scalpel or a small saw and processed with a horizontal grindstone (Struers DAP-7) and abrasive discs (Buehler micro cut discs, grain of 180, 320, 500, 1200, 2400 and 4000) progressively for a closer observation of the cut surface.

The histological analyses included two protocols: undecalcified and decalcified. For the undecalcified protocol, the samples were ground (using the same process as in cross-sectional surface analysis) to obtain thin sections, embedded in Pertex (Pertex, mounting medium for light microscopy. Histolab: Gotebourg, Sweden) and kept at ambient temperature for 72 h until the synthetic resin solidified and could be viewed with an optic microscope. For the decalcified protocol, gallstones were first fixed in formalin (v/v, PH 7–7.6, ratio 20:1 v/v) for 24 h and decalcified at room temperature in Decalc, 14% hydrochloric acid (Histo-Line Laboratories, Milan). However, the structural integrity of the stones collapsed during the xylene passage before fixing the samples in paraffin. Thus, the decalcified protocol could not be completed.

Finally, scanning electron microscopy was performed with a Cambridge Stereoscan 360 (Oxford, U.K.) with electron gun, vacuum pump, and image acquisition software, and energy dispersive spectrometry with detector from 138 eV to 5.9 keV (Oxford Link Pentafet, Oxford, UK) to provide the elemental composition of the stones.

3. Results and discussion

Each stone selected for analysis came from different extractions and thus from different individuals. Fourteen stones out of 270 calculi underwent undecalcified histological analysis and 9 underwent SEM-EDS analysis. A total of 8 gallstones underwent both histological and SEM-EDS analyses. The result of these analyses indicate that gallstones appear in a great variety of shapes, sizes and colors (Fig. 2) (Table 1). The stones observed appeared round, oval, multilobular or polygonal in shape, and presented varied external texture including smooth, porous, rough or dotted. Their coloration varied from grey, shades of white,

Fig. 2. Varying external morphology of gallstones.

yellow, brown, black and even green. The sizes varied from 0.2 to 4.9 cm.

The SEM-EDS analysis revealed the presence of numerous and unspecific chemical elements, including carbon, calcium, and phosphorus due to the organic and calcified proprieties of the material. The stones also contained sulfur, sodium, and magnesium, and occasionally, traces of chlorine, potassium, silicon, copper and iron (Table 1). Since assessing the presence of cholesterol and/or calcium bilirubinate requires a sophisticated laboratory technique, preferably using FTIR (Fourier Transformed Infrared Spectroscopy) (Qiao et al., 2013), which is often inaccessible to most researchers, it was excluded from this study.

Alternatively, a microscopic analysis of the cross-sectional surfaces

Table 1
Morphological, histological and elemental variations of gallstones.

Macroscopic Variation		Common Elements*	Rare Elements**	Microscopic Variation
Shape	Round/oval	Carbon	Chlorine	Radiating
	Multilobular	Calcium	Potassium	Concentric
	Polyedric	Phosphorus	Silicon	Organization of crystal deposits
Texture	Smooth	Sulfur	Copper	Amorphous
	Porous	Sodium	Iron	
	Rough	Magnesium		
	Dotted			
Color	Grey			
	Shades of white			
	Yellow			
	Brown			
	Black			
	Green			

* found repetitively.
** found in one or two instances.

Table 2
Classification of gallstones based on Kim et al (2003).

Cholesterol	
Pure	radial structure from center to periphery with pigment in the center (< 1/3 of the diameter of the cut surface)
Combination	two distinct layers: center of cholesterol surrounded by pigment or vice-versa (external layer > 1 mm)
Mixed	both concentric and radial structures superimposed
Pigment	
Brown	concentric layers
Black	amorphous aspect
Mixed	
usually two layers: center is larger and darker (amorphous)	

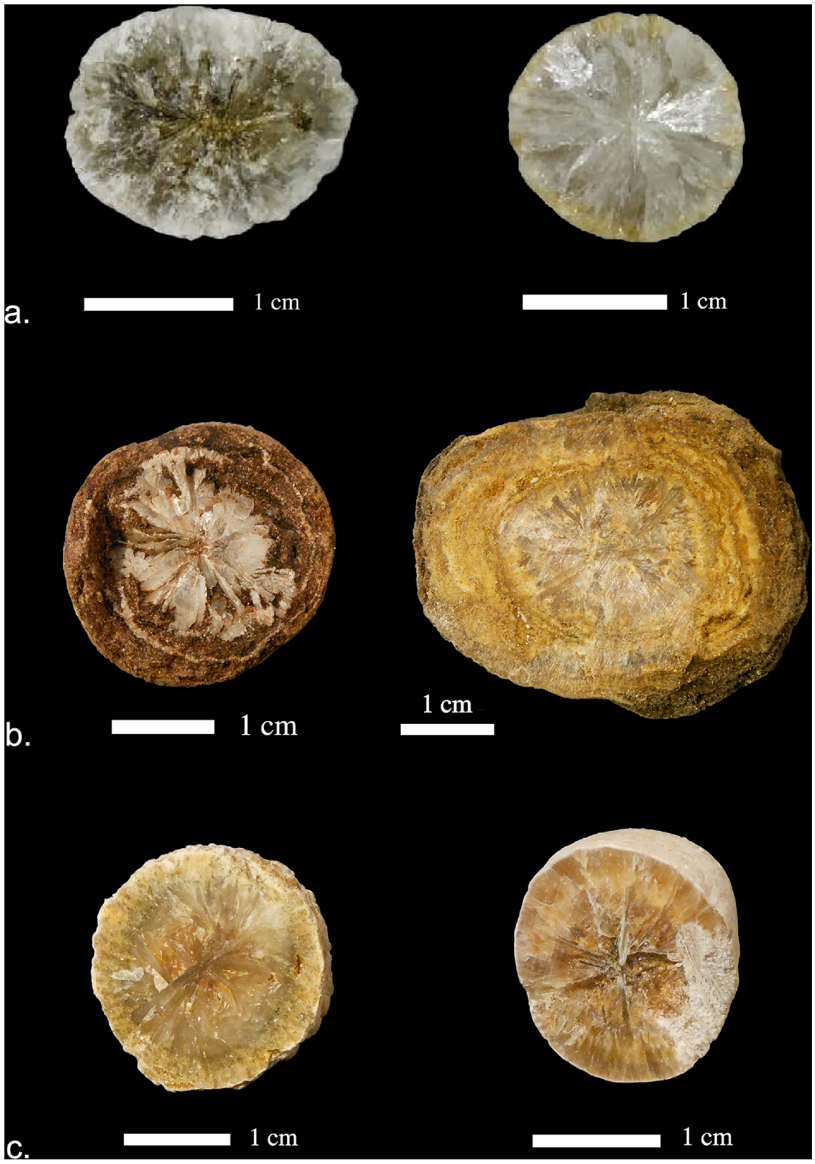

Fig. 3. Cholesterol gallstones (cut surfaces). **a:** pure cholesterol stones, note the radiating pattern from the center; **b:** combination cholesterol stones, note the two distinct layers with a center of cholesterol and the external crown of pigment; **c:** mixed cholesterol stones, note the superposition of the radiating and concentric patterns.

was undertaken. Based on the specific characteristics reported in Table 2, it was possible to classify each stone into cholesterol (Fig. 3), pigment (Fig. 4) or mixed stones (Fig. 5) and further categorize them within each group in accordance with Kim et al. (2003). Indeed, a radial pattern is specific to cholesterol stones, while brown pigment stones exclusively present concentric layers. No black pigment stone was available in our study, but their completely amorphous appearance, described and documented in the literature (Kim et al., 2003; Qiao et al., 2013) makes them easily identifiable.

The histological observations of undecalcified gallstones mirrored the variation noted in the cross-sectional macroscopic analysis, with radial, concentric, or amorphous patterns being clearly recognizable (Table 1). In addition, crystal cholesterols could also be directly observed, whether organized or disorganized, suggesting the presence of cholesterol gallstones (Fig. 6).

In spite of our success in noting variation and patterns within our gallstone specimens, it is important to note the limitations of this study. First, the literature reports that “the surface color of a gallstone can

Fig. 4. Brown pigment gallstone (cut surface). Note the characteristic concentric pattern. [No color in print].

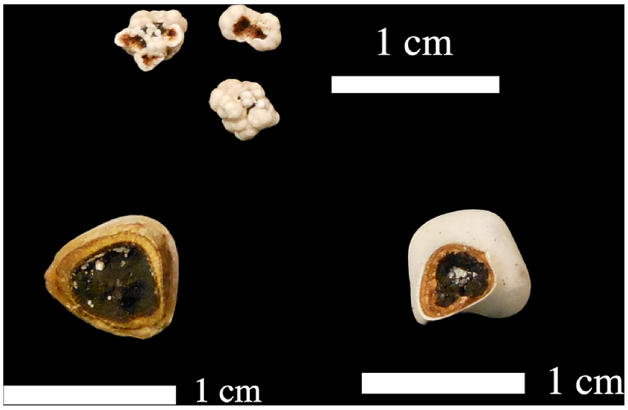

Fig. 5. Mixed gallstones (cut surfaces).

change after drying" (Kim et al., 2003). This was reflected in our study by the range of colors observed on dried "aged" gallstones. Other morphological features and considerations must therefore be taken into account when identifying and differentiating gallstones from geological stones. When bodies are not mummified, paleopathological differential diagnosis of cholelithiasis is essential and must include benign tumors (teratoma, dermoid cyst, leiomyoma), parasitic infections, tuberculosis calcifications, calcified lymph nodes, vascular calcifications, pleural plaques, hydatid cysts, psammoma bodies, calcified ovaries and lithopaedions (Armentano et al., 2012; Baud and Kramar, 1991; Komar and Buikstra, 2003). While the location, general shape (usually round or ovoid), size, solidity, absence of vascular impressions, and lack of skeletal indicators of neoplasm or tuberculosis can narrow the diagnosis to a biological stone, our macerated "aged" stones provide additional information that may be used for the differential diagnosis of unknown calcified objects in dry bone contexts.

4. Conclusion

Gallstones are common in modern populations and tend to be recurrent in affected individuals. Therefore, it is surprising that they are not found more frequently in archaeological contexts. It is possible that their similar appearance to rocks or geological concretions makes them difficult to identify. If this is so, the lack of gallstones in the paleopathological record does not reflect the frequency of the condition in the past, but rather our inefficiency in detecting their presence.

Hence, the aim of this paper was to document the variability of known gallstones from clinical samples in an effort to help archaeologists and paleopathologists recognize them in archaeological contexts. In this paper, we reported the morphological and histological aspects of "aged" gallstones. We found that gallstones take on many shapes, sizes, colors and textures, making it difficult to differentiate them from geological stones. Through cross-sectional surface analysis, however, useful classifications of gallstones were constructed. The microscopic histological analysis concurred with the macroscopic results, allowing direct observation of cholesterol crystal deposits. Finally, the SEM-EDS results, although not specific to the identification of gallstones, showed a large panel of chemical elements that comprise gallstones. Thus, we propose that careful macroscopic and histological evaluation, alongside the use of a SEM-EDS, will aid paleopathologists and bioarchaeologists in recognizing, and perhaps identifying, the presence of gallstones in the past.

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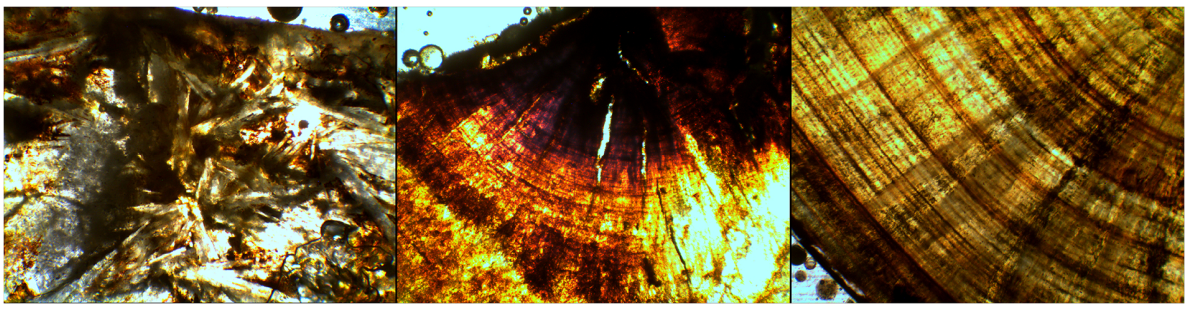

Fig. 6. Histological sections of gallstones (undecalcified protocol) magnification x4. **Far left**: note the cholesterol crystals deposited in a disorganized fashion; **middle:** central aspect with a radial structure and the periphery organized in concentric layers with varying coloration; **far right:** superposition of radial and concentric layers over the entire surface of the section.

References

Angel, J.L., 1973. Human skeletons from grave circles at Mycenae. Appendix in GE Milonas. The Grave Circle B of Mycenae. Archaeological Society of Athens, pp. 379–397.

Armentano, N., Subirana, M., Isidro, A., Escala, O., Malgosa, A., 2012. An ovarian teratoma of late Roman age. Int. J. Paleopathol. 2, 236–239. https://doi.org/10.1016/j.ijpp.2012.11.003.

Baud, C.A., Kramar, C., 1991. Soft tissue calcifications in paleopathology. In: Ortner, D.J., Aufderheide, A.C. (Eds.), Human Paleopathology: Current Syntheses and Future Options. Smithsonian Institution Press, Washington, DC, pp. 257–260.

Cesarani, F., Martina, M.C., Boano, R., Grilletto, R., D'Amicone, E., Venturi, C., Gandini, G., 2009. Multidetector CT Study of Gallbladder Stones in a Wrapped Egyptian Mummy. RadioGraphics 29, 1191–1194. https://doi.org/10.1148/rg.294085246.

Festi, D., Dormi, A., Capodicasa, S., Staniscia, T., Attili, A.F., Loria, P., Pazzi, P., Mazzella, G., Sama, C., Roda, E., Colecchia, A., 2008. Incidence of gallstone disease in Italy: results from a multicenter, population-based Italian study (the MICOL project). World J. Gastroenterol. 14, 5282–5289. https://doi.org/10.3748/wjg.14.5282.

Gray, P.H.K., 1967. Radiography of ancient Egyptian mummies. Med. Radiogr. Photogr. 43, 34–44.

Johnston, D.E., Kaplan, M.M., 1993. Pathogenesis and treatment of gallstones. N. Engl. J. Med. 328, 412–421.

Kim, I.S., Myung, S.J., Lee, S.S., Lee, S.K., Kim, M.H., 2003. Classification and nomenclature of gallstones revisited. Yonsei Med. J. 44, 561–570. https://doi.org/10.3349/ymj.2003.44.4.561.

Knoll, T., Schubert, A.B., Fahlenkamp, D., Leusmann, D.B., Wendt-Nordahl, G., Schubert, G., 2011. Urolithiasis Through the Ages: Data on More Than 200,000 Urinary Stone Analyses. J. Urol. 185, 1304–1311. https://doi.org/10.1016/j.juro.2010.11.073.

Komar, D.A., Buikstra, J.E., 2003. Differential diagnosis of a prehistoric biological object from the Koster (Illinois) Site. Int. J. Osteoarchaeol. 13, 157–164. https://doi.org/10.1002/oa.670.

Munizaga, J., Allison, M.J., Paredes, C., 1978. Cholelithiasis and cholecystitis in pre-Columbian Chileans. Am. J. Phys. Anthropol. 48, 209–212.

Qiao, T., Ma, R., Luo, X., Yang, L., Luo, Z., Zheng, P., 2013. The systematic classification of gallbladder stones. PLoS One 8, e74887. https://doi.org/10.1371/journal.pone.0074887.

Sanders, G., Kingsnorth, A.N., 2007. Gallstones. BMJ Br. Med. J. 335, 295–299. https://doi.org/10.1136/bmj.39267.452257.AD.

Shaffer, E.A., 2006. Epidemiology of gallbladder stone disease. Best Pract. Res. Clin. Gastroenterol. 20, 981–996. https://doi.org/10.1016/j.bpg.2006.05.004.

Siddiqui, A.A., 2016. Cholelithiasis, The Merck Manual for Health Professionals.

Smith, G.E., Dawson, W.R., 1924. Egyptian Mummies. G. Allen Unwin, ltd.

Steinbock, R.T., 1990. Studies in ancient calcified soft tissues and organic concretions. III: Gallstones (Cholelithiasis). J. Paleopathol. 3, 95–106.

Waldron, T., 2008. Palaeopathology. Cambridge University Press, Cambridge, United Kingdom.

Wei, O., 1973. Internal organs of a 2100-year-old female corpse. Lancet 302, 1198.

White, W.J., Dyson, T., 1988. Skeletal Remains From the Cemetery of St. Nicholas Shambles, City of London. London & Middlesex Archaeological Society, London, United Kingdom.

Ossification of costal cartilage

Costal cartilage allows the junction of the osseous part of the ribs to the sternum while providing a necessary degree of flexibility to the thorax for respiratory functions, movements and bending as well as shock absorption. With increasing age, costal cartilage undergoes changes in its microstructure including calcification and ossification. These skeletal changes are normal alterations associated with aging. The onset of costal cartilage mineralization is estimated in the second to third decade of life while ossification starts around 25 years of age and lamellar tissue is observed in individuals over 30 years (Kampen, Ciaassen and Kirsch, 1995; Rejtarova *et al.*, 2009).

Contrary to intramembranous ossification where bone is directly laid down in mesenchymal connective tissue, cartilage acts as a precursor for bone formation in endochondral ossification (Kusafuka *et al.*, 2001). The mechanisms responsible for cartilage ossification are not well understood. Histological studies have demonstrated that cartilage does indeed ossify, and that there is evidence of both perichondral and endochondral ossifications (King, 1939; Rejtarova *et al.*, 2009). Cartilage cells experience stages of proliferation and differentiation that ultimately culminate in mineralization of the matrix and chondrocyte hypertrophy (that is, the cartilage becomes calcified in the hypertrophic zone) and activation of the cells' osteogenic capability (start of the ossification process). Hypertrophic chondrocytes lay down collagen type X, release matrix vesicles including alkaline phosphatase and induce vascular invasion. While matrix vesicles mediate cartilage calcification, both collagen type X and alkaline phosphatase are essential for the ossification process (Bahrami *et al.*, 2001; Rejtarova *et al.*, 2009; Amizuka, 2012).

Different patterns of ossification exist depending on the ribs. Costal cartilage ossification is initiated with degenerative changes in the central area of the cartilage, but first rib cartilage ossification is a physiological process starting in the border and involving the center of the cartilage last (Rejtarova *et al.*, 2009). Moreover, the patterns and extent of costal cartilage ossification have been studied for their potential use for sex, age and ancestry estimations. Indeed, a study by Rejtovará and colleagues on 1044 chest and abdominal radiographs reports an association of peripheral ossification of rib cartilage with male sex with a 99.6% of probability and central ossification in women with 100% probability (Rejtarova *et al.*, 2004). However, ancestry estimations based on costal ossification gave contradictory results and the techniques for determination of age and sex have been considered not suitable for forensic or

anthropological practice (Barchilon *et al.*, 1996).

In this study, we document and confront histological analyses performed on dry bone ossifying cartilage obtained from individuals with two different post-mortem intervals: "fresh" remains macerated from autopsied cadavers and cemeterial skeletons buried for about 20 years.

In this research, 18 samples of ossifying costal cartilage from "fresh" and cemeterial cases were examined and compared. The "fresh" cases consist in cadavers autopsied at the medico-legal institute in Milan (Italy) and macerated in tap water for several months until the soft tissue could be completely removed from the skeleton, thus simulating dry bone (see part 4.1.4 Forensic cases). The cemeterial cases correspond to skeletons of the CAL Milano Cemetery Skeletal Collection (Cattaneo *et al.*, 2018), a modern and identified collection of 2127 unclaimed skeletons coming from the cemeteries of Milan in accordance with article 43 of the Italian National Police Mortuary Regulation (September 10, 1990, n°285) (see Chapter 4. Materials and Methods). Ossifying costal cartilage was selected from three "fresh" cases and three skeletons of the CAL Milano Cemetery Skeletal Collection (cases n°338, 389 and 971, Appendix I) with the only restriction of the exclusion of the first rib cartilage for its particularity. The individuals selected for study included three females and three males, with ages-at-death ranging between 35 and 90 years and a mean age of 67.5 years. Samples were cut with an electric nano circular saw and submitted to two protocols for histological analysis: undecalcified and decalcified. For the first protocol, samples of ossifying cartilage were ground with a horizontal grindstone (Struers DAP-7) and abrasive discs (Buehler micro cut discs, grains of 180, 320, 500, 1200, 2400 and 4000) used progressively, fixed in Pertex (Pertex, mounting medium for light microscopy. Histolab: Gotebourg, Sweden) and let to solidify for 72 hours in ambient temperature before observation with the optic microscope. For the second protocol, the samples were first fixed in formalin (v/v, PH 7-7.6, ratio 20:1) for 24 hours, decalcified at room temperature in Decalc 14% hydrochloric acid (Histo-Line Laboratories, Milan), rinsed in tap water for another 24 hours, dehydrated in alcoholic scale, and then embedded into paraffin. Finally, sections of 5-microns were cut from each block stained with Hematoxylin and Eosin (H&E) (see Chapter 4. Materials and Methods). Ten samples underwent undecalcified histological analysis, including three from "fresh" cases and seven from cemeterial skeletons. Eight samples underwent decalcified histological analysis: three from "fresh" cases and five from cemeterial skeletons.

In our investigation, histological assessment of mineralization of costal cartilage from cemeterial and "fresh" cases in both decalcified and undecalcified specimens showed overlapping pictures. In all specimens we observed a biphasic pattern composed of chondrocytes and osteons separated by a transitional zone, in which hypertrophic chondrocytes lost proteoglycans in their matrix and accumulated hydroxyapatite crystals that progressively formed lamellar bone tissue (Fig. 10a). Through polarized light it was possible to note mature bone formation adjacent to the cartilaginous tissue (Fig. 10b).

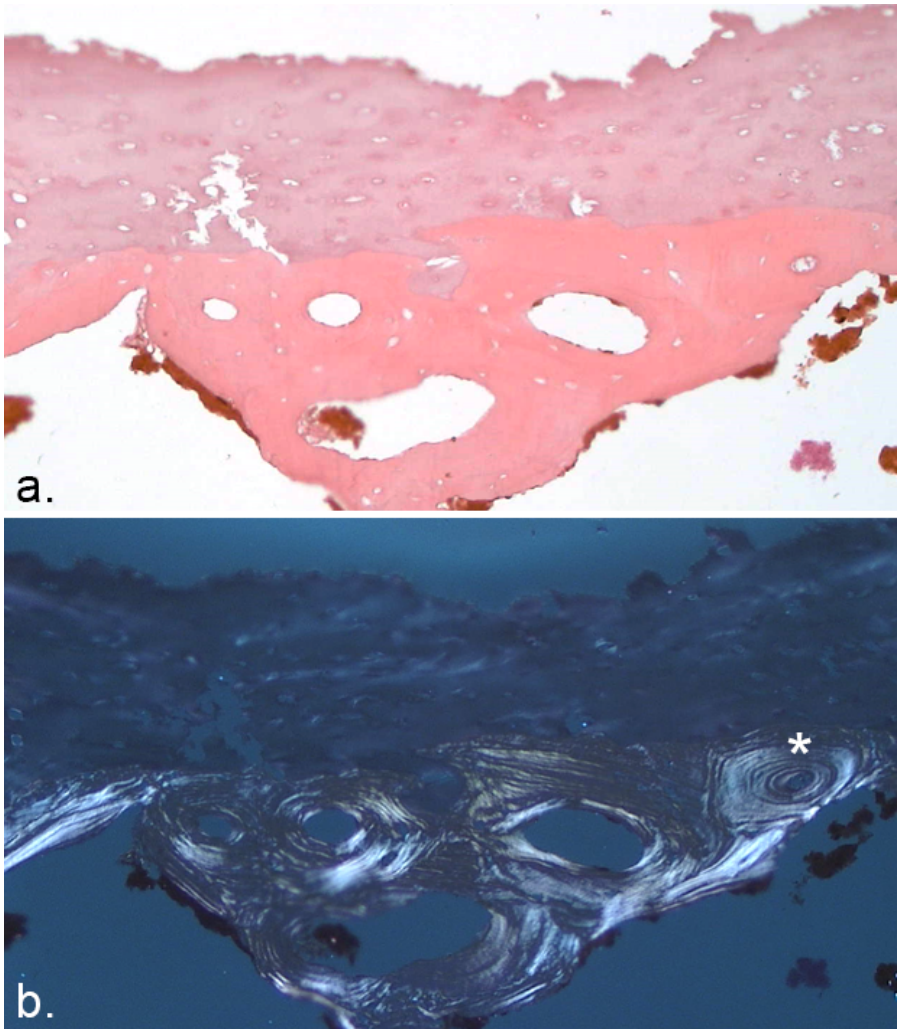

Figure 10: Decalcified histological sections of ossifying costal cartilage. a: unpolarized light; b: polarized light, note the presence of osteons (*). Magnification x10.

The non-decalcified sections showed the cellular details of the cartilaginous and bone components, namely chondrocytes and osteon islands (Fig. 11). By opposition, the cell nuclei were lost in the decalcified sections but the zone of progressive ossification was more clearly observed, the sections being thinner than in undecalcified samples.

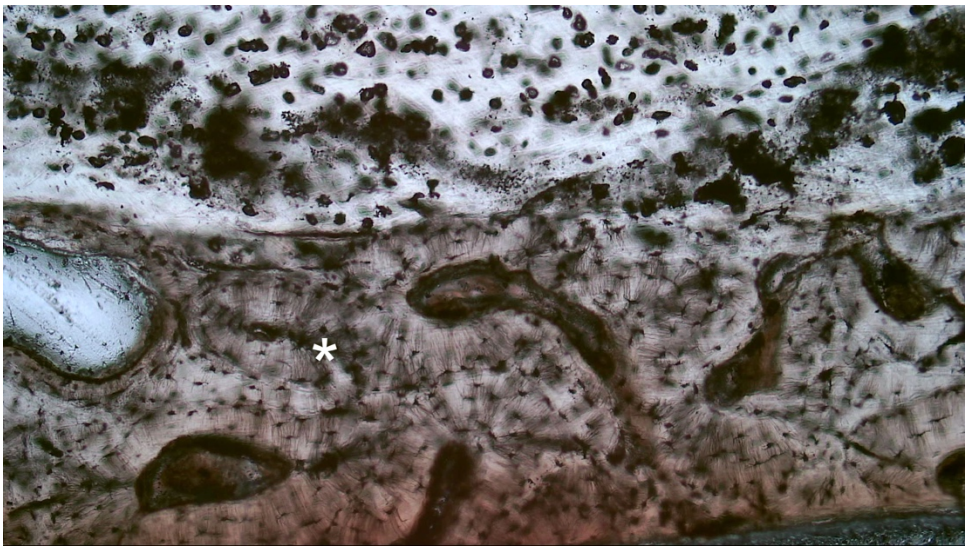

Figure 11: Undecalcified histological section of ossifying costal cartilage. Note the osteon (*). Magnification x10.

These results concur with the literature in the ossification of the mineralized matrix in costal cartilage (Kampen, Ciaassen and Kirsch, 1995; Rejtarova *et al.*, 2009). Interestingly in this study, both "fresh" and cemeterial samples provided similar histological pictures. This parallel indicates that regardless of the post-mortem interval, characteristic features of ossifying costal cartilage may be observed, even after 20 years of burial. This is particularly valuable for the identification of fragmented structures found in association with skeletal remains, as ossified costal cartilage may be recognized and identified after a long post-mortem interval.

In conclusion, we examined the histological composition of costal cartilage from individuals with two different post-mortem intervals: "fresh" samples from autopsied cadavers macerated to simulate dry bone, and cemeterial samples, from skeletons buried for about 20 years before entering the osteological collection. In both cases, similar results were obtained: the sections evidenced a biphasic structure of chondrocytes and osteon islands separated by a transitional zone, demonstrating the presence of both cartilaginous tissue and lamellar bone in a progressive ossification process. In addition, these results indicate that ossified cartilage may be identified on dry bone fragments, despite a very long post-mortem interval. In addition, these results provide criteria for the recognition of ossified cartilage and their distinction from other calcified structures that may be found among dry bone fragments, despite a very long post-mortem interval, thus supplying additional information to the biological profile in the analysis of skeletal remains.

Pleural plaques

In the forensic anthropology practice, the analysis of skeletal remains is not limited to the examination of bones, but also includes biological remains they may be associated with, such as calcification/ossification material. The recovery of such material can provide additional information to the construction of the biological profile as these elements may be indicators of advanced age or products of pathological conditions. However, before the forensic anthropology value of this material may be interpreted, they must first be identified among skeletal remains, even if mixed with soil, roots, dirt and bone fragments. Pleural plaques are delicate structures that may be easily disregarded; it is therefore important to know what pleural plaques macroscopically look like to become aware of them. To the best of our knowledge, no studies have been performed in this sense.

Pleural plaques are ivory-like, discrete, raised, irregularly shaped and sharply-circumscribed lesions of varying size and smooth or coarse texture, generally localized on the parietal pleura of the lateral chest wall, diaphragm or mediastinum (Hourihane, Lessof and Richardson, 1966; Roberts, 1971; Gevenois *et al.*, 1998; Clarke *et al.*, 2006). They are non-cancerous, most often asymptomatic lesions that do not require treatment but they are associated with a higher risk of pleural mesothelioma, pulmonary fibrosis and lung cancer (Hillerdal, 1994). The accepted consensus in the scientific community is that pleural plaques are specific indicators of long term asbestos exposure (after a latency of 20-30 years), especially if bilateral (Gevenois *et al.*, 1998; Clarke *et al.*, 2006). In addition, scientific studies have shown an existing relation between the increased number of asbestos bodies in the lungs and the probability to develop pleural plaques (Roberts, 1971). However, the presence of pleural plaques does not categorically prove a past asbestos exposure as other factors may cause their formation. In particular, pulmonary tuberculosis and trauma (e.g. rib fractures and hemothorax) may lead to pleural plaques, typically unilaterally. Calcifying fibrous pseudotumor, scleroderma (one case) and ankylosing spondylitis (two cases) are medical conditions that have been described in cases with pleural plaques and fibers exposure. In particular, erionite (a rare naturally occurring zeolite), silicates and man-made vitreous fibers have been found in epidemiological studies to be related to higher incidence of pleural plaques (Anton, 1967; Clarke *et al.*, 2006).

In this preliminary study, we macroscopically and histologically document two calcified pleural plaques found during the autopsy of a well-preserved cadaver and

macerated to simulate a dry bone scenario, in order to describe the characteristics of such plaques in a context of skeletonized remains, forensic or archaeological.

In September 2017, during the autopsy of a cadaver at the medico-legal institute of Milan, two pleural plaques were found bilaterally in the parietal pleura. The individual was an elderly male with unknown identity over 65 years of age, who died at the hospital after a coma-inducing left cerebral stroke and a terminal pulmonary edema. No evidence of asbestos exposure or pulmonary tuberculosis was found during the autopsy examination. One pleural plaque was harder than the other, indicating advanced calcification. Samples containing the plaques were taken and macerated in tap water for several months until the soft tissue could be completely removed and dried, thus simulating a dry bone scenario. They were macroscopically examined and submitted to two protocols for histological observation. Following the undecalcified protocol, four samples were ground with a horizontal grindstone (Struers DAP-7) and abrasive discs (Buehler micro cut discs, grains of 180, 320, 500, 1200, 2400 and 4000) used progressively, fixed in Pertex (Pertex, mounting medium for light microscopy. Histolab: Gotebourg, Sweden) and let to solidify for 72 hours in ambient temperature. For the decalcified protocol, two samples were first fixed in formalin (v/v, PH 7-7.6, ratio 20:1) for 24 hours, decalcified at room temperature in Decalc 14% hydrochloric acid (Histo-Line Laboratories, Milan), rinsed in tap water for another 24 hours, dehydrated in alcoholic scale, and embedded into paraffin. Then, sections of 5-microns were cut from each block stained with Hematoxylin and Eosin (H&E).

Macroscopically, pleural plaques were thin and flat plaques of various sizes with a yellowish color (Fig. 12), contrasting with the morphological features of other calcifications that may be found among skeletal remains; for instance, the concave-convex shape of atherosclerotic calcifications, the cylindrical form of Mönckeberg's arteriosclerosis or the round mass of gallstones, as seen in the results of previous research in this thesis.

Histomorphological examination did not evidence asbestos fibers in the lungs or in the pleural plaques, thus two hypotheses are possible: either there was asbestos exposure but no asbestos body were found, which is not a unique case in the literature (Roberts, 1971), or pleural plaque formation was due to another cause (tuberculosis can be excluded as no lesion suggestive of the infectious conditions was found during the autopsy).

Figure 12: Picture of the calcifications recovered from one of the two plaques examined

By opposition to the undecalcified protocol, the histological decalcification allowed the observation of the microscopic structure that constitutes the non-mineralized matrix of the plaque (Fig. 13). Histologically, the plaques were generally acellular, formed of laminated dense bands of avascular and hyaline collagen, deposited parallel to the surface of the plaque. The only detectable cellular component was the presence of sporadic spindle-shaped fibroblasts. The demarcation between the plaque and the pleura was clearly identifiable. Below the plaque, adipose tissue with vascular structures and an important population of lymphocytes and plasma cells were noted (Fig. 14).

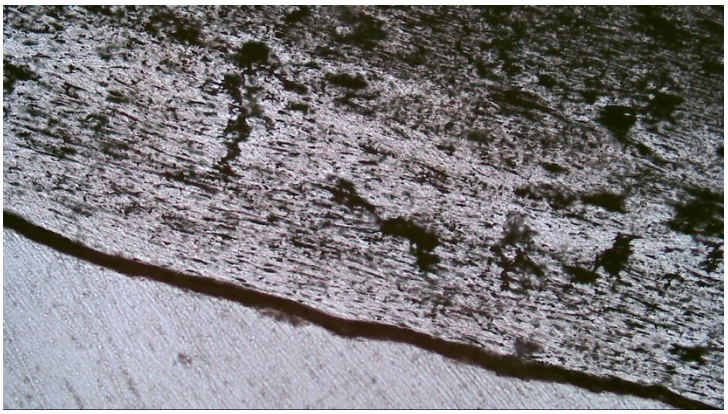

Figure 13: Undecalcified histological section of a pleural plaque sample showing mineralized matrix

The histological picture of the plaques is consistent with descriptions in the literature of plaques due to asbestos exposure (Roberts, 1971; Clarke *et al.*, 2006), despite the fact that asbestos could not be confirmed though lung histology.

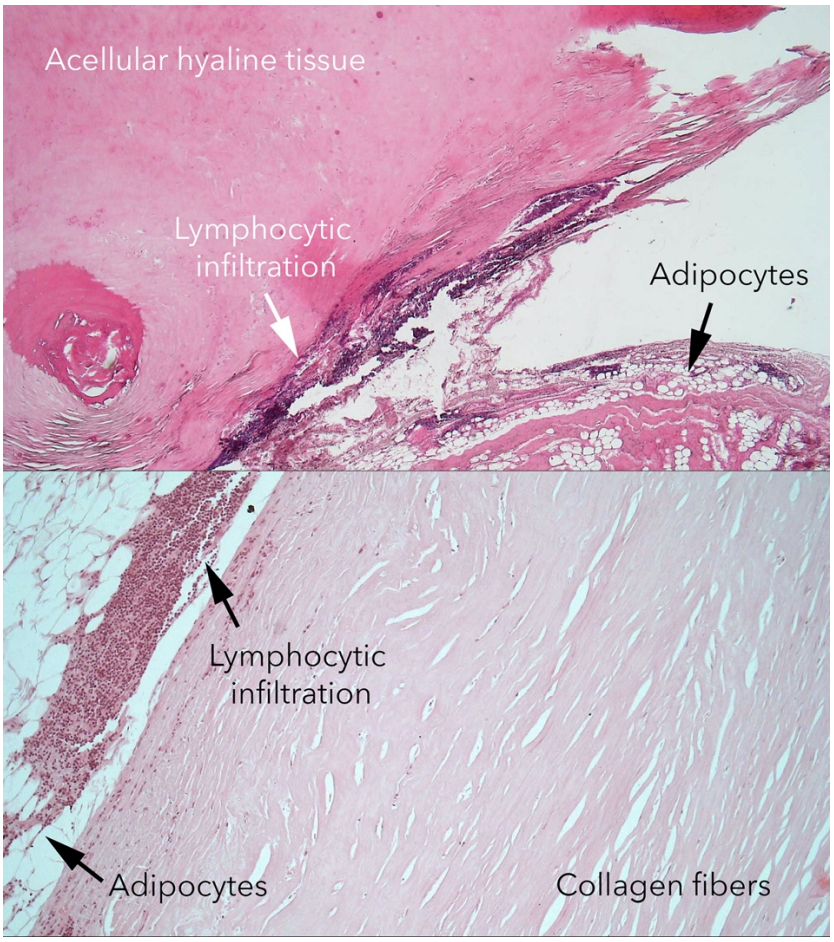

Figure 14: Decalcified histological sections of both pleural plaques (top image: harder plaque)

The detection of pleural fibrous plaques during autopsies represents a clinically significant entity and can allow the reconstruction of the occupational and pathological profile of the decedent. In addition, if found among skeletal remains, calcified pleural plaques can constitute a potential clue for identification if comparable antemortem information can be obtained. They can also provide critical data to the forensic anthropologist for the construction of the biological profile, given that they act as pathological markers and can survive decomposition processes.

In this study, we examined two pleural plaques of unknown causes extracted from an unidentified cadaver during autopsy and documented the macroscopic and histological appearance of these markers to help their recognition among skeletal remains.

5.3. The synergy between radiographic and macroscopic study of bone lesions

In this third research line, a comparative analysis was performed between macroscopic observation and radiographic imaging of the same bone lesions in various pathological conditions to investigate the strengths and pitfalls of both methods.

With this intent, plain radiographs were performed on fourteen skeletons of the CAL Milano Cemetery Skeletal Collection diagnosed with rheumatoid arthritis, diabetes, multiple myeloma, metastatic cancer, and osteomalacia to compare the macroscopic morphology and radiographic visualization of bone lesions. Over 200 osteolytic lesions and 65 areas of proliferative bone reaction (either spongiosclerotic or periosteal) were studied. "Comparative sets" of macroscopic photographs and radiographic imaging of the same osseous elements were realized to compare the detection and recognition of bone lesions. As a result, radiographic examination demonstrated that trabecular lesions may be lost through naked-eye observation, confirming data from the literature. However, many lesions, obvious macroscopically, were unperceived on radiographs due to contrast, including periosteal reactions, osteolytic lesions and spongiosclerosis. This article is the second research to also evidence the strengths of macroscopic examination in the analysis of bone lesions and the pitfalls of radiography in the observation of the same lesions, and the first one to do so for the conditions examined in the present study. This research argues in favor of a complementary approach between macroscopic observation and radiographic imaging for the recognition and diagnosis of bone lesions to avoid the loss of crucial information on bone disease and allow more informed diagnoses in forensic and archaeological cases.

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ORIGINAL ARTICLE

The synergy between radiographic and macroscopic observation of skeletal lesions on dry bone

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Abstract
The diagnosis of bone lesions is a fundamental part of the study of skeletal remains, both in the archeological and forensic context. On the one side, the literature proved the relevance of radiography for the detection of bone lesions; on the other side, the careful macroscopic observation of the morphology of bone lesions is often underestimated. For this study, we examined and performed plain radiography on 14 skeletons of the CAL Milano Cemetery Skeletal Collection diagnosed with rheumatoid arthritis, diabetes, multiple myeloma, metastatic cancer, and osteomalacia to compare the macroscopic morphology and radiographic visualization of bone lesions. At least 200 osteolytic lesions and 65 areas of proliferative bone reaction (either spongiosclerotic or periosteal) were studied. We realized "comparative sets" of macroscopic pictures and radiographic imaging of the same skeletal elements to allow comparisons of detection and recognition of bone lesions. As a result, while trabecular lesions may be lost through naked eye observation, many lesions can also be unperceived on radiographs due to contrast, including periosteal reactions, osteolytic lesions, and spongiosclerosis. The aim of this research was to investigate the strengths and pitfalls of digital radiography and macroscopic analysis and to demonstrate the synergy of a complementary approach between the two methods for lesion analysis in dry bone.

Keywords Forensic anthropology · Bone pathology · Radiographic imaging · Rheumatoid arthritis · Diabetes · Metastatic carcinoma

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Introduction

The study and recognition of bone pathologies is an important, although often underestimated, part of the forensic anthropology practice. When confronted with a skeletonized individual, the forensic anthropologist must construct an identikit or "biological profile" to help narrow down the potential pool of candidates for identification among a list of missing persons. This biological profile includes the estimation of sex, age, ancestry, and stature, the recording of anatomical variants as well as the analysis of pathological and/or traumatic lesions. In addition, as skeletal features that distinguish one individual from another, bone pathology can act as factors of individualization [1]. Despite their potential, the main issue with bone pathology is their recognition. As bones can only react in two ways to injuries (with either bone proliferation—osteoblastic activity—or bone remodeling—osteoclastic activity), bone reactions to different pathological conditions overlap. Complicating diagnosis even further are the non-specificity

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of most skeletal lesions and the variety of manifestations wrought by diseases.

Studies based on reference collections, as displayed in paleopathology manuals [2–5], can provide substantial information as they bypass the lack of personal history of archeological specimens and possess medical documentation associated to each individual. With this idea, the present authors have already published several articles on the macroscopic morphology and distribution of skeletal lesions in specific conditions based on a reference collection, the CAL Milano Cemetery Skeletal Collection, including rheumatoid arthritis [6], diabetes [7], multiple myeloma [8], and metastatic cancer [9–11]. However, not all lesions can be recognized through macroscopic observation.

Over the past 120 years, radiography provided a powerful tool for the diagnosis of bone diseases, allowing the visualization of changes in the internal bone structure in the clinical setting. Nonetheless, the sensitivity of radiography is not without limits. Indeed, a variation of about 40% in bone density is necessary to be visible through radiography [12], suggesting that some conditions may not be appropriately assessed with radiography. Even worse, some lesions can go completely undetected on radiographs, such as new bone deposition on the medial diaphysis of tibiae [12] or periosteal reactions on the visceral surfaces of ribs in intrathoracic chronic infections [13–17]. The emergence of modern imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI), and micro-CT permits a better observation of bone lesions, but their role in detecting bone diseases is not as well established as that of plain radiograms because access is generally inconvenient outside of a clinical context and they often lack standardized protocols [18].

In this article, we performed digital radiographs on skeletons studied in previous publications with antemortem clinical diagnoses of rheumatoid arthritis, diabetes, multiple myeloma, and metastatic cancer [6–11], as well as a skeleton with osteomalacia from the CAL Milano Cemetery Skeletal Collection, to compare the macroscopic morphology and radiographic visualization of bone lesions. While we know the relevance of radiography for the detection of bone lesions hidden from naked eye observation, are we really aware of the significance of a careful macroscopic observation of the morphology of bone lesions for their diagnosis? Indeed, in a study of osteoarthritis on 24 knee joints, Rogers and colleagues [19] report that abnormalities were detectable radiographically in only two specimens, while 16 showed evidence of macroscopic bone changes. The aim of this research is to explore not only the strengths of radiography in the study of bone diseases of skeletonized cases but also its pitfalls and to demonstrate the synergy of a complementary analysis between radiographs and macroscopic observation. To the best of our knowledge, this is the first study to perform a comparative analysis of bone lesions in this sense.

Material and methods

This research examined at least 200 osteolytic lesions and 65 areas of proliferative bone reaction (either spongiosclerotic or periosteal) from 14 skeletons of the CAL Milano Cemetery Skeletal Collection with specific pathological conditions. This collection is a contemporary and documented cemetery osteological collection of 2127 skeletons under study at the *Laboratorio di Antropologia e Odontologia Forense* (LABANOF), in the Department of Biomedical Sciences for Health, University of Milan, Italy [20]. All of the skeletons of the collection were first buried in a cemetery of Milan for a minimal duration of 10 years before being exhumed by cemetery workers with the use of heavy machinery. Each of the individuals of the collection is associated with a documentation including sex, age at death, dates of birth and death, cause of death, and additional pathologies clinically diagnosed before death. The years of birth of the individuals of the collection range from 1866 to 2000 and the dates of death from 1910 to 2001, but about 80% died after 1980 [20].

Among the individuals of the collection, 14 were selected for this study for the specific bone disease observable on their skeleton (Table 1). These include two skeletons with rheumatoid arthritis, four with diabetes, one with multiple myeloma, six with solid cancer, and one with osteomalacia. While the diagnoses of rheumatoid arthritis, diabetes, multiple myeloma, and cancer were performed clinically and recorded in the documentation associated to the individuals, the diagnosis of osteomalacia was realized anthropologically, based on the macroscopic study of the skeletal remains and by reference with the clinical and paleopathological literature [2, 3, 21–25]. Given the severe bending deformities observed on the bones of the lower legs (femora, tibiae, fibulae), left superior limb (humerus and scapula), and sternum (the remaining bones being either absent or heavily fragmented), the condition responsible could be narrowed to a metabolic disease. The differential diagnosis includes residual rickets and osteoporosis. Nine of the 14 individuals selected are females, and five are males. The age at death range was between 52 and 88 years, the years of birth between 1903 and 1940, and the years of death between 1990 and 1997. In each case, the year of diagnosis, duration of the disease, and potential treatment that might have influenced the course of the disease were not specified.

In a first step, each skeleton was carefully macroscopically examined for the presence of pathological lesions. Skeletal lesions were described using standard paleopathological terminology, measured with a Vernier caliper, and photographed for records. In a second step, plain X-ray radiographs were performed. Depending on the distribution of bone lesions (whether solitary or multiple), radiographs were carried out on particular bones (e.g., the lower legs in diabetes) to almost entire skeletons (e.g., cancer cases). Radiographic imaging

Table 1 Details of the individuals selected for the study

Case number	Sex	Age	Year of birth	Year of death	Diagnosis	References
277	F	76	1915	1991	Rheumatoid arthritis	[6]
1007	F	88	1903	1991		
20	F	82	1909	1991	Diabetes	[7]
25	M	52	1938	1990		
127	F	76	1914	1991		
395	M	76	1915	1991		
156	F	74	1916	1991	Multiple myeloma	[8]
366	M	85	1905	1991	Prostate cancer	[9]
897	M	56	1940	1997	Bladder cancer	[11]
422	F	59	1932	1992		
37	F	70	1920	1991	Breast cancer	[10]
459	F	83	1908	1992		
10	M	67	1923	1990	Liver cancer	–
7	F	79	1911	1991	Osteomalacia*	–

*Not clinically diagnosed

was realized in the service of radiology and medical imaging of the IRCCS Policlinico San Donato with a Siemens Luminos dRF Max1, with technical parameters ranging from 49.9 to 80.9 kVp and 0.2 to 2 mAs.

Five pathological conditions were studied in the present article, including rheumatoid arthritis, diabetes, multiple myeloma, metastatic cancer, and osteomalacia. Each of these conditions can affect the musculoskeletal system and will be defined below.

Rheumatoid arthritis

Rheumatoid arthritis is a chronic, progressive, systemic, and proinflammatory autoimmune condition affecting principally women (3:1 ratio) of about 30–50 years of age [26–28]. The erosive arthropathy is characterized by minimum to absent new bone deposition [2–4, 29]. It primarily affects the joints of the wrist and hands in a polyarticular, symmetric, and bilateral involvement, later spreading to more distant weight-bearing articulations including the elbows, shoulders, ankles, knees, temporomandibular joint, and atlantoaxial joint [28–33]. Contrary to the spondyloarthropathies, rheumatoid arthritis shows a sparing of the distal interphalangeal joints as well as sacroiliac and/or spinal fusion [2, 27, 34–36]. Skeletal erosions may lead to musculoskeletal complications including subluxation and dislocation of the atlantoaxial joint, "pencil-in-cup" deformities, ulnar deviation of the hand, and carpal and/or tarsal ankyloses [30, 37–39].

Diabetes mellitus

Diabetes mellitus is a disorder characterized by chronic hyperglycemia. Diabetes does not directly involve the skeleton, but diabetic complications may result in bone lesions. Indeed, complications in diabetes may be classified into microvascular and macrovascular. The latter constitute a major cause of morbidity and mortality in diabetes and may lead to peripheral artery disease and myocardial infarction or stroke [40–42]. The former include retinopathy, nephropathy, and neuropathy. While all three cause a range of clinical symptoms and complications, diabetic neuropathy is of particular interest in the study of bone lesions as it may lead to peripheral loss of the sense of touch, proprioception, temperature, and vibration, leaving the feet vulnerable to undetected microtrauma which may result in foot ulcerations and infections, fractures, dislocations, destructions, and deformities of the bones of the feet (such as Charcot's joints) [40, 41, 43]. The diagnosis of diabetes cannot be ascertained on dry bones, but lesions such as periosteal reactions, resorptions of the distal tufts, lytic lesions, evidence of trauma, osteochondritis dissecans, and vascular calcifications may be observed [7].

Multiple myeloma

Multiple myeloma is a hematological malignancy with a frequency of bone disease close to 100% [44, 45]. Skeletal lesions are exclusively osteolytic, spheroid, small to medium sized (5 to 2 cm in diameter), and with sharp or "punched-out" margins. Bone lesions in multiple myeloma are multifocal, scattered, and numerous, most commonly found on the vertebrae, sacrum, pelvis, ribs, proximal femur and humerus, skull, and sternum [2, 3, 46–49].

Metastatic solid cancers

Bone is a preferred site of metastasis from solid cancers as the bone microenvironment presents an excellent site for tumor survival, growth, and further extension in the body [50, 51]. Bone metastases may be osteolytic (bone resorption), osteoblastic (bone formation), or mixed (bone formation and

resorption), but generally, osteoclastic and osteoblastic components are both expressed, and skeletal metastases range from predominantly osteolytic to predominantly osteoblastic [44, 45, 52]. Osteolytic metastases may appear as coalescent porosity (coalescence of superficial pits) or round to oval osteolytic lesions in the trabecular bone but perforating the cortex with denticulate margins [46, 47, 53, 54]. Osteoblastic metastases may produce new bone deposits in the spongy bone thickening the existing trabeculae or "spongiosclerosis" [2], lay down on the cortical surface periosteal new bone either excrescent-coarse [55, 56], coral-like [15, 56], or, more rarely, spiculated [57, 58] fiber bone. Metastatic lesions from solid cancers tend to be multifocal rather than isolated [49, 59] and located in hematopoietic areas of the skeleton, especially the vertebrae, pelvis, ribs, skull, and ends of the long bones and are unusual distal to the knees and elbows [2, 3, 60].

Osteomalacia

Osteomalacia is a metabolic disorder caused by a deficit in vitamin D and resulting in bending deformities and pseudofractures. The latter correspond to small, linear fractures with the formation of an irregular callus. They are mostly found in the ribs, scapula, clavicle, ulna, pelvis, and femur. The former occur as a distortion of the bone under normal weight-bearing due to the weakening of the insufficiently mineralized bone and may affect the long bones, pelvis, and sternum and can cause kyphosis [21–25].

For easiness of reading, a few of the macroscopic terms used in the present article will be clarified here:

- Woven bone refers to an abnormal bone formation characterized by a distinct layer of immature periosteal new bone on the cortical shell and a porous-appearing surface. Woven bone typically indicates an early stage of development of the condition [2, 61].
- Over time, woven bone remodels into lamellar bone (also called remodeled bone) suggesting that the condition responsible has progressed in a later chronic stage or has healed. However, healed woven bone remodeled into lamellar bone may retain its sponge-like structure as evidenced by the presence of fine pores on its surface [2, 61].
- Bone atrophy is caused by an imbalance in the activity of osteoblasts and osteoclasts with increased bone resorption [2] and results in the resorption of part of a bone (an extremity or a process) altering the normal structure of the bone and potentially deforming the articulation (if the lesion involves a joint).
- Spongiosclerosis consists in subcortical osteoblastic activity within the space of the cancellous bone structure as "bone-in-bone"; the spongy bone visually appears abnormally thickened [2].
- Periosteal reactions refer to a deposition of new bone on the surface of the cortex and was observed in two various subtypes in this study: "coral-like" periosteal reaction is a thick new bone deposition, with small and dense spicules, giving an enlarged aspect to the bone [11, 15], whereas "sunburst" periosteal reaction appears as radiating spicules [58].

Results

As the objective of this research was to compare macroscopic and radiographic visualization of bone lesions, we realized "comparative sets" of macroscopic pictures and radiograms of the same skeletal elements/bone lesions, taken in the same position as far as possible, and assembled as one figure side by side. The analysis of the complementary potential of radiographic and macroscopic observation of bone lesions will be based on these comparative sets and divided per pathological condition (Table 2).

Rheumatoid arthritis

In the present research, two individuals had an antemortem clinical diagnosis of rheumatoid arthritis (Table 1).

Macroscopic observations

The left hand of case number 1007 is presented in Fig. 1, on the right. The second metacarpal (MC2) presented on its proximal base six round to oval perforations of the bone cortex connecting in a single osteolytic lesion, with sharp margins and deep trabecular involvement ranging in size from 2.1 to 6.9 mm. Marginal osteophytes were noted on proximal interphalangeal joints (PIPs) and distal interphalangeal joints (DIPs). Remodeled bone apposition was observed on the palmar and dorsal surface of the proximal half of the third intermediate phalanx, focalized on the lateral side; in the absence of articular erosion and any other alteration, the lesion was suggestive of an osseous healing reaction to a trauma. Alterations on the triquetral, MC1 and MC5 were caused by taphonomic factors.

On the left of Fig. 1, two comparative sets of case number 277 are presented: the left hand (on the top) and the right foot (excluding tarsals—on the bottom). On the left hand, osteolytic erosions are present on the base of MC1, MC2, MC3, and MC4 and the third proximal phalanx with remodeling, creating deformations (understood here as alterations of the normal shape) of the articulations. MC1, MC5, and the third intermediate phalanx suffered taphonomic changes. Similarly, on the right foot, the atrophy of all metatarsal (MT) heads and base of the first proximal phalanx completely

altered the metatarsophalangeal joints (MTPs). The bases of the proximal phalanges are widened (particularly noticeable on the fifth proximal phalanx) due to osteoclastic activity and slow remodeling, and the first proximal phalanx shows on the plantar surface an osteolytic lesion of 5.5 mm × 2.8 mm (not observable in Fig. 1). Taphonomic alterations are present on the first distal and fifth proximal phalanges and the base of all MT. Figure 2 shows the comparative sets of the right clavicle, axis, and ulnae of case number 277. The only taphonomic alteration was present on the acromial end (fragmented margin) of the clavicle. The remaining alterations consisted in the atrophy of the sternal end of the clavicle, the odontoid process of the second cervical vertebra, and the distal ends of the ulnae which resulted in the complete resorption of the styloid processes.

Radiographic observations

Minimal osteophytic bone formation was observable on the PIP and DIP of the left hand of case number 1007 (Fig. 1). The base of MC2 showed a clear radiolucent lesion at its base. This central articular erosion has a minimum diameter of 9.7 mm and a maximum diameter of 12.7 mm. Another radiolucent lesion can be seen on the base of the third intermediate phalanx of 12.6 × 10.3 mm, appearing as a subchondral cyst. In this case, the radiograph allows a better observation of the articular erosion and a greater understanding of the nature of the lesion on the third intermediate phalanx. Given that only proliferative reaction was observed through naked eye observation, the lesion seemed to be related to bone reaction to trauma. The plain X-ray, however, shows the lesion to be a subchondral cyst and not a fracture, which is consistent with the existing rheumatoid arthritis. Juxta-articular osteoporosis was also noted.

On the plain radiograph of the left hand of case number 277 (Fig. 1, top left), the morphostructural deformations of the bases of the metacarpals and third proximal phalanx are notable. In addition, radiodense areas can be seen on the distal third of the shaft of the third proximal phalanx, MC1 and MC2, and on the proximal third of the shaft of MC3. In light of the macroscopic observations, these radiodense areas can be attributed to slow bone remodeling consequent to the osteolytic erosions of the metacarpophalangeal and carpometacarpal joints. On the radiograph of the bones of the right foot of case number 277 (Fig. 1, bottom left), the atrophy of the MT and base of the proximal phalanx and the widening of the fifth proximal phalanx are clearly visible. The X-ray also shows radiolucent areas within the body of the first and fifth proximal phalanges, on the first distal phalanx and in the bases of the MT, as well as radiodensity along the cortical margins of MT1, MT2, and MT5. While the radiolucent areas of the first and fifth proximal phalanges, first distal phalanx, and bases of the MT may be attributed to taphonomic alterations, an oval radiolucent lesion with cortical involvement of 7.3 mm × 4.1 mm can be observed in the body of the first proximal phalanx distinct from postmortem damage. On the radiographic counterparts of the right clavicle, axis, and ulnae of case number 277 (Fig. 2), the atrophy of the processes (sternal end of the clavicle, odontoid process of the axis, styloid processes of the ulnae) was noted with very little, if any, cortical radiodensity on the remodeled margins.

Diabetes mellitus

Four skeletons with recorded diabetes were selected for this study (Table 1).

Macroscopic observations

The right foot of case number 20 (Fig. 3) showed the atrophies of the head of MT1 and base of the first proximal phalanx with a deep trabecular involvement and remodeling. In addition, an osteolytic erosion of the tuft of the second distal phalanx and concentric narrowing of the third and fourth distal phalanges were noted. Bone proliferation was also reported though marginal osteophytes on all metacarpophalangeal and interphalangeal joints as well as periosteal reaction on all MT and first proximal phalanx. The first metatarsal of the left foot of case number 20 (Fig. 3, right) presented a large para-articular osteolytic erosion of 15.4 mm × 13.1 mm and smaller articular erosions ranging from 2.4 to 4.9 mm in diameter on the atrophied head (not visible in Fig. 3) as well as healed woven bone on its plantar surface.

The fifth metatarsal of case number 395 (Fig. 4, top left) showed bone atrophy with complete resorption of the head and deformation of the normal shape of the proximal base, as well as woven and remodeled periosteal reactions spread along the cortical surface.

The third metatarsal of case number 127 (Fig. 4, top right) exhibited a round osteolytic lesion of 5.5 mm × 3.7 mm with remodeled margins and deep trabecular involvement. The morphological aspect of the lesion was highly consistent with osteomyelitis, the osteolytic perforation of the cortex representing the cloaca.

In the case number 25, the long bones of the lower leg were of particular interest (Fig. 4, bottom) as they presented substantial remodeled periosteal reaction along the entire cortical surfaces of both tibiae and fibulae. In addition, attachments of ligaments on the distal ends of the bones were ossified and presented as dense spicules of compact bone.

Radiographic observations

The structural alterations of the head of MT1 and base of the first phalanx of the right foot of case number 20 (Fig. 3) were clearly visible with bone remodeling and subchondral

Table 2 Pros and cons of macroscopic and radiographic analyses of bone lesions per pathological condition

	Macroscopy		Radiography		Summary
	Observed	Not observed	Observed	Not observed	
Rheumatoid arthritis	- Articular and para-articular osteolytic lesions - Bone atrophy - Distribution	- Trabecular osteolytic lesions such as cysts	- Articular and para-articular osteolytic lesions - Bone atrophy - Distribution - Trabecular osteolytic lesions		Macroscopy or radiography alone is enough for the diagnosis
Diabetes mellitus	- Articular and para-articular osteolytic lesions - Bone atrophy - Periosteal reactions		- Articular and para-articular osteolytic lesions - Bone atrophy	- Periosteal reactions	Important lesions go unnoticed with radiographs rendering macroscopic observation essential
Multiple myeloma	- Cortical osteolytic lesions	- Trabecular osteolytic lesions	- Cortical osteolytic lesions - Trabecular osteolytic lesions		Although not necessary for the recognition of the condition if enough osteolytic lesions are present, radiography shows the true extent of bone involvement
Metastatic cancer	- Cortical osteolytic lesions - Periosteal reactions	- Trabecular osteolytic lesions - Spongiosclerosis (observable only if the cortical bone is damaged)	- Cortical osteolytic lesions - Trabecular osteolytic lesions	- Periosteal reactions - Osteolytic and mixed lesions may be missed - Spongiosclerosis (observable only if the lesion is extensive)	Macroscopic observation is essential for the identification of periosteal and mixed lesions (and some osteolytic lesions) Radiography is essential for the detection of trabecular lesions
Osteomalacia	- Bending deformities - Periosteal reactions		- Bending deformities	- Periosteal reactions	Both analyses can identify bending deformities, but periosteal reactions may be missed on radiographs

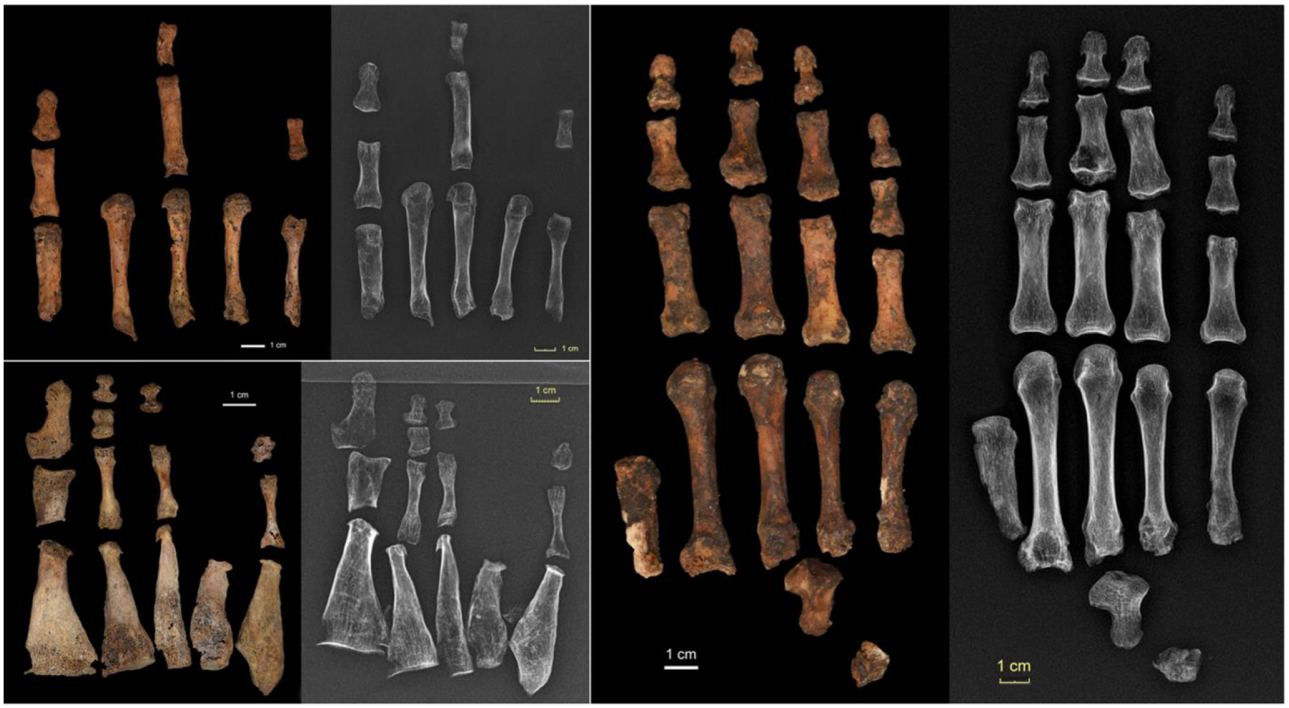

Fig. 1 *Rheumatoid arthritis*: comparative sets of the left hand (top left) and right foot (bottom left) of case number 277 and the left hand of case number 1007 (right)

sclerosis. The marginal osteophytes and periosteal reaction were also noted, but the lesions on the distal phalanges could not be recognized as the contrast on these small tubular bones is much reduced. The large para-articular osteolytic lesion of the head of the left MT1 was also very distinct on X-rays (measuring 13.9 mm); nonetheless, the periosteal reaction was identified macroscopically but not through radiography.

Similarly, the osteolytic lesions (bone atrophies) on the head and base of MT5 of case number 395 (Fig. 4, top left)

Fig. 2 *Rheumatoid arthritis*: comparative sets of the right clavicle (top left), axis (bottom left—right lateral view, superior is up), and ulnae (right) of case number 277

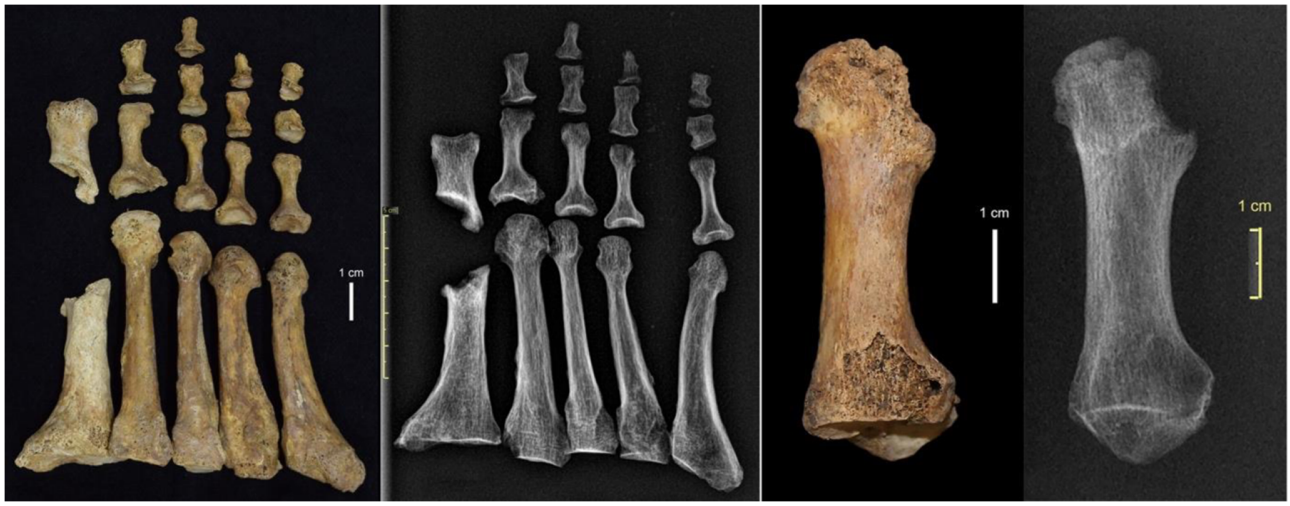

Fig. 3 *Diabetes mellitus*: comparative sets of the right foot (left) and right first metatarsal (right) of case number 20

were recognized on X-rays, but the periosteal reaction on the body was not perceived.

The radiological visualization of MT3 of case number 127 (Fig. 4, top right) proved surprising as the osteomyelitis was

Fig. 4 *Diabetes mellitus*: comparative sets of the left fifth metatarsal of case number 395 (top left—superior view, distal is right), the right third metatarsal of case number 127 (top right—superior view, distal is right), and the distal half of the fibulae and tibiae of case number 25 (bottom)

not recognized. An area of radiolucency of 10.1 mm × 2.9 mm was present within the trabecular bone and adjacent to the radiolucent lesion with cortical involvement (identified as cloaca macroscopically), but the appearance was distinct from a classic case of osteomyelitis. No clear sequestrum or involucrum could be identified.

The shafts of the tibiae and fibulae of case number 25 (Fig. 4, bottom) showed a thickening of the cortical layer by periosteal bone apposition (the medullary cavity was not affected), and periosteal reaction could be observed on the distal ends. Unlike the compact macroscopic observations, this periosteal new bone appeared disrupted and spiculated on X-rays.

Multiple myeloma

One skeleton with multiple myeloma was selected in this study (Table 1).

Macroscopic observations

The cranium (Fig. 5, top left) was not washed with water as the bones were particularly fragile due to taphonomic alterations. Nonetheless, numerous small spheroid osteolytic lesions with well-defined margins were found on the neurocranium (especially on the endocranial surface) and scattered across the skeleton: on the mandibular rami, sternal end of the clavicles, proximal ends of the humeri and femora, manubrium, ribs, bodies and neural arches of the vertebrae, sacrum (Fig. 5, bottom left), pubis, anterior and posterior ilia (Fig. 5, right), and around the glenoid fossa and spine of the scapulae. The morphology and distribution of these osteolytic lesions is typical of multiple myeloma.

Radiographic observations

Radiography showed an important number of small radiolucent lesions ("raindrop skull"), by far more numerous than observed macroscopically but with the same distribution, and diffuse osteopenia.

Metastatic cancer

Six cases with metastatic cancer were selected for this study, including one individual with prostate carcinoma, two with bladder carcinoma, two with breast carcinoma, and one with liver carcinoma (Table 1).

Macroscopic observations

In Fig. 6, the crania of case numbers 10, 459, and 37 are presented. The cranium of case number 10 exhibited two macroscopic osteolytic lesions on the frontal bone (including one large lesion of 21.9 mm × 31.9 mm visible on the top image of Fig. 6) and six on the parietals (not visible on the photograph) with denticulate margins, a greater involvement of the diploë compared with the tables and maximum diameters ranging from 5.1 to 31.9 mm. The cranium of case number 459 evidenced a single osteolytic lesion with denticulated margins of 13.3 mm × 17.3 mm on the right temporal bone. The morphological characteristics of the osteolytic lesions in both crania are typical of metastatic carcinoma [46, 47, 53, 54]. By opposition, the third cranium (case number 37) exhibited 20 lesions ranging in size from 2.4 to 15.2 mm and altered by taphonomic processes (white margins, soil within the lesions, superposed postmortem alterations). The diagnosis of the lesions was confusing, and in the absence of other indicators on the skeleton suggesting the presence of a metastatic cancer, pseudopathology would have remained a strong possibility.

Taphonomic alterations also resulted beneficial to the macroscopic observation of pathological lesions; indeed, the inferior and right lateral cortical layers of the manubrium of case number 422 (Fig. 7, top images) were fragmented and allowed the observation of three spongiosclerotic lesions in the trabecular bone: one large lesion can be viewed inferiorly, and two more discreet and deeper spongiosclerotic lesions can be noted after careful observation of the lateral side of the bone. The mandible of case number 422 (Fig. 7, bottom) showed osteolytic lesions on the mandibular rami with denticulate margins as well as small areas of trabecular bone with spongiosclerosis (not observable on the picture). Similar observations were made on the left innominate of case number 37 (Fig. 8, top left): multiple osteolytic lesions with deep trabecular involvement and scalloped margins were found ranging from 6.4 to 64.8 mm associated with proliferative reaction in the form of small areas of spongiosclerosis (not observable on the picture). The ilium of case number 422, by opposition, showed four macroscopic lesions: two osteolytic metastases and two mixed metastases with osteolytic lesions and spongiosclerosis of the exposed trabecular bone. The osteolytic lesions ranged in size from 5.0 to 37.1 mm.

The left innominate of case number 366 (Fig. 8, right) exhibited periosteal reaction on the whole surface of the anterior and posterior iliac fossa as well as around the greater sciatic notch. Bone proliferation was present as woven bone, remodeled bone, coral-like, and sunburst periosteal reaction. Sunburst periosteal reaction was located on the anterior and posterior iliac fossa, whereas coral-like periosteal reaction was focalized around the greater sciatic notch. The spicules of the sunburst reaction reached 3.1 mm.

The ribs of case number 897 presented osteolytic and osteoblastic lesions, including coral-like periosteal reaction (Fig. 9, top left). Small osteolytic lesions (ranging from 0.8 to 3.1 mm in diameter) were perforating the cortex of the ribs (head, angle, and body). The first, third, and fourth ribs (from

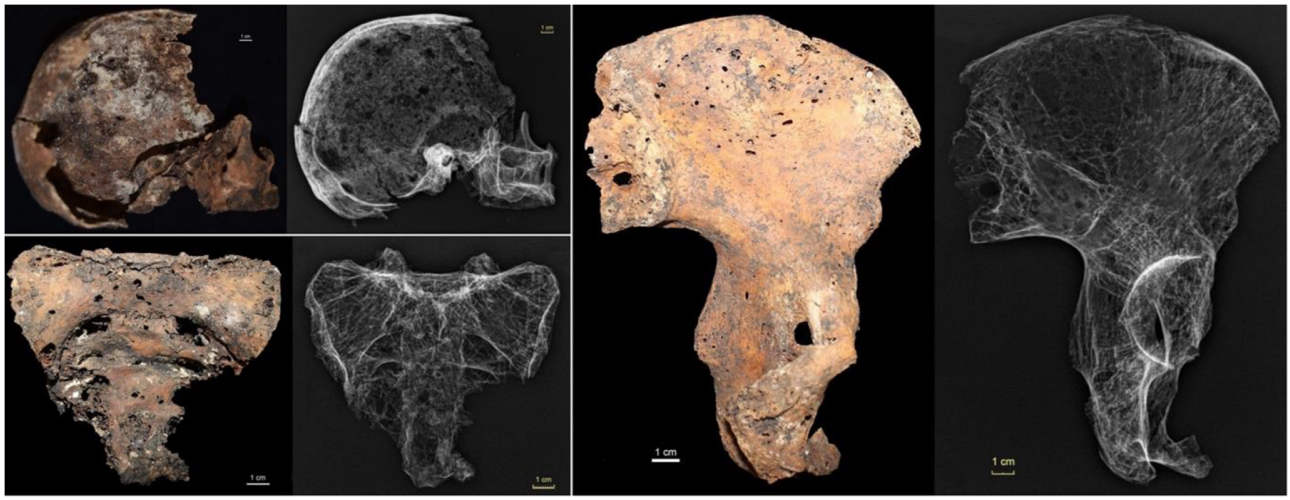

Fig. 5 *Multiple myeloma*: comparative sets of the cranium (top left—*norma lateralis*, right side), sacrum (bottom left), and right innominate (right) of case number 156

the top of the picture—the exact number of the rib was not determinable—the fourth rib was represented by a fragment of its body) manifested coral-like periosteal reaction on their bodies with a maximum thickness of 3.7 mm. The second rib (from the top of the picture) showed a thick layer of remodeled periosteal reaction not exceeding 0.3 mm in thickness, suggesting a coral-like periosteal reaction in its initial stages of development. The periosteal reactions were mainly located on the visceral surface of the ribs but were also present on the external layer, giving an enlarged aspect to the ribs in superior view.

In the skeleton of case number 897, 22 metastases were found including 17 mixed metastases (that is, metastases with both osteoblastic and osteoclastic components). The thoracic vertebrae T5 and T6 (Fig. 9, bottom left) and T7–T12 (Fig. 9, right) were fused together through ankylosis of the articular facets and osteophytic bridges. T7, T8, and T9 showed coalescent porosity (osteolytic activity on the bone surface creating a confluence of superficial pits) on their neural arches and woven periosteal reaction on the lateral sides of the neural arches. The same lesions were noted on T5 and T6 (coalescent porosity and periosteal reaction) as well as a large osteolytic lesion on the left side of the neural arch of T5 of 15.7 mm × 13.8 mm with spongiosclerosis in the underlying trabecular bone.

Radiographic observations

On the cranium of case number 10, at least 18 geographic well-defined radiolucent lesions with absent margins are visible, ranging from 3.0 to 35.0 mm in diameter. In this case, multiple myeloma constitutes a major differential diagnosis. Through naked eye observation, an osteolytic lesion was noted on the ectocranial surface of the right temporal bone of case number 459; however, if one does not already know exactly where to look, the lesion may appear invisible to the reader on the plain X-ray or may easily be confused with taphonomic alterations. The radiograph of the cranium of case number 37 was unencumbered by the secondary taphonomic alterations hindering macroscopic interpretations of the lesions and showed clear evidence of bone disease with the presence of multiple geographic radiolucent lesions with ill-defined margins ranging from 4.7 to 16.9 mm in size.

Four spongiosclerotic metastases can be counted on the sternum of case number 422 (Fig. 7, top images), while only three were perceived macroscopically. They presented as radiodense lesions with an ivory-like matrix, ill-defined margins, and a maximum diameter ranging from 9.2 to 18.9 mm. On the radiograph of the mandible of case number 422 (Fig. 7, bottom), radiolucent areas with ill-defined margins can be recognized, but no sclerosis was noted. Similarly, radiolucent areas with absent or ill-defined margins were even more numerous on the X-ray of the left innominate of case number 37 (Fig. 8, top left) than seen macroscopically, but no sclerosis was present. The radiograph of the ilium of case number 422 evidenced the presence of at least 25 lesions, contrasting with the four lesions observed macroscopically, including radiolucent areas with ill-defined margins and radiodense lesions (of 3.3 to 28.5 mm in diameter) with ivory-like matrix.

The left innominate of case number 366 (Fig. 8, right) showed a radiodensity with ill-defined margins on the superior area of the greater sciatic notch, coinciding in part with the location of the coral-like periosteal reaction observed macroscopically. A longitudinal ill-defined area of radiodensity was located on the iliac fossa, surrounded by two areas of radiolucency, one of them directly positioned where the sunburst periosteal reaction was found macroscopically. This is not the only time periosteal reaction, clearly observed macroscopically, never appeared on radiographs. While the spicules of

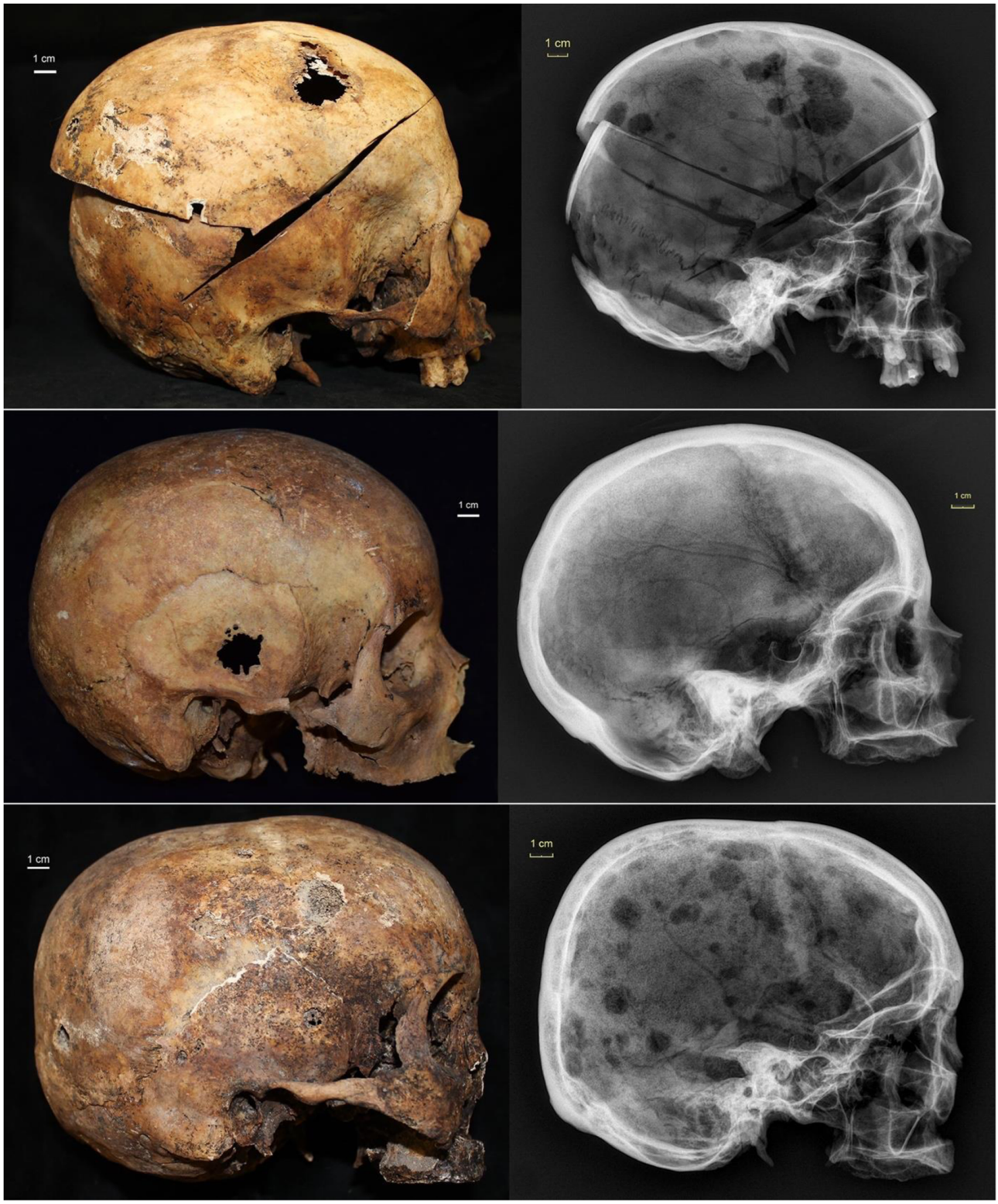

Fig. 6 *Metastatic carcinoma*: comparative sets of the cranium of case number 10 (top—*norma lateralis*, right side), case number 459 (middle—*norma lateralis*, right side), and case number 37 (bottom—*norma lateralis*, right side)

the first and third ribs of case number 897 (Fig. 9, top left) are clearly visible on the radiograph, the periosteal reaction of the second rib, giving an enlarged aspect to the bone, is completely absent on the X-ray, which shows only two discrete ill-defined

radiolucent lesions. The fourth rib, represented by only a fragment of its body, appears more altered by osteolytic lesions than proliferative ones on plain X-rays, and the marked coral-like periosteal reaction covering the bone is not recognized.

Mixed metastases manifesting as coalescent porosity associated with periosteal reaction (T5 and T6, Fig. 9, bottom left; T7–T12, Fig. 9, right) or osteolytic lesions exposing spongiosclerosis (T5 and T6, Fig. 9, bottom left) were macroscopically observed on the vertebrae of case number 897. Nonetheless, none of these lesions was visible on the radiographs of the same bones: ill-defined sclerotic areas could be seen on the superior margin of the vertebral body of T5 and the pedicles of T7 and T9, but their interpretation was unspecific.

Osteomalacia

The skeleton with osteomalacia in this study was not clinically diagnosed, but the pathological analysis of bone lesions was highly consistent with the condition (Table 1).

Macroscopic observations

Several severe bending deformities were noted on the femora, tibiae, fibulae, sternum, left humerus, and scapula (Fig. 10, left). The rest of the skeleton was too fragmented for pathological analysis. A callus was noted on the proximal left tibia and on the distal left radius corresponding to a Colles' fracture (Fig. 10, right).

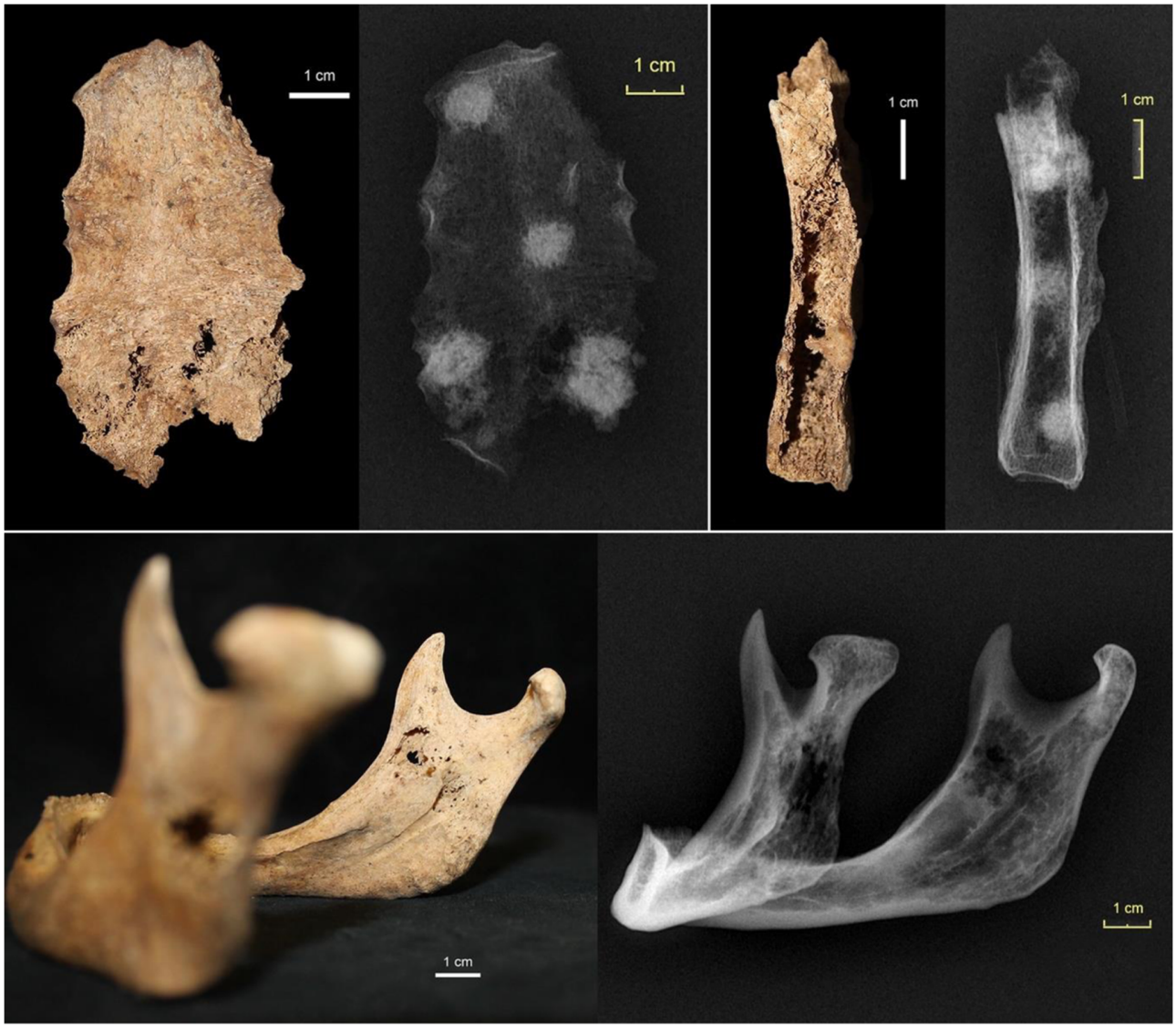

Fig. 7 *Metastatic carcinoma*: comparative sets of the sternum (top left: anterior view; top right: right lateral view) and mandible (bottom) of case number 422

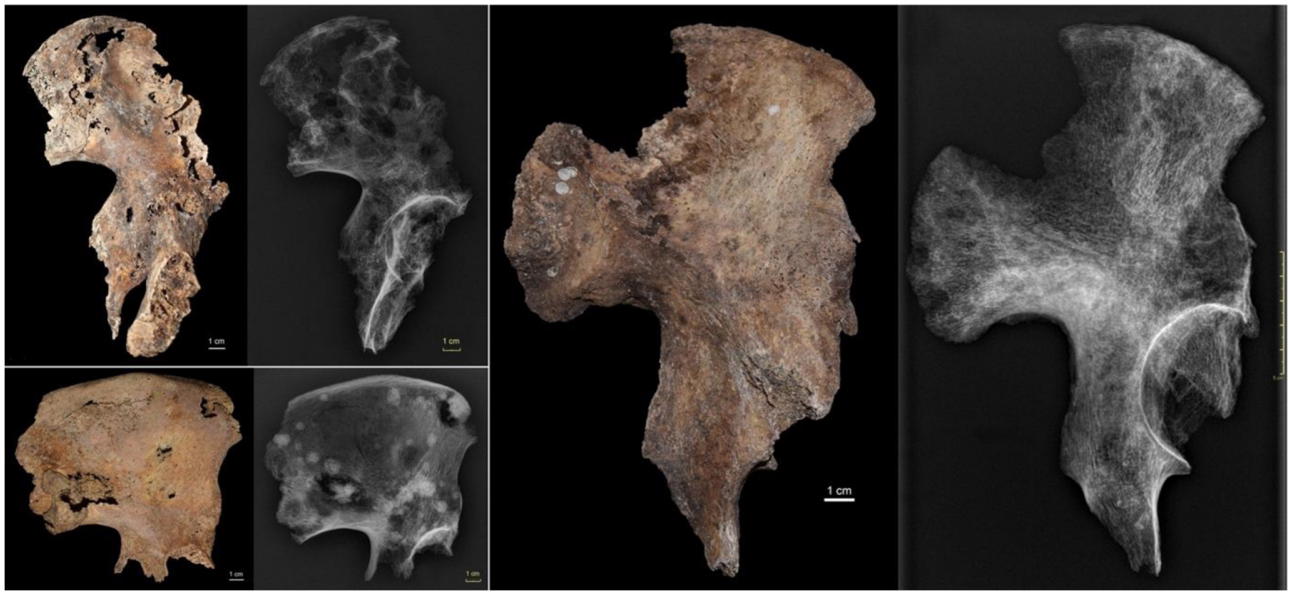

Fig. 8 *Metastatic carcinoma*: comparative sets of the left innominate of case number 37 (top left), case number 422 (bottom left), and case number 366 (right)

Radiographic observations

The severe morphostructural deformations with bending deformities were equally observed radiographically, as well as diffuse osteopenia (Fig. 10, left); however, no sign of fracture was noted on the distal left radius (Fig. 10, right).

Discussion and conclusion

In the present research, we comparatively examined the morphology of at least 200 osteolytic lesions and 65 areas of proliferative bone reaction (whether spongiosclerotic or periosteal) from 14 skeletons with rheumatoid arthritis (two indi-

Fig. 9 *Metastatic carcinoma*: comparative sets of the ribs (top left), 5th and 6th thoracic vertebrae (bottom left—lateral view), and 7th to 12th thoracic vertebrae (right—lateral view) of case number 897

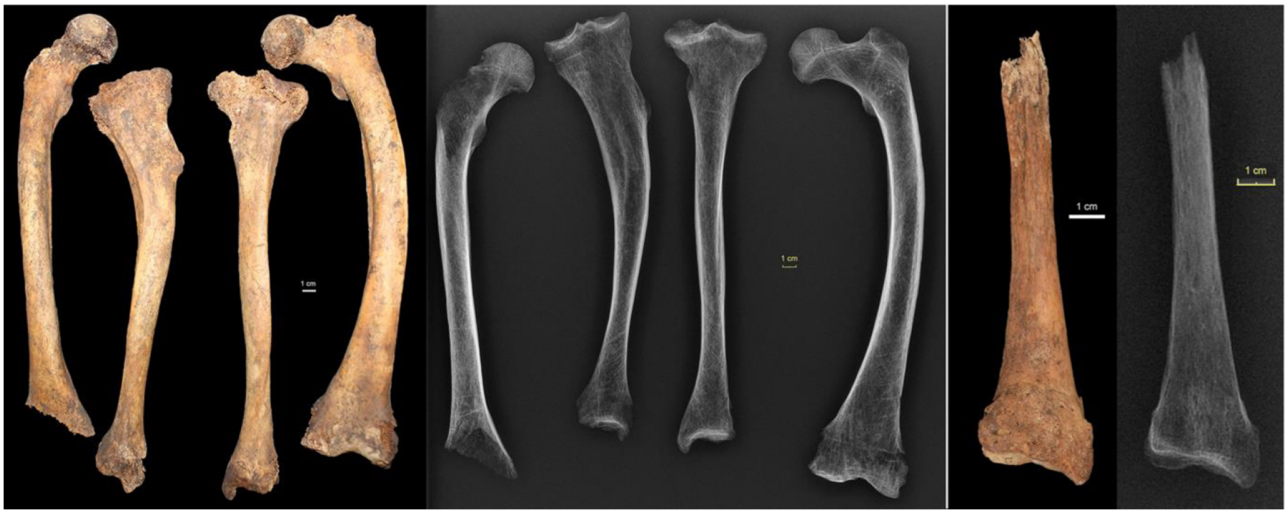

Fig. 10 *Osteomalacia*: comparative sets of the femora and tibiae (left) and left radius (right) of case number 7

viduals), diabetes mellitus (four individuals), multiple myeloma (one individual), metastatic carcinoma (six individuals), and osteomalacia (one individual) through macroscopic observation and radiographic imaging (Table 2).

Rheumatoid arthritis

In both cases of rheumatoid arthritis, the macroscopic analysis of bone lesions was essential for the recognition of the condition as the nature, location, and distribution of the lesions are typical of the autoimmune condition. Nonetheless, the subchondral cyst of the left third intermediate phalanx of case number 1007 (Fig. 1) was identified by X-rays but was not recognized by macroscopic observation of the cortical lesion. While the radiographs did provide additional information on the nature of the lesion (by showing areas of radiodensity in atrophic lesions), the extent of osteolytic erosions (left MC2 of case number 1007—Fig. 1), and allowed the observation of a subchondral cyst, they did not significantly help in the diagnosis of the arthropathy.

Diabetes mellitus

Several lesions were present in these diabetic cases, including periosteal reactions (case numbers 20, 25, and 395), osteomyelitis (case number 127), bone atrophy, and osteolytic lesions (case numbers 20 and 395). The osteolytic reactions were clearly identified on radiographs, but the osteomyelitis and periosteal reactions were not recognized as through macroscopy. This may be due to the sensitivity of radiography as a change of about 40% in bone density is necessary for detection on plain X-rays [12]. Consequently, if not observed macroscopically, some lesions in diabetes may be missed.

Multiple myeloma

Numerous small spheroid osteolytic lesions with well-defined margins were found scattered across the skeleton, including on the cranium, mandible, clavicles, sternum, ribs, vertebrae, pelvis, sacrum, scapulae, and proximal ends of the humeri and femora. The morphological characteristics and pattern of distribution of the osteolytic lesions found in case number 156 are typical of the hematological malignancy. While macroscopy would have been enough in this case for the diagnosis of multiple myeloma, radiographic imaging of the bones showed a much deeper osseous involvement and a better observation of the osteolytic lesions and provided a stronger argument for diagnosis.

Metastatic cancer

Three skeletons presented osteolytic lesions on the cranium (case numbers 10, 459, and 37) and resulted in very different radiographic images (Fig. 6). When looking at the radiograph of the cranium of case number 10, multiple myeloma remains an important possibility in the different diagnosis whereas the macroscopic appearance of the lesions is typical of osteolytic lesions in metastatic carcinoma [46, 47, 53, 54]. This is due to the loss of the morphological details of the margins of the lesions, one of the criteria distinguishing metastatic carcinoma from multiple myeloma [2, 3, 8, 10, 46, 47]. The only factor suggesting metastatic carcinoma as a stronger diagnosis is the dimension of lesions but as Rothschild and colleagues [47] conclude "size variation does not appear to be a sufficiently reliable criterion for distinguishing between the diseases." Although no diagnosis can be ascertained based solely on the macroscopic appearance of lesions, the morphological

details of the osteolytic lesions render the diagnosis of multiple myeloma inconsistent with the macroscopic observations. The comparative set of the cranium of case number 459 shows the importance of macroscopic observation as a large osteolytic lesion with morphological characteristics typical of metastatic carcinoma may be missed from the anthropological analysis if the bones are not macerated and carefully macroscopically examined. Finally, the comparative set of the cranium of case number 37 demonstrates the strengths of radiographic imaging with the detectability of lesions hidden from macroscopic observation (located in the trabecular bone/diploë) and the recognition of pathological lesions in spite of taphonomic alterations. The literature explains that one major complication of the radiographic imaging of skeletal remains (contrary to clinical cases) is the effect of taphonomy that may mimic pathological bone lesions on radiographs [18], but in this particular case, the X-ray helped clear the differential diagnosis.

As radiographs allow the visualization of the internal structure of bones, skeletal lesions located in the trabecular tissue can be recognized through plain X-rays whereas they remain unperceived through naked eye observation, concealed by the cortical shell. Spongiosclerotic osteoblastic metastases constitute such an example. The particular location of these lesions (within the trabecular bone) would render their macroscopic observation impossible but postmortem damage to the bone fragmented the cortical shell and some areas of spongy bone, allowing the observation of abnormal thickening of parts of the trabecular structure through the deposition of new bone within the existing trabeculae. Therefore, even spongiosclerotic lesions can be observed macroscopically, given the right conditions. Both situations were evidenced in these comparative sets: the presence of spongiosclerotic lesions hidden from macroscopic observation but caught on radiographs (sternum and left ilium of case number 422, Figs. 7 and 8) and the macroscopic finding of discrete spongiosclerosis around osteolytic lesions, unperceived by radiography (mandible of case number 422, Fig. 7 and left innominate of case number 37, Fig. 8).

The identification of a proliferative reaction in the lesion is not a minor detail, as it indicates healing, and thus informs the reader on the pathological process. Proliferative reactions in metastases may also manifest in the form of periosteal reactions: instead of osteoblastic activity occurring in the trabecular bone, the new bone deposits are laid down on the cortical surface of the skeletal element. However, periosteal reactions on the innominate of case number 366 and the ribs of case number 897 were not recognized as through macroscopy. The lack of identification of these periosteal reaction does not only mean that osteoblastic metastases may go unnoticed in radiographs but also that information on the aggressiveness of the lesions may be lost due to the sensitivity of radiographic imaging. Sunburst and coral-like bone lesions are interrupted periosteal reactions implying that the "speed of progression does not provide the periosteum adequate time for consolidation" [18] and thus indicate a more aggressive lesion.

Because of their location and the orientation of the bones, osteolytic metastases may be lost on radiographs (cranium of case number 459, Fig. 6) and the sensitivity of radiography may not allow perceiving osteoblastic metastases (mandible of case number 422, Fig. 7; left pelvic bones of case numbers 37 and 366, Fig. 8; ribs of case number 897, Fig. 9). The combination of these two factors may thus result in the loss of mixed metastases on plain X-rays, and the vertebrae of case number 897 are an example of this situation: an osteolytic lesion exposing a thickened trabecular bone (spongiosclerosis) and coalescing porosity associated to woven and remodeled periosteal reactions were macroscopically observed on T5–T6 and T7–T12 but not recognized on the X-rays of the same bones (Fig. 9).

Metastatic cancer may cause osteolytic, osteoblastic, or mixed skeletal lesions in the cortical and trabecular bone. Radiography has proven essential in the detection of bone lesions hidden from macroscopic observation, including osteolytic (cranium of case number 10, Fig. 6; cranium and left innominate of case number 37, Figs. 6 and 8) and spongiosclerotic osteoblastic metastases (sternum and ilium of case number 422, Figs. 7 and 8). However, many lesions clearly recognizable on dry bone were invisible on radiographs, including osteolytic metastases (cranium of case number 459, Fig. 6), aggressive periosteal reactions (left innominate of case number 366, Fig. 8 and ribs of case number 897, Fig. 9), spongiosclerotic lesions (mandible of case number 422, Fig. 7 and left innominate of case number 37, Fig. 8), and mixed metastases (vertebrae of case number 897, Fig. 9). Despite the strong potential of radiography for detection of bone metastases reported in the literature [62], a careful macroscopic analysis and description of bone lesions should not be neglected. In fact, in view of our results and the importance of the detection of all types of bone lesions, we argue for a complementary approach, considering both macroscopic observation and radiographic imaging, in the study of metastatic bone disease.

Osteomalacia

The severe bending deformities observed on the skeleton are highly consistent with a diagnosis of osteomalacia [2, 3, 21–25]. Radiography of the bones did not provide any additional information useful for the diagnosis. However, and surprisingly, the X-ray of the radius did not show any evident sign of trauma, demonstrating the importance of a macroscopic examination (Fig. 10).

As a result, macroscopic observation allows the analysis of the distribution of lesions and the recognition of osteolytic lesions, periosteal reactions, and bending deformities, but

lesions located in the trabecular bone cannot be seen unless the cortical shell is damaged. Thus, macroscopy alone permits the identification of diabetic bone lesions (although they are not pathognomonic of the condition) and the diagnosis of rheumatoid arthritis, multiple myeloma (if enough osteolytic lesions are visible on the cortical surface), and osteomalacia, but not always that of metastatic carcinoma. Most metastases are found in cancellous bone, hidden from macroscopic observation unless taphonomic alterations allow their visualization, meaning that many lesions in metastatic carcinoma are lost from the anthropological analysis, which can alter the diagnosis of the condition and potentially cause a misdiagnosis.

Cortical and trabecular osteolytic lesions, bone atrophies, and bending deformities can be identified on radiographs through changes in bone density and morphology. However, radiographic imaging is often unable to perceive periosteal reactions and may be blind to some osteolytic lesions as the identification of bone lesions is dependent upon the quality of the contrast. This implies that even though radiography is a sufficient tool for the diagnosis of rheumatoid arthritis, multiple myeloma, and osteomalacia, important lesions may be missed in diabetes and metastatic carcinoma, rendering macroscopic observation essential.

The literature has already demonstrated the importance of radiographic analysis for the detection of bone lesions [18, 62]. To the best of our knowledge, this article is one of the rare ones [19] to also evidence the strengths of macroscopic examination in the analysis of bone lesions and the pitfalls of radiography in the observation of the same lesions.

In light of the results of this study, we argue in favor of a complementary analysis between macroscopic observation and radiographic imaging for the recognition and diagnosis of bone lesions. In the analysis of bare bones, radiographs should be systematically performed in a forensic context and realized as far as possible in an archeological context if suggestive lesions are noted. In the case of an individual in a state of decomposition, macroscopic analysis of the bones should not be overlooked, radiographs should be performed, and recovery and maceration of the bones is advised in order to carefully analyze the skeletal elements for signs of pathologies. This complementary approach would avoid the loss of crucial information on bone disease and allow more informed diagnoses in forensic and archeological cases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

References

1. Cunha E (2006) Pathology as a factor of personal identity in forensic anthropology. In: Schmitt A, Cunha E, Pinheiro J (eds) . Springer, Forensic anthropology and medicine, pp 333–358
2. Ortner DJ (2003) Identification of pathological conditions in human skeletal remains, 3rd edn. Smithsonian Institution Press, New York
3. Aufderheide AC, Rodríguez-Martín C (1998) The Cambridge encyclopedia of human paleopathology. Cambridge University Press, Cambridge
4. Waldron T (2008) Palaeopathology. Cambridge University Press, Cambridge
5. Steinbock RT (1976) Paleopathological diagnosis and interpretation: bone diseases in ancient human populations. Charles Thomas Publisher, Springfield
6. Biehler-Gomez L, Cattaneo C (2018) The diagnostic implications of two cases of known rheumatoid arthritis from the CAL Milano Cemetery Skeletal Collection. J Forensic Sci 63:1880–1887. https://doi.org/10.1111/1556-4029.13799
7. Biehler-Gomez L, Castoldi E, Baldini E, Cappella A, Cattaneo C (2018) Diabetic bone lesions: a study on 38 known modern skeletons and the implications for forensic scenarios. Int J Legal Med 133:1–15. https://doi.org/10.1007/s00414-018-1870-0
8. Biehler-Gomez L, Giordano G, Cattaneo C (2019) Multiple myeloma bone lesions in skeletal remains: report of two known cases from the 20th century CAL Milano Cemetery Skeletal Collection. Int J Osteoarchaeol 29:101–107. https://doi.org/10.1002/oa.2721
9. Castoldi E, Cappella A, Gibelli D, Sforza C, Cattaneo C (2017) The difficult task of diagnosing prostate cancer metastases on dry bone. J Forensic Sci 63:672–682. https://doi.org/10.1111/1556-4029.13617
10. Biehler-Gomez L, Giordano G, Cattaneo C (2019) The appearance of breast cancer metastases on dry bone: implications for forensic anthropology. J Forensic Legal Med 61:5–12. https://doi.org/10.1016/j.jflm.2018.10.007
11. Biehler-Gomez L, Giordano G, Cattaneo C (2019) The overlooked primary: bladder cancer metastases on dry bone. A study of the 20th century CAL Milano Cemetery Skeletal Collection. Int J Paleopathol 24:130–140. https://doi.org/10.1016/j.ijpp.2018.10.005
12. Ortner DJ, Aufderheide AC (1991) Human paleopathology: current syntheses and future options. Smithsonian Institution Press, Washington, DC
13. Kelley MA, Micozzi MS (1984) Rib lesions in chronic pulmonary tuberculosis. Am J Phys Anthropol 65:381–386. https://doi.org/10.1002/ajpa.1330650407
14. Roberts CA, Lucy D, Manchester K (1994) Inflammatory lesions of ribs: an analysis of the Terry Collection. Am J Phys Anthropol 95:169–182. https://doi.org/10.1002/ajpa.1330950205
15. Santos AL, Roberts CA (2006) Anatomy of a serial killer: differential diagnosis of tuberculosis based on rib lesions of adult individuals from the Coimbra identified skeletal collection, Portugal. Am J Phys Anthropol 130:38–49. https://doi.org/10.1002/ajpa.20160
16. Matos V, Santos AL (2006) On the trail of pulmonary tuberculosis based on rib lesions: results from the human identified skeletal collection from the Museu Bocage (Lisbon, Portugal). Am J Phys Anthropol 130:190–200. https://doi.org/10.1002/ajpa.20309
17. Mays S (2018) How should we diagnose disease in palaeopathology? Some epistemological considerations. Int J Paleopathol 20:12–19. https://doi.org/10.1016/j.ijpp.2017.10.006
18. Chhem RK, Brothwell DR (2008) Paleoradiology: imaging mummies and fossils. Springer, Berlin
19. Rogers J, Watt I, Dieppe P (1990) Comparison of visual and radiographic detection of bony changes at the knee joint. BMJ 300:367–368

20. Cattaneo C, Mazzarelli D, Cappella A et al (2018) A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection. Forensic Sci Int 287: 219.e1–219.e5. https://doi.org/10.1016/j.forsciint.2018.03.041
21. Brickley M, Ives R (2010) The bioarchaeology of metabolic bone disease. Elsevier Academic Press, London
22. Brickley M, Mays S, Ives R (2005) Skeletal manifestations of vitamin D deficiency osteomalacia in documented historical collections. Int J Osteoarchaeol 15:389–403. https://doi.org/10.1002/oa.794
23. Brickley M, Mays S, Ives R (2007) An investigation of skeletal indicators of vitamin D deficiency in adults: effective markers for interpreting past living conditions and pollution levels in 18th and 19th century Birmingham, England. Am J Phys Anthropol 132:67–79. https://doi.org/10.1002/ajpa.20491
24. Ives R, Brickley M (2014) New findings in the identification of adult vitamin D deficiency osteomalacia: results from a large-scale study. Int J Paleopathol 7:45–56. https://doi.org/10.1016/j.ijpp.2014.06.004
25. Mays S, Brickley MB (2018) Vitamin D deficiency in bioarchaeology and beyond: the study of rickets and osteomalacia in the past. Int J Paleopathol 23:1–5. https://doi.org/10.1016/j.ijpp.2018.05.004
26. Choy E (2012) Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis. Rheumatology 51:v3–v11. https://doi.org/10.1093/rheumatology/kes113
27. Henrique da Mota LM, Afonso Cruz B, Viegas Brenol C et al (2013) Guidelines for the diagnosis of rheumatoid arthritis. Rev Bras Reumatol (English Ed) 53:141–157. https://doi.org/10.1016/S2255-5021(13)70019-1
28. Firestein GS (2000) Rheumatoid arthritis. In: Humes HD, HL DP, Gardner LB et al (eds) Kelley's Textbook of Internal Medicine, 4th edn. Lippincott Williams & Wilkins, pp 1347–1359
29. Rogers J, Waldron T, Dieppe P, Watt I (1987) Arthropathies in palaeopathology: the basis of classification according to most probable cause. J Archaeol Sci 14:179–193. https://doi.org/10.1016/0305-4403(87)90005-7
30. Brahee DD, Pierre-Jerome C, Kettner NW (2003) Clinical and radiological manifestations of the rheumatoid wrist, A comprehensive review. J Manip Physiol Ther 26:323–329. https://doi.org/10.1016/S0161-4754(03)00046-0
31. Fitzgerald RH, Kaufer H, Malkani AL (2002) Orthopaedics. Mosby, St Louis
32. Sommer OJ, Kladosek A, Weiler V, Czembirek H, Boeck M, Stiskal M (2005) Rheumatoid arthritis: a practical guide to state-of-the-art imaging, image interpretation, and clinical implications. RadioGraphics 25:381–398. https://doi.org/10.1148/rg.252045111
33. Vigorita VJ, Ghelman B (2016) Arthritis. In: Vigorita VJ, Ghelman B (eds) Orthopaedic pathology. Third. Lippincott Williams & Wilkins, Philadelphia, pp 710–768
34. Veale DJ, Fearon U (2015) What makes psoriatic and rheumatoid arthritis so different? RMD Open 1:e000025. https://doi.org/10.1136/rmdopen-2014-000025
35. Rothschild BM, Woods RJ (1991) Spondyloarthropathy: erosive arthritis in representative defleshed bones. Am J Phys Anthropol 85:125–134. https://doi.org/10.1002/ajpa.1330850202
36. Erickson AR, Cannella AC, Mikuls TR (2017) Clinical features of rheumatoid arthritis. In: Firestein GS, Budd R, Gabriel SE et al (eds) Kelley and Firestein's textbook of rheumatology, 10th edn. Elsevier Health Sciences, Philadelphia, pp 1167–1186
37. Kim HJ, Nemani VM, Riew KD, Brasington R (2015) Cervical spine disease in rheumatoid arthritis: incidence, manifestations, and therapy. Curr Rheumatol Rep 17:9. https://doi.org/10.1007/s11926-014-0486-8
38. Leden I, Forslind K, Svensson B (2012) Ankylosis of wrist and small joints of the hand occurs in rheumatoid arthritis: diagnostic implication in paleopathology. Int J Paleopathol 2:249–251. https://doi.org/10.1016/j.ijpp.2012.09.001
39. Zias J, Mitchell P (1996) Psoriatic arthritis in a fifth-century Judean Desert monastery. Am J Phys Anthr 101:491–502. https://doi.org/10.1002/(SICI)1096-8644(199612)101:4<491::AID-AJPA4>3.0.CO;2-Z
40. Brutsaert E (2017) Complications of diabetes mellitus—Merck Manual for Health Professionals. https://www.merckmanuals.com/professional/endocrine-and-metabolic-disorders/diabetes-mellitus-and-disorders-of-carbohydrate-metabolism/complications-of-diabetes-mellitus. Accessed 26 Mar 2019
41. Fowler MJ (2008) Microvascular and macrovascular complications of diabetes. Clin Diabetes 26:77–82
42. Beckman JA, Creager MA, Libby P (2002) Diabetes and atherosclerosis: epidemiology, pathophysiology, and management. JAMA 287:2570–2581. https://doi.org/10.1001/jama.287.19.2570
43. Crispin JC, Alcocer-Varela J (2003) Rheumatologic manifestations of diabetes mellitus. Am J Med 114:753–757. https://doi.org/10.1016/S0002-9343(03)00140-2
44. Coleman RE (1997) Skeletal complications of malignancy. Cancer 80:1588–1594. https://doi.org/10.1002/(SICI)1097-0142(19971015)80:8+<1588::AID-CNCR9>3.0.CO;2-G
45. Coleman RE (2001) Metastatic bone disease: clinical features, pathophysiology and treatment strategies. Cancer Treat Rev 27:165–176. https://doi.org/10.1053/ctrv.2000.0210
46. Strouhal E (1991) Myeloma multiplex versus osteolytic metastatic carcinoma: differential diagnosis in dry bones. Int J Osteoarchaeol 1:219–224. https://doi.org/10.1002/oa.1390010314
47. Rothschild BM, Hershkovitz I, Dutour O (1998) Clues potentially distinguishing lytic lesions of multiple myeloma from those of metastatic carcinoma. Am J Phys Anthropol 105:241–250. https://doi.org/10.1002/(SICI)1096-8644(199802)105:2<241::AID-AJPA10>3.0.CO;2-0
48. Roodman GD (2004) Mechanisms of bone metastasis. N Engl J Med 350:1655–1664. https://doi.org/10.1056/NEJMra030831
49. Randall RL (2015) Metastatic bone disease: an integrated approach to patient care. Springer
50. Paget S (1889) The distribution of secondary growths in cancer of the breast. Lancet 133:571–573. https://doi.org/10.1016/S0140-6736(00)49915-0
51. Talmadge JE, Fidler IJ (2010) AACR centennial series: the biology of cancer metastasis: historical perspective. Cancer Res 70:5649–5669. https://doi.org/10.1158/0008-5472.can-10-1040
52. Brothwell D (2008) Tumor and tumor-like processes. John Wiley & Sons
53. Marques C, Santos AL, Cunha E (2013) Better a broader diagnosis than a misdiagnosis: the study of a neoplastic condition in a male individual who died in early 20th century (Coimbra, Portugal). Int J Osteoarchaeol 23:664–675. https://doi.org/10.1002/oa.1294
54. Ragsdale BD, Campbell RA, Kirkpatrick CL (2018) Neoplasm or not? General principles of morphologic analysis of dry bone specimens. Int J Paleopathol 21:27–40. https://doi.org/10.1016/j.ijpp.2017.02.002
55. Andersen JG, Manchester K (1992) The rhinomaxillary syndrome in leprosy: a clinical, radiological and palaeopathological study. Int J Osteoarchaeol 2:121–129. https://doi.org/10.1002/oa.1390020204
56. Smith MO (2002) A probable case of metastatic carcinoma from the late prehistoric eastern Tennessee River Valley. Int J Osteoarchaeol 12:235–247. https://doi.org/10.1002/oa.618
57. Vilar J, Lezana AH, Pedrosa CS (1979) Spiculated periosteal reaction in metastatic lesions of bone. Skelet Radiol 3:230–233. https://doi.org/10.1007/BF00360941
58. Bloom RA, Libson E, Husband JE, Stoker DJ (1987) The periosteal sunburst reaction to bone metastases. A literature review and report of 20 additional cases. Skelet Radiol 16:629–634

59. Guise TA, Kozlow WM, Heras-Herzig A, Padalecki SS, Yin JJ, Chirgwin JM (2005) Molecular mechanisms of breast cancer metastases to bone. Clin Breast Cancer 5:S46–S53. https://doi.org/10.3816/CBC.2005.s.004
60. Lipton JF, Vigorita VJ (2016) Metastatic bone disease. In: Vigorita VJ, Ghelman B, Mintz D (eds) Orthopaedic pathology, 3rd edn. Lippincott Williams & Wilkin, Philadelphia, pp 594–619
61. Ortner DJ (2011) Human skeletal paleopathology. Int J Paleopathol 1:4–11. https://doi.org/10.1016/j.ijpp.2011.01.002
62. Rothschild BM, Rothschild C (1995) Comparison of radiologic and gross examination for detection of cancer in defleshed skeletons. Am J Phys Anthr 96:357–363. https://doi.org/10.1002/ajpa.1330960404

5.4. Discrepancies and inaccuracies in the description of bone lesions

Objective: The aim of this research is to analyze the similarities and discrepancies in the description of pathological bone lesions.
Materials and Methods: Ten participants (five forensic pathologists and five anthropologists) were asked to describe 30 bone lesions through visual and photographic observations, including character of the lesion, margins and periosteal reaction, according to recognized and accepted paleopathological terminology. Results were analyzed using statistical analysis and inter and intraobserver agreements were tested.
Results: The anthropologists showed slightly more consistent and accurate results compared to the forensic pathologists, and overall results were better when assessed by visual examination of the bone lesions. Lesion descriptions showed important contradictions and inaccuracies, particularly in the evaluation of the character of the lesion and periosteal reactions, with dramatic potential consequences for the diagnosis of bone disease.
Significance: This study shows the considerable pitfalls in the assessment of basic pathological bone manifestations and demonstrates the importance of continuing efforts in the standardization of paleopathological terminology.
Limitations: The small size of the study group limits the potential for interpretation.
Suggestions for Further Research: A larger pool of participants may strengthen and confirm trends in our results.

In the forensic anthropology practice, one of the main objectives is the construction of an informative and reliable biological profile, which includes the estimations of sex, age, ancestry and stature, the recording of anatomical variants and the analysis of traumatic injuries and pathological bone lesions. Although often neglected, the diagnosis of bone disease will provide additional information which may prove determinant in forensic cases; for instance, in narrowing down of a pool of missing persons for the identification of an unknown deceased. The macroscopic diagnosis of pathological conditions is based on the interpretation of the morphological appearance of bone lesions, their position on the bone and their distribution on the skeleton, compared with the clinical literature and previous published cases. The first step in the study of bone lesions consists in the recording of pathological bone abnormalities; indeed, as Roberts and Connell (2004) mention "the only way to attempt

any form of classification or diagnosis of disease in skeletal remains is with clear and objective description". Thus, a correct description of bone lesions using a comparable terminology understood by all is essential for an accurate diagnosis on dry bone, for the evaluation of proposed diagnoses by other researchers, and for the archiving of the documentation of bone lesions, especially in cases where the skeletal material will no longer be available (e.g. skeletons may be reburied).

From the 1980's, increased concern was raised relating to the use of inappropriate terms in describing normal and abnormal bone structures. The *Nomenclature in Paleopathology* (Manchester, Ogden and Storm, 2016), published in the Paleopathology Association Newsletter, represents one of the most important works aiming to standardize the terminology that may be used to describe bone structures to improve linguistic exactitude. Continuing efforts were realized to increase scientific rigor, consensus and accuracy in the documentation and diagnosis of bone disease, in particular with guidelines for the recording of pathological bone abnormalities (Buikstra and Ubelaker, 1994; Brickley and McKinley, 2004; Mitchell and Brickley, 2017) suggesting methodologies for an accurate description of bone lesions, and the adaptation of the "Istanbul Protocol Manual on the Effective Investigation and Documentation of Torture and other Cruel, Inhuman or Degrading Treatment or Punishment" (United Nations, 2004), used in forensic sciences to indicate the degree of certainty of the diagnosis of trauma suffered during torture, to its application in the diagnosis of bone disease (Appleby, Thomas and Buikstra, 2015). In addition, excellent articles have endeavored to standardize the terminology used to describe bone lesions (Rose *et al.*, 1991; Miller, Ragsdale and Ortner, 1996; Lovell, 2000; Ortner, 2003, chap. 4, 2012; Grauer, 2008; Buikstra, Cook and Bolhofner, 2017; Klaus, 2017; Mays, 2018; Ragsdale, Campbell and Kirkpatrick, 2018) and alerted on the importance of rigorous descriptions of skeletal lesions as the specific terms selected "describe the interlinked factors of the morphology and underlying pathological process(es) of abnormal new bone formation, abnormal bone loss, and abnormal bone size/shape" (Klaus, 2017) and thus constitute descriptive tools orienting the differential diagnosis. Nonetheless, there is still considerable variation in the description of bone lesions which may increase interobserver error and ambiguity and ultimately lead to misdiagnoses and misinterpretations among scientists. Consequently, continuing training in the recording of bone lesions is needed.

In this research, we subjected 10 participants (five forensic pathologists and five anthropologists) to a questionnaire for the description of 30 bone lesions according to terminology used in paleopathological reference works (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003, 2011b; Santos and Roberts, 2006; Buikstra, Cook and Bolhofner, 2017; Klaus, 2017; Mays, 2018; Ragsdale, Campbell and Kirkpatrick, 2018) and tested for inter- and intra-observer error. It is important to note that the diagnosis of the conditions responsible for the lesions was not evaluated. The aim of this research was to observe the similarities and discrepancies in the description of bone lesions.

The study consisted in the description of 30 bone lesions from 27 bones; in three instances, two lesions were located on the same bone but in different positions. Twenty-one bones were selected from skeletons of the CAL Milano Cemetery Skeletal Collection with antemortem diagnoses of disease (Cattaneo *et al.*, 2018) and six were archaeological bones from the CAL archaeological collection composed of about 5,000 skeletons from over 50 archaeological excavation sites in Italy. The lesions were undamaged by taphonomic alterations and clearly signaled on the bones, as the aim of this study was not to assess the detection of bone lesions but the variation in the description and classification of bone lesions according to recognized and accepted paleopathological terminology. These bone alterations included lesions due to tuberculosis, congenital syphilis, osteomyelitis, metastatic carcinoma, multiple myeloma, rheumatoid arthritis, diabetes, rickets, antemortem trauma and periosteal reactions on long bones. It is important to note that the diagnoses of the conditions were never asked, only the description of the lesions based on predefined answers.

Ten participants were subjected to a questionnaire assessing the types of abnormalities of 30 bone lesions: five forensic pathologists with experience in anthropology constituted the "forensic pathologists" and five experienced anthropologists (forensic anthropologists and bioarchaeologists) formed the "anthropologists". Each participant was asked to describe the 30 bone lesions twice, first based on photographic images on the computer (referred to as "computer assessment") and then through the visual observation of the real bone elements ("visual assessment"), to see if any difference was noted.

The description of bone lesions was based on a checklist with multiple predefined answers and divided in various sections, including character of the lesion, dimensions, degree of bone resorption (for osteolytic lesions), margins of the lesion (for osteolytic lesions), periosteal reaction and articular involvement. For the purpose of this

research, the results of three categories will be considered here: character of the lesion, margins and periosteal reaction, as presented in Table 2.

Table 2: Categories and possible answers submitted during the test and analyzed in the present study

Character of lesion	only osteoblastic
	mostly osteoblastic, but osteolytic component also present
	mostly osteolytic, but osteoblastic component also present
	only osteolytic
Margins*	rounded and remodeled
	no remodeling
Periosteal reaction	absent
	woven bone deposition (young periostitis)
	remodeled lamellar bone (remodeled periostitis)
	coarse spicules "coral-like"
	parallel spicules or "hair-on-end"
	radiating spicules or "sunburst"

*only applicable to osteolytic lesions

The description of bone abnormalities were based on standard and published paleopathological terminology (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003, 2011b; Santos and Roberts, 2006; Buikstra, Cook and Bolhofner, 2017; Klaus, 2017; Mays, 2018; Ragsdale, Campbell and Kirkpatrick, 2018; Biehler-Gomez, Giordano and Cattaneo, 2019). These categories were selected because they are particularly informative on the disease process and are therefore of great interpretative value. The correct recording of the character of the lesion (solely bone forming, solely bone destruction, or a combination of both components), the presence or absence of bone remodeling on the margins of an osteolytic lesion, and the presence and type of periosteal reaction, can not only indicate an attempt at bone healing but also informs on the rapidity of the process. As Ortner (2012) explains "in general, the slower the process, the more clearly defined the margins of a destructive lesion will be" on radiographs, due to bone remodeling. In osteolytic lesions, well-defined remodeled margins evidence a slow and chronic process, whereas poorly defined margins (without bone remodeling) suggest a rapid and aggressive process (Lovell, 2000; Ortner, 2012). Similarly, in osteoblastic lesions, woven bone (poorly organized porous fiber bone) is the result of a very rapid bone formation, which remodels overtime into smooth and dense compact bone, thus indicating a chronic and relatively slow process

(Lovell, 2000; Ortner, 2012). Finally, projecting spicules of bone reveal a very rapid and aggressive process of bone formation (Lovell, 2000; Ragsdale, Campbell and Kirkpatrick, 2018). The subcategories of periosteal reactions, and in particular the spiculated periosteal reactions, were selected based on recognized and accepted terminology and definitions (Ortner, 2003; Santos and Roberts, 2006; Ragsdale, Campbell and Kirkpatrick, 2018).

While only one answer was expected in the "character of lesion" and "margins" categories, several answers could be checked in the "periosteal reaction" category. Based on paleopathological literature (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003, 2011b; Santos and Roberts, 2006; Ragsdale, Campbell and Kirkpatrick, 2018), one of the authors (LBG) elaborated the "correct" results, used to calculate the accuracy rates of the participants.

Results were analyzed using statistical analysis. Inter-observer agreement was realized with Fleiss' Kappa in Excel (Microsoft® Excel® 2016), intra-observer agreement was performed with Cohen Kappa in SPSS (IBM SPSS Statistics for Windows, version 21), intra-observer agreements in periosteal reactions and accuracy rate calculations were done with Excel (Microsoft® Excel® 2016). Results were analyzed using statistical analysis. Inter-observer agreement was realized with Fleiss' Kappa in Excel (Microsoft® Excel® 2016), intra-observer agreement was performed with Cohen Kappa in SPSS (IBM SPSS Statistics for Windows, version 21), intra-observer agreements in periosteal reactions and accuracy rate calculations were done with Excel (Microsoft® Excel® 2016).

Inter-observer agreement

Based on Landis and Koch (1977), the results of the Fleiss' Kappa inter-observer agreements (Table 3) indicate that among the anthropologists, the inter-observer agreement is moderate (scoring 0.41-0.60) to substantial (scoring 0.61 to 0.80) in all categories except for one: the "computer assessment" of the "character of the lesion" which can be classified as a "fair" agreement (scoring 0.21-0.40). In addition, the agreement values are higher in all three categories of the "visual assessment", whereas the computer-based assessment shows less agreement. Among the forensic pathologists, all values lie within the fair agreement section.

Table 3: Fleiss' Kappa values for the groups of anthropologists and forensic pathologists, according to each category of description of lesions and divided per type of assessment

Category of lesion description	Anthropologists – Visual assessment	Anthropologists – Computer assessment	Forensic pathologists – Visual assessment	Forensic pathologists – Computer assessment
Character of lesion	0.5088	0.3781	0.2881	0.2594
Margins	0.6252	0.5856	0.3979	0.2782
Periosteal reaction	0.6301	0.5628	0.2501	0.2775
Overall	**0.5883**	**0.5088**	**0.3120**	**0.2717**

Overall, the Fleiss' Kappa values are higher in the "visual assessment" than in the "computer assessment", and in the group of anthropologists than among forensic pathologists (Fig 15). Fig. 15 also shows that the category "character of lesion" shows the poorest agreement, whereas the category "margins" presents the strongest agreement among participants.

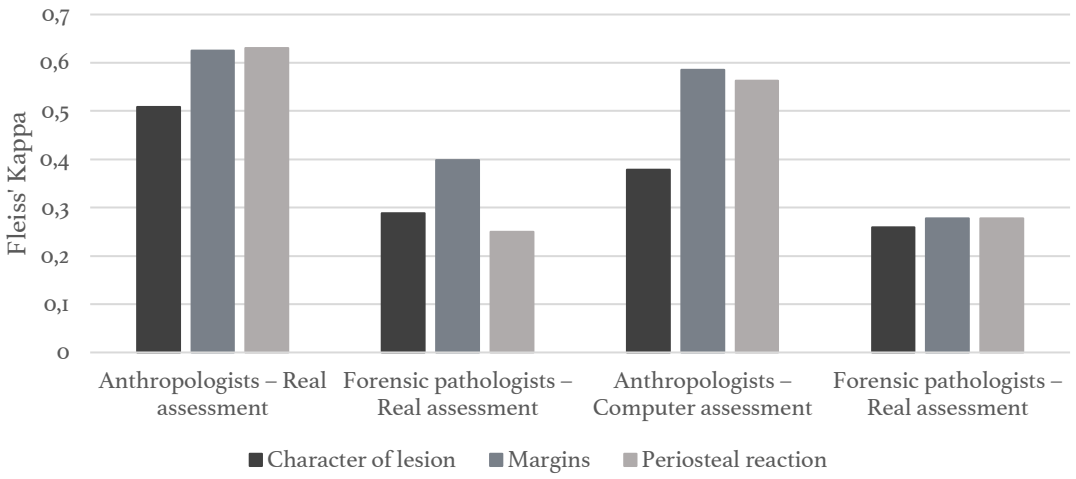

Figure 15: Inter-observer variance shown by Fleiss' Kappa between the groups of anthropologists and forensic pathologists according to the type of assessment of the bone lesions

Intra-observer agreement

Similarly, the Cohen Kappa intra-observer agreement results (Table 4) show a moderate to substantial intra-observer agreement between "visual" and "computer" assessments for both groups with variation between participants from fair to perfect agreements (Landis and Koch, 1977). The average values indicate a slightly greater agreement in the group of anthropologists and for the "margins" category.

Table 4: Cohen Kappa values for all participants per category of description of lesions. All Cohen Kappa values were significant with α = 0.05.

Category of lesion description	Participants of the group of anthropologists						Participants of the group of forensic pathologists *				
	1	2	3	4	5	**Avg.**	1	2	3	4	**Avg.**
Character of lesion	0.632	0.850	0.799	0.818	0.441	**0.544**	0.949	1.000	0.341	0.351	**0.660**
Margins	0.639	1.000	0.847	0.947	0.551	**0.743**	0.947	0.947	0.273	0.533	**0.675**
Periosteal reaction **	0.546	0.818	0.719	0.780	0.309	**0.634**	0.544	0.785	0.252	0.488	**0.517**
Total						**0.640**					**0.617**

* Participant n°5 did not perform the visual assessment of lesions and so the intra-observer agreement could not be calculated

** For the Cohen Kappa calculation of multiple answers in the "periosteal reaction" category, partial agreement was not considered, the answers had to be in the exact same combination to be seen as correct.

Accuracy rates

As seen in Table 5, the highest accuracies of both groups were obtained in the "margins" category and the lowest in the "periosteal reaction" category, regardless of the type of assessment used. Overall, visual assessment of bone lesions was more often correct than when computer-based and evaluations made by the anthropologists were slightly more accurate than in the group of forensic pathologists.

Table 5: Accuracy rates for the computer-based and visual assessments of the characteristics of bone lesions between the groups of anthropologists and forensic pathologists

Category of lesion description	**Anthropologists**		**Forensic pathologists**	
	Computer assessment (%)	Visual assessment (%)	Computer assessment (%)	Visual assessment (%)
Character of lesion	64.33	65.83	66.67	66.67
Margins	80.67	80.00	70.67	80.67
Periosteal reaction	62.19	57.19	56.17	66.94
Total	69.06	67.67	64.50	71.43

The accuracy rates of the positive identification of the different types of periosteal reactions (Table 6) show very poor results, close to 50%. This means that in average, one in two periosteal reactions was wrongly identified (e.g. a periosteal reaction was described when it was absent or vice-versa, or the wrong type of periosteal reaction was noted as present).

Table 6: Accuracy rates for the positive identifications in the subcategories of periosteal reactions between the groups of anthropologists and forensic pathologists

	Anthropologists		**Forensic pathologists**	
	Computer assessment (%)	Visual assessment (%)	Computer assessment (%)	Visual assessment (%)
Absent	77.50	76.25	60.00	70.31
Woven bone	46.67	55.56	40.00	44.44
Lamellar bone	57.78	62.22	57.78	44.44
"Coral-like"	0.00	10.00	30.00	37.50
"Hair-on-end"	20.00	60.00	40.00	75.00
"Sunburst"	80.00	80.00	40.00	25.00
Avg.	**46.99**	**57.34**	**44.63**	**49.45**

Consistency in the assessment of the lesions

Inconsistencies between the assessment of character of the lesion and their descriptions were noted. For instance, seven participants (four anthropologists and three forensic pathologists) noted some lesions as either osteolytic or osteoblastic (by opposition to manifesting both components) and then listed the presence of periosteal reactions/remodeled margins or bone resorption respectively, which contradicts the previous assessment of a lesion solely osteolytic or osteoblastic.

The inter-observer agreement analysis shows that anthropologists tended to agree more in their description of lesions than the forensic pathologists in our study (Table 2). In addition, they were slightly more correct in their description of bone lesions than the participants with a medico-legal background (Table 4). These results were expected as the anthropologists had more experience with skeletal remains and so higher probabilities to have been confronted to bone lesions, thus explaining a better accuracy in their assessment.

Participants generally tended to describe lesions similarly whether they performed the analysis based on photographs or by visual examination of the actual osseous elements (Table 3). Their answers were more similar on "visual" assessment and were slightly more accurate than when assessed on photographs (Tables 3 and 4). Again, these results were expected because details of bone lesions may be more difficult to recognize on photographs due to the constraint of definition and the lack of perspective. In addition, they demonstrate that visual observation should be preferred to photographs when recording bone lesions.

Participants tended to disagree on their assessment of the character of the lesions, without changing their answers between modalities of assessment (Tables 2 and 3). It is interesting to see that this category, which is the initial interpretation of the lesion and may significantly impact the diagnosis of the disease responsible for the lesion, showed so much variation between participants and such a low accuracy (varying between 64% and 67%, Table 4). Another interesting finding was that in a total of 19 instances, the participants evaluated lesions to be solely osteolytic or osteoblastic but also recorded the presence of bone production or bone resorption respectively in other categories. These variations, low accuracies and contradictory assessments testify to a need for continuous training in the description and recording of bone lesions.

The margin category was limited to a binary response: either rounded and remodelled or not remodelled (Table 1), it is therefore understandable that the results of this category were more similar between participants and modalities (Tables 2 and 3) and the most accurate results (Table 4). Nonetheless, they also show that the description of the pathological changes at the margin are the easiest to interpret.

Contrary to the margin category, the interpretation of periosteal reaction seems to have posed the most difficulties. Not only did the participants show variation in their results between themselves (in particular in the group of forensic pathologists, which may be due to the fact that they have less experience on dry bones than the group of anthropologists, resulting in more uncertainty) but they also considerably changed their results between modalities. This category is also the one with the poorest accuracy (ranging between 56% and 67% and with a 45% to 57% rate of positive identification). In fact, absent periostosis (that is, the absence of a new bone deposition on the cortical surface) was recognized in only 71% of cases. Overall, the different types of periosteal reaction were correctly identified about half of the time (49.6%) (Table 5). In two lesions in particular, we could understand that the participants mistook spongiosclerosis (observed in the trabecular bone exposed by an osteolytic perforation of the bone cortex) for the presence of a periosteal reaction. These results demonstrate a poor understanding of the terminology associated to the different types of periosteal reaction, which is particularly alarming considering that these may considerably orient the understanding of the disease process and the diagnosis of the causative agent responsible for the lesion. Ortner (2012) said "careful attention to the characteristics of the abnormal features created by the bone cells provides critical information basic to the description and diagnosis of all skeletal disorders". However,

and as highlighted in this study, there is still substantial variation, disagreement and inaccuracies in the description of bone lesions. Consequently, the understanding of the characteristics of bone lesions and the standardization of their description remain a crucial topic to address for the correct recording of bone lesions, the documentation of comparable data and the accurate diagnosis of bone disease.

Previous studies (Waldron and Rogers, 1991; Bridges, 1993) have already shown the existing inter-observer disagreement in the scoring and analysis of bone lesions (specifically, due to osteoarthritis) and alerted on the need for a standardized methodology in the analysis of bone lesions. In the present study, however, we also evidenced critical issues in lesion recording, in particular when assessing the character of lesion (bone forming or bone destruction) as well as the presence of periosteal bone forming lesions (regardless of the exact subcategory), namely, inconsistencies, confusions and very low accuracies. The results of this exploratory study thus argue in favor of continuing efforts in the training on the description of the characteristics of bone lesions.

The accurate description of bone lesions is an essential step for the correct diagnosis of bone disease. In this study, we submitted 30 bone lesions to ten anthropologist and forensic pathologist participants. As a result, anthropologists showed slightly more consistent and accurate results compared to the forensic pathologists and overall results were better when assessed by visual examination of the bone lesions. Lesion descriptions however showed important inaccuracies and contradictions, particularly in the evaluation of the character of the lesion and periosteal reactions, with dramatic potential consequences for the diagnosis of bone disease. We can only recommend continuing training and communication on the identification of the characteristics of bone lesions for the correct recording of bone lesions, the documentation of comparable data and the accurate diagnosis of bone disease. This study shows the considerable pitfalls in the description and classification of basic pathological bone manifestations and demonstrates the importance of continuing efforts in the standardization of paleopathological terminology.

Chapter 6. Discussion

"The dead cannot cry out for justice. It is a duty of the living to do so for them."
Lois McMaster Bujold

The main objective of this PhD thesis was to study bone diseases and their diagnosis based primarily on a reference collection, the CAL Milano Cemetery Skeletal Collection, composed of individuals with antemortem clinical diagnoses in their associated documentation. With this intent, the study was divided in four research lines, namely the macroscopic study of skeletons with clinically diagnosed conditions reported in their antemortem data; the histological analysis of non-skeletal calcified markers of disease including atherosclerotic calcifications, gallstones and that of ossifying cartilage and pleural plaques; the synergy between radiographic and macroscopic analysis in the study of skeletal lesions; and the discrepancies and inaccuracies in the description of bone lesions. In this chapter, the research lines examined in the present thesis will be critically discussed one by one.

6.1. Macroscopic study of skeletons with clinically diagnosed conditions

In this research line, seven pathological conditions were examined based on skeletons from the CAL Milano Cemetery Skeletal Collections with associated antemortem data including demographic information and antemortem clinical diagnoses of pathological conditions. In particular, the research included:

- 12 individuals diagnosed with a vasculopathy and 12 controls,
- two individuals with rheumatoid arthritis,
- 38 individuals with diabetes mellitus and 11 controls,
- nine individuals with HIV/AIDS,
- two individuals with multiple myeloma,
- 13 females with breast cancer,
- and 14 individuals with bladder cancer.

For the most part, this was the first time that these conditions were examined based on individuals with the condition clinically diagnosed before death (except for multiple myeloma). As discussed in the Introduction, in the lesion-based approach, dry bone diagnoses of disease are performed by comparison with data from the clinical literature and previous published cases. However, the clinical literature may not be

adequate to diagnose bone disease; indeed, bone lesions are not always described, sense of distribution is absent, and details are given through the prism of medical imaging which may not reflect macroscopic lesions. The paleopathological literature includes manuals of paleopathology (constituted largely of cases from pathological collections – with their own biases of representation of less extreme cases of disease) and archaeological specimens (for which the medical history is unknown) (See Chapter 1. Introduction). Reference collections thus offer an alternative source of information, presenting dry bone cases of clinically-diagnosed conditions. With the studies performed in this research line, we provided meticulous documentation of the variation of bone manifestations of rheumatoid arthritis, diabetes mellitus, HIV/AIDS, multiple myeloma as well as breast and bladder metastatic cancers based on skeletons with the conditions diagnosed antemortem. In particular, about rheumatoid arthritis, two opposite cases were presented: the first was a typical case of the condition, evidencing symmetric and bilateral osteolytic erosions, destructions and articular deformations; whereas the second showed secondary osteoarthrosis, challenging the diagnosis. The examination of all 38 cases of the collection with diabetes mellitus illustrated the range of lesions that may be observed. However, while these were consistent with the various complications of diabetes mellitus, none of the changes was specific to the condition. Indeed, because of the lack of specificity of diabetic lesions, its diagnosis on dry bones has never been defined. Nonetheless, our study demonstrated high frequencies of these unspecific lesions. Even though they are not pathognomonic for diabetes, the presence of these lesions in the feet (and in particular in the bones of the first ray) can suggest the condition in the differential diagnosis and lead to a reappraisal of the epidemiology of diabetes in the past. Similarly, nine skeletons with HIV/AIDS were examined and documented the possible lesions that may be observed in these individuals. All lesions were consistent with complications of HIV/AIDS and none was specific to the viral infection but this study illustrated for the first time what to expect in the skeletons of individuals suffering from HIV/AIDS. While the literature provides ample information on bone changes in multiple myeloma and metastatic cancer (see Chapter 3. Literature review), few dry bone cases have been documented (Capasso, 2005; David and Zimmerman, 2010; Riccomi, Fornaciari and Giuffra, 2019); with these studies, a total of 27 metastatic cancer and two multiple myeloma skeletons have been added to the literature. In fact, the two cases of multiple myeloma presented here are the third and fourth known cases in the

literature, after the two cases by Rothschild *et al.* (1998) (including one undocumented from the Mutter Museum, PA, USA) (Riccomi, Fornaciari and Giuffra, 2019). Moreover, the studies on metastatic cancer permitted to highlight differences and trends in the distribution and morphology of bone metastases of breast and bladder cancers. Finally, the research on atherosclerotic calcifications was not only the first study regarding atherosclerosis to be performed on a reference collection but it also demonstrated that atherosclerotic calcifications can be found and identified among skeletonized remains.

With this research, we answered the question of the macroscopic morphology of lesions on dry bone in rheumatoid arthritis, diabetes mellitus, HIV/AIDS, multiple myeloma, breast and bladder cancers as well as the macroscopic appearance of atherosclerotic calcifications, as it was based on known cases. In doing so, we implemented the literature with variation in the bone manifestations of these conditions. In addition, we discussed the diagnosis of these conditions in confrontation with the literature, strengthening the criteria for diagnosis already established (as in rheumatoid arthritis, multiple myeloma and metastatic cancer) and/or providing additional information for a reliable diagnosis (as in diabetes, HIV/AIDS, atherosclerosis and breast and bladder cancers).

However, several points remain to be studied and could be mentioned. First, the realization of systematic plain radiographs on the skeletons examined may have evidenced other bone lesions, hidden from naked-eye observation, and improved our understanding of bone diseases. Second, histological analyses of bone lesions may provide characteristic microscopic features for the conditions, allowing their distinction and recognition (Schultz, 2001; Schultz *et al.*, 2007). Such analyses could have potentially produced suggestive or pathognomonic criteria for the diagnosis of the conditions included in this research and could have significantly enhanced diagnostic methods on dry bone. Third, biomolecular analyses performed on samples with known conditions would allow to test and develop new techniques for detection and identification of pathological biomarkers as was demonstrated for tumoral diseases and bacterial and viral pathogens (Pérez-Martínez *et al.*, 2016). These techniques could have been tested and implemented for the skeletons studied from the CAL Milano Cemetery Skeletal Collection. Finally, while the antemortem documentation included diagnosed pathological conditions, no information regarding duration and potential treatments received were reported. Consequently, no

interpretations on the effects of treatments or the duration of the condition could be inferred regarding the presence, severity and morphology of bone lesions.
Nonetheless, radiographs were realized on selected skeletons studied macroscopically, as part of the third research line, and histological analyses were performed on atherosclerotic calcifications collected from cemeterial skeletal remains, in the second research line. In addition, a project on histological analysis of pathological bone markers is now planned to complete the current study. Similarly, a project on biomolecular analysis of bones from the collection, in particular searching for pathological DNA, and another on the toxicological analysis of skeletons with verified or suspected drug additions, intoxication or overdose in their associated documentation, were undertaken. One deficiency in this project that has not been addressed regards the paucity of antemortem information, in particular concerning duration and treatments. Thus, it would have been beneficial to access hospital records and obtain exhaustive medical information regarding the individuals studied in this project in order to further the interpretations and analyses of bone lesions. To remedy this issue, future undertakings include building a collaboration with the hospitals of Milan and obtaining the ethical approvals to access the hospital records of the individuals of the collection.

6.2. Histological study of non-skeletal calcified markers of disease

Non-skeletal calcified soft tissue material can provide considerable information to the biological profile as they may be indicators of advanced age or products of pathological conditions. However, they can also be easily mistaken and disregarded, especially if found mixed with soil and dirt. Consequently, in the second research line, histological analyses were carried out on samples of atherosclerotic calcifications, gallstones, ossifying costal cartilage and pleural plaques from cemeterial, autopsy and forensic cases, according to two protocols (decalcified and undecalcified), to provide specific documentation on this material and allow their recognition and identification.
In particular, 18 atherosclerotic calcifications, 18 gallstones, 18 fragments of ossifying costal cartilage and six samples of pleural plaques underwent histological analysis. Additionally, five atherosclerotic calcifications were submitted to SEM analysis and nine gallstones to SEM-EDS analysis.
With these studies, detailed documentations of the microscopic features of

atherosclerotic calcifications and gallstones were implemented. While histological studies (Hellings *et al.*, 2010; van Engelen *et al.*, 2013; Ababneh *et al.*, 2014) and SEM analysis (Schembri *et al.*, 2008) on fresh atherosclerotic plaques collected during surgeries have already been performed, we presented for the first time the histomorphological and SEM pictures of these vascular calcifications after death (autopsy samples) and a long post-mortem interval (about 20 years – cemeterial samples). Similarly, the research on gallstones implemented the existing literature on the elemental composition of gallstones (Ashok *et al.*, 2003; Chandran *et al.*, 2007; Athanasiadou *et al.*, 2013; Qiao *et al.*, 2013) and added one of the rare reports on the histological features of gallstones (Womack, Zeppa and Irvin, 1963). In addition, with light and polarized light microscopy, we illustrated the transition phase in ossifying costal cartilage between mineralized matrix and lamellar tissue and showed that ossifying costal cartilage may be recognized histologically even after 20 years of burial. The histological, structural and elemental study of these markers of disease (atherosclerotic calcifications and gallstones) as well as ossifying costal cartilage and pleural plaques, not only permitted a better understanding of their etiologies and a clear depiction of their composition and structure, but also allowed a specific documentation of their characteristic microscopic features and enables their identification and differentiation from other elements found among skeletal remains. Consequently, this second research line defines the microscopic composition and characteristic features of atherosclerotic calcifications, gallstones, ossifying costal cartilage and pleural plaques thus supplying decisive details for their distinction and recognition among skeletal remains. Moreover, this research provides novel data to the literature, with SEM images of atherosclerotic calcifications from autopsy cases and cemeterial skeletons buried for at least 20 years.

The main limitation in this research line is the reduced sample size used for this study (less than 10 samples per protocol). An increase in sample size would allow a greater reliability and precision of the results. This restriction is the debatable result of a difficult consensus between the scientific resolution to perform specific analyses (thin sections, SEM, SEM-EDS, etc.) and the necessity to preserve this unique and limited material from destructive analyses. In addition, immunohistochemistry was not implemented in the present study. Immunohistochemical staining may highlight the presence of specific molecular markers, characteristic of particular diseases, thus providing another mean for the diagnosis of pathological conditions on dry bones. The

use of immunohistochemical techniques is part of a larger project on pathological markers planned as a future research. For this project, other markers of disease and bone changes will be histologically examined as per previous research (Schultz, 2001; Schultz *et al.*, 2007) in an effort to identify characteristic features that may allow their diagnosis. Finally, atherosclerotic calcifications, gallstones, pleural plaques and costal cartilage are just three of numerous pathological bone markers and ossifying elements that may be found in association with skeletal remains. It may prove constructive to perform similar studies on kidney and bladder stones, thyroid cartilage and heterotopic ossifications such as *myositis ossificans* to compare and confront our results and eventually suggest microscopic discriminating features to improve diagnostic methods. However, this project on the histomorphological analysis of elements associated with skeletal remains is still ongoing with an expected significant increase in sample size and variety.

6.3. The synergy between radiographic and macroscopic study of bone lesions

In this research, we performed radiographs on skeletons with rheumatoid arthritis, diabetes, multiple myeloma, metastatic cancer and osteomalacia, and compared the detection and morphology of lesions with macroscopic examination. As a result, we evidenced lesions that were not noted with one technique or the other, reinforcing the literature on the strengths of radiographic imaging in the detection of bone lesions hidden from naked-eye observation (located in the trabecular bone structure) (Rothschild and Rothschild, 1995a; Chhem and Brothwell, 2008) but also demonstrating its pitfalls and the crucial role of a careful macroscopic examination. Indeed, radiographic imaging is often unable to perceive periosteal reactions and some osteolytic lesions because detection is dependent upon the quality of contrast. This implies that even though radiography is a sufficient tool for the diagnosis of rheumatoid arthritis, multiple myeloma, and osteomalacia, as demonstrated in the study, important lesions may be missed in diabetes and metastatic carcinoma, rendering macroscopic observation essential. Only one other research was published in the literature showing the potential of macroscopy: out of 24 knee joints evaluated, Rogers and colleagues (1990) found evidence of bone changes in 16 joints through macroscopic observation but in only 2 joints with radiographic analysis. Our study implemented the literature on the neglected subject of the importance of macroscopic

analysis. Consequently, in a case of a decomposed body, radiographic imaging may not be enough for an exhaustive appreciation of bone disease; considering our results, we argue that a thorough anthropological macroscopic examination of the bones recovered after maceration of the body may provide decisive information, such as bone lesions unnoticed on plain radiographs.

In this research line, we demonstrated the synergy of the application of both macroscopy and radiography in the study of bone disease, as each method counterbalances the limitations of the other; and alerted on the hazard to rely on only one method, as important bone changes can be unperceived by each technique. Finally, we emphasized the necessity to macerate decomposed bodies in order to collect as much data as possible, including pathological information.

Several limitations may be raised regarding this study. First, the limited number of individuals studied. Indeed, between one and four skeletons were examined per pathological condition. An augmentation in sample size would increase the reliability of our results and validate the trends observed; for instance, that radiographic imaging is often blind to periosteal reactions and some osteolytic lesions. However, this study included all the skeletons with the most relevant and marked lesions, as the point of this study was to compare radiographic and macroscopic observations of bone lesions. In addition, with the acquisition in the laboratory of a new digital portable x-ray machine, radiographic imaging of skeletons will become more systematic. Second, radiographic observations could have been compared with other imaging techniques such as magnetic resonance imaging and micro-computed tomography. These techniques would allow a more detailed observation of the lesions, potentially evidencing lesions that were missed from radiographic analysis. The reason why these techniques were not performed was that the aim of the study was not to demonstrate the strengths and pitfalls of the imaging technique, but the limitations in its application to the observation of bone lesions, and in the analysis of bones, plain radiography constitutes the technique routinely used. Thus, even if the limitations of digital radiography may be overcome by other techniques, they are not usually available in the forensic and archaeological practice and hence would not be reported. Third, the x-ray observations could have been compared with radiographs acquired on the living with the same conditions, in particular to evaluate the effect of the presence of soft tissue on the observation of bone lesions. Indeed, if some lesions were not perceived on radiographs due to contrast, it may be hypothesized that other lesions may be

missed due to the presence of soft tissues, adding contrast to the image. Such bias in the clinical assessment of bone lesions through radiographs may negatively influence the understanding, assessment of the severity and diagnosis of pathological conditions. A project is currently ongoing with the recovery of clinical images from various hospitals (in Milan, Italy; Nancy, France; and Columbus, Sri Lanka) to realize a study on the effect of soft tissues for the appreciation of bone lesions and provide an answer to this question.

6.4. Discrepancies and inaccuracies in the description of bone lesions

In this study, we submitted 30 bone lesions to a pool of ten participants divided in two: a group of five experienced anthropologists and a group of five forensic pathologists with training and experience in anthropology. All participants were asked to describe the skeletal lesions (clearly signaled on the bones) according to standard paleopathological terminology, by checking one of the proposed answers in three different categories, including character of the lesion (osteoblastic, osteolytic or mixed with both components), margins (either rounded and remodeled or not remodeled) and periosteal reaction (which could be absent or present, and if so as woven bone, lamellar bone, "coral-like", "hair-on-end" or "sunburst" periosteal reactions – more than one answer could be selected in this category). The obtained results were successively analyzed using statistical analysis, namely Fleiss' Kappa test for interobserver analysis, Cohen Kappa test for the intraobserver analysis, as well as accuracy and positive identification rates. In particular, results show inconsistencies between the interpretation of the character of the lesion and the subsequent descriptions; for instance, lesions evaluated as solely osteolytic or osteoblastic but associated with the presence of bone production or bone resorption respectively in other categories. This demonstrates the existence of a grave issue in the understanding of the terminology and an insufficient communication on the basic description of bone lesions. In addition, the categories character of lesions and periosteal reactions showed low accuracy rates (ranging between 56% and 67%), with a 45% to 57% rate of positive identification in periosteal reactions. In fact, absent periostosis (that is, the absence of a new bone deposition on the cortical surface) was recognized in only 71% of cases. Moreover, spongiosclerosis was consistently mistaken for a type of periosteal reaction. Not only do these results indicate that the different types of lesions are poorly

recognized but given that identification of anomalies and lesion description are the basis for the diagnosis of pathological conditions, they testify to the risk of misdiagnosis. Indeed, the inaccurate description of lesions generates considerable variation as well as interobserver error and ambiguity, ultimately leading to misinterpretations in the literature. With this pioneer study, we demonstrated that one of the prerequisites of the study of bone lesions, that is, their recognition and description, is not as assured as we would like to believe. Despite efforts in the standardization of the paleopathological terminology used to describe bone lesions (Buikstra, Cook and Bolhofner, 2017; Klaus, 2017; Mays, 2018; Ragsdale, Campbell and Kirkpatrick, 2018), variation and misunderstandings remain, proving the need for continuing effort in the communication, dissemination and training of the basic principles of the study of bone disease, starting with the description of skeletal lesions. Consequently, the last research line of this PhD project pointed out the issues in the current understanding and use of the paleopathological terminology for the description of bone lesions, stressing the need for continuing efforts in this area.

The main deficiency of this study concerns the limited size of the sample (30 bone lesions) and pool of participants (ten professionals in the field). As with previous research, an increase in sample and pool size could confirm and strengthen the results obtained in the present study. However, this was a pioneer and novel research that could be implemented in the future to reach a larger scale. Additionally, after evidencing the limitations in the recognition and comprehension of bone lesions, it would be interesting to investigate their impact on the diagnosis of bone diseases; for instance, by realizing a similar study and asking the participants to also perform a diagnosis. Such investigation could indicate the key issues resulting in misdiagnosis (e.g. how the misinterpretation of some characteristics of the lesions can negatively impact the final diagnosis) and eventually improve the diagnosis of skeletal diseases on dry bone. While these suggestions do not constitute one of the projects planned for future research, they are perspectives that could be considered for further studies.

Chapter 7. Conclusion and Future Directions

"The life of the dead is placed in the memory of the living."
Marcus Tullius Cicero

One of the objectives of the study of human remains is the pathological analysis, that is, the understanding of disease burden from the analysis of skeletal remains through the diagnosis of pathological conditions. The diagnosis of disease on bone remains is limited to the conditions satisfying the prerequisites for involvement of the skeleton with lesions specific enough to be attributed to a single condition. As explained in Chapter 1, the dry bone diagnosis of diseases consists in the analysis and interpretation of the morphology, position and distribution of lesions by comparison with the clinical literature and previous publications of similar cases. This method presents several limitations inherent to the material used for analysis (the skeletal elements under examination) and the material serving to perform the diagnosis (the clinical and paleopathological literature).

In this PhD project, we investigated the diagnosis of bone diseases based primarily on skeletons with clinical diagnoses from a reference collection, the CAL Milano Cemetery Skeletal Collection (Chapters 2). Indeed, documented reference collections constitute an underexploited resource of considerable value as the skeletal remains are associated with clinical or autopsy-based diagnoses of pathological conditions. Few studies have been published based on reference collections and could overcome the current difficulties in achieving reliable diagnoses by providing additional specific documentation on the morphology and distribution of bone lesions in skeletons with known conditions (Chapter 3).

Several pathological conditions were considered in this research including atherosclerosis, rheumatoid arthritis, diabetes mellitus, HIV, multiple myeloma and cancer. In addition, studies were also performed based on samples selected from routine autopsies (golden standard) and in particular on atherosclerosis (atherosclerotic calcifications), cholelithiasis (gallstones) and pleural plaques. Finally, material from forensic cases, namely ossifying costal cartilage and archaeological specimens with tuberculosis, rickets, congenital syphilis and antemortem trauma were also included for study. Depending on the objective of the different research lines, this material was subjected to different types of analyses including macroscopy, morphology, histology, SEM, SEM-EDS and radiography (Chapter 4).

As a result, we implemented the literature on the variation of the bone manifestations of pathological conditions, strengthening the criteria for diagnosis already established in rheumatoid arthritis, multiple myeloma and metastatic cancer and provided documentation for a reliable diagnosis of diabetes, HIV/AIDS, atherosclerosis and breast and bladder cancers. Moreover, we defined the microscopic structure and specific features of atherosclerotic calcifications, gallstones, ossifying costal cartilage and pleural plaques for their recognition and identification among skeletal remains. We also demonstrated the significant value of a meticulous macroscopic examination as well as the synergy of the application of both macroscopy and radiography in the study of bone disease. Finally, in a pioneer study, we evidenced crucial issues in the understanding of the paleopathological terminology for the description of bone lesions, proving the need for continuing efforts in the communication and training of the basic principles of the study of bone disease (Chapter 5). In addition to the contributions of the research realized in this PhD project, we also discussed the limitations and biases of the studies performed, arguing in particular the need for increased samples sizes and complementary research to improve the significance of our results (Chapter 6).

Future directions thus include the recovery of hospital records associated to the skeletons of the collections to evaluate in particular the impact of duration and treatment on the presence, morphology, severity and distribution of bone lesions; the realization of histological studies on other types of heterotopic ossifications for their recognition and distinction among skeletal remains; the undertaking of a larger project on the histological observation of other markers of disease to improve the understanding of the etiology and the diagnosis of bone lesions; the collection of medical imaging and in particular radiographs on the living with the same conditions to assess the influence of soft tissue on the observation of bone lesions; and lastly, the implementation of biomolecular analyses, specifically the research of pathological DNA and biomarkers of various diseases on dry bone to test and develop new methods for the diagnosis of disease. Consequently, this thesis does not mark the end of a project, but one milestone in a much larger project on the study of bone diseases, as much still remains to be done and the future research, although challenging, can only prove rewarding.

References

Aaron, J. E., Rogers, J. and Kanis, J. A. (1992) 'Paleohistology of Paget's disease in two medieval skeletons', *American Journal of Physical Anthropology*. Wiley Online Library, 89(3), pp. 325–331.

Ababneh, B. *et al.* (2014) 'Distribution of calcification in carotid endarterectomy tissues: comparison of micro-computed tomography imaging with histology.', *Vascular medicine (London, England)*. England, 19(5), pp. 343–350.

Abboud, I. A. (2008) 'Mineralogy and chemistry of urinary stones: patients from North Jordan', *Environmental Geochemistry and Health*, 30(5), pp. 445–463.

Abulafia, J. and Vignale, R. A. (1999) 'Leprosy: pathogenesis updated', *International Journal of Dermatology*. Wiley Online Library, 38(5), pp. 321–334.

Acsádi, G. and Nemeskéri, J. (1970) *History of human life span and mortality*. Budapest: Akademiai kiado.

Alamanos, Y. and Drosos, A. A. (2005) 'Epidemiology of adult rheumatoid arthritis', *Autoimmun Rev*, 4(3), pp. 130–136.

Alemán, I. *et al.* (2012) 'Brief communication: The Granada osteological collection of identified infants and young children', *Am J Phys Anthropol*. Wiley Online Library, 149(4), pp. 606–610.

Allison, M. J. *et al.* (1980) 'Metastatic tumor of bone in a Tiahuanaco female', *Bulletin of the New York Academy of Medicine*, 56(6), pp. 581–587.

Amizuka, N. (2012) 'Histology of epiphyseal cartilage calcification and endochondral ossification', *Frontiers in Bioscience*, E4(1), p. 2085.

Andersen, J. G. and Manchester, K. (1992) 'The rhinomaxillary syndrome in leprosy: A clinical, radiological and palaeopathological study', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 2(2), pp. 121–129.

Andersen, J. G., Manchester, K. and Roberts, C. A. (1994) 'Septic bone changes in leprosy: A clinical, radiological and palaeopathological review', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 4(1), pp. 21–30.

Anderson, T. (2001) 'A recently discovered mediaeval bladder stone from Norwich, with a review of British archaeological bladder stones and documentary evidence for their treatment', *BJU International*. Wiley Online Library, 88(4), pp. 351–354.

Anderson, T., Wakely, J. and Carter, A. (1992) 'Medieval example of metastatic carcinoma: A dry bone, radiological, and SEM study', *Am J Phys Anthropol*. Wiley Online Library, 89(3), pp. 309–323.

Anderson, W. F. *et al.* (2010) 'Male Breast Cancer: A Population-Based Comparison With Female Breast Cancer', *Journal of Clinical Oncology*. American Society of Clinical Oncology, 28(2), pp. 232–239.

Anton, H. C. (1967) 'Multiple Pleural Plaques', *The British Journal of Radiology*. The British Institute of Radiology, 40(477), pp. 685–690.

Appleby, J., Thomas, R. and Buikstra, J. (2015) 'Increasing confidence in paleopathological diagnosis – Application of the Istanbul terminological framework', *International Journal of Paleopathology*, 8, pp. 19–21.

Araújo, A., Reinhard, K. J. and Ferreira, L. F. (2015) 'Chapter Nine - Palaeoparasitology — Human Parasites in Ancient Material', in De Baets, K. and Littlewood, D. T. (eds) *Fossil Parasites*. Academic Press, pp. 349–387.

Arnett, F. C. *et al.* (1988) 'The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis', *Arthritis & Rheumatism*. John Wiley & Sons, Inc., 31(3), pp. 315–324.

Arnett, F. C. (2000) 'Spondyloarthropathies', in Humes, H. D. et al. (eds) *Kelley's Textbook of Internal Medicine*. 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, pp. 1364–1370.

Ashok, M. *et al.* (2003) 'Energy dispersive X-ray fluorescence analysis of gallstones', *Journal of Radioanalytical and Nuclear Chemistry*, 257(2), pp. 333–335.

Assis, S. and Codinha, S. (2010) 'Metastatic carcinoma in a 14th–19th century skeleton from Constância (Portugal)', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd., 20(5), pp. 603–620.

Athanasiadou, D. *et al.* (2013) 'New insights into the chemical and isotopic composition of human-body biominerals. I: Cholesterol gallstones from England and Greece', *Journal of Trace Elements in Medicine and Biology*, 27(2), pp. 79–84.

Aufderheide, A. C. and Rodríguez-Martín, C. (1998) *The Cambridge Encyclopedia of Human Paleopathology*.

Cambridge, United Kingdom: Cambridge University Press.

Baccino, E. *et al.* (2014) 'Technical note: The two step procedure (TSP) for the determination of age at death of adult human remains in forensic cases', *Forensic Science International*, 244, pp. 247–251.

Backwell, L. R. *et al.* (2012) 'Criteria for identifying bone modification by termites in the fossil record', *Palaeogeography, Palaeoclimatology, Palaeoecology*. Elsevier, 337–338, pp. 72–87.

Bahrami, S. *et al.* (2001) 'Endochondral ossification of costal cartilage is arrested after chondrocytes have reached hypertrophic stage of late differentiation.', *Matrix biology : journal of the International Society for Matrix Biology*. Netherlands, 19(8), pp. 707–715.

Banks, S. E. (2010) 'Erosive osteoarthritis: a current review of a clinical challenge', *Clinical Rheumatology*, 29(7), pp. 697–706.

Baraliakos, X., Coates, L. C. and Braun, J. (2015) 'The involvement of the spine in psoriatic arthritis', *Clin Exp Rheumatol*, 33(5 Suppl 93), pp. S31-5.

Baraybar, J. P. and Shimada, I. (1993) 'A possible case of metastatic carcinoma in a middle Sican burial from Batán Grande, Perú', *International Journal of Osteoarchaeology*. Wiley Online Library, 3(2), pp. 129–135.

Barchilon, V. *et al.* (1996) 'Factors affecting the rate and pattern of the first costal cartilage ossification', *American Journal of Forensic Medicine and Pathology*, 17(3), pp. 239–247.

Barnes, I. and Thomas, M. G. (2006) 'Evaluating bacterial pathogen DNA preservation in museum osteological collections', *Proceedings of the Royal Society B: Biological Sciences*. Royal Society, 273(1587), pp. 645–653.

Bass, W. (2005) *Human osteology: a laboratory manual and field manual*. 5th edn. Columbia, MO: Missouri Archaeological Society.

Bataille, R., Chappard, D. and Klein, B. (1992) 'Mechanisms of bone lesions in multiple myeloma', *Hematology/oncology clinics of North America*, 6(2), pp. 285–295.

Batson, O. V (1940) 'The function of the vertebral veins and their role in the spread of metastases', *Annals of Surgery*, 112(1), pp. 138–149.

Baud, C.-A. and Kramar, C. (1991) 'Soft tissue calcifications in paleopathology', in Ortner, D. J. and Aufderheide, A. C. (eds) *Human Paleopathology: Current Syntheses and Future Options*. Washington, DC: Smithsonian Institution Press, pp. 257–260.

Behrensmeyer, A. K. (1978) 'Taphonomic and ecologic information from bone weathering', *Paleobiology*. Cambridge University Press, 4(2), pp. 150–162.

Beighton, P. *et al.* (1993) 'Genetic skeletal dysplasias in the Museum of Pathological Anatomy, Vienna', *American Journal of Medical Genetics*. Wiley-Blackwell, 47(6), pp. 843–847.

Beighton, P. *et al.* (1994) 'Bone dysplasias of infancy in the Vienna collection', *Pediatric Radiology*, 24(6), pp. 384–386.

Berenbaum, F. (2013) 'Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!)', *Osteoarthritis and Cartilage*, 21(1), pp. 16–21.

Berry, J. L., Davies, M. and Mee, A. P. (2002) 'Vitamin D metabolism, rickets, and osteomalacia', in *Seminars in musculoskeletal radiology*. Thieme Medical Publishers, pp. 173–182.

Biehler-Gomez, L., Giordano, G. and Cattaneo, C. (2019) 'The overlooked primary: bladder cancer metastases on dry bone. A study of the 20th century CAL Milano Cemetery Skeletal Collection', *International Journal of Paleopathology*, 24, pp. 130–140.

Biermann, J. S. and Baker, L. H. (2000) 'Sarcomas of Soft Tissue and Bone', in Humes, H. D. et al. (eds) *Kelley's Textbook of Internal Medicine*. 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, pp. 1648–1653.

Binder, M. *et al.* (2014) 'On the antiquity of cancer: Evidence for metastatic carcinoma in a young man from ancient Nubia (c. 1200bc)', *PLoS One*, 9(3), pp. 1–11.

Bladé, J. and Rosiñol, L. (2007) 'Complications of Multiple Myeloma', *Hematology/Oncology Clinics of North America*, 21(6), pp. 1231–1246.

Bloom, R. A. *et al.* (1987) 'The periosteal sunburst reaction to bone metastases. A literature review and report of 20 additional cases', *Skeletal Radiol*, 16(8), pp. 629–634.

De Boer, H. H., Van der Merwe, A. E. and Maat, G. J. R. (2013) 'The diagnostic value of microscopy in dry bone palaeopathology: A review', *International Journal of Paleopathology*, 3(2), pp. 113–121.

Bonafe, L. *et al.* (2015) 'Nosology and classification of genetic skeletal disorders: 2015 revision', *American Journal of Medical Genetics Part A*. Wiley Online Library, 167(12), pp. 2869–2892.

Bouwman, A. S. and Brown, T. A. (2005) 'The limits of biomolecular palaeopathology: ancient DNA cannot be used to study venereal syphilis', *Journal of Archaeological Science*, 32(5), pp. 703–713.

Bradsher, R. W. (1992) 'Blastomycosis', *Clinical Infectious Diseases*. Oxford University Press, 14, pp. S82–S90.

Brahee, D. D., Pierre-Jerome, C. and Kettner, N. W. (2003) 'Clinical and radiological manifestations of the rheumatoid wrist. A comprehensive review', *Journal of Manipulative and Physiological Therapeutics*, 26(5), pp. 323–329.

Braun, H. J. and Gold, G. E. (2012) 'Diagnosis of osteoarthritis: Imaging', *Bone*, 51(2), pp. 278–288.

Braun, J. and Sieper, J. (2007) 'Ankylosing spondylitis', *The Lancet*, 369(9570), pp. 1379–1390.

Braunstein, E. M. (2017) *Evaluation of Anemia - Merck Manual For Health Professionals*. Available at: https://www.msdmanuals.com/professional/hematology-and-oncology/approach-to-the-patient-with-anemia/evaluation-of-anemia.

Breman, J. G. and Arita, I. (1980) 'The confirmation and maintenance of smallpox eradication'. Geneva, Switzerland: World Health Organization.

Brickley, M. and Ives, R. (2006) 'Skeletal manifestations of infantile scurvy', *American Journal of Physical Anthropology*. Wiley Online Library, 129(2), pp. 163–172.

Brickley, M. and Ives, R. (2010) *The Bioarchaeology of Metabolic Bone Disease*. London: Elsevier Academic Press.

Brickley, M., Mays, S. and Ives, R. (2005) 'Skeletal manifestations of vitamin D deficiency osteomalacia in documented historical collections', *International Journal of Osteoarchaeology*. Wiley Online Library, 15(6), pp. 389–403.

Brickley, M., Mays, S. and Ives, R. (2007) 'An investigation of skeletal indicators of vitamin D deficiency in adults: Effective markers for interpreting past living conditions and pollution levels in 18th and 19th century Birmingham, England', *American Journal of Physical Anthropology*. Wiley Online Library, 132(1), pp. 67–79.

Brickley, M., Mays, S. and Ives, R. (2010) 'Evaluation and interpretation of residual rickets deformities in adults', *International Journal of Osteoarchaeology*. Wiley Online Library, 20(1), pp. 54–66.

Brickley, M. and McKinley, J. I. (2004) *Guidelines to the standards for recording human remains*. Southampton: British Association for Biological Anthropology and Osteoarchaeology and Institute of Field Archaeologists.

Bridges, P. S. (1993) 'The effect of variation in methodology on the outcome of osteoarthritic studies', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 3(4), pp. 289–295.

Brooks, S. and Suchey, J. M. (1990) 'Skeletal age determination based on the os pubis: A comparison of the Acsádi-Nemeskéri and Suchey-Brooks methods', *Human Evolution*, 5(3), pp. 227–238.

Brothwell, D. (2008) 'Tumor and tumor-like processes', in Pinhasi, R. and Mays, S. (eds) *Advances in human palaeopathology*. John Wiley & Sons, pp. 253–281.

Brothwell, D. (2012) 'Tumors: Problems of differential diagnosis in paleopathology', in Grauer, A. L. (ed.) *A Companion to Paleopathology*. Oxford: Wiley-Blackwell, pp. 420–433.

Bruintjes, T. and Panhuysen, R. (1992) 'The paleopathological diagnosis of seronegatieve spondylarthropathies', in *9th European Meeting of the Paleopathology Association*. Barcelona, Spain, pp. 73–77.

Buckberry, J. L. and Chamberlain, A. T. (2002) 'Age estimation from the auricular surface of the ilium: A revised method', *American Journal of Physical Anthropology*. John Wiley & Sons, Ltd, 119(3), pp. 231–239.

Buikstra, J. E. (2019) *Ortner's Identification of Pathological Conditions in Human Skeletal Remains*. Academic Press.

Buikstra, J. E., Cook, D. C. and Bolhofner, K. L. (2017) 'Introduction: Scientific rigor in paleopathology', *International Journal of Paleopathology*, 19(July), pp. 80–87.

Buikstra, J. E. and Ubelaker, D. H. (1994) *Standards for Data Collection from Human Skeletal Remains*. Fayetteville, Arkansas: Arkansas Archaeological Survey Report Number 44.

Bussard, K. M., Gay, C. V and Mastro, A. M. (2008) 'The bone microenvironment in metastasis; what is special about bone?', *Cancer and Metastasis Reviews*, 27(1), pp. 41–55.

Canoso, J. J. (2000) 'Infiltrative, Neoplastic, and Endocrinologic Causes of Musculoskeletal Complaints', in Humes, H. D. et al. (eds) *Kelley's Textbook of Internal Medicine*. 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, pp. 1431–1436.

Capasso, L. L. (2005) 'Antiquity of cancer', *International Journal of Cancer*. Wiley Online Library, 113(1), pp. 2–13.

Cappella, A. *et al.* (2017) 'The Issue of Age Estimation in a Modern Skeletal Population: Are Even the More Modern Current Aging Methods Satisfactory for the Elderly?', *Journal of Forensic Sciences*, 62(1), pp. 12–17.

Cardoso, H. F. V (2006) 'Brief communication: The collection of identified human skeletons housed at the Bocage Museum (National Museum of Natural History), Lisbon, Portugal', *Am J Phys Anthropol*. Wiley Online Library, 129(2), pp. 173–176.

Carey, M. C. (1993) 'Pathogenesis of gallstones', *The American Journal of Surgery*, 165(4), pp. 410–419.

Carrara, N., Scaggion, C. and Holland, E. (2018) 'The Tedeschi collection: A collection of documented and undocumented human skeletal remains at the Museum of Anthropology, Padua University (Italy)', *American Journal of Physical Anthropology*, 166(4), pp. 930–933.

Castoldi, E. *et al.* (2017) 'The Difficult Task of Diagnosing Prostate Cancer Metastases on Dry Bone', *J Forensic Sci*.

Cattaneo, C. *et al.* (2018) 'A modern documented Italian identified skeletal collection of 2127 skeletons: the CAL Milano Cemetery Skeletal Collection', *Forensic Science International*. Elsevier, 287, pp. 219.e1-219.e5.

Cawley, W. D. and Paine, R. R. (2015) 'Skeletal indicators of reactive arthritis: A case study comparison to other skeletal conditions, such as rheumatoid arthritis, ankylosing spondylitis, ankylosing seronegative SpA, and DISH', *International Journal of Paleopathology*, 11, pp. 70–74.

Chandran, P. *et al.* (2007) 'An extended chemical analysis of gallstone', *Indian Journal of Clinical Biochemistry*. New Delhi: Springer India, 22(2), pp. 145–150.

Chantelau, E. and Onvlee, G. J. (2006) 'Charcot foot in diabetes: farewell to the neurotrophic theory', *Horm Metab Res*, 38(6), pp. 361–367.

Chew, F. S. (2012) *Skeletal Radiology: the Bare Bones*. 3rd edn. Philadelphia, PA: Lippincott Williams & Wilkins.

Chhem, R. K. and Brothwell, D. (2008) *Paleoradiology: Imaging mummies and fossils*. Berlin (Germany): Springer.

Choy, E. (2012) 'Understanding the dynamics: pathways involved in the pathogenesis of rheumatoid arthritis', *Rheumatology*, 51(suppl_5), pp. v3–v11.

Christensen, A. M., Passalacqua, N. V and Bartelink, E. J. (2014) *Forensic anthropology: current methods and practice*. Elsevier.

Clarke, C. C. *et al.* (2006) 'Pleural Plaques: A Review of Diagnostic Issues and Possible Nonasbestos Factors', *Archives of Environmental & Occupational Health*. Routledge, 61(4), pp. 183–192.

Cockshott, P. and MacGregor, M. (1958) 'Osteomyelitis Variolosa.', *Quarterly Journal of Medicine*, 27(107), pp. 369–387.

Coe, F. L., Parks, J. H. and Asplin, J. R. (1992) 'The Pathogenesis and Treatment of Kidney Stones', *New England Journal of Medicine*. Massachusetts Medical Society, 327(16), pp. 1141–1152.

Coleman, R. E. (1997) 'Skeletal complications of malignancy', *Cancer*. John Wiley & Sons, Inc., 80(S8), pp. 1588–1594.

Coleman, R. E. (2001) 'Metastatic bone disease: clinical features, pathophysiology and treatment strategies', *Cancer Treatment Reviews*, 27(3), pp. 165–176.

Coleman, R. E. (2006) 'Clinical Features of Metastatic Bone Disease and Risk of Skeletal Morbidity', *Clinical Cancer Research*, 12(20), p. 6243.

Corron, L. *et al.* (2017) 'Using Classifications to Identify Pathological and Taphonomic Modifications on Ancient Bones: Do "Taphognomonic" Criteria Exist?', *BMSAP*, 29(1–2), pp. 1–18.

Crubézy, É. and Trinkaus, E. (1992) 'Shanidar 1: A case of hyperostotic disease (DISH) in the middle paleolithic', *American Journal of Physical Anthropology*. John Wiley & Sons, Ltd, 89(4), pp. 411–420.

Cunha, Eugenia (2006) 'Pathology as a factor of personal identity in forensic anthropology', in Schmitt, A., Cunha, Eugénia, and Pinheiro, J. (eds) *Forensic anthropology and medicine*. Springer, pp. 333–358.

Cunningham, C., Scheuer, L. and Black, S. (2016) *Developmental Juvenile Osteology*. Academic Press.

David, A. R. and Zimmerman, M. R. (2010) 'Cancer: an old disease, a new disease or something in between?', *Nat Rev Cancer*. Nature Publishing Group, 10(10), p. 728.

Davidson, J. C. and Palmer, P. E. S. (1963) 'Osteomyelitis Variolosa', *The Journal of Bone and Joint Surgery*.

British volume. The British Editorial Society of Bone & Joint Surgery, 45-B(4), pp. 687–693.

Dayal, M. R. *et al.* (2009) 'The history and composition of the Raymond A. Dart Collection of Human Skeletons at the University of the Witwatersrand, Johannesburg, South Africa.', *American journal of physical anthropology*. United States, 140(2), pp. 324–335.

Delgado, J. *et al.* (2005) 'Lung Cancer Pathogenesis Associated With Wood Smoke Exposure', *Chest*. Elsevier, 128(1), pp. 124–131.

Derry, D. E. (1911) 'Damage done to Skulls and Bones by Termites', *Nature*. Nature Publishing Group, 86, p. 245.

DeVita, V. T. and Chu, E. (2008) 'A History of Cancer Chemotherapy', *Cancer Research*, 68(21), p. 8643.

Dhir, V. and Aggarwal, A. (2013) 'Psoriatic arthritis: a critical review', *Clin Rev Allergy Immunol*. 2012/02/02, 44(2), pp. 141–148.

Dominick, K. L. and Baker, T. A. (2004) 'Racial and ethnic differences in osteoarthritis: prevalence, outcomes, and medical care', *Ethn Dis*, 14(4), pp. 558–566.

Donoghue, H. D. *et al.* (2004) 'Tuberculosis: from prehistory to Robert Koch, as revealed by ancient DNA', *The Lancet infectious diseases*. Elsevier, 4(9), pp. 584–592.

Dorfman, H. D. and Czerniak, B. (1998) *Bone tumors*. Mosby.

Duhig, C., Strouhal, E. and Němečková, A. (1996) 'Case of a secondary carcinoma of Anglo-Saxon period from Edix Hill, Barrington, Cambridgeshire, England', *Journal of Paleopathology*, 8, pp. 25–32.

Edwards, C. M., Zhuang, J. and Mundy, G. R. (2008) 'The pathogenesis of the bone disease of multiple myeloma', *Bone*, 42(6), pp. 1007–1013.

Eeckels, R., Vincent, J. and Seynhaeve, V. (1964) 'Bone Lesions due to Smallpox', *Archives of disease in childhood*, 39(208), pp. 591–597.

Ehrlich, G. E. (2001) 'Erosive osteoarthritis: Presentation, clinical pearls, and therapy', *Current Rheumatology Reports*, 3(6), pp. 484–488.

Eichelmann, K. *et al.* (2013) 'Leprosy. An Update: Definition, Pathogenesis, Classification, Diagnosis, and Treatment', *Actas Dermo-Sifiliográficas (English Edition)*, 104(7), pp. 554–563.

Eknoyan, G. (2004) 'History of urolithiasis', *Clinical Reviews in Bone and Mineral Metabolism*, 2(3), pp. 177–185.

Eliopoulos, C., Lagia, A. and Manolis, S. (2007) 'A modern, documented human skeletal collection from Greece', *HOMO - Journal of Comparative Human Biology*, 58(3), pp. 221–228.

Emadi, A. and Law, J. (2018) *Overview of Leukemia - Merck Manual for Health Professionals*. Available at: https://www.merckmanuals.com/professional/hematology-and-oncology/leukemias/overview-of-leukemia.

van Engelen, A. *et al.* (2013) 'Automated segmentation of atherosclerotic histology based on pattern classification.', *Journal of pathology informatics*, 4(Suppl), p. S3.

Entezami, P. *et al.* (2011) 'Historical Perspective on the Etiology of Rheumatoid Arthritis', *Hand clinics*, 27(1), pp. 1–10.

Erickson, A. R., Cannella, A. C. and Mikuls, T. R. (2017) 'Clinical Features of Rheumatoid Arthritis', in Firestein, G. S. et al. (eds) *Kelley and Firestein's Textbook of Rheumatology*. Tenth. Philadelphia, PA: Elsevier Health Sciences, pp. 1167–1186.

Faget, G. H. and Mayoral, A. (1944) 'Bone Changes in Leprosy: A Clinical and Roentgenologic Study of 505 Cases', *Radiology*. Radiological Society of North America, 42(1), pp. 1–13.

Fain, O. (2005) 'Musculoskeletal manifestations of scurvy', *Joint Bone Spine*, 72(2), pp. 124–128.

Falasca, G. F. (2006) 'Metabolic diseases: gout', *Clinics in Dermatology*, 24(6), pp. 498–508.

Faraji, F. and Eissenberg, J. C. (2013) 'Seed and soil: A conceptual framework of metastasis for clinicians', *Missouri Medicine*, 110(4), pp. 302–308.

Farnsworth, N. and Rosen, T. (2006) 'Endemic treponematosis: review and update', *Clinics in Dermatology*, 24(3), pp. 181–190.

Felson, D. T. (2013) 'Osteoarthritis as a disease of mechanics', *Osteoarthritis and Cartilage*, 21(1), pp. 10–15.

Ferreira, M. T. *et al.* (2014) 'A new forensic collection housed at the University of Coimbra, Portugal: The 21st century identified skeletal collection', *Forensic Science International*, 245(Supplement C), pp. 202.e1-202.e5.

Fidler, I. J. (1989) 'Origin and biology of cancer metastasis', *Cytometry*. Wiley Online Library, 10(6), pp. 673–680.

Finzel, S. *et al.* (2011) 'A comparative study of periarticular bone lesions in rheumatoid arthritis and psoriatic arthritis', *Ann Rheum Dis*, 70(1), pp. 122–127.

Firestein, G. S. (2000) 'Rheumatoid Arthritis', in Humes, H. D. et al. (eds) *Kelley's Textbook of Internal Medicine*. 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, pp. 1347–1359.

Firestein, G. S. (2003) 'Evolving concepts of rheumatoid arthritis', *Nature*, 423(6937), pp. 356–361.

Firestein, G. S. *et al.* (2017) *Kelley and Firestein's Textbook of Rheumatology*. Tenth. Philadelphia, PA: Elsevier.

Fitzgerald, R. H., Kaufer, H. and Malkani, A. L. (2002) *Orthopaedics*. St Louis: Mosby.

Galasko, C. S. B. (1986) 'Skeletal Metastases', *Clin Orthop Relat Res*, 210, pp. 18–30.

Garber, J. R. *et al.* (2012) 'Clinical Practice Guidelines for Hypothyroidism in Adults: Cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association', *Thyroid*. Mary Ann Liebert, Inc., publishers, 22(12), pp. 1200–1235.

García-Arias, M., Balsa, A. and Mola, E. M. (2011) 'Septic arthritis', *Best Practice & Research Clinical Rheumatology*, 25(3), pp. 407–421.

Garvin, H. M., Sholts, S. B. and Mosca, L. A. (2014) 'Sexual dimorphism in human cranial trait scores: effects of population, age, and body size.', *American journal of physical anthropology*. United States, 154(2), pp. 259–269.

Gault, M. H. and Chafe, L. (2000) 'Relationship of frequency, age, sex, stone weight and composition in 15,624 stones: comparison of results for 1980 to 1983 and 1995 to 1998', *The Journal of Urology*, 164(2), pp. 302–307.

Gehweiler, J. A., Capp, M. P. and Chick, E. W. (1970) 'Observations on the roentgen patterns in blastomycosis of bone: a review of cases from the blastomycosis cooperative study of the Veterans Administration and Duke University Medical Center', *American Journal of Roentgenology*. Am Roentgen Ray Soc, 108(3), pp. 497–510.

Gevenois, P. A. *et al.* (1998) 'Asbestosis, pleural plaques and diffuse pleural thickening: three distinct benign responses to asbestos exposure', *European Respiratory Journal*, 11(5), p. 1021.

Ghosh, Kanjaksha and Ghosh, Kinjalka (2007) 'Pathogenesis of anemia in malaria: a concise review', *Parasitology Research*, 101(6), pp. 1463–1469.

Giuffra, V. *et al.* (2008) 'Giant Bladder Stone in a Natural Mummy of the Early 19th Century', *Urology*, 72(4), pp. 780–781.

Giuliani, N., Rizzoli, V. and Roodman, G. D. (2006) 'Multiple myeloma bone disease: pathophysiology of osteoblast inhibition', *Blood*, 108(13), pp. 3992–3996.

Giurato, L. and Uccioli, L. (2006) 'The diabetic foot: Charcot joint and osteomyelitis', *Nucl Med Commun*, 27(9), pp. 745–749.

Gjestland, T. (1955) 'The Oslo study of untreated syphilis; an epidemiologic investigation of the natural course of the syphilitic infection based upon a re-study of the Boeck-Bruusgaard material.', *Acta dermato-venereologica. Supplementum*, 35(Suppl 34), p. 3.

Gocha, T. P. *et al.* (2015) 'Testing the applicability of six macroscopic skeletal aging techniques on a modern Southeast Asian sample.', *Forensic science international*. Ireland, 249, pp. 318.e1–7.

Gold, R. H., Hawkins, R. A. and Katz, R. D. (1991) 'Bacterial osteomyelitis: findings on plain radiography, CT, MR, and scintigraphy.', *American Journal of Roentgenology*. American Roentgen Ray Society, 157(2), pp. 365–370.

Golden, M. P. and Vikram, H. R. (2005) 'Extrapulmonary tuberculosis: an overview.', *American family physician*. United States, 72(9), pp. 1761–1768.

Grauer, A. L. (2008) 'Macroscopic analysis and data collection in palaeopathology', in Pinhasi, R. and Mays, S. (eds) *Advances in Human Palaeopathology*. Wiley Online Library, pp. 57–76.

Grauer, A. L. (2012) *A Companion to Paleopathology*. John Wiley & Sons.

Gruber, R. (2012) 'Reader's digest of the pathophysiology of bone metastases', *Wiener Medizinische Wochenschrift*, 162(17), pp. 370–373.

Grupe, G. (1988) 'Metastasizing carcinoma in a medieval skeleton: Differential diagnosis and etiology', *Am J Phys Anthropol*. Wiley Subscription Services, Inc., A Wiley Company, 75(3), pp. 369–374.

Guise, T. A. *et al.* (1996) 'Evidence for a causal role of parathyroid hormone-related protein in the pathogenesis of human breast cancer-mediated osteolysis.', *The Journal of Clinical Investigation*. The American Society for Clinical Investigation, 98(7), pp. 1544–1549.

Guise, T. A. (2002) 'The vicious cycle of bone metastases', *Journal of Musculoskeletal and Neuronal Interactions*. Hylonome, 2(6), pp. 570–572.

Guise, T. A. *et al.* (2006) 'Basic Mechanisms Responsible for Osteolytic and Osteoblastic Bone Metastases', *Clinical Cancer Research*, 12(20), pp. 6213–6216.

Guise, T. A., Yin, J. J. and Mohammad, K. S. (2003) 'Role of endothelin-1 in osteoblastic bone metastases', *Cancer*. Wiley Online Library, 97(S3), pp. 779–784.

Gulia, A., Fried, I. and Massone, C. (2010) 'New insights in the pathogenesis and genetics of leprosy', *F1000 medicine reports*. Medicine Reports, 2, p. 30.

Gupta, G. P. and Massagué, J. (2006) 'Cancer Metastasis: Building a Framework', *Cell*, 127(4), pp. 679–695.

Gupta, R. (1993) 'A short history of neuropathic arthropathy', *Clin Orthop Relat Res*, (296), pp. 43–49.

Haas, C. J. *et al.* (2000) 'Detection of Leprosy in Ancient Human Skeletal Remains by Molecular Identification of Mycobacterium leprae', *American Journal of Clinical Pathology*, 114(3), pp. 428–436.

Hackett, C. J. (1975) 'An introduction to diagnostic criteria of syphilis, treponarid and Yaws (treponematoses) in dry bones, and some implications', *Virchows Archiv A*, 368(3), pp. 229–241.

Hackett, C. J. (1976) *Diagnostic Criteria of Syphilis, Yaws and Treponarid (Treponematoses) and Some Other Diseases in Dry Bones*. Berlin (Germany): Springer-Verlag.

Hall, R. and Scanlon, M. F. (1979) 'Hypothyroidism: Clinical features and complications', *Clinics in Endocrinology and Metabolism*, 8(1), pp. 29–38.

Hannu, T. (2011) 'Reactive arthritis', *Best Practice & Research Clinical Rheumatology*, 25(3), pp. 347–357.

Harper, K. N. *et al.* (2011) 'The origin and antiquity of syphilis revisited: An Appraisal of Old World pre-Columbian evidence for treponemal infection', *American Journal of Physical Anthropology*. Wiley Online Library, 146(S53), pp. 99–133.

Harper, K. N., Zuckerman, M. K. and Armelagos, G. J. (2013) 'Correspondence: A Possible (But not Probable?) Case of Treponemal Disease', *International Journal of Osteoarchaeology*. Wiley Online Library, 23(5), pp. 626–627.

Harvey, H. A. and von Reyn Cream, L. (2007) 'Biology of Bone Metastases: Causes and Consequences', *Clinical Breast Cancer*, 7(Supplement 1), pp. S7–S13.

Hauser, G. *et al.* (1989) *Epigenetic variants of the human skull*. Stuttgart, Germany: E. Schweizerbart'sche Verlagsbuchhandlung.

Hay, R. J. (2006) 'Fungal infections', *Clinics in Dermatology*, 24(3), pp. 201–212.

Heathcote, G. M. *et al.* (1998) 'On Treponemal Disease in the Western Pacific: Corrections and Critique', *Current Anthropology*. The University of Chicago Press, 39(3), pp. 359–368.

Hefner, J. T. (2009) 'Cranial Nonmetric Variation and Estimating Ancestry*', *Journal of Forensic Sciences*. John Wiley & Sons, Ltd, 54(5), pp. 985–995.

Hellings, W. E. *et al.* (2010) 'Composition of Carotid Atherosclerotic Plaque Is Associated With Cardiovascular Outcome', *A Prognostic Study*, 121(17), pp. 1941–1950.

Helliwell, P. and Taylor, W. (2005) 'Classification and diagnostic criteria for psoriatic arthritis', *Annals of the Rheumatic Diseases*, 64(Suppl 2), pp. ii3–ii8.

Henderson, D. A. (1999) 'Smallpox: clinical and epidemiologic features', *Emerging infectious diseases*. Centers for Disease Control, 5(4), pp. 537–539.

Henrique da Mota, L. M. *et al.* (2013) 'Guidelines for the diagnosis of rheumatoid arthritis', *Revista Brasileira de Reumatologia*, 53(2), pp. 141–157.

Hens, S. M., Rastelli, E. and Belcastro, M. G. (2008) 'Age Estimation from the Human Os Coxa: A Test on a Documented Italian Collection*', *Journal of Forensic Sciences*. Wiley Online Library, 53(5), pp. 1040–1043.

Hershkovitz, I. *et al.* (1997) 'Recognition of sickle cell anemia in skeletal remains of children', *American Journal of Physical Anthropology*, 104(2), pp. 213–226.

Hershkovitz, I. *et al.* (1998) 'Clues to recognition of fungal origin of lytic skeletal lesions', *American Journal of Physical Anthropology*. Wiley Subscription Services, 106(1), pp. 47–60.

Hershkovitz, I. *et al.* (2002) 'Serpens endocrania symmetrica (SES): A new term and a possible clue for identifying intrathoracic disease in skeletal populations', *American Journal of Physical Anthropology*. Wiley Online Library, 118(3), pp. 201–216.

Hillerdal, G. (1994) 'Pleural Plaques and Risk for Bronchial Carcinoma and Mesothelioma', *Chest*, 105(1), pp. 144–150.

Ho, E. L. and Lukehart, S. A. (2011) 'Syphilis: using modern approaches to understand an old disease', *The Journal of Clinical Investigation*. The American Society for Clinical Investigation, 121(12), pp. 4584–4592.

Hochberg, M. C. (2000) 'Osteoarthritis and Polychondritis', in Humes, H. D. et al. (eds) *Kelley's Textbook of Internal Medicine*. 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, pp. 1359–1364.

Holden, W., Orchard, T. and Wordsworth, P. (2003) 'Enteropathic arthritis', *Rheumatic Disease Clinics of North America*, 29(3), pp. 513–530.

Holgate, R. L. V and Steyn, M. (2016) 'Diffuse idiopathic skeletal hyperostosis: Diagnostic, clinical, and paleopathological considerations', *Clinical Anatomy*. Wiley Online Library, 29(7), pp. 870–877.

Holloway, K. L. *et al.* (2013) 'Skeletal Lesions in Human Tuberculosis May Sometimes Heal: An Aid to Palaeopathological Diagnoses', *PLoS One*. Public Library of Science, 8(4), p. e62798.

Hopewell, P. C. (1994) 'Overview of clinical tuberculosis', in Bloom, B. R. (ed.) *Tuberculosis: pathogenesis, protection, and control*. Washington, DC: American Society for Microbiology, pp. 25–46.

Hourihane, D. O., Lessof, L. and Richardson, P. C. (1966) 'Hyaline and Calcified Pleural Plaques as an Index of Exposure to Asbestos', *British Medical Journal*, 1(5495), pp. 1069–1074.

Hruska, K. A. and Teitelbaum, S. L. (1995) 'Renal Osteodystrophy', *New England Journal of Medicine*. Massachusetts Medical Society, 333(3), pp. 166–175.

Huchet, J.-B. *et al.* (2011) 'Taphonomic evidence of a human skeleton gnawed by termites in a Moche-civilisation grave at Huaca de la Luna, Peru', *International Journal of Osteoarchaeology*. Wiley Online Library, 21(1), pp. 92–102.

von Hunnius, T. E. *et al.* (2007) 'Digging deeper into the limits of ancient DNA research on syphilis', *Journal of Archaeological Science*, 34(12), pp. 2091–2100.

Hunt, D. R. and Albanese, J. (2005) 'History and demographic composition of the Robert J. Terry anatomical collection.', *American journal of physical anthropology*. United States, 127(4), pp. 406–417.

Hutchinson, J. (1887) *Syphilis*. London, UK: Cassell & Company Limited.

Ioannou, S. *et al.* (2016) 'Diagnosing congenital syphilis using Hutchinson's method: Differentiating between syphilitic, mercurial, and syphilitic-mercurial dental defects', *American Journal of Physical Anthropology*. Wiley Online Library, 159(4), pp. 617–629.

Iscan, M. Y. and Loth, S. R. (1986) 'Estimation of age and determination of sex from the sternal rib', *Forensic osteology*, pp. 68–89.

Ives, R. and Brickley, M. (2014) 'New findings in the identification of adult vitamin D deficiency osteomalacia: Results from a large-scale study', *International Journal of Paleopathology*, 7, pp. 45–56.

Jackes, M. K. (1983) 'Osteological evidence for smallpox: A possible case from seventeenth century Ontario', *American Journal of Physical Anthropology*. John Wiley & Sons, Ltd, 60(1), pp. 75–81.

Jacobson, H. G. *et al.* (1958) 'The vertebral pedicle sign: a roentgen finding to differentiate metastatic carcinoma from multiple myeloma.', *The American journal of roentgenology, radium therapy, and nuclear medicine*, 80(5), pp. 817–821.

Jacobson, J. A. *et al.* (2008) 'Radiographic Evaluation of Arthritis: Degenerative Joint Disease and Variations', *Radiology*. Radiological Society of North America, 248(3), pp. 737–747.

Jadon, D. R. and McHugh, N. J. (2014) 'Other seronegative spondyloarthropathies', *Medicine*, 42(5), pp. 257–261.

Jain, R., Sawhney, S. and Berry, M. (1993) 'Computed tomography of vertebral tuberculosis: patterns of bone destruction.', *Clinical radiology*, 47(3), pp. 196–199.

Jantz, L. M. and Jantz, R. L. (1999) 'Secular change in long bone length and proportion in the United States, 1800–1970', *American Journal of Physical Anthropology*. Wiley Online Library, 110(1), pp. 57–67.

Jantz, R. L. and Jantz, L. M. (2016) 'The Remarkable Change in Euro-American Cranial Shape and Size', *Human Biology*. Wayne State University Press, 88(1), pp. 56–64.

Jaskowiec, T. C. *et al.* (2017) 'No stone unturned: The presence of kidney stones in a skeleton from 19th century Peoria, Illinois', *International Journal of Paleopathology*, 19, pp. 18–23.

Johnson, V. L. and Hunter, D. J. (2014) 'The epidemiology of osteoarthritis', *Best Practice & Research Clinical Rheumatology*, 28(1), pp. 5–15.

Johnston, D. E. and Kaplan, M. M. (1993) 'Pathogenesis and Treatment of Gallstones', *New England Journal of Medicine*. Massachusetts Medical Society, 328(6), pp. 412–421.

Joyce, M. J. (2012) *Metastatic Bone Tumors - Merck Manual for Health Professionals*. Available at:

https://www.merckmanuals.com/professional/musculoskeletal-and-connective-tissue-disorders/tumors-of-bones-and-joints/metastatic-bone-tumors.

Jurik, A. G. (2011) 'Imaging the spine in arthritis—a pictorial review', *Insights into Imaging*. Berlin/Heidelberg: Springer-Verlag, 2(2), pp. 177–191.

Kampen, W. U., Ciaassen, H. and Kirsch, T. (1995) 'Mineralization and osteogenesis in the human first rib cartilage', *Annals of Anatomy*, 177(2), pp. 171–177.

Kelley, M. A. and El-Najjar, M. Y. (1980) 'Natural variation and differential diagnosis of skeletal changes in tuberculosis', *American Journal of Physical Anthropology*. Wiley-Blackwell, 52(2), pp. 153–167.

Kelley, M. A. and Micozzi, M. S. (1984) 'Rib lesions in chronic pulmonary tuberculosis', *American Journal of Physical Anthropology*. Wiley-Blackwell, 65(4), pp. 381–386.

Kim, H. J. *et al.* (2015) 'Cervical spine disease in rheumatoid arthritis: incidence, manifestations, and therapy', *Curr Rheumatol Rep*, 17(2), p. 9.

Kim, I. S. *et al.* (2003) 'Classification and nomenclature of gallstones revisited', *Yonsei Med J*, 44(4), pp. 561–570.

Kim, K. Y. *et al.* (2003) 'A literature review of the epidemiology and treatment of acute gout', *Clinical Therapeutics*, 25(6), pp. 1593–1617.

Kim, P. S., Klausmeier, T. L. and Orr, D. P. (2009) 'Reactive Arthritis: A Review', *Journal of Adolescent Health*, 44(4), pp. 309–315.

King, J. B. (1939) 'Calcification of the Costal Cartilages', *The British Journal of Radiology*. The British Institute of Radiology, 12(133), pp. 2–12.

Kirkpatrick, C. L., Campbell, R. A. and Hunt, K. J. (2018) 'Paleo-oncology: Taking stock and moving forward', *International Journal of Paleopathology*, 21, pp. 3–11.

Klales, A. R., Ousley, S. D. and Vollner, J. M. (2012) 'A revised method of sexing the human innominate using Phenice's nonmetric traits and statistical methods', *American Journal of Physical Anthropology*. John Wiley & Sons, Ltd, 149(1), pp. 104–114.

Klaus, H. D. (2017) 'Paleopathological rigor and differential diagnosis: Case studies involving terminology, description, and diagnostic frameworks for scurvy in skeletal remains', *International Journal of Paleopathology*, 19, pp. 96–110.

Knoll, T. (2010) 'Epidemiology, Pathogenesis, and Pathophysiology of Urolithiasis', *European Urology Supplements*, 9(12), pp. 802–806.

Knoll, T. *et al.* (2011) 'Urolithiasis Through the Ages: Data on More Than 200,000 Urinary Stone Analyses', *The Journal of Urology*, 185(4), pp. 1304–1311.

Komar, D. A. and Buikstra, J. E. (2003) 'Differential diagnosis of a prehistoric biological object from the Koster (Illinois) Site', *International Journal of Osteoarchaeology*. Wiley Online Library, 13(3), pp. 157–164.

Krakow, D. and Rimoin, D. L. (2010) 'The skeletal dysplasias', *Genetics In Medicine*. The American College of Medical Genetics, 12, p. 327.

Kumar, S. K. *et al.* (2017) 'Multiple myeloma', *Nature Reviews Disease Primers*, 3, pp. 1–20.

Kusafuka, K. *et al.* (2001) 'Ossification of tracheal cartilage in aged humans: a histological and immunohistochemical analysis.', *Journal of bone and mineral metabolism*. Japan, 19(3), pp. 168–174.

L'Abbé, E. N., Loots, M. and Meiring, J. H. (2005) 'The Pretoria Bone Collection: A modern South African skeletal sample', *HOMO - Journal of Comparative Human Biology*, 56(2), pp. 197–205.

de la Rúa, C., Baraybar, J. P. and Etxeberria, F. (1995) 'Neolithic case of metastasizing carcinoma: Multiple approaches to differential diagnosis', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd., 5(3), pp. 254–264.

LaFond, R. E. and Lukehart, S. A. (2006) 'Biological Basis for Syphilis', *Clinical Microbiology Reviews*, 19(1), pp. 29 LP – 49.

Lagia, A., Eliopoulos, C. and Manolis, S. (2007) 'Thalassemia: macroscopic and radiological study of a case', *International Journal of Osteoarchaeology*. Wiley Online Library, 17(3), pp. 269–285.

Lahu, A. *et al.* (2015) 'Modes of Presentation of Reactive Arthritis Based on the Affected Joints', *Medical Archives*, 69(1), pp. 42–45.

Laiho, K. and Kauppi, M. (2002) 'The cervical spine in patients with psoriatic arthritis', *Annals of the Rheumatic Diseases*, 61(7), pp. 650–652.

Landis, J. R. and Koch, G. G. (1977) 'The measurement of observer agreement for categorical data', *Biometrics*. JSTOR, pp. 159–174.

Lee, D. M. and Weinblatt, M. E. (2001) 'Rheumatoid arthritis', *Lancet*, 358(9285), pp. 903–911.

Lefort, M. and Bennike, P. (2007) 'A case study of possible differential diagnoses of a medieval skeleton from Denmark: leprosy, ergotism, treponematosis, sarcoidosis or smallpox?', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 17(4), pp. 337–349.

Levy, M. J. and Valabhji, J. (2008) 'The diabetic foot', *Surgery (Oxford)*, 26(1), pp. 25–28.

Lew, D. P. and Waldvogel, F. A. (1997) 'Osteomyelitis', *New England Journal of Medicine*. Massachusetts Medical Society, 336(14), pp. 999–1007.

Lewis, M. (2017) *Paleopathology of children: Identification of pathological conditions in the human skeletal remains of non-adults*. Academic Press.

Lichenstein, L. (1953) 'Histiocytosis X; integration of eosinophilic granuloma of bone, Letterer-Siwe disease, and Schuller-Christian disease as related manifestations of a single nosologic entity.', *A.M.A. archives of pathology*, 56(1), pp. 84–102.

Lipton, J. F. and Vigorita, V. J. (2016) 'Metastatic Bone Disease', in Vigorita, V. J., Ghelman, B., and Mintz, D. (eds) *Orthopaedic Pathology*. Third edit. Philadelphia: Lippincott Williams & Wilkin, pp. 594–619.

Lovejoy, C. O. *et al.* (1985) 'Chronological metamorphosis of the auricular surface of the ilium: A new method for the determination of adult skeletal age at death', *Am J Phys Anthropol*. Wiley Online Library, 68(1), pp. 15–28.

Lovell, N. C. (2000) 'Paleopathological description and diagnosis', in Saunders, S. R. and Katzenberg, A. M. (eds) *Biological Anthropology of the Human Skeleton*. 1st edn. Wiley-Liss New York, pp. 217–248.

Lowenberg, B., Downing, J. R. and Burnett, A. (1999) 'Acute Myeloid Leukemia', *New England Journal of Medicine*. Massachusetts Medical Society, 341(14), pp. 1051–1062.

Luna, L. H. *et al.* (2008) 'A case of multiple metastasis in Late Holocene hunter-gatherers from the Argentine Pampean region', *International Journal of Osteoarchaeology*. Wiley Online Library, 18(5), pp. 492–506.

Lupi, O., Tyring, S. K. and McGinnis, M. R. (2005) 'Tropical dermatology: Fungal tropical diseases', *Journal of the American Academy of Dermatology*, 53(6), pp. 931–951.

Majithia, V. and Geraci, S. A. (2007) 'Rheumatoid Arthritis: Diagnosis and Management', *The American Journal of Medicine*, 120(11), pp. 936–939.

Malkina, A. (2018) *Chronic Kidney Disease - Merck Manual for Health Professionals*. Available at: https://www.msdmanuals.com/professional/genitourinary-disorders/chronic-kidney-disease/chronic-kidney-disease.

Manchester, M., Ogden, A. and Storm, R. (2016) *Nomenclature in Paleopathology*, *Paleopathology Association Newsletter*.

Mankin, H. J. (1974) 'Review Article, Rickets, Osteomalacia, and Renal Osteodystrophy', *Journal of Bone & Joint Surgery*. Lippincott Williams & Wilkins, 56(1), pp. 101–128.

Mann, R. W. and Hunt, D. R. (2005) *Photographic regional atlas of bone disease*. 3rd ed. Springfield, IL: Charles Thomas Publisher.

Manthey, L. *et al.* (2017) 'Secular change of sexually dimorphic cranial variables in Euro-Americans and Germans.', *International journal of legal medicine*. Germany, 131(4), pp. 1113–1118.

Mariotti, V. *et al.* (2015) 'Skeletal evidence of tuberculosis in a modern identified human skeletal collection (Certosa cemetery, Bologna, Italy)', *American Journal of Physical Anthropology*. Wiley Online Library, 157(3), pp. 389–401.

Marks, M. K. and Hamilton, M. D. (2007) 'Metastatic carcinoma: palaeopathology and differential diagnosis', *International Journal of Osteoarchaeology*. Wiley Online Library, 17(3), pp. 217–234.

Marks, M., Solomon, A. W. and Mabey, D. C. (2014) 'Endemic treponemal diseases', *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 108(10), pp. 601–607.

Marlow, E. J. and Pastor, R. F. (2011) 'Sex determination using the second cervical vertebra--A test of the method.', *Journal of forensic sciences*, 56(1), pp. 165–169.

Marques, C. *et al.* (2018) 'Absence of evidence or evidence of absence? A discussion on paleoepidemiology of neoplasms with contributions from two Portuguese human skeletal reference collections (19th–20th century)', *International Journal of Paleopathology*, 21, pp. 83–95.

Marques, C., Santos, A. L. and Cunha, E. (2013) 'Better a Broader Diagnosis Than a Misdiagnosis: The Study of a Neoplastic Condition in a Male Individual who Died in Early 20th Century (Coimbra, Portugal)', *International Journal of Osteoarchaeology*. Wiley Online Library, 23(6), pp. 664–675.

Marshall, M. *et al.* (2013) 'Erosive osteoarthritis: a more severe form of radiographic hand osteoarthritis

rather than a distinct entity?', *Annals of the Rheumatic Diseases*, 74(1), pp. 136–141.

Martrille, L. *et al.* (2007) 'Comparison of Four Skeletal Methods for the Estimation of Age at Death on White and Black Adults*', *Journal of Forensic Sciences*. Blackwell Publishing Inc, 52(2), pp. 302–307.

Marx, S. J. (2000) 'Hyperparathyroid and Hypoparathyroid Disorders', *New England Journal of Medicine*. Massachusetts Medical Society, 343(25), pp. 1863–1875.

Mas, A. J. and Rotés-Querol, J. (2007) 'Erosive osteoarthritis of the feet: Description of two patients', *Joint Bone Spine*, 74(3), pp. 296–298.

Mastro, A. M., Gay, C. V and Welch, D. R. (2003) 'The skeleton as a unique environment for breast cancer cells', *Clinical & Experimental Metastasis*, 20(3), pp. 275–284.

Matos, V. and Santos, A. L. (2006) 'On the trail of pulmonary tuberculosis based on rib lesions: Results from the human identified skeletal collection from the Museu Bocage (Lisbon, Portugal)', *American Journal of Physical Anthropology*. Wiley Online Library, 130(2), pp. 190–200.

Mays, S. *et al.* (1996) 'A case of metastatic carcinoma of medieval date from Wharram Percy, England', *Journal of Paleopathology*, 8, pp. 33–42.

Mays, S. *et al.* (2001) 'Paleopathological and biomolecular study of tuberculosis in a medieval skeletal collection from England', *American Journal of Physical Anthropology*. John Wiley & Sons, Ltd, 114(4), pp. 298–311.

Mays, S. (2018) 'How should we diagnose disease in palaeopathology? Some epistemological considerations', *International Journal of Paleopathology*, 20, pp. 12–19.

Mays, S. and Brickley, M. (2018) 'Vitamin D deficiency in bioarchaeology and beyond: The study of rickets and osteomalacia in the past', *International Journal of Paleopathology*, 23, pp. 1–5.

Mays, S., Brickley, M. and Ives, R. (2006) 'Skeletal manifestations of rickets in infants and young children in a historic population from England', *American Journal of Physical Anthropology*. Wiley Online Library, 129(3), pp. 362–374.

Mays, S. and Vincent, S. (2013) 'Correspondence: Probable and Possible Cases of Treponematosis: A Response to the Comment "A Possible (But not Probable) Case of Treponemal Disease" by Harper, Zuckerman and Armelagos', *International Journal of Osteoarchaeology*. Wiley Online Library, 23(5), pp. 628–629.

Mays, S., Watt, I. and Loe, L. (2016) 'An Unusual Erosive Arthropathy from Medieval England', *International Journal of Osteoarchaeology*. Wiley Online Library, 27(4), pp. 693–699.

McIlvaine, B. K. (2015) 'Implications of Reappraising the Iron-Deficiency Anemia Hypothesis', *International Journal of Osteoarchaeology*. Wiley Online Library, 25(6), pp. 997–1000.

McInnes, I. B. and Schett, G. (2011) 'The Pathogenesis of Rheumatoid Arthritis', *New England Journal of Medicine*. Massachusetts Medical Society, 365(23), pp. 2205–2219.

Meindl, R. S. and Lovejoy, C. O. (1985) 'Ectocranial suture closure: A revised method for the determination of skeletal age at death based on the lateral-anterior sutures', *Am J Phys Anthropol*. Wiley Online Library, 68(1), pp. 57–66.

Melikian, M. (2006) 'A case of metastatic carcinoma from 18th century London', *International Journal of Osteoarchaeology*. Wiley Online Library, 16(2), pp. 138–144.

Merczi, M. *et al.* (2014) 'Skeletal Metastatic Carcinomas from the Roman Period (1st to 5th Century AD) in Hungary', *Pathobiology*, 81(2), pp. 100–111.

Meyer, C. *et al.* (2002) 'Syphilis 2001 a palaeopathological reappraisal', *HOMO-Journal of Comparative Human Biology*. Urban & Fischer, 53(1), pp. 39–58.

Micarelli, I. *et al.* (2019) 'A possible case of mycosis in a post-classical burial from La Selvicciola (Italy)', *International Journal of Paleopathology*, 24, pp. 25–33.

Miler, S. F., Thomas, C. Y. and Shiozawa, Y. (2017) 'Molecular Involvement of the Bone Marrow Microenvironment in Bone Metastasis', in *Introduction to Cancer Metastasis*. Academic Press, pp. 263–276.

Miller, E., Ragsdale, B. D. and Ortner, D. J. (1996) 'Accuracy in Dry Bone Diagnosis: A Comment on Palaeopathological Methods', *International Journal of Osteoarchaeology*, 6(3), pp. 221–229.

Miller, L. H. *et al.* (2002) 'The pathogenic basis of malaria', *Nature*. Nature Publishing Group, 415, p. 673.

Miller, T. T. (2008) 'Bone Tumors and Tumorlike Conditions: Analysis with Conventional Radiography', *Radiology*. Radiological Society of North America, 246(3), pp. 662–674.

Milner, G. R. and Smith, V. G. (1989) 'Carnivore alteration of human bone from a late prehistoric site in illinois', *American Journal of Physical Anthropology*. Wiley Online Library, 79(1), pp. 43–49.

Mitchell, P. D. and Brickley, M. (2017) *Updated guidelines to the standards for recording human remains*. Chartered Institute for Archaeologists.

Mitjà, O., Asiedu, K. and Mabey, D. (2013) 'Yaws', *The Lancet*, 381(9868), pp. 763–773.

Moe, S. M. *et al.* (2007) 'Chronic Kidney Disease–Mineral-Bone Disorder: A New Paradigm', *Advances in Chronic Kidney Disease*, 14(1), pp. 3–12.

Moe, S. M. and Chen, N. X. (2008) 'Mechanisms of Vascular Calcification in Chronic Kidney Disease', *Journal of the American Society of Nephrology*, 19(2), pp. 213 LP – 216.

Molines, L., Darmon, P. and Raccah, D. (2010) 'Charcot's foot: Newest findings on its pathophysiology, diagnosis and treatment', *Diabetes & metabolism*, 36(4), pp. 251–255.

Moll, J. M. H. and Wright, V. (1973) 'Psoriatic arthritis', *Seminars in Arthritis and Rheumatism*, 3(1), pp. 55–78.

Møller-Christensen, V. *et al.* (1952) 'Changes in the anterior nasal spine and the alveolar process of the maxillary bone in leprosy', *Internat. J. Leprosy*, 20, pp. 335–340.

Møller-Christensen, V. (1953) *Ten lepers from Næstved in Denmark: a study of skeletons from a Medieval Danish leper hospital*. Danish Science Press.

Møller-Christensen, V. (1961) *Bone Changes in Leprosy.*, *Bone Changes in Leprosy*. Copenhagen: Munksgaard, Denmark.

Monu, J. U. V and Pope Jr, T. L. (2004) 'Gout: a clinical and radiologic review', *Radiologic Clinics of North America*, 42(1), pp. 169–184.

Moore, R. M. and Green, N. E. (1982) 'Blastomycosis of bone. A report of six cases.', *JBJS*, 64(7).

Moore, Z. S., Seward, J. F. and Lane, J. M. (2006) 'Smallpox', *The Lancet*, 367(9508), pp. 425–435.

Mundy, G. R. (1997) 'Mechanisms of bone metastasis', *Cancer*. Wiley Online Library, 80(S8), pp. 1546–1556.

Mundy, G. R. (2002) 'Metastasis: Metastasis to bone: causes, consequences and therapeutic opportunities', *Nat Rev Cancer*, 2(8), pp. 584–593.

Myers, W. A., Gottlieb, A. B. and Mease, P. (2006) 'Psoriasis and psoriatic arthritis: clinical features and disease mechanisms', *Clinics in Dermatology*, 24(5), pp. 438–447.

Nathoo, N. *et al.* (2011) 'History of the Vertebral Venous Plexus and the Significant Contributions of Breschet and Batson', *Neurosurgery*, 69(5), pp. 1007–1014.

National Cancer Institute (2017) *Cancer Statistics*. Available at: https://www.cancer.gov/about-cancer/understanding/statistics.

Navega, D. *et al.* (2015) 'AncesTrees: ancestry estimation with randomized decision trees', *International Journal of Legal Medicine*, 129(5), pp. 1145–1153.

Nerlich, A. G. *et al.* (1997) 'Molecular evidence for tuberculosis in an ancient Egyptian mummy', *The Lancet*. Elsevier, 350(9088), p. 1404.

Nerlich, A. G. *et al.* (2006) 'Malignant tumors in two ancient populations: An approach to historical tumor epidemiology.', *Oncology reports*, 16(1), pp. 197–202.

Nerlich, A. G. *et al.* (2008) 'Plasmodium falciparum in ancient Egypt', *Emerging infectious diseases*. Centers for Disease Control and Prevention, 14(8), pp. 1317–1319.

Nuki, G. and Simkin, P. A. (2006) 'A concise history of gout and hyperuricemia and their treatment', *Arthritis Res Ther*, 8 Suppl 1, p. S1.

O'Sullivan, G. J., Carty, F. L. and Cronin, C. G. (2015) 'Imaging of bone metastasis: An update', *World Journal of Radiology*. Baishideng Publishing Group Inc, 7(8), pp. 202–211.

Olivieri, I. *et al.* (2002) 'Seronegative spondyloarthritides', *Best Practice & Research Clinical Rheumatology*, 16(5), pp. 723–739.

Oostra, R. *et al.* (1998) 'Congenital anomalies in the teratological collection of Museum Vrolik in Amsterdam, The Netherlands. II: Skeletal dysplasias', *American Journal of Medical Genetics*. Wiley Online Library, 77(2), pp. 116–134.

Ordi, J. *et al.* (2006) 'The Severe Gout of Holy Roman Emperor Charles V', *New England Journal of Medicine*. Massachusetts Medical Society, 355(5), pp. 516–520.

Ortner, D. J. *et al.* (2001) 'Evidence of probable scurvy in subadults from archeological sites in North America', *American Journal of Physical Anthropology*. John Wiley & Sons, Ltd, 114(4), pp. 343–351.

Ortner, D. J. (2003) *Identification of Pathological Conditions in Human Skeletal Remains*. 3rd edn. New York: Smithsonian Institution Press.

Ortner, D. J. (2011a) 'Human skeletal paleopathology', *International Journal of Paleopathology*, 1(1), pp. 4–11.

Ortner, D. J. (2011b) 'What skeletons tell us. The story of human paleopathology', *Virchows Archiv*, 459(3), pp. 247–254.

Ortner, D. J. (2012) 'Differential diagnosis and issues in disease classification', in *A Companion to Paleopathology*. Wiley Online Library, pp. 250–267.

Ortner, D. J. and Aufderheide, A. C. (1991) *Human Paleopathology: Current Syntheses and Future Options*. Washington, DC: Smithsonian Institution Press.

Ortner, D. J. and Hotz, G. (2005) 'Skeletal manifestations of hypothyroidism from Switzerland', *American Journal of Physical Anthropology*. Wiley Online Library, 127(1), pp. 1–6.

Ortner, D. J. and Mays, S. (1998) 'Dry-bone manifestations of rickets in infancy and early childhood', *International Journal of Osteoarchaeology*. Wiley Online Library, 8(1), pp. 45–55.

Ousley, S. D. and Jantz, R. L. (2012) 'Fordisc 3 and statistical methods for estimating sex and ancestry', in Dirkmaat, D. C. (ed.) *A companion to forensic anthropology*. New York: Wiley-Blackwell, pp. 311–329.

Oxenham, M. F. and Cavill, I. (2010) 'Porotic hyperostosis and cribra orbitalia: the erythropoietic response to iron-deficiency anaemia', *Anthropological Science*, 118(3), pp. 199–200.

Özdemir, K., Akyol, A. A. and Erdal, Y. S. (2015) 'A Case of Ancient Bladder Stones from Oluz Höyük, Amasya, Turkey', *International Journal of Osteoarchaeology*. Wiley Online Library, 25(6), pp. 827–837.

Paget, S. (1889) 'The distribution of secondary growths in cancer of the breast', *The Lancet*. Elsevier, 133(3421), pp. 571–573.

Pálfi, G. *et al.* (2012) 'Juvenile cases of skeletal tuberculosis from the Terry Anatomical collection (Smithsonian Institution, Washington, DC, USA)', *Acta Biologica Szegediensis*, 56(1), pp. 1–12.

Peeling, R. W. and Hook, E. W. (2006) 'The pathogenesis of syphilis: the Great Mimicker, revisited', *The Journal of Pathology*. Wiley Online Library, 208(2), pp. 224–232.

Pérez-Martínez, C. *et al.* (2016) 'Usefulness of protein analysis for detecting pathologies in bone remains', *Forensic Science International*, 258, pp. 68–73.

Phenice, T. W. (1969) 'A newly developed visual method of sexing the os pubis', *Am J Phys Anthropol*. Wiley Online Library, 30(2), pp. 297–301.

Pigrau-Serrallach, C. and Rodríguez-Pardo, D. (2013) 'Bone and joint tuberculosis', *European Spine Journal*, 22(4), pp. 556–566.

Pinhasi, R. and Mays, S. (2008) *Advances in human palaeopathology*. John Wiley & Sons.

Prates, C. *et al.* (2011) 'Prostate metastatic bone cancer in an Egyptian Ptolemaic mummy, a proposed radiological diagnosis', *International Journal of Paleopathology*, 1(2), pp. 98–103.

Preminger, G. M. (2016) *Urinary calculi - Merck Manual for Health Professionals*. Available at: https://www.msdmanuals.com/professional/genitourinary-disorders/urinary-calculi/urinary-calculi.

Preston, S. H. (1995) 'Human mortality throughout history and prehistory', in *The state of humanity*. Oxford: Blackwell, pp. 30–36.

Punzi, L., Ramonda, R. and Sfriso, P. (2004) 'Erosive osteoarthritis', *Best Practice & Research Clinical Rheumatology*, 18(5), pp. 739–758.

Qiao, T. *et al.* (2013) 'The Systematic Classification of Gallbladder Stones', *PLoS One*. San Francisco, USA: Public Library of Science, 8(10), p. e74887.

Raab, M. S. *et al.* (2009) 'Multiple myeloma.', *Lancet*, 374(9686), pp. 324–39.

Rabino Massa, E., Cerutti, N. and Marin D. Savoia, A. (2000) 'Malaria in Ancient Egypt: Paleoimmunological Investigation on Predynastic Mummified Remains', *Chungará (Arica)*. scielocl, pp. 7–9.

Ragsdale, B. D., Campbell, R. A. and Kirkpatrick, C. L. (2018) 'Neoplasm or not? General principles of morphologic analysis of dry bone specimens', *International Journal of Paleopathology*, 21, pp. 27–40.

Randall, R. L. (2015) *Metastatic bone disease: an integrated approach to patient care*. Springer.

Raychaudhuri, S. P. and Deodhar, A. (2014) 'The classification and diagnostic criteria of ankylosing spondylitis', *Journal of Autoimmunity*, 48–49, pp. 128–133.

Redman, J. E. *et al.* (2009) 'Mycocerosic acid biomarkers for the diagnosis of tuberculosis in the Coimbra Skeletal Collection', *Tuberculosis*, 89(4), pp. 267–277.

Reinhard, K. J. *et al.* (2013) 'Food, parasites, and epidemiological transitions: A broad perspective', *International Journal of Paleopathology*, 3(3), pp. 150–157.

Rejtarova, O. *et al.* (2004) 'Costal cartilages--a clue for determination of sex.', *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia*. Czech Republic, 148(2), pp. 241–243.

Rejtarova, O. *et al.* (2009) 'Age and sexually dimorphic changes in costal cartilages. A preliminary microscopic study.', *Forensic science international*, 193(1–3), pp. 72–78.

Resende, G. G. *et al.* (2013) 'Enteropathic arthritis in Brazil: data from the Brazilian registry of spondyloarthritis', *Revista Brasileira de Reumatologia*, 53(6), pp. 452–459.

Resnick, D. and Niwayama, G. (1976) 'Radiographic and Pathologic Features of Spinal Involvement in Diffuse Idiopathic Skeletal Hyperostosis (DISH)', *Radiology*. Radiological Society of North America, 119(3), pp. 559–568.

Retief, F. P. and Cilliers, L. (2011) 'Breast cancer in antiquity Ancient concept of tumours Breast cancer : historical overview', *South African Medical Journal*, 101(8), pp. 513–515.

Rezai, A. R. *et al.* (1995) 'Modern management of spinal tuberculosis.', *Neurosurgery*. United States, 36(1), pp. 87–88.

Riccomi, G., Fornaciari, G. and Giuffra, V. (2019) 'Multiple myeloma in paleopathology: A critical review', *International Journal of Paleopathology*, 24, pp. 201–212.

Richette, P. and Bardin, T. (2010) 'Gout', *The Lancet*, 375(9711), pp. 318–328.

Rimoin, D. L. *et al.* (2007) 'The Skeletal Dysplasias', *Annals of the New York Academy of Sciences*. Wiley Online Library, 1117(1), pp. 302–309.

Rindfleisch, J. A. and Muller, D. (2005) 'Diagnosis and management of rheumatoid arthritis', *Am Fam Physician*, 72(6), pp. 1037–1047.

Rissech, C. and Steadman, D. W. (2011) 'The demographic, socio-economic and temporal contextualisation of the Universitat Autònoma de Barcelona collection of identified human skeletons (UAB collection)', *International Journal of Osteoarchaeology*. Wiley Online Library, 21(3), pp. 313–322.

Robbins, G. *et al.* (2009) 'Ancient Skeletal Evidence for Leprosy in India (2000 B.C.)', *PloS One*. Public Library of Science, 4(5), p. e5669.

Roberts, C. A. and Connell, B. (2004) 'Guidance on recording palaeopathology', in *Guidelines to the standards for recording human remains.* Southampton: British Association for Biological Anthropology and Osteoarchaeology and Institute of Field Archaeologists, pp. 34–39.

Roberts, C. A., Lucy, D. and Manchester, K. (1994) 'Inflammatory lesions of ribs: An analysis of the Terry Collection', *American Journal of Physical Anthropology*. Wiley-Blackwell, 95(2), pp. 169–182.

Roberts, C. A. and Manchester, K. (2007) *The Archaeology of Disease*. Third. Ithaca, New York: Cornell University Press.

Roberts, G. H. (1971) 'The pathology of parietal pleural plaques', *Journal of Clinical Pathology*, 24(4), pp. 348–353.

Roches, E. *et al.* (2002) 'Microscopic evidence for Paget's disease in two osteoarchaeological samples from early northern France', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 12(4), pp. 229–234.

Rodríguez-Martín, C. (2006) 'Identification and differential diagnosis of traumatic lesions of the skeleton', in Schmitt, A., Cunha, E., and Pinheiro, J. (eds) *Forensic Anthropology and Medicine*. Springer, pp. 197–221.

Rogers, J. *et al.* (1987) 'Arthropathies in palaeopathology: The basis of classification according to most probable cause', *Journal of Archaeological Science*, 14(2), pp. 179–193.

Rogers, J. (2000) 'The palaeopathology of joint disease', in Cox, M. and Mays, S. (eds) *Human osteology in archaeology and forensic science*. Cambridge University Press, pp. 163–182.

Rogers, J. and Waldron, T. (1995) *A field guide to joint disease in archaeology*. Wiley Chichester.

Rogers, J. and Waldron, T. (2001) 'DISH and the monastic way of life', *International Journal of Osteoarchaeology*. Wiley Online Library, 11(5), pp. 357–365.

Rogers, J., Waldron, T. and Watt, I. (1991) 'Erosive osteoarthritis in a medieval skeleton', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 1(2), pp. 151–153.

Rogers, J., Watt, I. and Dieppe, P. (1981) 'Medical History: Arthritis in Saxon and mediaeval skeletons', *Br Med J (Clin Res Ed)*, 283(6307), pp. 1668–1670.

Rogers, J., Watt, I. and Dieppe, P. (1990) 'Comparison of visual and radiographic detection of bony changes at the knee joint', *BMJ (Clinical research ed.)*, 300(6721), pp. 367–368.

Roodman, G. D. (2004) 'Mechanisms of Bone Metastasis', *New England Journal of Medicine*, 350(16), pp.

1655–1664.

Rose, J. C. *et al.* (1991) 'Skeletal database committee recommendations'.

Rosenthal, D. I. (1997) 'Radiologic diagnosis of bone metastases', *Cancer*. Wiley Online Library, 80(S8), pp. 1595–1607.

Rothschild, B. M. *et al.* (1997) 'Recognition of leukemia in skeletal remains: Report and comparison of two cases', *American Journal of Physical Anthropology*. Wiley Online Library, 102(4), pp. 481–496.

Rothschild, B. M. *et al.* (2002) 'Letter to the Editor', *American Journal of Human Biology*. John Wiley & Sons, Ltd, 14(4), pp. 417–420.

Rothschild, B. M. (2002) 'Not the Lucy, not the one', *Clin Exp Rheumatol*, 20(6), pp. 741–743.

Rothschild, B. M. (2012) 'Extirpolation of the mythology that porotic hyperostosis is caused by iron deficiency secondary to dietary shift to maize', *Advances in Anthropology*. Scientific Research Publishing, 2(03), p. 157.

Rothschild, B. M., Hershkovitz, I. and Dutour, O. (1998) 'Clues potentially distinguishing lytic lesions of multiple myeloma from those of metastatic carcinoma', *American Journal of Physical Anthropology*. Wiley Online Library, 105(2), pp. 241–250.

Rothschild, B. M. and Rothschild, C. (1995a) 'Comparison of radiologic and gross examination for detection of cancer in defleshed skeletons', *Am J Phys Anthropol*. Wiley Online Library, 96(4), pp. 357–363.

Rothschild, B. M. and Rothschild, C. (1995b) 'Treponemal Disease Revisited: Skeletal Discriminators for Yaws, Bejel, and Venereal Syphilis', *Clinical Infectious Diseases*, 20(5), pp. 1402–1408.

Rothschild, B. M., Ruhli, F. and Rothschild, C. (2002) 'Skeletal clues apparently distinguishing Waldenstrom's macroglobulinemia from multiple myeloma and leukemia', *American Journal of Human Biology*. Wiley Online Library, 14(4), pp. 532–537.

Rothschild, B. M., Witzke, B. J. and Hershkovitz, I. (1999) 'Metastatic cancer in the Jurassic', *Lancet*, 354(9176), p. 398.

Rothschild, B. M. and Woods, R. J. (1991) 'Spondyloarthropathy: Erosive arthritis in representative defleshed bones', *American Journal of Physical Anthropology*. Wiley-Blackwell, 85(2), pp. 125–134.

Rothschild, C., Rothschild, B. M. and Hershkovitz, I. (2002) 'Clues to recognition of kidney disease in archaeologic record: characteristics and occurrence of leontiasis ossium.', *Reumatismo*, 54(2), pp. 133–143.

Rougé-Maillart, C. *et al.* (2009) 'Development of a method to estimate skeletal age at death in adults using the acetabulum and the auricular surface on a Portuguese population', *Forensic Science International*, 188(1), pp. 91–95.

Ruffer, M. A. (1910) 'Note on the presence of "Bilharzia Haematobia" in Egyptian mummies of the twentieth dynasty [1250-1000 BC]', *British Medical Journal*. BMJ Publishing Group, 1(2557), p. 16.

Rühli, F. J., Hotz, G. and Böni, T. (2003) 'Brief communication: The Galler Collection: A little-known historic Swiss bone pathology reference series', *American Journal of Physical Anthropology*. Wiley Online Library, 121(1), pp. 15–18.

Rule, A. D., Krambeck, A. E. and Lieske, J. C. (2011) 'Chronic Kidney Disease in Kidney Stone Formers', *Clinical Journal of the American Society of Nephrology*, 6(8), pp. 2069 LP – 2075.

Rybak, L. D. and Rosenthal, D. I. (2001) 'Radiological imaging for the diagnosis of bone metastases', *Q J Nucl Med*, 45(1), pp. 53–64.

Saleem, S. N. and Hawass, Z. (2014) 'Brief Report: Ankylosing Spondylitis or Diffuse Idiopathic Skeletal Hyperostosis in Royal Egyptian Mummies of the 18th–20th Dynasties? Computed Tomography and Archaeology Studies', *Arthritis & Rheumatology*. Wiley Online Library, 66(12), pp. 3311–3316.

Salo, W. L. *et al.* (1994) 'Identification of Mycobacterium tuberculosis DNA in a pre-Columbian Peruvian mummy', *Proceedings of the National Academy of Sciences*, 91(6), pp. 2091–2094.

Sanabria-Medina, C. *et al.* (2016) 'A contemporary Colombian skeletal reference collection: A resource for the development of population specific standards', *Forensic Science International*, 266(Supplement C), pp. 577.e1-577.e4.

Santos, A. L. and Roberts, C. A. (2001) 'A picture of tuberculosis in young Portuguese people in the early 20th century: A multidisciplinary study of the skeletal and historical evidence', *American Journal of Physical Anthropology*. Wiley Online Library, 115(1), pp. 38–49.

Santos, A. L. and Roberts, C. A. (2006) 'Anatomy of a serial killer: Differential diagnosis of tuberculosis based on rib lesions of adult individuals from the Coimbra identified skeletal collection, Portugal',

American Journal of Physical Anthropology. Wiley Online Library, 130(1), pp. 38–49.

Sarnak, M. J. *et al.* (2003) 'Kidney Disease as a Risk Factor for Development of Cardiovascular Disease', *Circulation*. American Heart Association, 108(17), pp. 2154–2169.

Sarzi-Puttini, P. and Atzeni, F. (2004) 'New developments in our understanding of DISH (diffuse idiopathic skeletal hyperostosis)', *Current Opinion in Rheumatology*, 16(3).

Sawyers, C. L. (1999) 'Chronic Myeloid Leukemia', *New England Journal of Medicine*. Massachusetts Medical Society, 340(17), pp. 1330–1340.

Schamall, D. *et al.* (2003) 'Changes in trabecular bone structure in rickets and osteomalacia: the potential of a medico-historical collection', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 13(5), pp. 283–288.

Schembri, L. *et al.* (2008) 'Scanning Electron Microscopy Examination and Elemental Analysis of Atherosclerotic Calcifications in a Human Carotid Plaque', *Circulation*, 117(23), pp. e479–e480.

Scheuer, L. and Black, S. (2004) *The Juvenile Skeleton*. Elsevier.

Schultz, M. (2001) 'Paleohistopathology of bone: A new approach to the study of ancient diseases', *American Journal of Physical Anthropology*. Wiley Online Library, 116(S33), pp. 106–147.

Schultz, M. *et al.* (2007) 'Oldest known case of metastasizing prostate carcinoma diagnosed in the skeleton of a 2,700-year-old Scythian king from Arzhan (Siberia, Russia)', *International Journal of Cancer*. Wiley Online Library, 121(12), pp. 2591–2595.

Scott, D. L., Wolfe, F. and Huizinga, T. W. (2010) 'Rheumatoid arthritis', *Lancet*, 376(9746), pp. 1094–1108.

Šefčáková, A. *et al.* (2001) 'Case of metastatic carcinoma from end of the 8th–Early 9th century Slovakia', *American Journal of Physical Anthropology*. Wiley Online Library, 116(3), pp. 216–229.

Sehgal, V. (1987) 'Reactions in Leprosy Clinical Aspects', *International Journal of Dermatology*. John Wiley & Sons, Ltd (10.1111), 26(5), pp. 278–285.

Selmi, C. and Gershwin, M. E. (2014) 'Diagnosis and classification of reactive arthritis', *Autoimmun Rev*, 13(4–5), pp. 546–549.

Shanley, D. J. (1995) 'Tuberculosis of the spine: imaging features.', *American Journal of Roentgenology*. American Roentgen Ray Society, 164(3), pp. 659–664.

Siddiqui, A. A. (2016) *Cholelithiasis - Merck Manual for Health Professionals*. Available at: https://www.msdmanuals.com/professional/hepatic-and-biliary-disorders/gallbladder-and-bile-duct-disorders/cholelithiasis.

Siegel, R. L., Miller, K. D. and Jemal, A. (2018) 'Cancer statistics, 2018', *CA: A Cancer Journal for Clinicians*. Wiley Online Library, 68(1), pp. 7–30.

Sieper, J. *et al.* (2002) 'Ankylosing spondylitis: an overview', *Annals of the Rheumatic Diseases*, 61(suppl 3), p. iii8.

Silman, A. J. and Pearson, J. E. (2002) 'Epidemiology and genetics of rheumatoid arthritis', *Arthritis Research & Therapy*, 4(3), p. S265.

Simmons, T., Jantz, R. L. and Bass, W. (1990) 'Stature Estimation from Fragmentary Femora: A Revision of the Steele Method BT', *Journal of Forensic Sciences*, 35(3), pp. 628–636.

Singer, F. (2016) 'Paget's disease of bone', in *Endotext [Internet]*. MDText. com, Inc.

Singh, A. E. and Romanowski, B. (1999) 'Syphilis: Review with Emphasis on Clinical, Epidemiologic, and Some Biologic Features', *Clinical Microbiology Reviews*, 12(2), pp. 187 LP – 209.

Šlaus, M., Novak, M. and Čavka, M. (2012) 'Four cases of ankylosing spondylitis in medieval skeletal series from Croatia', *Rheumatology International*, 32(12), pp. 3985–3992.

Smith-Guzmán, N. E. (2015) 'The skeletal manifestation of malaria: An epidemiological approach using documented skeletal collections', *American Journal of Physical Anthropology*. Wiley Online Library, 158(4), pp. 624–635.

Smith, I. (2003) 'Mycobacterium tuberculosis Pathogenesis and Molecular Determinants of Virulence', *Clinical Microbiology Reviews*, 16(3), pp. 463 LP – 496.

Smith, M. O. (2002) 'A probable case of metastatic carcinoma from the late prehistoric eastern Tennessee River Valley', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd., 12(4), pp. 235–247.

Smolen, J. S., Aletaha, D. and McInnes, I. B. (2016) 'Rheumatoid arthritis', *The Lancet*, 388(10055), pp. 2023–2038.

Spradley, K. and Jantz, R. L. (2011) 'Sex Estimation in Forensic Anthropology: Skull Versus Postcranial Elements', *Journal of Forensic Sciences*. Wiley Online Library, 56(2), pp. 289–296.

Spradley, K., Stull, K. E. and Hefner, J. T. (2016) 'Craniofacial Secular Change in Recent Mexican Migrants.', *Human biology*, 88(1), pp. 15–29.

Stanley, J. C. and Collier, A. M. (2008) 'Charcot osteo-arthropathy', *Current Orthopaedics*, 22(6), pp. 428–433.

Steeg, P. S. (2006) 'Tumor metastasis: mechanistic insights and clinical challenges', *Nature Medicine*. Nature Publishing Group, 12, p. 895.

Steinbock, R. T. (1976) *Paleopathological diagnosis and interpretation: bone diseases in ancient human populations*. Springfield, IL: Charles Thomas Publisher.

Steinbock, R. T. (1990a) 'Studies in ancient calcified soft tissues and organic concretions. II: Urolithiasis (renal and urinary bladder stone disease)', *Journal of Paleopathology*, 3(1), pp. 39–59.

Steinbock, R. T. (1990b) 'Studies in ancient calcified soft tissues and organic concretions. III: Gallstones (Cholelithiasis)', *Journal of Paleopathology*, 3(2), pp. 95–106.

Steyn, M. *et al.* (2013) 'The changing face of tuberculosis: Trends in tuberculosis-associated skeletal changes', *Tuberculosis*, 93(4), pp. 467–474.

Steyn, M. and Buskes, J. (2016) 'Skeletal manifestations of tuberculosis in modern human remains', *Clinical Anatomy*. Wiley Online Library, 29(7), pp. 854–861.

Strouhal, E. (1991) 'Myeloma multiplex versus osteolytic metastatic carcinoma: Differential diagnosis in dry bones', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd., 1(3–4), pp. 219–224.

Strouhal, E. (1993) 'A case of metastatic carcinoma from Christian Sayala (Egyptian Nubia).', *Anthropologischer Anzeiger; Bericht uber die biologisch-anthropologische Literatur*. Germany, 51(2), pp. 97–115.

Talmadge, J. E. and Fidler, I. J. (2010) 'AACR Centennial Series: The Biology of Cancer Metastasis: Historical Perspective', *Cancer Research*, 70(14), pp. 5649–5669.

Tefferi, A. (2003) 'Anemia in Adults: A Contemporary Approach to Diagnosis', *Mayo Clinic Proceedings*, 78(10), pp. 1274–1280.

Theriault, R. L. and Theriault, R. L. (2012) 'Biology of bone metastases', *Cancer Control*, 19(2), pp. 92–101.

Tierney, D. and Nardell, E. A. (2018) *Tuberculosis (TB) - Merck Manual For Health Professionals*. Available at: https://www.merckmanuals.com/professional/infectious-diseases/mycobacteria/tuberculosis-tb (Accessed: 6 September 2019).

Tkocz, I. and Bierring, F. (1984) 'A medieval case of metastasizing carcinoma with multiple osteosclerotic bone lesions', *Am J Phys Anthropol*. Wiley Online Library, 65(4), pp. 373–380.

Trepman, E., Nihal, A. and Pinzur, M. S. (2005) 'Current topics review: Charcot neuroarthropathy of the foot and ankle', *Foot Ankle Int*, 26(1), pp. 46–63.

Trotter, M. and Gleser, G. C. (1952) 'Estimation of stature from long bones of American Whites and Negroes', *American journal of physical anthropology*. Wiley Online Library, 10(4), pp. 463–514.

Trotter, M. and Gleser, G. C. (1958) 'A re-evaluation of estimation of stature based on measurements of stature taken during life and of long bones after death', *Am J Phys Anthropol*. Wiley Online Library, 16(1), pp. 79–123.

Ubelaker, D. H. (2014) 'Osteology reference collections', in Smith, C. (ed.) *Encyclopedia of Global Archaeology*. New York: Springer, pp. 5632–5641.

Umbelino, C., Cunha, E. and Silva, A. M. (1996) 'A possible case of poliomyelitis in a Portuguese skeleton dated from the 15th century', *Salud, Enfermedad YMuserte en el Pasado Consucuencìas biológicas del esters y lapatología. Fundación Uriach*, 1838, pp. 229–235.

United Nations (2004) *Istanbul Protocol Manual on the Effective Investigation and Documentation of Torture and Other Cruel, Inhuman or Degrading Treatment or Punishment. Office of The United Nations High Commissioner for Human Rights*. New York / Geneva.

Vanderpump, M. P. J. and Tunbridge, W. M. G. (2002) 'Epidemiology and Prevention of Clinical and Subclinical Hypothyroidism', *Thyroid*. Mary Ann Liebert, Inc., publishers, 12(10), pp. 839–847.

Vassiliou, V., Chow, E. and Kardamakis, D. (2013) *Bone metastases: a translational and clinical approach*. Springer Science & Business Media.

Veale, D. J. and Fearon, U. (2015) 'What makes psoriatic and rheumatoid arthritis so different?', *RMD Open*. BMJ Publishing Group, 1(1), p. e000025.

Vigorita, V. J. and Ghelman, B. (2016) 'Arthritis', in Vigorita, V. J. and Ghelman, B. (eds) *Orthopaedic Pathology*. Third edit. Philadelphia: Lippincott Williams & Wilkins, pp. 710–768.

Vilar, J., Lezana, A. H. and Pedrosa, C. S. (1979) 'Spiculated periosteal reaction in metastatic lesions of bone', *Skeletal Radiology*, 3(4), pp. 230–233.

Vincent Rajkumar, S. (2009) 'Multiple Myeloma', *Current problems in cancer*, 33(1), pp. 7–64.

Voulgari, P. V (2011) 'Rheumatological manifestations in inflammatory bowel disease', *Annals of Gastroenterology : Quarterly Publication of the Hellenic Society of Gastroenterology*. Hellenic Society of Gastroenterology, 24(3), pp. 173–180.

Wakely, J. *et al.* (1998) 'Case of a Malignant Tumour from Abingdon, Oxfordshire, England', *Journal of Archaeological Science*, 25(10), pp. 949–955.

Wakely, J., Anderson, T. and Carter, A. (1995) 'A Multidisciplinarian Case Study of Prostatic(?) Carcinoma from Mediaeval Canterbury', *Journal of Archaeological Science*, 22(4), pp. 469–477.

Waldron, T. (1997) 'A nineteenth-century case of carcinoma of the prostate, with a note on the early history of the disease', *International Journal of Osteoarchaeology*. Wiley Online Library, 7(3), pp. 241–247.

Waldron, T. (2008) *Palaeopathology*. Cambridge, United Kingdom: Cambridge University Press.

Waldron, T. and Rogers, J. (1991) 'Inter-observer variation in coding osteoarthritis in human skeletal remains', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd, 1(1), pp. 49–56.

Walker, E. G. (1983) 'Evidence for prehistoric cardiovascular disease of syphilitic origin on the Northern Plains', *Am J Phys Anthropol*. Wiley Online Library, 60(4), pp. 499–503.

Walker, P. L. (2005) 'Greater sciatic notch morphology: Sex, age, and population differences', *American Journal of Physical Anthropology*. Wiley Online Library, 127(4), pp. 385–391.

Walker, P. L. (2008) 'Sexing skulls using discriminant function analysis of visually assessed traits', *American Journal of Physical Anthropology*. Wiley Online Library, 136(1), pp. 39–50.

Walker, P. L. *et al.* (2009) 'The causes of porotic hyperostosis and cribra orbitalia: A reappraisal of the iron-deficiency-anemia hypothesis', *American Journal of Physical Anthropology*, 139(2), pp. 109–125.

Walker, S. L. and Lockwood, D. N. J. (2006) 'The clinical and immunological features of leprosy', *British Medical Bulletin*, 77–78(1), pp. 103–121.

Wapler, U., Crubézy, É. and Schultz, M. (2004) 'Is cribra orbitalia synonymous with anemia? Analysis and interpretation of cranial pathology in Sudan', *American Journal of Physical Anthropology*. Wiley Online Library, 123(4), pp. 333–339.

Wasterlain, S. N., Ascenso, B. F. and Silva, A. M. (2011) 'Skeletal metastatic carcinoma: A case from 15th–20th century Coimbra, Portugal', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd., 21(3), pp. 336–346.

Watts, R. A. *et al.* (2013) *Oxford textbook of rheumatology*. Fourth. New York, USA: Oxford University Press.

Webster, A. C. *et al.* (2017) 'Chronic Kidney Disease', *The Lancet*, 389(10075), pp. 1238–1252.

Wedel, V. L. and Galloway, A. (2013) *Broken bones: anthropological analysis of blunt force trauma*. Charles C Thomas Publisher.

Weinstein, I. B. and Case, K. (2008) 'The history of cancer research: Introducing an AACR centennial series', *Cancer Research*, 68(17), pp. 6861–6862.

Weiss, E. and Jurmain, R. (2007) 'Osteoarthritis revisited: a contemporary review of aetiology', *International Journal of Osteoarchaeology*. John Wiley & Sons, Ltd., 17(5), pp. 437–450.

Weisz, G. M. *et al.* (2011) 'The ossification diathesis in the Medici family: DISH and other features', *Rheumatology International*, 31(12), pp. 1649–1652.

Wells, C. (1963) 'Ancient egyptian pathology', *The Journal of Laryngology & Otology*. Cambridge University Press, 77(03), pp. 261–265.

White, T. D. and Folkens A., P. (2005) *The Human Bone Manual*. San Diego, United States: Elesvier Academic Press.

WHO (2017) *Cancer World Health Organization Statistics*.

Whyte, M. P. (2006) 'Paget's Disease of Bone', *New England Journal of Medicine*. Massachusetts Medical Society, 355(6), pp. 593–600.

Wickramasinghe, S. N. and Abdalla, S. H. (2000) 'Blood and bone marrow changes in malaria', *Best Practice & Research Clinical Haematology*, 13(2), pp. 277–299.

Womack, N. A., Zeppa, R. and Irvin, G. L. (1963) 'The Anatomy of Gallstones', *Annals of Surgery*, 157(5), pp. 670–686.

Wood, J. W. *et al.* (1992) 'The Osteological Paradox: Problems of Inferring Prehistoric Health from Skeletal Samples [and Comments and Reply]', *Current Anthropology*. The University of Chicago Press,

33(4), pp. 343–370.

World Health Organization (2018) *WHO releases new International Classification of Diseases (ICD 11)*. Available at: https://www.who.int/news-room/detail/18-06-2018-who-releases-new-international-classification-of-diseases-(icd-11) (Accessed: 8 March 2019).

Wukich, D. K. and Sung, W. (2009) 'Charcot arthropathy of the foot and ankle: modern concepts and management review', *Journal of Diabetes and its Complications*, 23(6), pp. 409–426.

Zaka, R. and Williams, C. J. (2006) 'New developments in the epidemiology and genetics of gout', *Current Rheumatology Reports*, 8(3), pp. 215–223.

Zimmerman, M. R. and Kelley, M. A. (1982) *Atlas of human paleopathology*. New York: Praeger Publishers.

Zochling, J. and Smith, E. U. R. (2010) 'Seronegative spondyloarthritis', *Best Practice & Research Clinical Rheumatology*, 24(6), pp. 747–756.

Zuckerman, M. K. (2016) 'More harm than healing? Investigating the iatrogenic effects of mercury treatment on acquired syphilis in post-medieval London', *Open Archaeology*, 2(1), pp. 42–55.

Appendices

Appendix 1: Description of the individuals selected for the project

Research line	Case n°	Sex	Age	Year of birth	Year of death	Cause of death
Bladder cancer	**39**	M	76	1914	1991	Bladder neoplasia, neoplastic cachexia
Bladder cancer	**149**	M	60	1930	1991	Bladder neoplasia, cardiac arrest
Bladder cancer	**164**	M	77	1913	1990	Bladder neoplasia, myocardial infarctus
Bladder cancer	**310**	M	58	1933	1991	Bladder cancer, hepatic and lymphatic metastases, cardiac arrest
Bladder cancer	**350**	M	85	1906	1991	Bladder cancer, pulmonary metastases, cardiac arrest
Bladder cancer	**389**	M	76	1916	1992	Bladder neoplasia, endoabdominal metastases, cardiac arrest
Bladder cancer	**407**	F	86	1904	1991	Probable bladder neoplasia, secondary anemia, cardiac arrest
Bladder cancer	**422**	F	59	1932	1992	Bladder cancer, multiple metastases, terminal cachexia
Bladder cancer	**456**	M	71	1920	1992	Bladder, hepatic metastases, cardiac arrest
Bladder cancer	**490**	F	82	1909	1991	Bladder neoplasia, hepatic and pelvic metastases, neoplastic cachexia
Bladder cancer	**897**	M	56	1940	1997	Bladder cancer and deterioration, stroke, cardiac arrest
Bladder cancer	**1039**	M	60	1931	1991	Bladder heteroplasia, hydroelectric imbalance, quadriplegia, cardiac insufficiency
Bladder cancer	**1141**	M	78	1913	1992	Left bronchopulmonitis, respiratory insufficiency, bladder neoplasia, cardiac arrest
Breast cancer	**37**	F	70	1920	1991	Breast neoplasia, diffuse bone metastases, neoplastic cachexia
Breast cancer	**89**	F	86	1905	1991	Metastatic breast cancer, digestive hemorrhage, varicose ulcer left inferior limb, cardiac arrest
Breast cancer	**326**	F	75	1933	1991	Ovarian and right breast cancer, hepatic metastases, peritoneal carcinoma, neoplastic cachexia
Breast cancer	**337**	F	78	1913	1992	Right ulcerated breast neoplasia, neoplastic cachexia, cardiac arrest
Breast cancer	**425**	F	61	1928	1991	Breast cancer, bone metastases, neoplastic cachexia
Breast cancer	**459**	F	83	1908	1992	Right breast cancer, hepatic metastases, cardiac arrest
Breast cancer	**535**	F	69	1916	1985	Right breast cancer, multiple metastases, neoplastic cachexia
Breast cancer	**735**	F	68	1929	1997	Cyrrhosis, rupture of esophageal varices, left mastectomy for malign tumor, cardiac arrest
Breast cancer	**912**	F	54	1937	1991	Left breast cancer, diffuse metastases, cardiac arrest
Breast cancer	**971**	F	90	1900	1991	Left breast cancer, diffuse metastases, neoplastic cachexia, aortic cardiopathy

Breast cancer	**977**	F	89	1902	1991	Right breast cancer, diffuse metastases, massive tumoral abdominal mass, cardiac arrest
Breast cancer	**1197**	F	91	1900	1991	Breast cancer and neoplastic cachexia
Breast cancer	**1200**	F	77	1914	1991	Operated left breast cancer, metastases, cachexia, cardiac arrest
Breast cancer and Diabetes Mellitus	**923**	F	78	1912	1991	Breast cancer and diabetes mellitus, respiratory insufficiency, cardiac arrest
Cancer	**280**	F	62	1928	1991	Acute myeloid leukemia, cerebral ictus
Cancer	**312**	M	57	1934	1992	Adenocarcinoma, esophageal cancer
Cancer	**332**	F	74	1934	1992	Gastric neoplasia, lymphonodular metastases, neoplastic cachexia
Cancer	**351**	F	55	1934	1991	Colorectal cancer (adenocarcinoma sigma), hepatic and abdominal metastases, cardiac arrest
Cancer	**361**	F	83	1908	1991	Uterine neoplasia, diffuse metastases, cardiac arrest
Cancer	**365**	M	58	1932	1991	Pancreatic cancer, gastric ulcer, acute respiratory insufficiency
Cancer	**378**	F	86	1904	1991	Kidney cancer, peritonitis, cardiac arrest
Cancer	**387**	F	81	1909	1991	Gastric cancer, cachexia
Cancer	**431**	M	81	1910	1991	Stroke, intestinal cancer, heart failure and cardiac arrest
Cancer	**446**	M	82	1910	1992	Pulmonary carcinoma, cachexia, cardiac arrest
Cancer	**529**	F	88	1897	1985	Rectum cancer, diffuse metastases, neoplastic cachexia
Cancer	**587**	M	70	1915	1985	Diabetes mellitus and diabetic neoplasia, heart failure, cardiac arrest
Cancer	**623**	M	75	1910	1986	Cyrrhosis, gastric neoplasia, cardiac arrest
Cancer	**726**	M	75	1912	1988	Colon neoplasia, recurrence with metastases, neoplastic cachexia, cardiac arrest
Cancer	**910**	F	68	1923	1992	Pulmonary neoplasia, acute respiratory insufficiency, cardiac arrest
Cancer	**929**	M	69	1922	1991	Pancreatic cancer, acute pancreatitis, cardiac arrest
Cancer	**932**	M	63	1927	1991	Left pulmonary carcinoma, diffused metastases, cardiac arrest
Cancer	**948**	M	78	1920	1991	Intestinal neoplasia, cerebral metastases, cardiac arrest
Cancer	**954**	F	68	1923	1991	Uterine cancer with metastases, cachexia, hypertension
Cancer	**956**	F	71	1919	1991	Vulvar neoplasia, anemia, cardiac arrest
Cancer	**1008**	M	79	1912	1992	Lung carcinoma, pleural metastases, respiratory insufficiency
Cancer	**1010**	M	72	1919	1991	Oropharyngeal carcinoma, hepatic metastases, hemorragic shock
Cancer	**1019**	M	75	1916	1992	Cancer, uncontrolled cirrhosis, cardiac arrest
Cancer	**1030**	F	68	1923	1992	Metastatic neoplasia, deep vein thrombosis, cardiac arrest
Cancer	**1043**	M	80	1911	1991	Penis cancer, heart failure, cardiac arrest
Cancer	**1045**	M	76	1915	1991	Neoplasia of the pancreas, neoplastic cachexia

Cancer	**1056**	M	83	1908	1991	Hepatic neoplasia, hemoperitoneum, cardiac arrest
Cancer	**1062**	F	63	1928	1991	Parieto-occipital glioblastoma, cardiac and respiratory arrests
Cancer	**1080**	M	74	1917	1991	Pulmonary neoplasia, diffused metastases, cachexia
Cancer	**1091**	F	63	1928	1992	Undifferentiated carcinoma, pulmonary and hepatic metastases, cardiac arrest
Cancer	**1109**	F	73	1917	1991	Chronic lymphatic leukemia, grave osteoporosis, cardiac arrest
Cancer	**1160**	M	76	1915	1991	Hepatic and pulmonary metastases from unknown primary, neoplastic cachexia
Cancer	**1162**	M	60	1931	1991	Pulmonary carcinoma, respiratory insufficiency
Cardiovascular Diseases	**172**	M	42	1948	1991	Chronic bronchopneumonia, hepatic cirrhosis and respiratory failure
Cardiovascular Diseases	**282**	M	83	1908	1991	Cerebral vasculopathy, cachexia and malignant hyperthermia
Cardiovascular Diseases	**298**	F	87	1905	1992	Cerebral vasculopathy, atrial fibrillation, coronaropathy and cardiac arrest
Cardiovascular Diseases	**308**	M	85	1906	1992	Vasculosclerosis, senile deterioration and cardiac arrest
Cardiovascular Diseases	**371**	F	84	1907	1992	Atherosclerotic vasculopathy, renal insufficiency and cardiac arrest
Cardiovascular Diseases	**381**	M	81	1910	1991	Atherosclerotic vasculopathy and heart failure
Cardiovascular Diseases	**386**	F	102	1889	1991	Ischemic cardiopathy, cerebral vasculopathy and cardiac arrest
Cardiovascular Diseases	**410**	M	87	1904	1992	Arterial hypertension, arteriosclerosis, cerebral stroke, cardiac and respiratory arrest
Cardiovascular Diseases	**423**	F	76	1915	1991	Chronic cerebral vasculopathy, cachexia and cardiac arrest
Cardiovascular Diseases	**493**	F	85	1907	1992	Cerebral vasculopathy, senile dementia and cardiac arrest
Cardiovascular Diseases	**499**	F	82	1909	1991	Cerebral vasculopathy and cerebral coma
Cardiovascular Diseases	**625**	M	80	1905	1986	Chronic encephalovasculopathy, cerebral stroke and cardiac arrest
Control	**182**	F	47	1943	1991	Kidney failure in diabetic therapy, pulmonary edema and cardiac arrest
Control	**304**	M	85	1917	1992	Cerebral hemorrhage and cardiac arrest
Control	**338**	F	82	1909	1992	Chronic ischemic cardiopathy, syncopal episode, definitive pace-maker and cardiac arrest
Control	**397**	M	81	1910	1992	Heart failure and cardiac arrest
Control	**403**	F	95	1896	1992	Arterial hypertension, Parkinson's diseased cardiac arrest
Control	**477**	F	56	1934	1991	Acute necrotic pancreatitis, septic state, metabolic coma and cardiac arrest
Control	**489**	F	83	1908	1991	Intestinal occlusion, acute pancreatitis and cardiac arrest
Control	**513**	M	69	1914	1984	Acute cerebral vasculopathy and cardiac arrest
Control	**602**	F	86	1900	1986	Heart failure and cardiac arrest

Control	**1063**	F	39	1924	1963	Non-specified
Diabetes Mellitus	**20**	F	82	1909	1991	Arrhytmic-hypertensive cardiopathy, bronchopulmonitis, diabetes and acute heart insufficiency
Diabetes Mellitus	**25**	M	52	1938	1990	Bilateral interstitial lung disease, diabetes and severe respiratory insufficiency
Diabetes Mellitus	**38**	F	86	1905	1991	Arterial hypertension, diabetes and chronic obstructive pulmonary disease
Diabetes Mellitus	**40**	M	80	1911	1991	Ischemic cerebral vasculopathy, diabetes, cerebral ictus with seizure, active duodenal gastric ulcer
Diabetes Mellitus	**53**	F	80	1910	1991	Sclerohypertensive cardiopathy, diabetes and chronic cardiac insufficiency
Diabetes Mellitus	**83**	F	87	1904	1991	Inferior limb gangrene, insulin-dependent diabetes, chronic arteriopathy and suspected pulmonary embolism
Diabetes Mellitus	**86**	M	68	1923	1991	Diabetes, gangrène on inferior limbs and hypovolemic shock
Diabetes Mellitus	**126**	M	76	1914	1990	Cerebral ictus, cerebral vasculopathy, diabetes and arterial hypertension
Diabetes Mellitus	**127**	F	76	1914	1991	Cerebral ictus on right hemisphere, diabetes mellitus and hypertension
Diabetes Mellitus	**135**	F	83	1908	1991	Encephalovasculopathy, diabetes and cerebral ictus
Diabetes Mellitus	**170**	M	70	1920	1991	Ischemic cardiopathy, diabetes mellitus type II, chronic obstructive pulmonary disease, pulmonary edema, cardiac and respiratory insufficiencies
Diabetes Mellitus	**275**	M	75	1916	1991	Dilated cardiomyopathy post-ischemic, low tension, diabetes, chronic renal insufficiency, acute pulmonary edema
Diabetes Mellitus	**315**	F	74	1916	1991	Diabetes mellitus and comatose state after brain injury
Diabetes Mellitus	**323**	M	61	1929	1991	Diabetes mellitus, acute pancreatitis and sepsis
Diabetes Mellitus	**336**	M	70	1921	1992	Diabetes mellitus and diabetic gangrene on right foot
Diabetes Mellitus	**368**	M	77	1915	1992	Diabetes mellitus, ictus right hemisphere, bilateral seizure, acute pulmonary edema, atrial fibrillation
Diabetes Mellitus	**426**	F	68	1923	1992	Bronchopulmonitis, diabetes mellitus and sepsis
Diabetes Mellitus	**452**	F	68	1924	1992	Hypertension, diabetes and myocardiac infarctus
Diabetes Mellitus	**481**	F	85	1906	1992	Renal insufficiency, diabetes mellitus type II and previous acute myocardiac infarctus
Diabetes Mellitus	**517**	F	71	1916	1987	Chronic obstructive bronchopneumopathy, diabetes and respiratory insufficiency
Diabetes Mellitus	**545**	F	77	1909	1986	Generalized vasculopathy, diabetes mellitus type I and senile marasmus
Diabetes Mellitus	**561**	F	74	1910	1984	Uncontrolled diabetes mellitus, intestinal occlusion and acute renal and cardiac insufficiencies
Diabetes Mellitus	**570**	M	74	1910	1985	Ischemic cardiopathy, diabetes mellitus and cardiac insufficiency

Diabetes Mellitus	**573**	F	79	1907	1987	Cerebral vasculopathy, diabetes mellitus type II and hyperosmolar coma
Diabetes Mellitus	**587**	M	77	1915	1985	Diabetic neoplasia and cardiac insufficiency
Diabetes Mellitus	**646**	M	78	1907	1986	Chronic renal insufficiency, miocardiocoronarosclerosis, diabetes and peptic ulcer
Diabetes Mellitus	**851**	F	66	1930	1997	Severe diffuse arteriopathy, diabetes mellitus type III and amputation left thigh
Diabetes Mellitus	**1014**	M	80	1911	1992	Renal insufficiency, diabetes and brain natriuretic peptide (basal, right)
Diabetes Mellitus	**1068**	F	74	1916	1991	Dilated cardiomyopathy, diabetes mellitus and cardiac insufficiency
Diabetes Mellitus and Cancer	**10**	M	67	1923	1990	Cyrrhosis, hepatic neoplasia and diabetes mellitus, terminal hepatic coma and cardiac arrest
Diabetes Mellitus and Cancer	**61**	F	83	1907	1991	Left pulmonary neoplasm and insulino-dependent diabetes, physical decline, cardiac arrest
Diabetes Mellitus and Cancer	**66**	F	58	1932	1991	Gastric neoplasm and diabetes mellitus, hepatic metastases, cardiac arrest
Diabetes Mellitus and Cancer	**151**	M	54	1936	1990	Pulmonary tumor and diabetes mellitus, respiratory insufficiency, cardiac arrest
Diabetes Mellitus and Cancer	**366**	M	85	1905	1991	Anuria in prostatic cancer, heart insufficiency and diabetes mellitus, cardiac and respiratory arrests
Diabetes Mellitus and Cancer	**395**	M	76	1915	1991	Cirrhotic cancer with bone metastases, metabolic compensation in diabetes mellitus and diabetic neuropathy, terminal collapsus syndrome and cardiac arrest
Diabetes Mellitus and Cancer	**712**	M	88	1897	1985	Prostate cancer and diabetes mellitus, chronic cardiopathy, cardiac arrest
Diabetes Mellitus and Cancer	**893**	M	70	1927	1997	Lymphoma and diabetes mellitus, neoplastic cachexia, cardiac arrest
HIV	**347**	M	31	1960	1991	HIV infection, pneumonia, hepatic failure
HIV	**774**	M	28	1962	1991	HIV infection, pulmonitis, cirrhosis, cardiac arrest
HIV	**782**	M	49	1943	1992	AIDS, bronchopulmonitis, cardiac arrest
HIV	**854**	F	41	1954	1995	HIV infection (10 years), AIDS, wasting syndrome (4 months) and right pneumothorax
HIV	**855**	M	35	1961	1996	HIV infection, hepatic insufficiency, encephalitis (5 years); hepatic, cerebral and respiratory complications (1 year), AIDS and cardiac arrest
HIV	**857**	M	29	1966	1995	HIV infection (10 years), cerebral toxoplasmosis (13 months), encephalitis (2 months) and chorioretinitis (8 months) due to cytomegalovirus
HIV and Cancer	**289**	M	47	1944	1991	HIV infection, AIDS, bronchogenic spinocellular carcinoma
HIV and Cancer	**856**	F	41	1954	1995	Cerebral lymphoma, HIV complications (6 months), Pseudomonas aeruginosa infection, Mycobacterium cytomegalovirus, neoplastic cachexia and infective complications

HIV and Cancer	**858**	M	55	1941	1995	HIV infection (8 years), Kaposi sarcoma, disseminated cytomegalovirus infection, atypical mycobacterium (1 year), interstitial pulmonitis (9 days)
Multiple Myeloma	**156**	F	74	1916	1991	Multiple myeloma, anemia, cardiac arrest
Multiple Myeloma	**471**	F	61	1930	1991	Multiple myeloma, left bronchopulmonitis, irreversible cardiac arrest
Osteomalacia	**7**	F	79	1911	1991	Respiratory insufficiency, acute pulmonary edema, cardiac arrest
Rheumatoid Arthritis	**277**	F	76	1915	1991	Rheumatoid arthritis, luxation of the cervical column, cardiac arrest
Rheumatoid Arthritis	**1007**	F	88	1903	1991	Rheumatoid arthritis, renal insufficiency, irreversible pulmonary edema, cardiac arrest
Rheumatoid Arthritis and Cancer	**557**	M	89	1899	1987	Senile sclerotic vasculopathy - Myocardiosclerosis, rheumatoid arthritis, prostatic adenoma and cardiac syncope