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ANALYSIS OF MARKERS OF DISEASE AND HEALTH STATUS ON HUMAN SKELETONS

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Abstract

One of the main aims of the forensic anthropological practice regards the proper identification of human remains. Years of studies and research provided many instruments for the reconstruction of the so-called "biological profile" (e.g. age at death, sex, stature, race); nevertheless, and despite the numerous information that it can provide, the pathological investigation of skeletons still displays many uncertainties. The identification of pathological signs from bones can in fact narrow the field of research in case of the recovery of unknown human remains, and can provide valuable data for the definition of a possible cause of death. However, the difficulties encountered when analyzing skeletal remains, due to taphonomic and environmental variables, non-specificity of the bone reaction and the monotony of bone tissue manifestation to stimuli, make pathological identification of diseases one of the most difficult fields of forensic anthropology. The aim of this research project is that of trying to narrow and clarify some aspects of skeletal pathology and forensic anthropology, by analyzing skeletons from three cemeteries of Milan, which are part of the Milano Cemetery Skeletal Collection. The main feature of this collection regards the availability, for many of its individuals, of ISTAT death certificates that make the observers aware of the main cause of death and related pathological conditions of such subjects. After collecting this information the attention then focused on the macroscopic investigation of specific pathologies, those that were more widespread in the available sample, to include: cancer metastases, diabetes, Cardiovascular Disease (CVD) and Human Immunodeficiency Virus (HIV) related to drug abuse. The skeletons of this research were selected and cleaned in a proper way; subsequently, the specific pathologic investigation was carried on after an accurate literature search, aimed at finding the most characteristic features that permit the correct identification of the pathology from bones. The analyses performed allowed the demonstration of the difficulties encountered in unequivocally describe and identify such diseases, despite the previous studies conducted, mainly due to the aspecific and often altered structures acquired by the stimulated areas on bones. The modifications caused by taphonomy, together with the subjectivity of the responses and the paucity of available reference data made the investigation quite difficult. However, the available causes of death permitted a baseline to better understand the observations in identifying and describing pathologic-related skeletal lesions, and to set the basis for a more detailed and comprehensive macroscopic pathologic investigation. Such macroscopic investigation, however, has it limits, as it can not go over a certain level of certainty; for this reason, additional investigation methods (such as x-rays, biochemistry, histology and so on) or additional antemortem data are necessary for increasing the amount of information we can gain from the examination of bare skeleton.

Chapter 1

INTRODUCTION

Forensic Anthropology

In the medicolegal field, many disciplines are acquiring more and more value and specialization in the analysis of decedents. For example, in several scenarios, the pathologist might not have proper training to recover and deal with human remains (1). For a better understanding of the dynamics of death the pathologist therefore needs a group of specialists whose cooperation can highlight fundamental information necessary for the reconstruction of the events surrounding the case. One such specialists is the forensic anthropologist.

The American Board of Forensic Anthropology[1] offers the following definition of "forensic anthropology":

> *Forensic anthropology is the application of the science of physical anthropology to the legal processes. The identification of skeletal, badly decomposed, or otherwise unidentified human remains is important for both legal and human reasons. Forensic anthropologists apply standard scientific techniques developed in physical anthropology to identify human remains, and to assist in the detection of crime. Forensic anthropologists frequently work in conjunction with forensic pathologists, odontologists, and homicide investigators to identify a decedent, discover evidence of foul play, and/or the postmortem interval. In addition to assisting in locating and recovering suspicious remains, forensic anthropologists work to suggest age, sex, ancestry, stature, and unique features of a decedent from the skeleton.*

Commonly, forensic anthropology deals with the analysis of fully or semi skeletonized human remains (2). Just like the pathologist deals with the human cadaver from the death scene to establish time and cause of death, in the same manner the anthropologist, when nothing remains of a victim but bones, must deal with the search and proper retrieval of the skeleton and with issues such as identification and detection of trauma which may lead to establish cause and manner of death (3). Additionally, the post-mortem interval, ante-mortem pathology and trauma, as well as post-mortem artifacts associated with the scene are asked to be determined. In many occasions remains are commingled, fragmentary or even charred, and the forensic anthropologist is called to decide whether they are of human origin as well as to assess the biological characteristics (2).

When dealing with human remains, it is necessary to collect a set of data, called the "biological profile". This term refers to the identification of all the personal characteristics, e.g. sex, age,

[1] "American Board of Forensic Anthropology". http://www.theabfa.org/

ancestry and stature, that provide the basic steps for the process of identification. In fact, when dealing with the remains of an individual (after determining that they are human), the determination of sex is an important early step, as it eliminates approximately 50% of the population from further consideration on a missing person list. In addition, many of the additional tests of identity (stature and age estimation) are sex-specific, with different regression formulae and standards for males and females (4).

Personal identity additionally relies on the presence of idiosyncratic features and on the observation of distinctive skeletal characteristics that may be the only feature that permits distinguishing one person from another (1). Such features can be calluses due to antemortem trauma, specific physiologic features of the subject, and pathologic alterations caused by specific diseases. Over the years, the problem of personal identification has become increasingly important, due to large-scale migrations, wars, mass disasters, accidents and homicides, but also concerns individuals who dies far from home, without any identification documents that can tell who the person was. Nowadays much information and many instruments are available for identifying an unknown decedent, but when only badly preserved human remains are the object of investigation forensic anthropology is the only discipline that can aid with the entire biological profile.

Therefore, in the forensic anthropological practice, parameters such as sex, age at death, race, and stature, which allow biological identity and which are easily attained thanks to numerous studies previously conducted, are not sufficient. Indeed, for positive identification, such an identity is often not enough because the same biological profile can be shared among various individuals. As previously mentioned, one has to rely on unique skeletal features: bone lesions (5), both of pathologic and traumatic origin, are one of the main instruments. Furthermore, observations on pathology and other abnormal conditions not only can assist identification, but can give insights into the cause and manner of death (2, 5).

Bone pathology

Skeletal pathology has always been a tough subject. Despite the fact that the skeleton can sometimes be the only source of information available, since it is the most resilient part of the body after death, it is always less informative than a fresh cadaver. Furthermore, in most instances, the skeleton is the ultimate body system to react to a pathologic “aggression”. This means that there are many diseases that do not leave any sign on bones, and those that do leave a sign tend to be the chronic conditions. Therefore, when dealing with bone pathology, “the absence of evidence is not evidence of absence” (5). A further and not less important limitation of the interpretation of diseases from skeletal remains concerns the uniformity and monotony of bone reaction. Bone cells, when “attacked”, only have two ways to react: by means of bone formation (osteoblasts) or bone destruction (osteoclasts), or both phenomena. Consequently, the same bone manifestation can be linked to a variety of etiologies, which makes the correct diagnosis difficult (5). The accurate interpretation of skeletal pathology is therefore one of the most controversial and arduous aspects of the forensic anthropological practice.

The study of skeletal diseases is usually associated with the study of ancient pathologies (the discipline for archeological and historical material is called “palaeopathology”), aimed at reconstructing some aspect of human or nonhuman biological processes in the past (6). However, although paleopathologists can deal directly with the remains of disease, no positive control supporting the diagnosis is available. Thus, it is generally impossible for palaeopathologists to determine the cause of death of those they examine, which is a great pity because the knowledge of how the causes of death and pathologies might have changed over the centuries would add greatly to our understanding of the history of disease. Furthermore, most paleopathologists are not medical doctors and so are not always inclined to base their diagnosis on clinical evidence (7). Nevertheless, the accurate pathological knowledge and investigation of ancient samples can reveal itself to be a useful tool also for much wider topics, like sex and age determination in samples that do not display clear characteristics, as demonstrated by Belcastro *et al.* (8) who used Hyperostosis Frontalis Interna (HFI) as marker for sex determination. Additionally, also pseudopathologies need to be considered. The term “pseudopathology” refers to post-mortem skeletal changes that may be mistakenly diagnosed as ante-mortem pathological conditions. Soil, for example, is often responsible for warping bones and erosion of the bone cortex, which can easily be mistaken for specific pathological conditions, like rickets or trauma, and cribra orbitalia or periostitis respectively (9-11).

As Waldron (7) reported, many studies on the accuracy of diagnosis, most frequently by comparing a clinical diagnosis with that determined at autopsy, were performed. In one study of fifty-three autopsies on previously published reports, it was found that up to half of all clinical diagnoses were seriously in error, and that in up to one-fifth, the error was sufficiently serious that the patient received the wrong treatment. This proves that if clinicians, with a host of information at their disposal get their diagnoses wrong so frequently, how much more likely is it that palaeopathologists will fare any better when they have so little information on which to base their conclusions?

In case of skeletal pathology in fact the diagnosis is performed solely on the direct examination of the skeleton, or of part of it, sometimes supplemented by radiography, histology and ancient DNA (aDNA) analysis (7). Therefore only morphology and distribution of the changes found in the skeleton are the material available. It is therefore on such material and such type of investigation that the palaeopathological and forensic anthropological practice need to focus. Skeletal pathology can indeed provide an enormous amount of information that can strongly increase and boost the modern common approach to such disciplines. Demographic changes in centuries, the spread and diffusion of specific diseases, personal identification and cause of death are all subjects that need and can be deepened thanks to a more accurate and careful approach to skeletal pathology.

Chapter 2

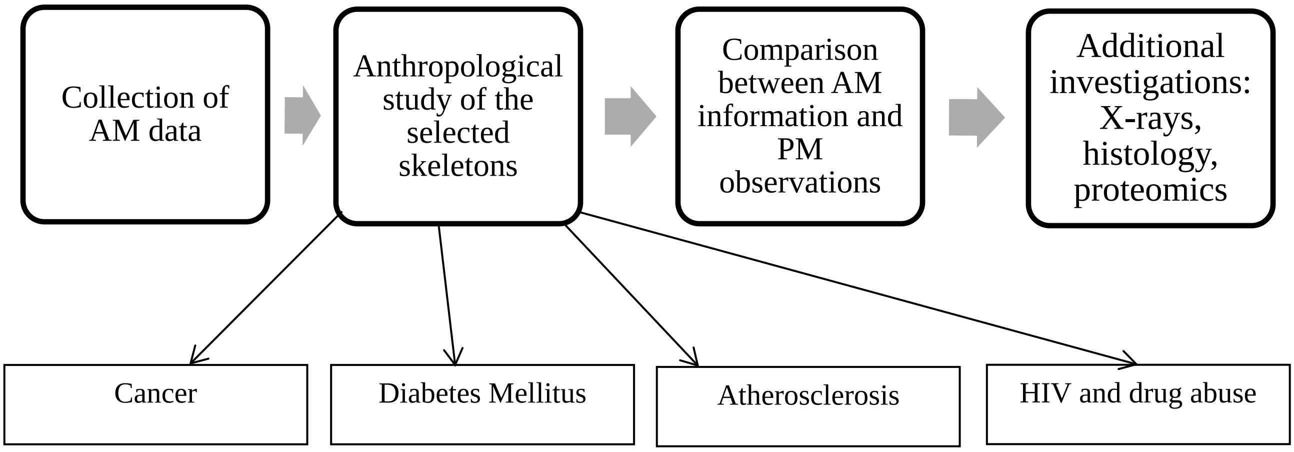
PURPOSE OF THE RESEARCH PROJECT

The aim of the current research project focuses on the identification of pathological markers on skeletons, in order to provide a more precise knowledge of how some pathologies appear on bones and whether they are recognizable or not. As previously mentioned, the utility of this analysis is particularly evident in the forensic anthropological (and archaeological) practice since it can provide additional information for the positive identification of a decedent and could help clarify some aspects of skeletal pathology that are still poorly understood. As the skeleton is often the last part of the body to react to a pathology, but at the same time its most resilient part, the possibility to define if a person was affected by a specific disease from bones is mandatory for getting closer to the identity of recovered people.

The project is structured according to the information collected in the first period of the Ph.D. Thanks to the availability of data regarding the causes of death of the individuals of the collection, as well as of the related pathological condition (all obtained in the first months of the PhD), the main pathologies affecting such individuals were identified and selected. The project therefore started (during the first year) on the analysis of metastatic signs on the skeleton, in order to identify the parameters that univocally characterize a bone metastasis from a macroscopic point of view. According to the initial information collected in this analysis, during the second year the attention was then focused on the variety of manifestations of prostate cancer metastases. In parallel, also the skeletal manifestation of diabetes was investigated, together with the research of the presence and the analysis of the aspect of vascular calcifications, consequent to athero- and arteriosclerotic processes. In the last year of the PhD project, some cases of HIV deaths were investigated.

The work has been carried on the LABANOF known Skeletal Collection within the Laboratory of Forensic Anthropology and Odontology (LABANOF) of the University of Milan, Its organization is described and explained in Graph 2.1.

The thesis, due to convenience reasons, is organized in different paragraphs, one for each pathology considered. For each of them an introduction on the specific topic will be presented, followed by the description of the methods used for the analysis, the explanation of the results obtained and their discussion. First of all, however, a description of the collection from which the material in analysis was taken is performed. In fact, it is only after the recovery of all the skeletons of the collection and of the causes of death relative to them that the study became possible.



Graph 2.1: Schematic representation of the structure and organization of the project ongoing. After the collection of the antemortem (AM) data, some the main pathologies were identified and studied. The information collected (postmortem data, PM) were compared to the antemortem ones in order to investigate the manifestation of such pathologies on bones. The diseases investigated are in the boxes (Cancer, Diabetes, Atherosclerosis and HIV).

Chapter 3

THE MILANO CEMETERY SKELETAL COLLECTION: Labanof I-MASC

(Labanof's Identified Milano Anthropological Skeletal Collection)

In the forensic anthropological practice, the recovery and study of human skeletal remains have a mandatory role (12). The importance of osteological collections is in fact widely recognized, both in the fields of forensic anthropology, bioarcheology, paleopathology, and human paleontology (13). As a matter of fact, all the available information used for performing the diagnoses requested in the forensic and archaeological practice were obtained by studying large numbers of skeletons, in order to identify the characteristics that allow us to distinguish a female from a male (14-17), different ranges of ages (18-22), pathologies (23-24), and morpho-metrical features (25-26). Their recognised usefulness is however well-known also for far different fields, such as pathology, molecular biology, physiology, and specific medical disciplines (13, 27-28).
The term “reference” or “documented” skeletal collection usually refers to a group of skeletons in which the basic documentary data (sex, age at death, biological origin) are known for each individual (29). The importance of such data is testified by the possibility of developing studies and research projects aimed at testing and improving the existing methods, as well as at identifying new useful parameters and standards. These collections were traditionally composed of human skeletal remains acquired through archaeological excavations; nevertheless, the need for skeletons with associated information, which would allow researchers to develop proven estimation methods for sex, age or stature (among other characteristics), originated skeletal collections composed of bones from hospitals, autopsies, or cemeteries, in order to gain such information (28).
Furthermore, although the application of sexing, aging, and pathology identification methods are commonly used on account of their renown satisfactory usefulness, the specificity of each population is becoming more and more evident. It is indeed common knowledge that osteometric methods for the determination of sex from the skeleton are population specific, and therefore researchers from around the world started to conduct studies in order to establish group specific standards of assessment (27, 30). It is hence important to consider also the geographical standardisation of the parameters for the contextualization of the material in analysis. As a consequence, there already exists a discrete number of skeletal assemblages, distributed in different parts of the world (12-13, 27-29, 31-40).
At the Department of Biomedical Sciences for Health of the University of Milan, the LABANOF (Laboratory of Forensic Anthropology and Odontology) is currently in possession of a huge osteological and skeletal collection. In fact, over the past six years, the LABANOF, in close collaboration with the city of Milan and the "*Cimitero Maggiore*" (the main cemetery of Milan), successfully succeeded in concluding an agreement for the creation and the assemblage of a new and contemporary cemeterial skeletal collection and the gathering, in collaboration with the ASL

(*Azienda Sanitaria Locale*), of its related documentation: ISTAT death certificates (concerning the causes of death and the pathological conditions that led to it), autopsy reports, and demographic data. The acquisition of the entire skeletal collection was carried out in full accordance with the rules of the National Police Mortuary Regulation: the Article 43 of the Presidential Decree of the Italian Republic (DPR) n.285 of September 10th, 1990 gives in fact to the cemetery the permission of assigning unclaimed skeletal remains to Universities for education and research purposes.

The collection of skeletons began in January 2012, with the acquisition of the first 265 individuals. Since then, the collection had a rapid numerical increase. Over the past four years its expansion reached a substantial number of complete or almost complete skeletons, reaching a total number of 2127, coming from the "*Cimitero Maggiore*" (Milan) (n= 1692, 79%), the Cemetery of Lambrate (Milan) (n= 294, 14%), and the Cemetery of Baggio (n= 141, 7%). New individuals are however continuously added, as the collection is in constant growth. The distinctiveness of such skeletal remains is that the majority of them (65%) belong to modern individuals, who died after 1990, and the 80% after 1980 (the exact distribution of the ages of death are summarised in Fig. 3.1).

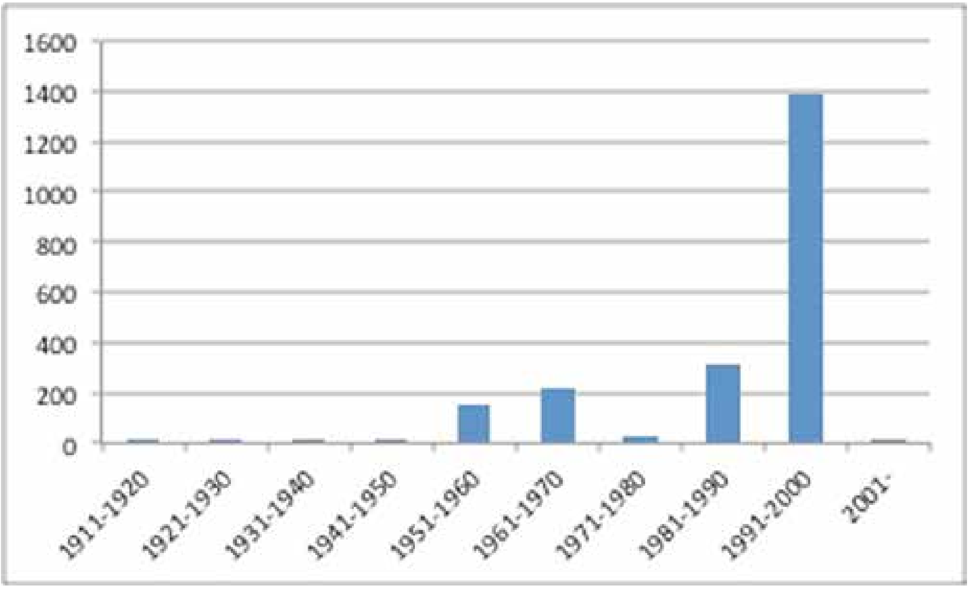


Figure 3.1: Distribution of individuals of the Milan Cemetery Collection per years of death.

All the remains, once they were completely skeletonised, arrived at the Laboratory stored in metal boxes, equipped with an identifier plate on which name, date of birth, and date of death are recorded; in addition, each metal box (containing a unique individual) was provided with a cemetery form where personal data and information regarding the burial and the exhumation (modality, date and place, number of the grave and of the cemeterial field) were reported. The demographic information of each individual, which are sensitive data according to law, were treated in agreement with the regulations and with prior authorization from the National Data Protection Supervisor. All individuals were therefore stored and archived after gathering the data of interest (name and surname, gender, age, date of birth and death, grave and field numbers), and made anonymous through the acquisition of a progressive number.

Description of the cemetery collection

Currently, the cemetery collection is composed of 2127 skeletons of both sexes, with ages at death ranging between 0 and 104 years. Most of the collection is constituted by adult individuals (91%, n= 1938), but there is also a valuable representation of sub-adults (9%, n= 189). Dates of death range between 1910 and 2001 and the exhumations were performed starting from 2007. Years of birth range between 1874 and 2000. Sexes are both equally represented. The female sample constitutes 51% (n= 1092) of the collection, with ages at death ranging from 0 to 104 years old; the male sample, of 1013 individuals (48%), ranges between 0 and 101 years old. The remaining 1% is represented by unknown people (i.e., people for which name or birth and death information were not available).

Among these data, 254 individuals were added to the collection because of the available photograph of the person alive. These skeletons were expressly selected among others with regard to the complete preservation of the skull and the good quality and resolution of the photography, which are an excellent instrument for future studies on face morphology and facial identification.

Fig. 3.2: example of a skeleton of the collection at the opening of the metal box in which it is stored.

The skeletal preservation varies among the collection, ranging from almost complete skeletons, with just few bones of hands and feet missing, to skeletons with only few skeletal regions still available (Fig. 3.2). The loss of some skeletal districts was however expected as the bodies were buried in the soil for almost 15 years. Additionally, the recovery of the skeletons was carried on by cemetery workers by means of heavy vehicles, a step that was performed without any forensic anthropological attendance, which could have caused the fortuitous loss of some skeletal elements.

Among the various data at disposal, it is important to highlight the availability of the national death certificates, in Italy named

“ISTAT certificates”. These documents have become an obligation under the Article 1 of the D.P.R. of September 10, 1990 n.285 of the Italian Mortuary Police Regulation. In this article, the denunciation of the death of an individual has to be communicated to the people in charge on specific forms, in order to univocally ascertain the cause of death for each deceased. These investigations into the causes of death intend to survey the total health information and demo-social data for sanitary, epidemiological and statistical purposes. On the ISTAT certificates the medical doctor who ascertains the death must indicate, in cases of natural death, the morbid sequence that caused it, as well as any other relevant diseases that could have led to the terminal one; in cases of violent death, instead, the modality, description and complications which occurred have to be reported. By far it has been possible to retrieve ISTAT certificates for 1366 individuals (64% of the entire collection), among which 1315 are adults (96%), and 51 subadults (4%). Part of the remaining corresponds to individuals who died before 1980, which represent to 22% (n= 459) of the collection, for which the death certificates were not written up, as well as emigrant people, or people expired in other districts (different from Milan, were the collection of the data was conducted). The collection of those information is however still ongoing. The main cause of death pointed out, regarding the adults, is cardiac arrest, often arising as the final consequence of other pathologies ongoing, and cancer. Of great importance is also the presence of a consistent percentage of trauma, represented by cases of traffic accidents, downfall, gunshot, choking, etc. Such death certificates also offer the chance to gain important information about the presence of relevant pathological states. The highest percentage is represented by neoplastic conditions, but also numerous cases of diabetes, pneumonia and HIV have been recovered, as well as few cases of rheumatoid arthritis, tuberculosis and syphilis. These data hence provide an invaluable control material for the study and diagnosis of diseases which are, in anthropology, solely based on the presence of pathological signs on bones, often non-specific (Table 3.1).

Another precious source of information is represented by the available autopsy reports, kept at the Institute of Legal Medicine of Milan. Such reports of course exist only for those individuals who were subjected to autopsy prior burial at the aforementioned Institute, most of which died of a violent death or/and in suspicious circumstances. In the autopsy reports a variety of data about the person (such as height, weight, health status, and the last time seen alive) are described, as well as a detailed description of the external examination and the analysis conducted on the organs. Finally, cause and manner of death are specifically reported, and so are the injuries involving soft tissues and skeletal elements, when present. Such information acts as a valuable source for interpreting same trauma in two different decomposition stages: the observation of a body at autopsy and the subsequent analysis of the skeletal remains after 15 years of inhumation represents a distinct resource for taphonomy studies, as well as for comparing and inquiring the aspect of skeletal lesions and pathologies with the superimposition of environmental factors.

Cause of death/other relevant pathologies	**N. of individuals**
Cardiac arrest	870
Cancer	500
Trauma	110
Diabetes	94
Pneumonia, BPCO	63
Respiratory failure	59
Ictus	36
HIV	35
Pulmonary oedema	25
Renal failure	25
Intracranial haemorrhage	20
Sepsis	18
Internal bleeding	16
Liver failure	13
Rheumatoid arthritis	7
Intoxication	7
Aneurysm	7
TBC	6
Quadriplegia	5
Coma	5
Multiple Sclerosis	3
Embolism	3
Aspiration pneumonia	3
Meningitis	3
Mielodisplasia	1
Poliomyelitis	1
Huntington's disease	1
Down syndrome	1
Paget syndrome	1
Syphilis	1

Table 3.1: List of the main causes of death and of other relevant pathologies affecting the individuals of the Milano Cemetery Skeletal Collection, and the corresponding number of individuals affected (as reported on ISTAT death certificates).

Until now, 123 autopsy reports have been collected. Of course, as previously said, just a small number of individuals of the collection were subjected to the postmortem analysis, and some of them were also performed on other Legal Medicine Institutes: this information are therefore not available. The survey is still in process as new individuals subjected to autopsy are found and studied.

The anthropological study of the collection is continuously ongoing. By far, more than 300 individuals have been studied (275 adults and 41 subadults). These subjects were selected in part randomly from the entire collection, and in part by reason of their well-known cause of death or pathologies and of the availability of autopsy reports/ISTAT certificates, in order to perform focused studies.

Discussion

The development of the Milano Cemetery Skeletal Collection Project will help to deepen the knowledge of the skeletal features of the contemporary population of Milan, with also a specific look into the accuracy of methods concerning sex, age and pathological estimations, which are of great importance in local forensic investigations. The availability of an Italian skeletal collection is in fact a powerful tool for the development of the forensic anthropological practice, especially for the possibility to expand and enhance its potentialities in this specific geographical area. The presence of known demographic information, such as age, gender, ethnicity, and date of death, proves invaluable for its use in the corroboration of anthropological methods, which is of pivotal importance for creating an identikit (key stage in the study of human remains of judicial relevance) and for validating the existing methodologies. It is also of great value the contribution provided by the available antemortem data in the study of subadults. In particular, the opportunity to know the exact subadult age in terms of months or days (sometimes also the foetus age prior birth) can help to improve the accuracy of the aging methods for living children. Furthermore, as for the adults, the presence and aspect of pathological and traumatic signs can be inquired, well-aware of what should be sought. This aspect is of particular importance, since it could be helpful also in contexts such as child abuse and neglect.

The informative usefulness of the Collection is therefore not limited only to demography, but is extended to much more ambitious aims of the discipline, such as the study of taphonomy, pathology and trauma, which is possible thanks to the presence of detailed autopsy reports (albeit just for a limited number of individuals) as well as of the ISTAT death certificates. In this perspective, the available material is remarkably important because of the possibility to have 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. Very little is known on how traumatic lesions are altered by the superimposition of taphonomic variables or on how the environmental intervention misleads the anthropologist in the diagnosis of an injury, mimicking the evidence of a foul play. Some studies were already conducted in order to try to answer those questions (41-47); nevertheless, a real context of trauma and taphonomy, sustained by the awareness of the presence (or absence) of lesions and their location, is one of a kind.

The first studies aimed at investigating these aspects, comparing data from the autopsy analysis and the information achievable from the same skeletonized body after 15 years of inhumation have been recently conducted on the Milano Cemetery Skeletal Collection. These studies attempted to detect the survival of traumatic signs on bones and the possibility to recognize them after the interference of taphonomic variables, as well as to test the reliability of the macro-morphological parameters commonly used for distinguishing between perimortem and postmortem lesions (48-49). The obtained results succeeded in demonstrating the substantial informative value provided by the availability of skeletal population for which the causes of death (and in particular the autopsy reports) are accessible, thus providing an efficient clarification of the usefulness and limits of the forensic anthropological practice in studying human remains.

The study of skeletal morphology is also well-known for furnishing information regarding the comprehension of pathological signs, when present (6). The ISTAT death certificates collected offer an invaluable tool for understanding the aspect and pathogenesis of those pathologies that may be recovered on the bone tissue, offering a control when analysing a skeleton from a pathological point of view. Besides that, the chance to know which disease a person suffered in life or at death could allow the anthropologist to confirm the existing diagnostic methodologies and to test new ones, among which, for example, radiographic images, histological methods, isotopic analysis (50), biomolecular analysis (51), and the identification of pathogenic DNA (52-54).

Many more are however the possible research clues that can arise from such material. For example, as mentioned above, the recent acquisition of photographs portraying many individuals of the collection will allow us to improve another field strictly connected to the forensic anthropological one: the facial morphology and cranio-facial superimposition one. It will be therefore possible to test the accuracy of this identification method from the skull, and better identify the cranio-facial characters that allow to define and predict the aspect of a face.

Age estimation can also be further inquired, as already done in some dissertations aimed at testing the reliability of the method based on the aspect of the acetabulum and auricular surfaces.

Furthermore, the action of taphonomy on the preservation of the bone tissue and the effect of different types of soils on it (pH, composition, humidity, etc.) could be investigated. This is in fact possible because the specific location of the grave of each individual in each cemetery is recorded, and for more or less every skeleton a sample of soil is available (since it was preserved into the metal box, together with the bones).

All the studies concluded, ongoing, and in program thus demonstrate the importance and the potentialities of the Milano Cemetery Skeletal Collection, which is proven to be an informative tool of pivotal significance for the study of both ancient and modern skeletal remains, as well as for the better interpretation of modern forensic cases.

A scientific article regarding this topic is being prepared.

Chapter 4

CANCER

Among all the pathological conditions affecting the individuals of the Milano Cemetery Skeletal Collection, cancer is one of the most widespread, affecting almost 500 subjects (23,5%) for which the cause of death was recovered.

It was immediately clear that cancer represents a proper disease for skeletal pathologic investigations. Primary bone tumors are usually uncommon and benign tumors are much more common than malignant. Malignant bone tumors may be primary or secondary. Cancer metastasis to bone from other organs is by far the most common cause of tumors affecting the skeleton (primary malignant tumors account for less than 1% of all bone malignant tumors) (7, 55). Metastatic bone tumors, which are those considered in the current study, spread from the primary site to the skeleton occurs via four main routes: through the blood vascular system, both arterial and venous; by direct spread from a tumor adjacent to a bone; through the lymphatic system; and through the cerebrospinal fluid in those with tumors of the brain (7). Liotta and Kohn (56) suggested that, although the distribution of metastases in distant organs can be predicted by the anatomic distribution of blood flow from the primary site in 30% of cases, specific properties of the tumor cell and features of the metastatic site determine where the metastasis occurs in the majority of cases. In fact, over a century ago, Stephen Paget was the first who observed a non-random pattern of metastases to certain organs by analyzing autopsy records of 735 women who died of breast cancer. He proposed the “seed and soil” hypothesis, according which the formation of metastasis results only when the “seed” and “soil” are compatible, i.e., osteotropic cancer cells possess certain properties that enable them to grow in bone, and the bone/bone marrow microenvironment provides a fertile soil above which such cells can grow (57).

Therefore, although virtually any malignant tumor can metastasize to bone, just some of them show a greater propensity than others. Carcinomas of the breast, lung, prostate, kidney and thyroid are particularly liable to do so. In males, carcinoma of the prostate and of the lungs account for the majority of bone involvements, while in females carcinoma of the breast is by far the most common cause (7).

Secondary bone tumors tend to be predominantly lytic or sclerotic, which means that they stimulate either the osteoclast, osteoblast, or both. Of metastatic tumors to bone 75% are osteolytic, 15% are osteoblastic and the remaining 10% are mixed osteoblastic and osteolytic (58). The classification is however still unclear. According to Waldron (7), breast and lung metastases are mainly lytic, whereas prostatic ones are mainly sclerotic. Ortner (6) reports that the most common causes of osteolytic lesions are carcinoma of the kidney, lung, breast, gastrointestinal tract, and thyroid; osteoblastic lesions are commonly caused by prostate carcinoma in men and carcinoma of the breast, uterus and ovary in women; mixed lesions can be associated with any type of metastatic tumor but are most common in carcinoma of the lung and breast.

The most commonly involved skeletal regions in metastatic carcinoma are the spine and sacrum, the proximal metaphysis and epiphysis of femurs, ribs, sternum, skull, pelvis and proximal humerus. This area, rich in spongy bone, represent the location of the hematopoietic marrow in the adult (6-7, 59). In the spine, the most frequently involved vertebrae are the lower thoracic, followed in decreasing frequency by the lumbar, sacral and cervical vertebrae. The metastases are prevailingly located in the vertebral bodies, but neural arches and spinous processes may also be affected. Compression fractures are therefore common. In the cranial vault the marked destruction is in the diploe, whereas the outer and inner tables are often spared (6).

Once the tumor cells reach the bone marrow, they start growing and replacing the marrow without changing of the bone architecture. The continued growth of the tumor cells leads to destruction and osteoclastic resorption of cancellous bone and internal scalloping or complete destruction of cortical bone (osteolytic metastases). Lytic metastases therefore progressively destroy cancellous bone and erode the cortex of long bones from within. At least microscopically, however, some reactive bone formation is often seen in some of the lesions and/or in their vicinity. In other cases, a strong osteoblastic response to the presence of the tumor cells is observed, consisting of new bone deposits replacing old trabeculae. In general, fast-growing tumors are mainly osteolytic, whereas slow-growing ones elicit an osteoblastic response (6).

Although the study of bone metastases is already on its way, some problems still remain unsolved. The first problem regards the possibility to clearly differentiate a metastasis from other events (pathologic, traumatic or taphonomic). Marks and Hamilton (60) assessed that metastatic carcinoma is often indistinguishable from other tumor types and osseous disorders. For example, one problem is to distinguish between breast cancer and multiple myeloma: in the skeleton these two types of cancer reflect a morphological gradient, making differential diagnosis impossible in some cases. Indeed, there is a suite of metabolic and reactive bone diseases that can mimic the neoplastic process within both cortical and periosteal layers (6). Furthermore, the monotony in the reaction of the bone tissue to an insult, as well as the degradation and modification caused by environmental and decompositional occurrences after death can mislead the anthropological assessment; diagnosis is in fact further complicated in ancient skeletal remains given the destructive effects of post-depositional processes. Secondly, once assessed the presence of a metastatic cancer, despite the pattern of distribution of metastases form the primary tumor has been defined in the different cases, the narrowness of the skeletal locations involved and the often random distribution of the metastases (particularly in the early stages of invasion) and the scarcity of variability in the bone response, make it difficult to identify the original cancer, which is mandatory for assessing a more precise personal identification.

Materials and methods

A group of skeletons, for which the presence of tumor as pathological condition was signaled, was analyzed. All these skeletons had already been cleaned and studied form an anthropological point of view (bone inventory, sex, age, race, stature, pathologies and eventual trauma). According to Ortner (6) recommendations, after the disposition of the skeleton in the anatomical position, the inspection of each bone was performed, in order to inquire and record the distribution of lesions within the whole skeleton. The location of abnormalities on each bone was provided, together with a description based on specific features, such as dimension, shape, color, aspect of the margins and aspect of the erosion in the trabecular portion. All this information was reported in 2 different forms: the first form is the representation of a skeletal model, on which each lesion was pointed out on the correspondent skeletal district in different colors, in order to distinguish between pathologic, taphonomic and dubious marks. The term "dubious" refers to those signs for which a clear pathological assessment was impossible to be done according solely to their macroscopic appearance. The second form is a table in which, for each skeletal district of each skeleton, each lesion was described reporting the main features: location on the skeleton, dimensions, shape, color and aspect of the margins (with eventual presence of remodeling), aspect of the erosion in the trabecular portion, presence of smaller areas of erosion nearby, and anthropological evaluation (pathologic, taphonomic or dubious). These two forms together permit to have a pattern of distribution of the lesion on the skeleton, an approximate quantity of the evident bone metastases and a description of them, which are identifying parameters that permit to tag a bone hole or a defect as a metastatic sign and not as a taphonomic one.

14 skeletons were studied with this purpose, which are listed in Table 4.1.

Case N.	Sex	Age	Cause of death	Type of tumor	Metastases pointed out
1	M	85	Prostate cancer; neoplastic cachexia	Prostate	No
2	M	67	Hepatic cirrhosis; liver neoplasia, diabetes mellitus type I; terminal hepatic coma	Liver	No
3	M	83	Hepatic neoplasia; neoplastic cachexia; C.O.P.D.	Liver	No
4	M	60	Bronchial adenocarcinoma; diffused metastases; cardiac arrest	Lungs	Yes
5	F	70	Breast cancer; diffused bone metastases; neoplastic cachexia	Breast	Yes
6	M	85	Prostate cancer; multiple skeletal metastases; cardiac arrest	Prostate	Yes
7	M	79	Prostate cancer; anemy, spinal metastasis; cardiac arrest	Prostate	Yes
8	M	85	Anuria in prostatic cancer; cardiac unbalance; cardiac arrest; diabetes mellitus	Prostate	No
9	M	76	Hepatic cancer with bone metastases; metabolic deficit in diabetes mellitus and diabetic kidney disease; terminal collapse	Liver	Yes
10	F	61	Breast cancer; bone metastases; neoplastic cachexia	Breast	Yes
11	M	56	Bladder cancer; ictus cerebri; cardiac arrest; cachexia	Bladder	No
12	M	73	Prostate cancer; diffuse metastases, cardiac arrest	Prostate	Yes
13	M	79	Prostate cancer; bone metastases; cardiac arrest	Prostate	Yes
14	M	80	Ischemic cerebral vasculopathy; stroke, convultion; gastric and duodenal ulcer; diabetes mellitus	None	No

Table 4.1: list of the individuals studied for the investigation on the manifestation of bone metastases.

Results

A list of the cases investigated and of the observations is given below.

Case 1

Male, 85 years old. No skeletal metastases reported in the cause of death.

The skeleton is incomplete: albeit the skull and part of the upper left limb, the pelvis is abundantly eroded, as well the lower limbs.

It was however possible to identify an area as a possible metastasis (Fig. 4.1): it corresponds to a finely remodeled lytic area in the internal part of the right femoral head (dimensions 16x15mm, 10mm deep) (Fig. 4.1), displaying a smooth and regular surface and easily recognizable shape.

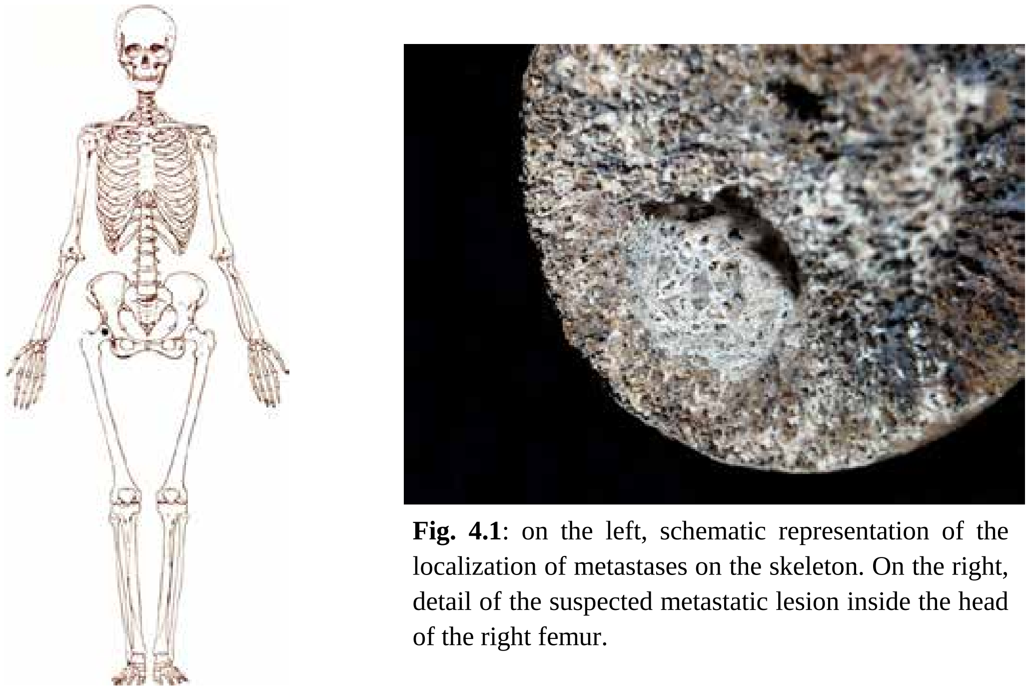


Fig. 4.1: on the left, schematic representation of the localization of metastases on the skeleton. On the right, detail of the suspected metastatic lesion inside the head of the right femur.

Case 2

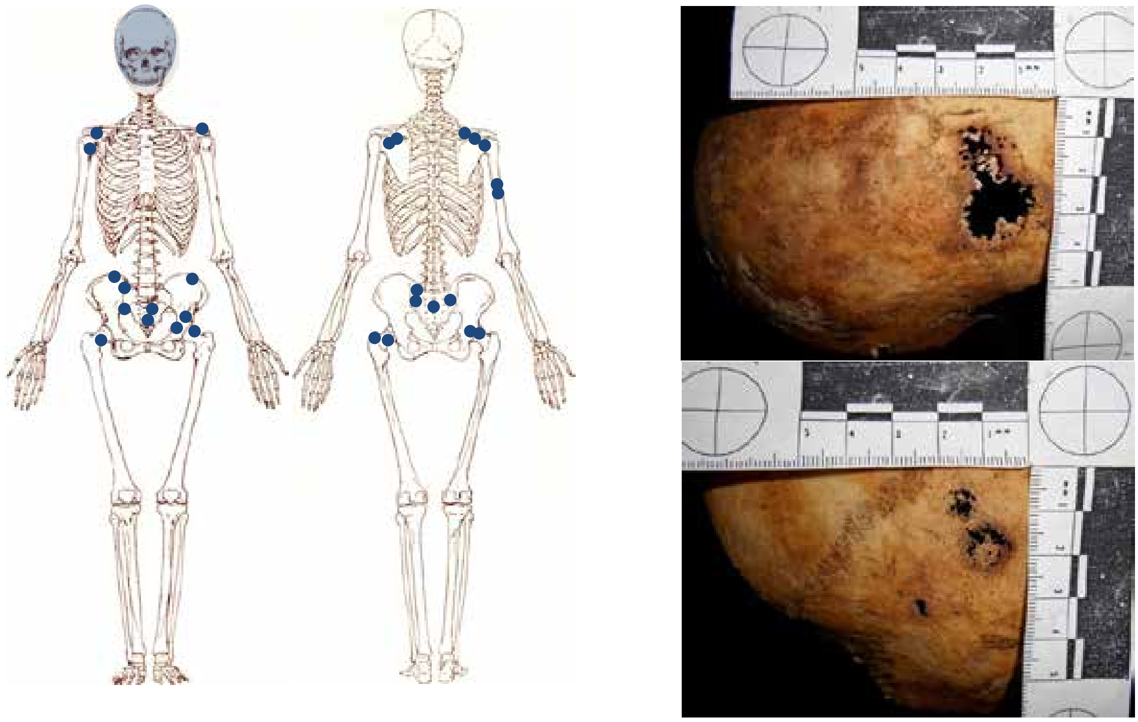


Fig. 4.2: on the left, schematic representation of the localization of metastases on the skeleton. On the right, detail of the same metastatic lytic “denticulated” lesions on the cranium.

Male, 67 years of age, suffering of liver neoplasia. At the anthropological analysis it was possible to notice a consistent number of metastases, mainly located on the skull. Such metastases display the typical lytic morphology: oval or circular shape, with dimensions raging from few millimeters to 5cm, with denticulated or lacy edges and numerous small circular lesions nearby, producing a sort of “porotic aspect” (Fig. 4.2). Such metastases were observed mainly on the skull, where in some cases brought event to the complete destruction of the involved areas (like the left zygomatic process), but also in the sacrum, innominate bones, scapulae, left clavicle, left humerus, femurs, ribs and vertebrae.

In all such cases, where the medullary part was thick enough for being investigated, it could be noticed a deeper lysis of this part of the bone than the cortical one, thus proving that it was the portion of the bone that was firs attacked and substituted by the metastatic new tissue.

Case 3

Male, 83 years old, affected by hepatic neoplasia.

Fig. 4.3: on the left, schematic representation of the localization of metastases on the skeleton. On the right, detail of the lytic metastasis on the left transverse arch of T1.

The only pathologic signs of the cancer were observed on the left transvers process of the I thoracic vertebra (Fig. 4.3), and on the upper part of the body of another vertebra (unidentified). The regular and remodeled margins of such lesions (as seen also at the stereomicroscope), together with the deep erosion of the trabecular underlying tissue permitted to assess them as cancer metastases. No other lesions, despite the taphonomic ones, were observed.

Case 4

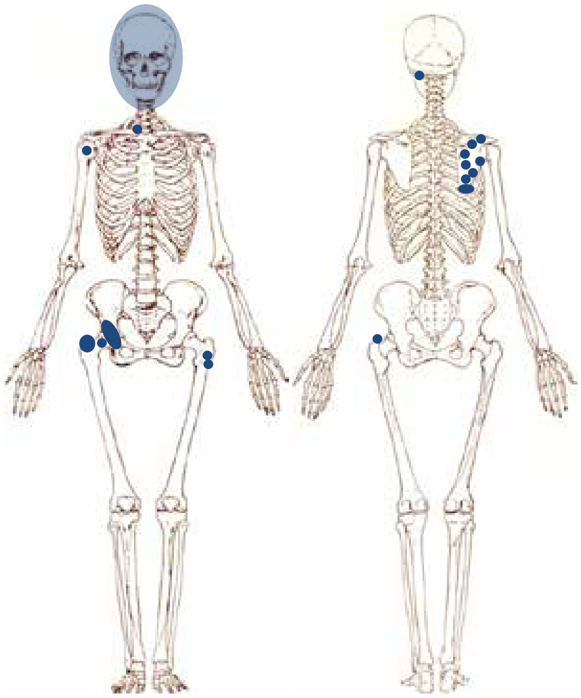
Male, 60-year old, affected by bronchial adenocarcinoma. At the anthropological analysis, only a lytic lesion on the right iliac fossa was observed. However, such lesion displays however dubious characteristics, also because of the localization, where the cortical tissue is thin and easily modified by taphonomy (Fig. 4.4). Nevertheless, an accurate inspection with the stereomicroscope permitted to observe the finely remodeled margins of the lesion, thus testifying for the pathologic origin of the inquired area.

Fig. 4.4: on the left, schematic representation of the localization of metastasis on the skeleton. On the right, detail of the suspected metastasis on the right iliac fossa.

Case 5

Female, 70 years of age. According to the cause of death, she suffered of breast cancer in life. At the anthropological analysis, it was possible to identify 8 different metastatic signs on the cranium, all lytic and of almost 1cm of diameter. Most of them display regularly eroded margins and a deeper loss of the trabecular tissue in correspondence of the area of interest. However, the taphonomic intervention often altered the margins of some other lesions, thus not permitting to identify in them the typical characteristics that determine a metastatic invasion (Fig. 4.5).

Other areas interested by the pathology are the pelvis, the upper and lower limbs



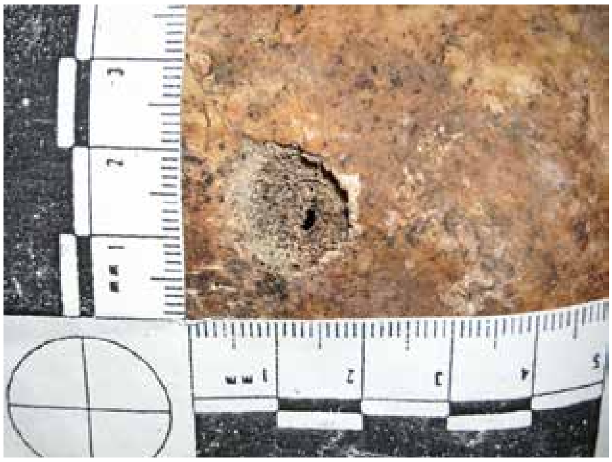


Fig. 4.5: on the left, schematic representation of the localization of metastases on the skeleton (lytic). On the right, example of a dubious mark on the skull.

Case 6

Male, 85 years of age, affected by prostate cancer, with multiple skeletal metastases reported with the cause of death. Despite that, no metastatic signs were noticed on the skeleton. The unique areas that display uncertain characteristics are the heads of both femurs: due to taphonomy, which caused the erosion of the cortical tissue, the trabeculae are exposed, displaying an aspect which looks thicker than normal, with deposits of finely trabeculated bone filling the spaces between the spongy bone trabeculae (Fig. 4.6). Nevertheless, such an appearance is not clear enough for being addressed as a consequence of the metastatic invasion. Nothing relevant was observed on the rest of the skeleton.

Fig. 4.6: Head of the left femur.

Case 7

Male, 79 years old, affected by prostate cancer. No skeletal metastases reported in the cause of death.

At the anthropological analysis the presence of more than 50 areas identified as metastases and 40 areas of uncertain diagnosis were noticed. As shown in Figure 4.7, the pathological lesions are mainly located in the axial skeleton (vertebrae and ribs) and some bone apposition is visible also on the proximal left femur, sacrum and sternum. However, although not common sites for prostate metastases, it has been possible to find modifications also on the clavicles, scapulae and proximal epiphysis of the right humerus. Most of the lesions observed have been addressed as “mixed”, since they display both lytic and proliferative features. The lytic lesions appear as small (dimensions range from 0.3 and 3cm) almost rounded areas, with a deep loss of the underlying spongy tissue and curved regular remodeled margins. They are located mainly in the upper part of the body (scapulae, clavicle and sternum, blue dots in Figure 4.7). On the contrary, the proliferative ones involve femur, vertebrae and ribs. On vertebrae and ribs both types of manifestation are evident: they in fact display areas of new bone deposit, mostly evident as protruding bone spicules, above areas of bone lysis, evident as small holes in the bone under the bone apposition. A particularly interesting sign is visible on the left arch of the first lumbar vertebra, where a rounded lesion (1x1.1 cm) displays both a lytic central area and small protruding bone spicules on the margins (Fig. 4.7).

Despite the presence of an abnormal bone growth on the column, forming bridges across all the contiguous vertebral bodies and arches of the thoracic and lumbar vertebrae (possible consequence of ankylosing spondylitis), the modifications caused by the metastases are still well-recognizable, with irregularly oriented bone streaks and protruding spicules.

Areas of uncertain diagnosis were found on ribs, scapulae and pelvis. In this last area they mainly have a proliferative appearance, whereas in the other ones they display both aspects. On the ribs, for example, areas of diffuse porosity as well as areas of streaked new bone apposition are noticeable, although not clear enough to assign them a metastatic origin.

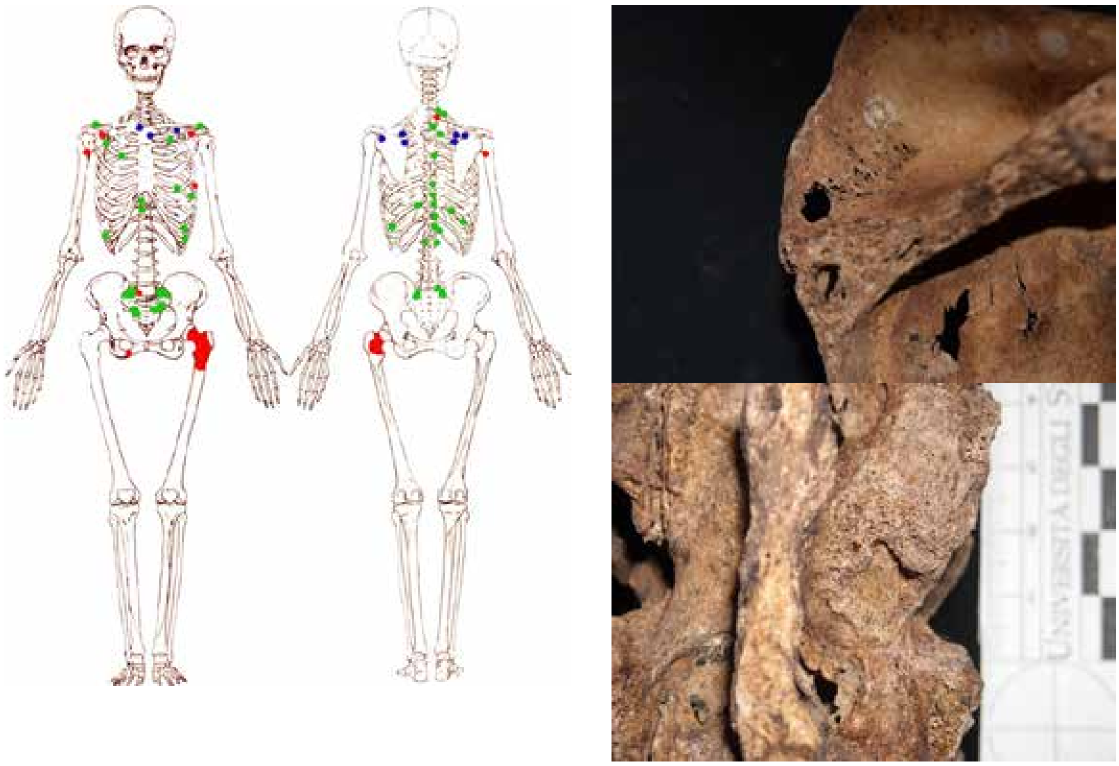


Fig. 4.7: on the left, schematic representation of the localization of metastases on the skeleton (red, proliferative; blue, lytic; green, mixed). On the right, example of lytic lesions on the right scapula, and of a mixed lesion on the right arch of a lumbar vertebra.

Case 8

Male, 85 years old. The cause of death points out the presence of prostatic cancer and diabetes mellitus, but not the presence of metastases.

The anthropological analysis permitted to identify signs of the neoplastic condition affecting the subject, particularly evident on the left os coxae (Fig. 4.8). A diffuse spiculated new bone apposition is evident on the whole ilium, acetabulum and on part of the ischium. The bone appears streaked, with new-formed bone tissue irregularly growth, providing to the surface of the iliac fossa a “lace” appearance. This particular “lace/net” aspect is also provided by the presence of small lytic areas (few millimeters of diameter) along the trend of the streaked new bone apposition. Locally, small bone spicules are visible also in the posterior side of the bone, although less developed than the ventral side (Fig. 4.8). A small area of spiculated bone apposition is evident also in the internal portion of the right acetabulum. Of particular interest is also a well identifiable metastasis on the left transversal process of the VII cervical vertebra, evident as “sun-burst” reaction (Fig. 4.8). This is a rare finding since the cervical area of the axial skeleton is rarely involved in the metastatic invasion from prostate cancer, which normally involves the lumbar portion. Indeed, also the lumbar vertebrae display moderate signs that could

be linked to the metastatic condition: the spongy tissue, visible thanks to the taphonomic modification of the skeleton, locally displays a thickening caused by deposits of bone tissue, filling the spaces between the spongy bone trabeculae (the so-called “bone in bone”(61)).
Uncertain areas are visible on the pelvis, proximal femurs and lumbar vertebrae. All such areas display a rough bone apposition, but its regular arrangement makes them hardly identifiable as metastases or as a consequence to other pathologic processes.

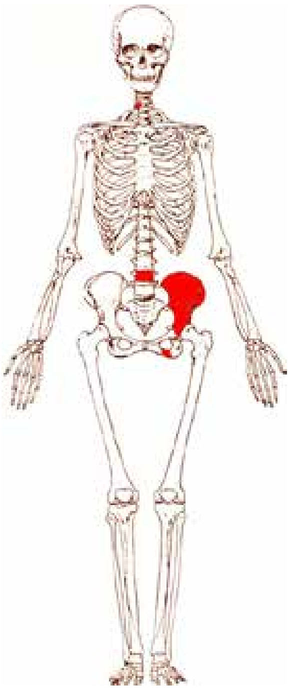


Fig. 4.8: on the left, schematic representation of the localization of metastases on the skeleton (all proliferative). On the right, example of bone spicules on the iliac fossa left.

Case 9

Male, 76 years of age, affected by hepatic cancer. Skeletal metastases are reported on the ISTAT certificate, without any mention to the specific localization. Regular and finely remodeled rounded lesions were observed on the cranium, on the external part of the left sphenoid. However, the most characteristic area that can be addressed as pathologic form a macroscopic point of view is localized on the left hip bone, posteriorly to the acetabulum. This oval area displays a deep loss of the trabecular area, which displays irregular but remodeled margins, with bone apposition “in layers”. Such margins appear in fact “stratified”, with bone apposition on the external part and bone spicules in the medial portion (Fig. 4.9).

Fig. 4.9: on the left, schematic representation of the localization of metastases on the skeleton (blue, lytic; green; mixed). On the right, the metastatic lesion on the left hip bone.

It is interesting to notice also a consistent remodeling interesting the right scapula, which displays a complex remodeled area consequent to an antemortem fracture. Acute osteoarthrosis is widely diffused on the whole spine.

Case 10

Female, 61 years of age, affected by breast cancer. Bone metastases are highlighted in the cause of death, without mention to the localization.

The macroscopic investigation did not permit to identify evident signs of the pathology on the skeleton. However, this case is particularly interesting because of the presence of multiple areas where it is possible to observe the so-called “bone in bone”. In particular, thanks to the bad preservation of ribs, it was possible to observe the fine apposition of bone tissue among the trabeculae. An example of such areas are exposed in Figure 4.10.

Fig. 4.10: Examples of the anomalous bone apposition in the internal structure of two ribs.

Case 11

56 year-old male, affected by bladder cancer. On this subject, such pathology displays a variety of manifestations (Fig. 4.11). For example, on the left scapula clear signs of lytic metastases are evident, with well-defined rounded shape, remodeled margins, smaller lytic areas all along the border, and a deep erosion of the bone tissue inside the bone itself. However, areas of bone apposition are evident on the edges of such lytic areas, evident as periostitis. Furthermore, where the inner part is exposed, small areas of thicker bone apposition are also evident. Bone apposition can be observed also on the sternum and vertebrae (from the thoracic to the lumbar). However, the skeletal district where the pathology displays its stronger manifestation is the rib cage. Both small lytic areas and diffuse bone tissue apposition can be observed, mostly on the left side (Fig. 4.11).

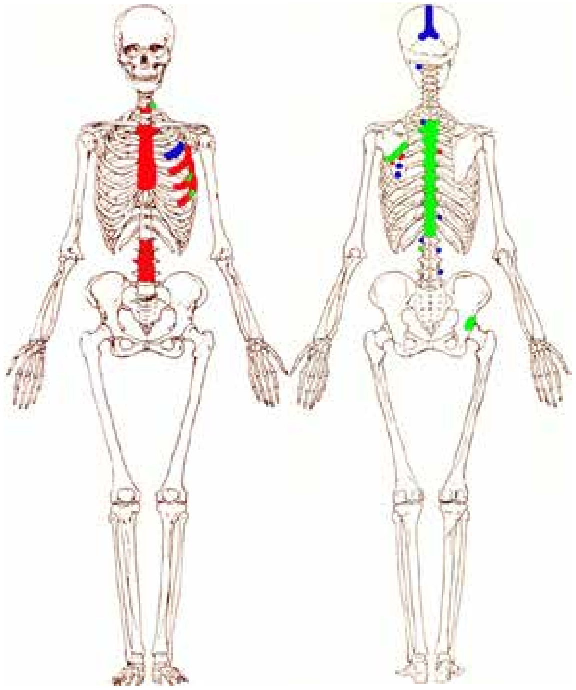


Fig. 4.11: on the left, schematic representation of the localization of metastases on the skeleton (green, mixed; red, proliferative; blue, lytic). On the right, a left rib of the subject, displaying diffuse protruding bone spicules as consequence to bone apposition.

Case 12

Male, 73 years old, affected by prostate cancer. “Diffuse metastases” are reported in the cause of death, without any mention to the direct implication of the skeleton.

According to the anthropological investigation, a total amount of over 60 pathologic metastases was noticed, together with other signs of uncertain diagnosis (Fig. 4.12). Skeletal metastases can be observed on almost all the bone elements of the axial skeleton (all the thoracic and lumbar vertebrae and most of the ribs), on the sacrum, both os coxae, and on the proximal epiphysis of the right femur and of the left humerus. All these metastases display a so-called "mixed" appearance, e.g. with both lytic and proliferative activity. In fact, the aspect of such metastases is characterized by the presence of eroded areas, above which new bone tissue apposition provides the skeletal elements a spiculated appearance. Where this process in particularly advanced, the presence of such protruding bone spicules is highly evident, thus conferring the bone with a sun-burst aspect. Particularly noticeable is the presence of a metastasis to the skull. In particular, both the deep frontal portions of the orbital cavities display an abundant bone apposition, which acquires a sun-burst aspect in the right orbit. Porosity and bone apposition are visible also on the external portion of the sphenoid, frontal, and occipital bone, although to a less developed stage.

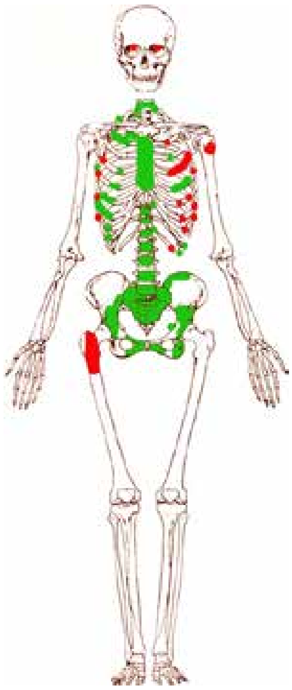


Fig. 4.12: on the left, schematic representation of the localization of metastases on the skeleton (green, mixed; red, proliferative). On the right, a metastatic proliferative lesion on a vertebral body, with well visible protruding spicules.

Case 13

Male, 79 years old, affected by prostate cancer. Bone metastases are pointed out in the cause of death, without any indication regarding number and location.

At the anthropological analysis metastatic signs have been found only in the iliac fossa (both left and right) and right ischium (Fig. 4.13). Such signs have uniquely a lytic appearance, mostly rounded in shape, with "lacy" borders due to the presence of numerous small (<1mm) lytic areas all along the margins and a deep erosion of the trabecular tissue below (Fig. 4.13). The dimensions range from 0.4cm to more than 5cm in length (as on the lateral margin of the left os coxae, between the anterior superior and inferior iliac spine). Typical lytic lesions are also visible on the spine, but just on two thoracic vertebrae (T2 and T8).

Uncertain lytic areas affect the pelvis and the vertebral body of L4 and L5. Such areas display small rounded lesions with remodeled regular margins. Nevertheless, the characteristics of such areas are not clear enough for a clear diagnosis among prostate cancer metastasis or degenerative disease.

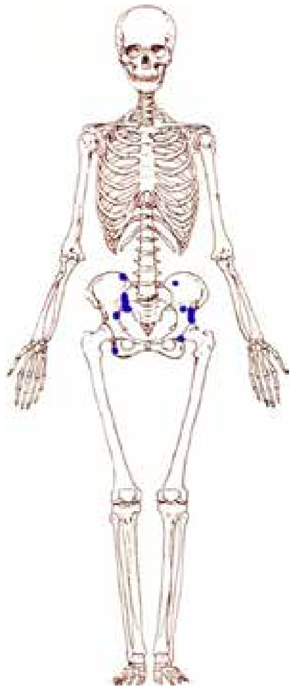


Fig. 4.13: on the left, schematic representation of the localization of metastases on the skeleton (lytic). On the right, a metastatic lesion on a vertebral body.

Case 14 (control)

Male, 80 years old. The cause of death reports ischemic cerebral vasculopathy, stroke and convultion as primary pathological conditions affecting the subject, together with gastric and duodenal ulcer and diabetes mellitus; the final cause of death is cardiac arrest. The subject was selected according to sex and age, concordant with the other subject considered, and to the absence of relevant diseases affecting the skeleton. The only pathological conditions expected are therefore degenerative ones. In fact no relevant pathologies, excepted osteoarthrosis, have been detected, as well as no signs that could be related to metastases from prostate cancer, neither lytic nor proliferative.

The unique area that displays dubious characteristics is a rounded lytic lesion on the right inferior articular facet of L5 (0.4x0.3cm), which displays a deeper erosion of the underlying trabecular tissue. No other dubious areas were pointed out.

Table 4.2 summarizes the data obtained by the analysis performed. In most of cases secondary metastatic lesions appear to be lytic, and all of the primary tumors considered appear to provide such manifestation. Proliferative lesions were noticed in breast, prostate and bladder cancer; finally, mixed lesions have been observed in case of prostate, liver and bladder cancer. In particular, in case of prostate and cancer metastases all the three types of possible manifestation were present.

Type of primary tumor	**Number of cases considered**	**Lytic lesions**	**Proliferative**	**Mixed**
Breast	2	1	1	
Prostate	6	3	4	2
Liver	3	3		1
Bladder	1	1	1	1
Lungs	1	1		

Table 4.2: summary of the collected data.

Discussion

According to the observations performed, the typical aspect of bone metastases, as described on books and literature, was observed in more than one case. In particular, three different and well-defined types of skeletal metastases can be categorized.

Osteolytic lesions display an almost circular or oval outline, often with denticulated or lacy edges (62). According to the observations performed, it is in fact common to find small (diameter <1mm) circular lesion around the main one and sometimes it is also visible a proliferative reaction of the bone tissue around the lytic area. The damage affecting the underlying diploe or spongy bone is almost always wider and deeper below the cortical surface above. Differently from taphonomic modifications, however, the loss of spongy bone is well defined and seems regularly controlled. In the most severe cases, the spread of the metastatic cells undermines both outer and inner lamina (in the skull) and can cause pathological fractures by the interruption of the continuity of the bone itself. From the observation conducted, it was possible to observe that the invasion by lytic metastases progressively destroys the cancellous bone, eroding the cortex of bones from within. Before perforating the cortex, however, the metastatic mass develops between the trabeculae, stimulating the osteoclastic activity and freeing space for its own growth. The involvement of the outer layer seems to start as one (sometimes multiple) small round defect, which progressively enlarges or merges with the adjacent ones. It forms therefore a main defect encircled by smaller ones. This merging, particularly in those cases in which the small holes on the margins are still well identifiable, provides to the bone lesion its typical so-called “lacy” appearance. Regarding the skull, by far it has not been possible to notice a preference for a first attack to the outer or inner layer. In order to better understand the exact development of destruction of the bone would be however interesting to observe how these defects appear in a real condition, where the metastatic mass is still present.

The identification of metastases is however not always so clear. This typical structure is mainly observed on the skull and sometimes on the other skeletal districts, where the defects appear mainly as cavities on the surface of the bone. Nevertheless, the 15 years of permanence in the burial, together with the normal decomposition processes, could have masked some metastatic defects by altering their margins, but could also have formed some taphonomic lesions which can be easily confused with pathologic ones, as it was noticed in many “dubious” cases. Many are in fact the bone defects that could not be assessed neither as pathologic nor as taphonomic just on the base of their macroscopic appearance. Such marks display round but indented margins or regular-border holes with irregular shape and a scarce loss of the underlying spongy tissue. This is a feature particularly common on those bones with a very thin cortical layer in relation to the thickness of the trabecular portion (vertebral bodies, hip bones, sometimes skull) or on those more easily attacked by the environmental modifications (ribs and sacrum).

For depicting proliferative metastatic cases, prostate cancer proves itself very useful. Prostate cancer is in fact widely known for being one of the tumors with the highest percentage of involvement of the skeleton as a primary site of metastasis (61, 63-65), and such metastases have always been addressed as osteoblastic (which means, able to provoke a proliferative reaction in the skeletal areas where they develop). This proliferative reaction is usually defined “sunburst”, due to its particular morphology. Skeletons affected by this specific reaction in fact display widespread roughened new bone deposit, which is usually evident in the first stages as rough periostitis and, in the most advanced ones, as small protruding bone spicules. An example of such a manifestation is represented by Case 8, whose iliac fossa is completely covered by small bone needles protruding frontally from the bone surface. Despite such clear aspect, proliferative metastases are not always so easily detected: in some cases, in fact, just a rough periostitis is evident, probably as initial stage; in some other cases streaked new bone apposition lengthwise the trend of the bone itself was observed. However, the most interesting observation done regards the so-called “bone in bone”, as described by Heuck (61). Such name refers to a specific thickening of the trabecular tissue, which appears filled in its structure by newly formed spongy bone tissue. In multiple cases such manifestation was observed; however, the most significant case is Case 10 (breast cancer), where such specific structures (of millimetric dimensions) were well-visible in the damaged ribs of the subject. This specific observation demonstrates the singularity of this reaction, which is evident only in cases of damaged bones, where the inner portion is exposed. A hypothesis is that such manifestation represents an initial stage of the metastatic development, which produces those "bone tissue masses". However, the oddity is due to the fact that breast cancer produces lytic metastases, and not proliferative; therefore, although such reaction can appear justifiable in other cancer cases, its explanation is more demanding in case of breast cancer. Further investigations are therefore necessary.

Another manifestation observed is the so-called "mixed" one, which displays, in the same district, both bone resorption and bone tissue apposition. In the cases inquired, the individuals who displayed proliferative metastases presented also an extensive lytic activity in the areas affected by the metastases themselves, thus displaying protruding bone spicules above an underlying lytic area. The appearance of such lytic areas differs from that of purely metastatic lytic lesions, since the margins and the whole surface of the cavity, when present, are finely remodeled. Most commonly, such lytic areas have a "lacy" appearance, the erosion of the trabecular tissue is not as deep as that of the lytic lesions, and the cavity that is formed provides a "lining net" above which the spicules develop. The explanation of such expansive reaction can be probably provided by a study promoted by Keller and Brown (66), which states that when prostate cancer cells metastasize to bone they initially induce osteoclastogenesis with consequent bone resorption; subsequently, once that the bone breaks down, the extracellular matrix releases a variety of growth factors that act on the tumor cells and diminish their osteoclastogenetic ability, while promoting their ability to grow and induce osteoblastic activity. All such manifestations therefore confirm and strengthen the information known from literature regarding the lytic component necessary for the subsequent bone apposition in cases of prostate cancer metastases (66-67). Relevant of Case 7 is also that it displays both types of reactions. Lysis involves the upper part of the body (scapulae and sternum, mainly), whereas bone apposition is evident on femur, pelvis and ribs. In most cases, both manifestations merge, thus displaying mixed lesions.

As for Case 10, where an unexpected manifestation of breast cancer was observed, also prostate cancer provided interesting surprises. Beyond proliferative and mixed manifestations, in fact, also purely lytic activity was observed. As literature teaches, the unique and rare case in which lytic bone metastases are observed as consequence of prostatic cancer is known to be the relatively uncommon neuroendocrine tumor of the prostate (68). Despite that, in the present analysis 2 cases displayed such reaction. What has been seen on the skeleton of Case 13 are a series of rounded lytic areas, with undercut “lacy” margins and a wide erosion of the underlying trabecular tissue. Dimensions range from few millimeters to more than 5cm. Such areas are widespread in the pubis, and in particular in the iliac area, but have been noticed also on vertebrae (thoracic and lumbar). This specific type of reaction, which creates regular and remodeled holes starting from the trabecular bone tissue and that subsequently involves the overhead cortical one is typical of other types of cancer, such as breast, lung, kidney, thyroid and gastrointestinal tract (6), and is therefore a rare finding consequent to prostate cancer. The unique reference to similar manifestations in literature is provided by Mundy (69), who points out the possibility of patients with prostate cancer to develop osteolytic lesions similar to those seen in patients with metastatic breast cancer. In this context it results particularly evident that the possibility to clearly associate the presence of specific metastases on the skeleton to a well-definite tumor is mandatory.

The information obtained in the current investigation demonstrates the difficulty in clearly identifying the manifestation of cancer on the skeleton due to its mixed expression. Metastatic cells have in fact once again been proven to be able to promote both lysis and proliferation in the bone tissue, in most of the occasions on the same area. In general, lytic lesions on the skeleton represent a tricky issue as they need to be differentiated from other causes of holes in the bone (like post-mortem damage and trauma) (7). Similarly, periostitis can be both an initial stage of a proliferative metastasis, or the reaction to an inflammation caused by other factors. The framework is therefore still not clear. Solely on the macroscopic analysis performed and on the data so far available from literature regarding the manifestation and diffusion of different cancer metastases, it would be impossible to correctly perform a differential diagnosis. In this perspective, since the macroscopic analysis alone cannot provide a sufficient instrument for the specific diagnosis, additional methodologies (such as histology or biochemical investigations) should be developed and applied in order to differentiate on the dry bone the metastases of a tumor from those caused by another one. Cases of “anomalous” metastatic manifestations have in fact already been reported in literature, as in the case of an osteoblastic multiple myeloma, commonly known for producing small lytic rounded lesion, testified by Li *et al*. (70).

Interesting observations arose from the analysis of the only case considered of bladder cancer. Bladder cancer is not famous for being one of the common osteotropic tumors, although authors (71-72) pointed out the frequent involvement of the skeleton by this type of tumor. The metastases have been described as mixed (osteolytic and osteoblasitc) (73-74), as observed also in the case we had at disposal: both lysis and apposition of new bone tissue are present, often on the same skeletal district (mainly ribs, vertebrae and scapula), displaying an erosion above which new bone deposits are evident as protruding spicules and rough heap of newly formed bone tissue. The interesting case observed and the particular manifestation of the pathology, which could be compared with that of prostate cancer (according to the type of manifestation and the localization on the skeleton) deserves a deeper investigation.

It is still unclear whether there could be a correlation among the location on the skeleton or the stage of diffusion of the pathology and a specific manifestation of the metastasis. It is however known that the location does not provide useful information for cancer identification, since in almost all cases of metastatic carcinoma the bones most commonly involved are those of the axial skeleton, including the spine and sacrum, the proximal metaphysis and epiphysis of the femur, ribs, sternum, skull, pelvis, and proximal humerus (6), which are also those richer in hematopoietic tissue. The current study confirms what so far is known from literature: the first areas where metastases spread are the pelvis and the rib cage, and at a more advanced stage of the pathology also the shoulder girdle and, occasionally, the skull. However, many cases of well identifiable metastases have been observed also on the scapula, which is not commonly described as an involved area.

This initial investigation into the skeletal manifestation of metastatic cancer, regardless of its limitations, permitted to highlight not only the difficulties encountered in performing this type of diagnosis but also the usefulness of investigating the dry bone directly, with at least some antemortem information available. Despite the lack of clinical data on the progression of the pathology, the availability of the ISTAT certificates on causes of death revealed themselves to be a fundamental instrument for the investigation conducted. There is however the need to further inquire a wider number of cases, in order to better understand the manifestation of such pathology on larger numbers and different primary tumors, with the possibility to access to the clinical data of such individuals. In addition, for further studies, the possibility to detect the presence of specific proteins, characteristic of each primary cancer, absorbed to bone hydroxyapatite (as has been proven in literature), may be a valuable instrument for confirming the diagnosis of tumorous diseases by specific tumor markers (75). This tool would be of great help also for identifying all those skeletal lesions of uncertain origin and differentiate pathologic lesions from the taphonomic ones. In fact, in this context it is important to highlight also that the ISTAT certificates pointed out more than once the presence of bone metastases, which were not always observable on the dry skeleton. It has been hypothesized that such metastases developed only within the spongy structure of the bone, without breaking the cortical layer, and resulting therefore unnoticeable macroscopically. Therefore, X-ray analysis would be necessary in order to confirm or contradict such hypothesis. This will also provide information on how the development of metastases, since the earliest steps, takes place, which is an aspect poorly investigated and that could help to better contextualize dubious defects as pathological or taphonomic.

Regardless of future developments, the present study for the first time illustrates the macroscopic aspect of this pathology on dry bone of individuals who are known to have suffered from this disease, as well as the intricate diagnostic issues. Characterizing and identifying pathological conditions will assist the forensic anthropologist constructing more reliable biological profiles in the event that skeletal health impacts certain analyses.

A scientific article regarding this topic was published:

Castoldi E, Cappella A, Gibelli D, Sforza C, Cattaneo C. 2017 *The difficult task of diagnosing prostate cancer metastases on dry bone*. J For Sci; doi: 10.1111/1556-4029.13617.

Chapter 5

DIABETES

Diabetes mellitus is one of the most widespread diseases worldwide, currently affecting more than 415 million people and expected to involve 642 million people by 2040 (76). This disease, deeply investigated from a clinical and radiological point of view, is however still scarcely known and described from an forensic anthropological and palaeopathological one.

Diabetes is represented by a group of chronic diseases, all characterized by high blood glucose levels (77). Three main types of diabetes are known: Type 1, characterized by an immunologically mediated damage to beta cells, resulting in progressive loss of insulin production, on a genetic basis; Type 2, the most common, is associated with insulin resistance combined with non-immunological inadequate compensatory insulin secretion response; Type 3 is called “gestational diabetes”, diagnosed when hyperglycemia is detected for the first time in pregnancy and it is associated with a high risk of type 2 diabetes in older life (78-79).

Long lasting high blood glucose levels are known for leading to a series of serious complications, such as diabetic retinopathy, nephropathy, neuropathy, peripheral arterial disease and reduced resistance to infections (80). It is also known that patients with diabetes mellitus have various skeletal disorders, both primitive and secondary to some of the other complications listed above, including osteopenia or osteoporosis, Charcot’s arthropathy, osteomyelitis, and the diabetic foot syndrome (81). Type 1 diabetes is in fact associated with modest reduction in bone mineral density (BMD) and bone loss, whereas in case of Type 2 there is an elevated BMD and a reduction in the bone strength (82-84). However, both conditions lead to increased risk of osteoporosis and fragility fractures (77, 81-82, 85-90).

The diabetic foot syndrome is characterized by a modification in the foot structure, caused by an altered physiology and immune response to trauma and infections. The main causes are peripheral neuropathy, peripheral arterial disease, infection secondary to trauma or ulceration, and soft tissue and bone deformity (80). In particular, infections of bacterial and fungal origin can develop from an ulcer or from an infected soft tissue thus involving the adjacent bones, causing osteomyelitis and septic arthritis, all complications that highly increase the risk of lower extremity amputations (91-94). Furthermore, soft tissue and bone deformity can result in Charcot’s arthropathy, a progressive disease affecting bones, joints and soft tissues on the background of peripheral neuropathy. It is a “neuropathic inflammatory sarco-osteoarthropathy”, which causes destruction of the skeletal architecture, bone erosions, joint instability, dislocations and ulcerations (95-100). The most commonly affected districts are the foot and ankle (101), but recent data describe cases affecting the knee (102-106), hand, wrist (107-108) and spine (109-110).

In literature all these effects are well described from a physio-pathological, clinical and radiological perspective; however, the macroscopic aspects of the consequences of diabetes on the skeleton from an anthropological point of view are still extensively unknown. Radiological analysis of living patients, which are usually performed for diagnostic purposes, describe in detail the bone changes related to diabetes through plain radiographies (PR) and magnetic resonance imaging (MRI) (111-113). As a matter of fact, all these imaging techniques can help the current study in the identification of the most typical lesions (including amputations) and their most frequent localization on the bones, although a study aimed at directly observing and identifying these specific signs on bones is still lacking.

Aim of the current part of the study conducted is therefore to identify and recognize, from an anthropological point of view, macroscopic signs on the skeleton, particularly on dry bones, caused by a diabetic condition, or at least its complications. The usefulness of this research would be evident both in the forensic field, in order to enrich the biological profile of an individual with new information about the state of health and in providing new tools for facilitating the identification of unknown people, and in the archeological one, e.g. in demographic studies, providing a more accurate interpretation of diseases, lifestyle and socio-economic status of specific populations of the past.

Materials and Methods

20 skeletons of adult individuals, 19 of which suffered from this disease during their life (and 1 control case), were analyzed. Such individuals are, once again, part of the Milano Cemetery Skeletal Collection and were selected according to their known cause of death. Table 5.1 describes the demographic characteristics of the subjects in analysis.

Case N.	**Sex**	**Age**	**Cause (and contributing causes) of death**
1	M	67	Decompensated liver cirrhosis; Liver cancer, Type I Diabetes Mellitus; Terminal hepatic coma
2	F	82	Hypertensive-arrhythmic cardiopathy; Bronchopneumonia; Acute heart failure; Diabetes Mellitus
3	F	86	Blood hypertension; C.O.P.D.; Diabetes Mellitus; C.C. Arrest
4	F	80	Sclero-hypertensive cardiopathy; Chronic heart failure; C.C. Decompensation; Diabetes Mellitus
5	M	68	Decompensated diabetes mellitus + Gangrene of the lower limbs; Hypovolemic shock; C.C. Arrest
6	F	76	Right hemispheric brain stroke; Hypertension; Heart failure + Diabetes
7	M	54	Lung cancer; Respiratory failure; C.C. Arrest. + Diabetes Mellitus
8	M	70	Ischemic cardiopathy; Chronic C.O.P.D.; Cardiac decompensation; Pulmonary edema; Cardio-Respiratory failure and arrest; Type II Diabetes Mellitus
9	F	48	Renal failure In diabetic therapy; Acute pulmonary edema; C.C. Arrest
10	M	70	Diabetes; Diabetic gangrene of the right foot; C.C. Arrest
11	F	82	Chronic ischemic cardiopathy; Previous acute myocardial infarction; Syncope; Permanent Pace Maker; C.R. Arrest.; Diabetes Mellitus
12	M	85	Anuria in prostatic cancer; C.C. decompensation; C.R. Arrest; Diabetes Mellitus
13	M	77	Right hemispheric brain stroke; Seizures in bilateral hemispheric brain stroke; Acute pulmonary edema; Circulatory arrest; Diabetes Mellitus; Atrial fibrillation
14	M	76	Epatocellular carcinoma with bone metastases; Metabolic decompensation in Diabetes Mellitus; Diabetic nephropathy; Terminal collassive syndrome
15	F	68	Bronchopneumonia; Sepsis; C.C. arrest; Diabetes Mellitus
16	F	68	Diabetes; Hypertension; Myocardial infarction
17	F	79	Diabetic coma; Left bronchopneumonia; Brain stroke
18	F	66	Type II Diabetes Mellitus; Severe generalized arthropathy; C.C. arrest; Left transfemoral amputation
19	M	52	Bilateral pulmonary interstitiopathy; Severe respiratory failure; Terminal C.C. collapse + Diabetes Mellitus
Control	M	81	Brain stroke; Intestinal carcinoma; Circulatory decompensation; Cardiac arrest

Table 5.1. Demographic data concerning the 20 skeletons of the sample.

The procedure for the investigation performed was once again the same previously described: the selected subjects were cleaned with cold water and brushes, and placed in anatomical position, in order to assess the bone inventory and the biological profile of each subject. After a deep and accurate bibliographical review, the specific pathological analysis was carried out. Such specific investigation was performed macroscopically, identifying the most frequent pathologic sings linked to diabetes and their main areas of involvement, according to the directions given by clinical and radiological literature. The bone changes considered as possible indicators of diabetes mellitus are:

- lytic lesions,
- erosions,
- remodeling,
- periostitis,
- deformation (without bone loss),
- marginal lipping,
- porosity
- eburnation (osteoarthritis).

Although some of the lesions can be best described by a multistage sequence of changes, for the purpose of the present analyses they were all dichotomized to absent and present.

The data obtained were inserted into excel spreadsheets, meticulously describing the modifications observed for each bone, in order to differentiate between the taphonomic lesions and the non taphonomic ones. The non taphonomic lesions, as expected to be linked to diabetes in accordance to the information collected, were inserted into new tables, sorted, for each skeleton, according to the type of injury (lytic lesion, erosion, deformation, periostitis, remodeling, osteoarthritis) and to the district involved (hand/upper limb - foot/lower limb - spine). At the same time, an accurate photographic documentation of the whole skeletons and of the single bone elements (and lesions) was done.

At the end of this analysis, an assessment about the presence or absence of the characters mentioned above was made, considering their frequency according to the district and to the type of the affected bone. Furthermore, a differential diagnosis was undertaken through a comparison between the presence and the feature of lesions in the skeletons and similar cases from the paleopathological literature.

Results

The 19 skeletons selected for the study are of both sexes, with ages and death ranging between 48 and 86 years. All the skeletons were mostly well preserved, although single bones or entire skeletal districts could be missing and/or ruined by taphonomic factors.
Below, each case will be analyzed in detail.

Case 1

Male, 67 years old, suffering from Type I Diabetes Mellitus and Liver Cancer. The anthropological analysis revealed several bone lytic lesions, probably due to bone metastases, on skull, femurs and pelvis (Fig. 5.1). This skeleton, that was almost completely preserved, especially for what regards the lower limbs, did not display any of the typical signs that could be related to a diabetic condition.

Fig 5.1. The bones of the feet of subject 1, without any significant lesion.

Case 2

Female, 82 years old, suffering from Diabetes Mellitus. According to the anthropological analysis it was possible to observe, on the right foot, erosion and remodeling of the 1° right metatarsal and the 1° right proximal phalanx, with total resorption of the head and subtotal resorption of the proximal end, respectively, consequent to lytic processes (Fig. 5.2).

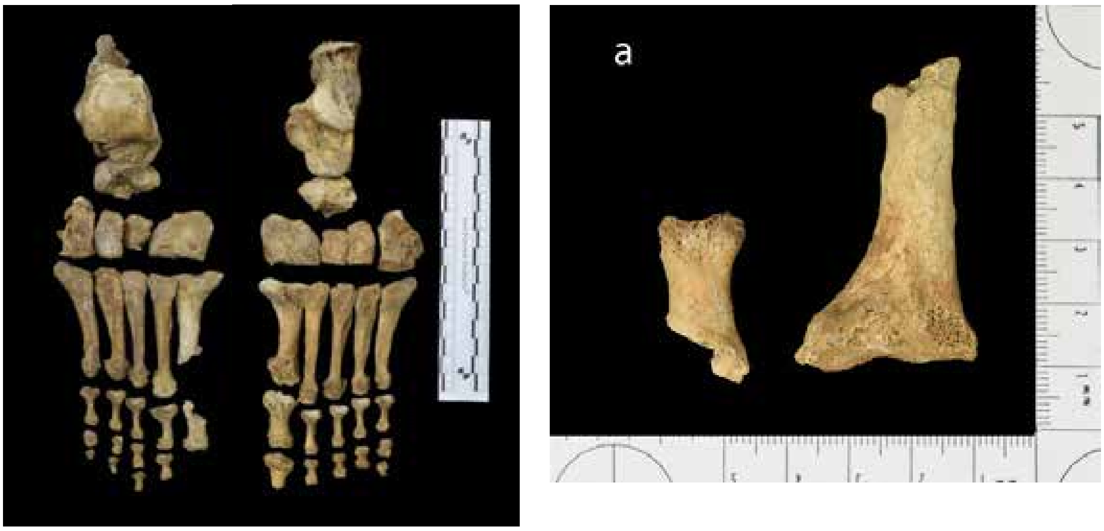


Fig 5.2. On the left, the bones of the feet of subject 2. (a) Totally resorbed head of the 1° right metatarsal bone with erosion and lytic activity, and partial resorption of the base of the 1° right proximal phalanx, with erosion and lytic activity.

On the other foot, the 1° left metatarsal bone and the 1° left proximal phalanx both display erosive cavities and lytic processes with remodeling and partial destruction of the head and of the proximal sub-chondral plate, respectively. Additionally, also the 1° right metacarpal bone exhibits lytic activity, eburneation, and marginal lipping of the proximal articular surface. Spinal osteoarthritis with porosity and osteophytes of the cervical vertebrae was also found.

Case 3

Female, 86 years old. The anthropological analysis revealed evidence of erosion and multiple deep circular lytic cavities on the heads of the 1° right and left metatarsal bones. These lesions are located along the margins of the distal articular surfaces, approximately 2mm in diameter each, and they are characterized by well-defined and rounded margins (Fig. 5.3). Osteoarthrosis of the spine, right and left humerus, right tibia and fibula and right patella was also present.

Fig.5.3: on the left, the bones of the feet of subject 3. On the right, detail of the rounded lesions on the I metatarsal.

Case 4

Female, 80 years old. The anthropological analysis revealed mild osteoarthritis of both feet, with osteophytosis of the distal phalanxes and the fusion between the 5° distal and the 5° intermediate phalanxes (Fig. 5.4). This skeleton, that was almost totally preserved, especially in the lower limbs, did not display the majority of the signs commonly related to a diabetic condition.

Fig. 5.4: the bones of the feet of subject 4.

Case 5

Male, 68 years old, suffering from decompensated Diabetes Mellitus, with gangrene of the lower limbs. At the anthropological analysis both feet, tibias and fibulas were impossible to be analyzed because they were not inside the box. Thus, nothing could be said about this subject.

Case 6

Female, 76 years old. The main feature observed is a deep circular lytic lesion (3mm diameter), with rounded, well defined reshaped margins on the superior surface (middle third) of the diaphysis of the III right metatarsal bone. This lesion involves the medullary cavity of the bone, and it is probably the consequence of an osteomyelitic process (Fig. 5.5). Additionally, evidences of spinal osteoarthritis are visible, as well as a prosthesis of the neck of the right femur.

Fig. 5.5: on the left, the bones of the feet of subject 4; above, the osteolytic lesion on the III right metatarsal.

Case 7

Male, 54 years old, suffering from Diabetes Mellitus and Lung Cancer. According to the anthropological analysis, some circular erosive cavities near the medial margin of the distal articular surface of the 1° right metatarsal bone were found, as well as longitudinal erosion, marginal lipping and osteophytes on the distal end of the 1° left distal phalanx (Fig. 5.6). There are also evidences of spinal osteoarthritis. No other signs possibly related to the diabetic condition were present.

Fig. 5.6: the bones of the feet of subject 7.

Case 8

Male, 70 years old, suffering from Type II Diabetes Mellitus. The anthropological analysis yelded pronounced lipping, osteophytosis, eburneation, porosity, erosions and lytic activity on the heads of the 1° right and left metatarsal bones. Similar lesions were also found on the proximal articular surfaces of the right and left 1° proximal phalanges (Fig. 5.7).

There is evidence of spinal, feet and knee osteoarthritis, especially pronounced on the articular surfaces between right femur and tibia, with eburneation, porosity and marginal lipping.

Fig. 5.7: on the left, the bones of the feet of subject 8; on the right, a detail of the proliferative lesions on the first metatarsal and phalanges.

Case 9

Female, 48 years old, suffering from renal failure in diabetic therapy. The anthropological analysis revealed only taphonomic changes and mild spinal osteoarthritis. Although the feet were present and complete, they did not show any significant alteration (Fig. 5.8).

Fig. 5.8: the bones of the feet, subject 9.

Case 10

Male, 70 years old, suffering from Diabetes Mellitus with gangrene of the right foot. The anthropological analysis revealed the presence of pronounced erosion, lytic activity, remodeling and subtotal resorption of the 5° right metatarsal head (Fig. 5.9). The proximal articular surface of the 5° right proximal phalanx showed subchondral bone erosion and destruction. The distal end of the 1° right distal phalanx showed erosion and marginal lipping. The left foot, tibia and fibula are not present because of a transfemoral amputation at the middle third. The amputated extremity shows bony hooklike projections on the posterior face (probably due to the insertions of the adductor muscle), signs of remodeling and a patent diaphyseal hole.

Fig. 5.9: the bones of the right foot of subject 10; on the right, detail of the resorption of the head of the V metatarsal bone.

Case 11

Female, 82 years old. The anthropological analysis revealed non-taphonomic erosions with well-defined margins on the distal end of the 1° and 5° distal phalanxes of the right foot (Fig. 5.10). The left foot, tibia and fibula are not present because of a transfemoral amputation at the middle third. Also in this case, the femoral extremity amputee showed signs of remodeling and bony hooklike projections on the posterior face, probably due to the insertions of the adductor muscles. No significant alterations on the right leg and foot were found. There were also signs of spinal osteoarthritis with marked porosity, destruction and osteophytes on the superior and inferior body surfaces of C5 and C7.

Fig 5.10: the bones of the right foot of subject 11.

Case 12

Male, 85 years old, suffering from Diabetes Mellitus and Prostatic Cancer. The anthropological analysis revealed erosions and periostitis of the 1° right metatarsal head and a longitudinal erosion with well-defined margins on the distal end of the 1° left distal phalanx. No other significant alterations were found (Fig. 5.11).

Fig 5.11: the bones of the feet of subject 12.

Case 13

Male, 77 years old. The anthropological analysis revealed remodeling on the medial surface of the right foot, in particular on the medial side of the 1° metatarsal bone, probably as a consequence of an ante-mortem trauma (Fig. 5.12). Eburneation, lytic cavities with rounded margins and marginal lipping of the proximal articular surface of the 1° right metacarpal bone were found. There were also evidences of DISH and osteoarthritis.

Fig 5.12: the bones of the feet of subject 13.

Case 14

Male, 76 years old, suffering from metabolic decompensation in Diabetes Mellitus, Diabetic Nephropathy and Epatocellular carcinoma with bone metastases. The anthropological analysis showed porosity and eburneation on the head of the right 1° metatarsal bone. On the left 5° metatarsal bone a subtotal resorption of the head was observed, with remodeling, erosions and lytic activity (Fig. 5.13). The proximal epiphysis, as well as the shaft, presented reshaping and periostitis, that was also found on the right and left femurs and on the left tibia and fibula, with porous and eroded surface. Lastly, spinal osteoarthritis was found.

Fig 5.13: on the left, the bones of the feet of subject 14. On the right, a detail of the V left metatarsal bone.

Case 15

Female, 68 years old, suffering from Diabetes Mellitus and sepsis. The anthropological analysis showed, on the right foot, the presence of remodeling of the distal end of the 2° proximal phalanx, probably due to an ante-mortem trauma, the deformation of the body of the 5° metatarsal bone, with greater medial convexity, and sings of porosity, erosive cavities and eburneation on the distal articular surface of 1° metatarsal bone (Fig. 5.14).

Fig 5.14: the bones of the feet of subject 14, on the left; on the right, a detail of the II, III and IV proximal left phalanges, displaying erosion and lytic activity.

The left foot, additionally, displays thinning, remodeling, and subtotal resorption, with erosion and lytic activity, of the distal ends of the 2°, 3°, 4° proximal phalanxes, and of the head of the 5° metatarsal bone (Fig. 5.14). Also the 1° metatarsal bone exhibits signs of porosity, marginal lytic cavities with rounded margins, marginal lipping and eburneation of the distal articular surface.

Signs of periostitis on the medial side of the distal third of the left fibula were found. Additionally, a deep oval lytic lesion (8 mm width, 4,5 mm height) with well-defined rounded margins on the palmar surface of the distal epiphyses of the right radius was observed. This lesion is mighty deep, with the involvement of the medullary cavity and it is supposed to be due to an osteomyelitic process. Spinal and knee osteoarthritis were found, especially marked on the superior and inferior faces of the body of C4 and C5 and on both femurs, with lipping, osteophytes, erosions, eburneation, porosity and lytic processes.

Case 16

Female, 68 years old. The anthropological analysis revealed mild osteoarthritis of both feet (fusion of the 5° intermediate phalanx with the 5° distal one, Fig. 5.15) and of the spine and femurs. There were also porosity, lytic cavities, deformation and eburneation on the proximal articular surface of the 1° left metacarpal bone. The left fibula showed periostitis and marginal lipping of the distal epiphysis. The head of the right humerus displayed osteoproduction, marginal lipping and eburneation, probably due to an ante-mortem trauma.

Fig 5.15: the bones of the feet of subject 16.

Case 17

Female, 79 years old, died due to a diabetic coma condition. The anthropological analysis did not show any sign of the diabetic conditions (Fig. 5.16). The only pathologic signs observable from the skeletal remains are porosity, lytic activity and eburneation of the proximal articular surface of the I left metacarpal bone.

Fig 5.16: the bones of the feet of subject 17.

Case 18

Female, 66 years old, suffering from Type II Diabetes Mellitus and severe generalized arthropathy. Both feet are absent, and a left transfemoral amputation at the middle third is evident, with remodeling and bony hooklike projections on the posterior side of the amputated extremity (Fig. 5.17). Spinal osteoarthritis was also present.

Fig 5.17: the right femur of subject 18, displaying a transfemoral amputation.

Case 19

Male, 52 years old. The anthropological analysis showed marked periostitis on both lower limbs. The periosteal reaction is especially evident on the anterior surface of both femurs, with a porous, striated and thickened surface, resulting in a slight flaring of the proximal third (Fig. 5.18). It is also particularly marked on the medial sides of both fibulae and on the lateral sides of both tibiae, at the distal third. The surface is characterized by an extensive periosteal reactive bone formation with elevated spicules and irregular indented bony proliferations. A mild periostitis was evident also on the calcaneus and the superior surfaces of the metatarsals on both feet. Spinal osteoarthritis was also found.

Fig 5.18: the bones of the feet of subject 19.

Case 20 (control)

Male, 81 years old, died of brain stroke. Diabetes is not mentioned among the related pathological conditions in the ISTAT certificate of death. According to the anthropological analysis the skeleton did not seem to suffer from particular diseases. It showed mild spinal osteroarthritis, a slightly raised bone apposition on the distal medial surface of the right fibula and the fusion of the 5° intermediate phalanx with the 5° distal phalanx bilaterally on both feet (Fig. 5.19). Thus, typical diabetic signs were not evident in any of the districts investigated.

Fig 5.19: the bones of the feet of subject 19, the control case.

The lesions that were most frequently observed are osteoarthritis, found in 90% of cases, lytic lesions and erosions, found respectively in 50% and 40% of cases. Although with a lower frequency, also remodeling (30%), periostitis (25%) and deformations (5%) were found (Tab 5.2). The sample includes an equal number of male and female, but there is not a significant prevalence between them for any of the parameters considered. As regards the localization and distribution of such lesions, feet are involved in 55% of cases, and in 45% the tibia, fibula and femurs. Other areas that display the characteristics considered are the spinal column (85%), hands (20%), and arms (5%), although to a lesser extent.

Type of lesion	Cases involved	%
Lytic lesions	10	50
Erosions	8	40
Deformations	1	5
Periostitis	5	25
Osteoarthritis	18	90
Remodeling	6	30

Tab. 5.2. List of all the lesions observed and the number of cases in which were found (also in percentage of the total).

Better considering each district, the lower limbs are the most interested. The lesions on the foot in most of the cases involve the I metatarsal, often bilaterally, in the region of the head of the bone and of the metatarso-phalangeal joint, whilst the III and V metatarsals are affected in a minority of cases, as well as the proximal phalanges. On these bones the most frequent injuries are lytic lesions, erosions followed by remodeling and a minority of osteoarthritis and periostitis. Distal phalanges are mainly affected by osteoarthritis and erosive activity (Table 5.3); in a similar way, also proximal phalanges are often affected.

Anatomical district	Lytic lesions	Erosions	Deformation	Periostitis	Osteoarthritis	Remodeling
Foot	19	24	1	4	13	11
Leg				10	5	3
Hand	4				4	
Arm	1					
Column					16	

Tab. 5.3. Type of lesion according to the anatomical region concerned.

Discussion

Diabetes Mellitus is known for being a chronic metabolic disease characterized by hyperglycemia, appearing as a global health problem. If not adequately controlled this disease causes several systemic complications, such as the involvement of the skeletal system, much less known and investigated than the other complications.

The study showed the presence of several bone changes that may refer to the consequences of diabetes and their frequency, as shown in Table 5.2. The data exposed in the results confirm the fact that the involvement of bones as consequence of diabetes in the majority of cases affects the lower limbs as primary site, and subsequently the rest of the body, thus confirming the information known from literature (101). In fact, because of diabetic peripheral neuropathy (which reduces vascularization and sensitivity), hardly healable skin lesions frequently appear in sites subjected to a high pressure (and therefore most affected to trauma), as the lower limbs. These wounds, especially the deeper ones, can get infected, until affecting the bone and developing important inflammatory processes which can lead to periostitis, osteomyelitis and gangrene (80, 91-94). The results additionally show that the effects caused by these pathologic processes were frequently found on the samples examined.

Among the cases considered, some appear particularly significant in order to better understand the pathogenesis of diabetes. In fact, almost all of the suspicious lesions were found in 4 cases, while in other 4 cases, none of them was found. An example is represented by Case 2, which shows lytic processes on the I metatarsal and proximal phalanx bilaterally. These lesions could be due to the presence of inflammatory or infectious phenomena underlying skin ulcers, which could have led to osteomyelitis. Moreover, these lesions, as seen also in other cases, most frequently affect the I and V metatarsal head and the distal phalanx of the great toe, since they are the points of the foot subjected to the greatest pressure and are most frequently affected by trauma (80, 91, 114-117).

Another example: Case 10 shows lytic activity of the head of the right V metatarsal. Similarly to Case 2, also in this case there is probably an osteomyelitic process underlying a skin ulcer. Particularly significant results the reporting, on the ISTAT form, of a gangrene condition to the right foot, which goes to confirm our findings. To further support this hypothesis, the left foot, tibia and fibula are absent, as a consequence to a left transfemoral amputation, probably due to major infections and gangrene of the left leg. In Case 14 there are signs of periostitis on the left distal fibula and on the left V metatarsal, which also shows lytic activity of the head as in previous cases. Lastly, Case 15 is one of the most interesting analyzed, since it displays the involvement of both feet. In the left foot there is deformation and remodeling of the II proximal phalanx, probably due to an ante-mortem fracture. Indeed, one of the features of the bone involvement in diabetes is the increased risk of fracture due to bone loss and decreased bone strength (77, 79, 82). The V right metatarsal shows a deformed shape without bone loss. In the left foot there is a lytic activity and remodeling of the head not only of the V metatarsal, but also of the I-III proximal phalanxes. The head of the I metatarsal has multiple lytic cavities and there are signs of periostitis on the distal end of the left fibula. These lesions could suggest a condition of deformation, ulceration and infection of the lower limbs, mainly ankles and feet, highly compatible with a diabetic complications (101). Furthermore, this example also permits to highlight how comorbidity can make the specific identification of the pathology a hard work to perform.

According to the information available, more than 50% of all lower-extremity amputations are performed in diabetic patients, and the incidence of amputation among diabetic patients is 10 times higher that of the non-diabetic population (118). The factors that determine the need of an amputation are a more or less extensive soft-tissue impairment, with ulcers, infections, decreased blood supply and gangrene. Among the investigated cases, three show transfemoral amputations at the middle third. Case 10 displays a left femoral amputation with gangrene of the right foot, as described in the ISTAT report. A similar amputation can be observed in Case 11, although on the right foot only small erosions of the distal phalanges have been noticed. Finally, Case 18 also displays a left transfemoral amputation at the middle third without any of the characteristic signs on the right lower limb. In many cases, as Case 5, where one or more parts of the lower limbs are absent, it is difficult to determine the presence of amputation, as one or more of the missing parts may have gone lost as the consequence of poor storage and recovery, but also because of single or multiple amputations. For this reason it is not possible to affirm with certainty if these skeleton present or not significant features related to diabetes.

Another important aspect that emerged from the anthropological analysis is the presence of osteoarthritis in almost all of the investigated cases. The presence of this pathology is normal in a sample of individuals all over 48-50 years of age, but it is known from literature that diabetes may cause, in addition to increased risk of osteoporosis and fragility fractures, an important arthropathy of the weight-bearing joints such as those of the foot, the knee, the hand and the spine (101-110). These conditions appear on bones as marked osteoarthritis and bone and joint destruction, especially in people with a more severe foot involvement. In the sample of the study there are 4 suspect cases with a similar aspect of the I carpo-metacarpal joint (Cases 2, 13, 16 and 17) and 1 case (Case 3) with a marked knee involvement. These findings may be thus related to the presence of an advanced stage diabetes, but considering the age of the subjects, in this case osteoarthritis is not a parameter that can be considered, especially alone, reliable in determining this pathological state.

The results of the study permit us to recognize the presence of diabetes following the detection of different specific types of lesions, or combinations of these. Especially focusing our attention on the lower limbs, the evidence of a major amputation, alone but mostly along with lesions of the other lower limb, point us towards a very likely diagnosis of advanced diabetes. Similarly, lytic processes, remodeling and erosions of metatarsals (especially the I and the V) and phalanxes, produced due to osteomyelitis. This is the set of signs that most likely make us suspect about the presence of diabetes. Small and isolated lesions can also be found, although not enough for performing an accurate diagnose.

It is important to consider that there are several diseases that affect the skeleton and a differential diagnosis may not always be possible, though ongoing research in orthopedic pathology, skeletal radiology, clinical experience and, as in the present study, physical anthropology are giving their contribution to clarify their skeletal manifestations (6). In this sense a difficult aspect of the forensic anthropological practice is the fact that many diseases leave similar signs on the skeleton. It is therefore necessary to identify such signs in detail in order to perform an accurate differential diagnosis between diabetes and other pathologies. For example, osteological changes of Rheumatoid Arthritis usually involve multiple joints symmetrically (6, 119-123), Tuberculosis affects with higher frequency the lower spine or the large joints (6, 119, 124-128) and Treponematosis causes typical cranial lesions (6, 121, 129-130), although each of this pathologies can affect, less frequently, the postcranial skeleton. The most characteristic changes in Gout are shown in feet, but their appearance is highly distinctive and easily ascribable to this pathology (6, 131-132). Sarcoidosis affects the feet, but also other districts rare in diabetes and the differential diagnosis may be impossible on dry bone alone (6). The most difficult situation is represented by osteomyelitis and periostitis, because they can be recollected to an infectious process involving bones or joints, and the causes of this situation can start from the infection of adjacent soft tissues, such as diabetic ulcers, or due to a simple peripheral arterial disease, or infections on surgical or traumatic wounds, such as open fractures, or even remote septic foci that cause the hematogenous spread in the body (6).

In conclusion this study describes the features that make us suspect the presence of diabetic disease. Getting additional information, it would be possible to create a method that allows to make a more accurate diagnosis of this pathology. However, the macroscopic study of skeletal lesions of such diseases leads to a greater understanding of it and allows to collect data useful also for the modern medicine. Clinical and surgical practice of the traditional medicine have the chance to better understand the bone lesions of certain diseases and also an important part of forensic medicine, forensic anthropology, would develop through this. The information collected in this study could enrich our knowledge about the biological profile of a particular individual, providing additional information useful for personal identification. From these circumstances it emerges the importance of the current study, which investigates for the first time the effects of diabetes from an anthropological point of view. This widespread disease in fact, in an advanced stage of evolution, with disabling bone lesions, is definitely a very well distinctive trait of the individual that cannot be lost.

A scientific article regarding this topic is being prepared.

Chapter 6

CARDIOVASCULAR DISEASES (CVD)

Cardiovascular diseases (CVD) are the first cause of death worldwide, responsible for an estimated 17.5 million deaths per year (133-134). Atherosclerosis is described as a progressive chronic inflammatory and fibroproliferative disease, which creates plaques in the walls of any large and medium-sized artery (133). The atherogenic process counts five successive phases (135-138): first, the accumulation and oxidation of low-density lipoprotein (LDL) particles in a hemodynamic site of preference in the arterial wall, called a "fatty streak", produces the initiation of the lesion; second, the consequent inflammation calls monocytes in the intima, which proliferate and differentiate into macrophages; third, the macrophages take the oxydated LDL, forming a "foam cell" and undertake apoptosis creating a necrosis; fourth, the smooth muscle cells form a fibrous plaque accumulating around the necrotic core; finally, after a varying period of time, this necrotic core calcifies in the intima and advanced lesions can appear (137-140).

According to Towler (141), different types of vascular calcification can be distinguished: atherosclerotic intimal calcification, aortic valve calcification and medial artery calcification. Arteriosclerosis can impair any artery (most commonly the femoral, tibial and uterine arteries), increasing the arterial wall stiffness and reducing its elastance. It is in fact often linked to lower extremity amputations in individuals with diabetes mellitus and cardiovascular morbidity and mortality (142-143).

However, information concerning the macroscopic appearance of these calcifications is still scarce: arteriosclerosis is known for creating circumferential and contiguous non-luminal obstructive calcification, whereas atherosclerotic calcifications have a tubular shape (141-142, 144-146). The process of formation of calcifications is known to begin during the second decade of life and, in an unpredictable way, it takes decades to develop (136, 147). It is however an active and organized mechanism, finely controlled, similar to bone formation (147-150). A hypothesis states 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 (148).

Atherosclerotic calcifications have been divided into two major categories: microcalcifications (<2mm) and macrocalcifications (>2mm). 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 (140), as a physiologic defense to maintain the integrity of the arterial wall (151). Microcalcifications, on the contrary, result from plaque rupture due to the inflammation and are responsible for thrombosis (140).

Clinical and epidemiological studies based on forensic pathology analyses and CT-imaging were performed on recent population samples and demonstrated a correlation between increasing age and diffuse pattern of atherosclerotic calcification, as well as a shift over time in the ratio of male to female affliction (152-153). While atherosclerosis seems to be prevalent in males under 60, females over 55-60 years are the most affected group. The main hypothesis accounting for this observation consists of a protective effect of female hormones, especially estrogen, in the atherogenic process.

Until recently, CVD were 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 (134). However, different analyses preformed on mummies demonstrated the finding of calcified plaques (154-157). 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 (156). Other mentioned factors include the arterial degeneration over time and the genetic predisposition for CVD (156). Nevertheless, results of conducted studies brought to describe atherosclerosis to be as frequent 3000 years ago as today (154). All the studies conducted were however performed on mummies, where luminal narrowing, thickening of an arterial wall and atheromatous findings could be “easily” identified with common technologies; the problem is of different nature when dealing with skeletonized individuals (145).

The investigation of atherosclerotic calcifications as markers for CVD proves to be crucial not only in paleopathology, but also for modern forensic purposes. Indeed, in case of skeletonized or advanced decomposed cadavers, as previously already said, a correct interpretation of pathological data provides important instruments for the construction of an anthropological biological profile, which could help in the identification of unknown deceased. Nonetheless, and before stating a diagnosis, a careful and meticulous excavation/exhumation/recovery is necessary in order to collect any findings such as calcifications.

In this perspective, this study aims to investigate the presence and the significance that calcifications may provide when recovered in skeletal remains. In particular, it focuses on the macroscopic identification of any calcified material that anthropologists can potentially find when studying skeletal remains. This research is the first that attempts to study calcifications on skeletal remains and their correlation to CVD, hopefully highlighting the importance of recovering and interpreting calcified material that can act as markers of the disease.

Materials and methods

With this intent, 24 exhumed modern and known skeletons were selected among those belonging to the Milano Cemetery Skeletal Collection. Such sample includes 12 individuals whose associated documentation indicates vasculopathy or atherosclerosis/arteriosclerosis as a pathological condition, and a control sample of 12 individuals, with no mention of vasculopathy in their ISTAT certificate. Age range and sex distribution are shown in Table 6.1. The individuals were selected in order to study the relation between vascular calcifications and three specific factors: sex, age and the presence or absence of a diagnosed vascular disease. The skeletons were therefore blindly studied without any knowledge of the associated documentation. The preparation of the skeletal material is the same used also for the other pathological cases considered; the only difference consists in the accurate recovery and sifting, with a 1.5mm woven wire, of the content of the box, of the cranial vault (when the state of preservation allowed elements to be kept in it) and of clothing elements, such as tights or socks, when present, in order to separate calcified material (or suspected calcified material) from other elements. The identification of the calcified material was made possible by direct comparison with a known autopsy collection of vascular calcifications from the LABANOF (Fig.6.1) and with images from literature (144).

Fig. 6.1: morphological comparison between atherosclerotic calcifications recovered in the study (left) and autopsy atherosclerotic calcifications.(right).

Case N.	**Sample**	**Sex**	**Age**	**Cause of death**	**Related pathological conditions**	**State of preservation of the remains**
1	Vasculopathy	F	76	Cardiac arrest	Chronic cerebral vasculopathy, Cachexia	Almost complete
2	Vasculopathy	F	82	Cerebral coma	Cerebral vasculopathy	Almost complete
3	Vasculopathy	F	84	Cardiac arrest	Atherosclerotic vasculopathy, Renal insufficiency	Almost complete
4	Vasculopathy	F	85	Cardiac arrest	Cerebral vasculopathy, Senile dementia	Mostly complete
5	Vasculopathy	F	87	Cardiac arrest	Cerebral vasculopathy, Atrial fibrillation, Coronaropathy	Almost complete
6	Vasculopathy	F	102	Cardiac arrest	Ischemic cardiopathy, Cerebral vasculpathy	Mostly complete
7	Control	F	39	Non-specified		Mostly complete
8	Control	F	47	Cardiac arrest	Kidney failure in diabetic therapy, Pulmonary Edema	Fragmented
9	Control	F	56	Cardiac arrest	Acute necrotic pancreatitis, Septic state, Metabolic coma	Mostly complete
10	Control	F	83	Cardiac arrest	Intestinal occlusion, Acute pancreatitis	Fragmented
11	Control	F	86	Cardiac arrest	Heart failure	Badly preserved
12	Control	F	95	Cardiac arrest	Arterial hypertension, Parkinson's disease	Fragmented
13	Vasculopathy	M	69	Cardiac arrest	Acute cerebral vasculopathy	Badly preserved
14	Vasculopathy	M	80	Cardiac arrest	Chronic encephalovasculopathy, Cerebral stroke	Mostly complete
15	Vasculopathy	M	81	Heart failure	Atherosclerotic vasculopathy	Fragmented
16	Vasculopathy	M	83	Malignant hyperthermia	Cerebral vasculopathy, Cachexia	Mostly complete
17	Vasculopathy	M	85	Cardiac Arrest	Vasculosclerosis, Senile deterioration	Almost Complete
18	Vasculopathy	M	87	Cardiac and respiratory arrest	Arterial hypertension, Arteriosclerosis, Cerebral stroke	Almost Complete
19	Control	M	31	Hepatic failure	HIV infection, Pneumonia	Almost Complete
20	Control	M	42	Respiratory failure	Chronic bronchopneumonia, Hepatic cirrhosis	Almost Complete
21	Control	M	56	Cardiac arrest	Bladder cancer, Stroke, Cancer deterioration	Mostly complete
22	Control	M	81	Cardiac arrest	Heart failure	Fragmented

23	Control	M	81	Cardiac arrest	Stroke, K-intestinal, Heart failure	Fragmented
24	Control	M	85	Cardiac arrest	Cerebral hemorrhage	Badly preserved

Table 6.1: Details of the study sample.

After cleaning the bones with cold water and soft brushes, and the reconstruction of the biological profile, a macroscopic observation of the recovered calcified material was performed. Such analysis consists of a description based on specific parameters: number, location (if found in a sock, the limb was specified; “unknown” referred to a non-specific location, such as the soil residues from the bottom of the box), shape, color, range size, maximum length and maximum thickness. Such data were then interpreted for identifying the parameters that permit to distinguish arteriosclerotic form atherosclerotic calcifications, and provide therefore guidelines for their recognition and their usefulness in paleopathologic and forensic purposes.

Results

According to the type of material in analysis and the observations performed, each single case will be displayed in Tables 6.2.

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

Table 6.2: distribution of the recovered calcifications.

Regardless of their typology, 735 calcifications were recovered and studied in 20 out of 24 skeletons (83% of the study sample), in both pathologic and control samples. Results concerning the macroscopic observations conducted on all the findings collected in the skeletons are reported in Table 6.3. As it is observable in the table, calcifications differ in morphology, color and location. Based on these variabilities and by comparison with literature (141, 144) and the known autopsy collection, it was possible to achieve a categorization of four types of calcifications as a base for identification. In this table, ossified cartilage description serves as a distinctive material, to differentiate and identify vascular calcifications. Also, the non-identified calcifications are of unknown origin and morphologically discernable from the atherosclerotic and arteriosclerotic types.

Criteria	Atherosclerosis		Arteriosclerosis	Ossified cartilage	Non-identified
Location	post-cranium	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					

Table 6.3: categorization of the different types of calcifications recovered and compared to ossified cartilage.

As a matter of fact, 293 calcifications were identified as atherosclerotic, 205 as arteriosclerotic, 5 are suspected atherosclerotic calcifications and 232 are non-identified (Table 6.2). Atherosclerotic calcifications are present in 83% of the female sample (10 out of 12 skeletons) and in 66% of the male sample (8 out of 12). Despite the fact that both males and females are almost equally affected by the disease, out 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 acquired only in the female sample (Figures 6.2 and 6.3). No calcifications were found in the 4 individuals under 56 years of age. Considering the presence of calcifications in relation with age groups of the other subjects, 18 out of 20 individuals presented atherosclerotic calcifications (8 males and all 10 females over 56 years). Furthermore, intimal artery calcifications were collected in both vasculopathy (173 calcifications out of 293, or 59%) and control samples (120 calcifications out of 293, or 41%).

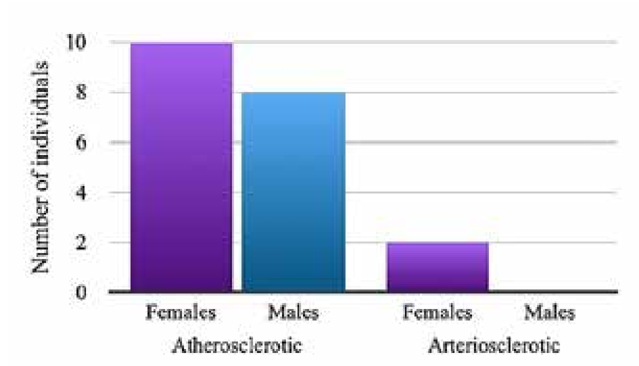


Fig. 6.2: distribution of the skeletal remains in which vascular calcifications were found according to sex.

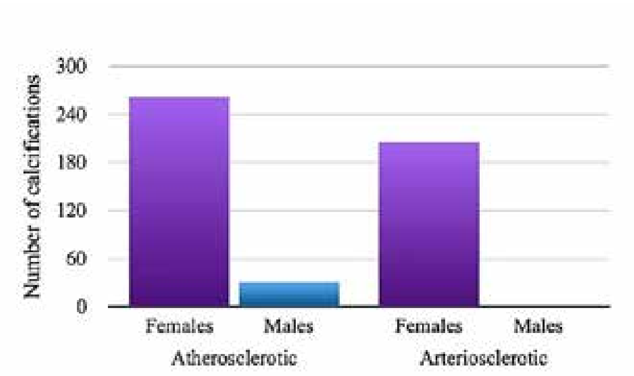


Fig. 6.3: distribution of the recovered vascular calcifications according to the sex of the individuals.

Discussion

The main purpose of this part of the research project was to evaluate the possibility of finding calcified material associated with skeletal remains. Through the study of the 24 exhumed skeletal remains, whose related pathological conditions and biological information are known, a total number of 735 calcified materials were collected. It is important to highlight that, according to the recovery procedures performed in cemeteries, the amount of calcifications present in the recovered material cannot be considered an actual representation of the amount of calcifications in the individual, but only a "minimum" representation.

All calcifications collected have been thoroughly studied through a macroscopic observation and then categorized in different types of calcifications. Atherosclerotic calcifications are of two different types: post-cranial and cranial. Post-cranial 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 (which in autopsy cases is uniformly light yellow), which can be related to taphonomic processes. Cranial atherosclerotic calcifications, however, 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 arterial calcifications, are long hollow cylinders formed by an uniformly brown and extremely fragile layer of irregular margins. Their long tubular shape is the most identifiable criterion, described in literature as "circumferential and contiguous" without obstruction of the lumen (141-142). Even though medial arterial calcifications can affect any artery, they are more common in the femoral, tibial and uterine arteries (143), which is why the cranial calcifications previously described can be considered atherosclerotic, especially since cranial atherosclerotic calcifications are a common finding (152). Only 5 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 they were not definitely identified as atherosclerotic or arteriosclerotic, it was possible to affirm that they were due to another kind of ossification/calcification mechanism. These non-vascular 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 materials of unknown origin recovered in skeletal remains that could provide important evidence of other pathological conditions (such as, 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 2.5mm to 23mm. Since all atherosclerotic calcifications collected have a dimension superior to 2mm, they can all be considered macrocalcifications. 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 whole calcifications.

However, since no soft tissues (and so no arteries) are preserved in our sample, the affirmation of the diagnosis of atherosclerosis on the base of sole calcifications is biased. Thus, literature showed multiple cases in which one arterial calcification was considered to be enough for the diagnosis of the disease (148, 155, 157-158). As a consequence, atherosclerotic calcifications found in skeletal remains and identified macroscopically, can act as markers for the pathological diagnosis of CVD.

Another objective of this research was to study the relation between the collection of vascular calcifications and specific factors including sex, age and diagnosed vasculopathy. As for the sex factor, a little difference was observed between the number of males and females affected by the disease (Figure 6.2), despite previous studies demonstrating that females over 60 years are more subject to atherosclerosis than males (152-153). Nevertheless, females have about 8.5 times more atherosclerotic calcifications than males (Figure 6.3). Analogously, arteriosclerotic calcifications were recovered only in females. Concerning age, atherosclerotic calcifications were recovered only in individuals over 56 years. Indeed, the atherogenic process can start as early as the second decade of life but it corresponds to a soft tissue stage. Yet, only materials that survive decomposition and all other taphonomic processes can be recovered, which means calcified or material. It is then harder to find evidence of the disease in skeletal remains of young individuals, since the atherosclerotic plaques, if any, would not be calcified yet. Thus, the absence of calcification does not imply the absence of the disease. Consequently, the age of the individual is related to the recovery of calcifications in skeletal remains. Nonetheless, the amount of calcifications does not present a linear correlation with age. In literature, pathological and epidemiological studies as well as paleopathological researches agree on a strong relation between age and atherosclerotic calcification process (147, 149, 152-153 155-157), but other studies also specify that aging has no direct effect on the vascular calcification mechanism (147, 151). This information corroborates our findings and explains the nonlinearity observed in the study sample, between age and number of calcifications.

The presence of a control sample proved itself to essential in the investigation of the relation between the diagnosis of vasculopathy in life and the recovery of vascular calcifications. 59% of the calcifications collected were found in the vasculopathy sample and 41% in the control sample. However and considering that the control sample also included the 4 skeletal remains under 56 years in which no calcification was found, the difference in number of findings between both samples 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 since clinical studies demonstrated a high presence of vascular calcifications in asymptomatic/randomly selected individuals (152-153).

The lack of evidence of disease in the paleopathological and forensic practice cannot be seen as proof of its non-existence. 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 identification of vascular calcifications is 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. This difference in numbers of calcifications has never been studied before and could be of significant interest in the understanding of the disease. Since 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. Indeed and as proved possible in the present study in case of inhumation and high post-mortem interval, it should be possible to find more evidence of CVD in archaeological 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.

A scientific article regarding this topic was published:

Biehler-Gomez L, Cappella A, Castoldi E, Matrille L, Cattaneo C. 2017. *Survival of atherosclerotic calcifications in skeletonized material: forensic and pathological implications.* Journal of Forensic Sciences; doi: 10.1111/1556-4029.13592

Chapter 7

HIV AND DRUG ABUSE

HIV and drug abuse have been an epidemic event, which had a particularly important development and expansion in the ‘90s of the last century. The term “HIV” refers to a specific virus, the human immunodeficiency virus, that, with the passing of time, causes the onset of the acquired immunodeficiency syndrome (AIDS). One of the most common ways in which HIV propagated was due to unprotected sex and through infected needles for drug injection. According to Nicolosi *et al.* (159) in Italy, during the 90’s IVDU (intravenous drug users) were the group most frequently affected by AIDS, accounting for 5974 of 8837 adult cases reported up to March 1991. The city of Milan had in that period the highest rate of reported AIDS cases in Italy (27,9/100000 residents from 1981 to 1990). In 2001 Quaglio *et al.* (160) conducted a study to ascertain the causes of deaths among a very large cohort of heroin injecting drug users (IDUs) who, from 1985 to 1998, attended 36 Public Health Authority Centers for Drug Users (PCDUs) in north-eastern Italy. What they found is that of 2708 deaths, overdose was found to be the major cause (37%), followed by AIDS (32.5%) and road accidents (9.4%). The percentage of deaths due to AIDS increased steadily from 2.7% in 1985 to 42.2% in 1996.

One of the most problematic consequences of HIV infection is the onset of opportunistic diseases and immune derangement, together with metabolic, cardiovascular, liver, bone and kidney complications. According to information acquired from literature, HIV is not known for leaving specific marks on the skeleton. However, studied demonstrated that the most common alteration observed in HIV-infected patients is reduced bone mineral density (BMD) (161-162). In particular, the bone disorders observed are osteopenia (bone mineral density reduction), osteoporosis (bone strength reduction with increased fracture risk), osteomalacia (softening of the bone due to defective bone mineralization) and osteonecrosis (bone death due to poor blood supply) (163).

The Milano Cemetery Skeletal Collection proves itself to be a valuable and appropriate instrument of study for this specific circumstance. Most of its subject, indeed, died in the ‘90s of the last century and, thanks to the available causes of death, it is known that it houses a good representation of individuals with such an infection, or known for being drug addicted at the moment of death. Such two aspects were put together because of the connection that there was among them in those years; however, the fact that a subject suffered from HIV does not mean that he or she was drug addicted. The anthropological analysis is in fact focused also in clarifying such aspects, when possible.

Drug abuse is commonly associated with significant detrimental psychological, nutritional, and social changes, any of which can markedly affect the general and oral health of the individual user. Dental management of the drug-addicted patients may prove frustrating because of the tendency to recurrent caries and periodontal disease associated with any drug abuse (164). For example, according to Shaner (165) caries pattern associated with chronic methamphetamine abuse initially involve the buccal surface of the posterior teeth and the interproximal of the anterior teeth progressing to complete destruction of the coronal portion of the tooth. A persistent dryness of the mouth is the reason given by addicts for the ingestion of copious amounts of refined carbohydrates. In fact dental symptoms of MA are xerostomia and clenching or grinding (bruxism). The oral signs of MA abuse are rampant caries, gingival inflammation (periodontitis) and occlusal wear. The combination of xerostomia, frequent sipping of carbonated soft drinks to relive the sensation of dry mouth, extremely high dental plaque levels and nonexistent or inadequate oral hygiene (165). Transient xerostomia is also common in heroin abusers. This condition is probably due to: 1) the action of opiates in diminishing secretions, and 2) the psychic factors related to the use of heroin. Abstinence from heroin reverses the condition. Therefore, a transient xerostomia which leads to plaque (food debris and bacteria) formation, plus large amounts of refined carbohydrates results in the development of rampant caries in the heroin addict. The excessive wear and fractured teeth noted might be associated with the fact that opiate derivatives seem to produce spastic tooth grinding (166). The heroin addict tends to neglect his oral health due to his physical and emotional dependence on the drug. The high incidence of dental disease seems to be due to local environmental factors coupled with the systemic effects of heroin, rather than the drug itself. The desire to ingest refined carbohydrates seems to be more physiological than anything else (167).

Cocaine abuse has been associated with a variety of cardiac disorders that include angina pectoris and myocardial infarction (168-170), and sudden cardiac death may occur due to acute cocaine toxicity (168, 171). Snorting of cocaine powder intranasally often results in irritation of the nasal mucosa, causing sneezing, sniffing, rhinitis, and ulceration or perforation of the nasal septum following heavy long term use (172). Cocaine intoxication may be associated with cervical abrasion of teeth and gingival laceration due to overly vigorous tooth brushing and flossing while “high”. Severe bruxism and flattened cuspal inclines may also be more common among cocaine addicts, accompanied by increased frequency of temporomandibular joint disorders. Yukna (173) also presented a series of case reports describing gingival and alveolar bone damage due to chronic gingival application of cocaine (174).

Since the information of drug addiction are not reported in the ISTAT death certificates (except few cases), and aware of the connection among HIV and drug abuse typical of the historical period to which the individuals of the Milano Cemetery Skeletal Collection belong, we selected some individuals known for suffering from HIV in life. According to all these information, the study therefore focused on finding signs in the oral region of the skeleton that can be recollected to drug assumption**.**

Materials and methods

As for the previously described cases, the method of preparation of the material in analysis follows the same procedure. The 8 subjects in analysis have been selected according to the reported cause of death, as displayed in Table 6.1.

Case N.	**Age**	**Sex**	**Cause of death**
1	31	M	HIV; Pneumonia; Hepatic insufficiency
2	28	M	HIV; Pneumonia, hepatic cirrhosis; Cardiac arrest
3	49	M	AIDS; Bronchial Pneumonia; cardiac and respiratory arrest.
4	41	F	HIV (10 years); AIDS; Wosting syndrome (4 months); Pneumothorax (right)
5	35	M	HIV; Hepatic insufficiency; encephalitis (5 years); hepatic, cerebral and respiratory complications (1 year); AIDS, Cardiac arrest.
6	41	F	Cerebral lymphoma, HIV complications (6 months); Pseudomonas aeruginosa infection, mycobacteriosis (cytomegalovirus); neoplastic cachexia; infective complications
7	29	M	HIV (10 years); cerebral toxoplasmosis (1 year, 1 month); encephalitis (2 months); chorioretinitis
8	55	M	HIV (8 years); Kaposi's sarcoma, cmv infection; atypical mycobacteriosis (1 year); pneumonia (9 days)

Table 6.1: list of the subjects involved in the study, with related demographic and pathologic information.

Such individuals were therefore cleaned with cold water and soft brushes (after the recovery and the removal of any taphonomic element attached to the single bone elements) and put in anatomical position. After assessing sex, age, race and stature, the specific pathologic investigation was done. As previously mentioned, an accurate bibliographic review, aimed at identifying all the modifications produced by drug addiction, was carried on before the direct investigation on the skeletons. According to the information collected, HIV does not produce any bone modification or typical lesion; only osteopenia is reported as a possible consequence. On the contrary, drug addiction is known for producing a series of complications that specifically involve the cranio-facial area. In particular, destructive caries, infections, periodontitis and toothwear are known for being the most diffuse oral complications in drug addicted. Based only on such information it is impossible to define the type of drug assumed; however, since many modifications are common among different drugs, the study aimed at first at identifying such pathologic changes. In detail, they are:

- Toothwear
- Caries
- Inflammation and abscesses
- Calculus
- Periodontitis.

Results

Below, each investigated case will be described.

Case 1

Male, 31 years of age, HIV positive, without any information about drug addiction. What was possible to observe at the anthropological analysis is the presence of macroporosity on the glabella, sovraorbital margin and occipital bone. On the frontal bone, in correspondence of the left side of the metopic suture and right above the naso-frontal and maxillary-frontal sutures, it is evident an area of remodeling, oval-shaped, without well-defined margins; the main hypothesis regarding its origin deals with an underlying infection of the frontal sinuses. Another oval lytic area can be observed on the infero-medial margin of the frontal bone, in correspondence of the medial portion of the right orbit (in correspondence of the maxillary-frontal suture) (Fig. 6.1). Also a perimortem fracture of the left nasal bone can be noticed (Fig. 6.1).

Fig 6.1: on the left, cranium of Case 1. On the right, detail of the frontal area, where the remodeling region, macroporosity, lytic area and perimortem fracture of the nasal bone can be seen.

The subject displays an almost compete loss of the dental elements. All the teeth of the upper jaw are lost antemortem, as testified by the strong remodeling of all the alveoli. On the mandible, the loss of the elements in the III quarter looks more recent than that in the IV quarter. A dental form for the subject in analysis is displayed in Figure 6.2. Periodontisis is also visible.

On the rest of the body, it was possible to observe an antemortem fracture of the spinous process of T4 and T5, and of the transverse processes of L3. Furthermore, diffuse periostitis was observed on the external margin of left ribs VI (lower margin), VII (all) and VIII (upper margin).

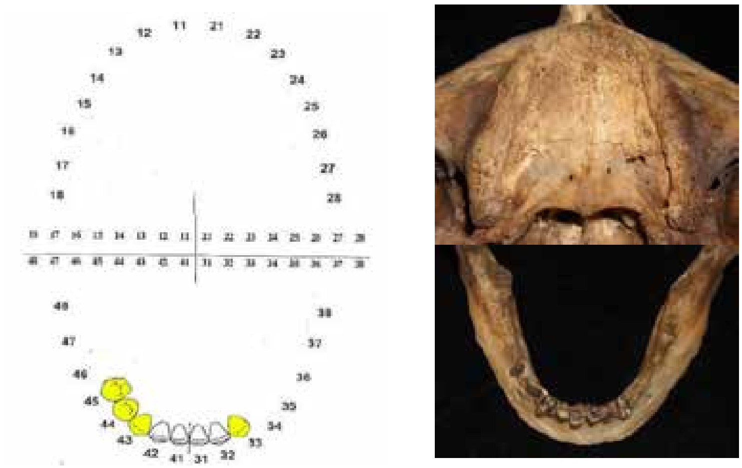


Fig 6.2: dental form of Case 1. Teeth 31,32, 41 and 42 display caries of neck and crown (lingual side); of teeth 33, 43,44 and 45 just a roof residual is left (due to destructive caries, in yellow).

Case 2

Male, 28 years of age, HIV positive, with pneumonia. The anthropological analysis displayed an antemortem fracture of the nasal spine, with deviation of the nasal septum (Fig. 6.3); another antemortem fracture can be observed on the sovraorbital margin, and at the III distal of right radius. Also in this case, macroporosity on the glabella and on the sovraorbital margin is visible.

According to the aspect of the cutting lesions observed on vertebrae, a marrow sample during autopsy can be supposed.

Fig 6.3: on the left, cranium of Case 2. On the right, detail of the nasal spine.

Also in this case, the subject displays an almost compete loss of the dental elements. The few elements still present are radicular residuals, as consequences of destructive caries (in yellow in Figure 6.4). Widespread areas of inflammation can be observed on many alveolar margins, probably due to previous abscesses, as well as diffuse periodontotis.

Fig 6.4: dental form of Case 2. Teeth 21, 32 and 33 display destructive caries (in yellow); in black is highlighted the presence of tartar, whereas the red lines point out the alveoli where signs of inflammation are visible. A detail of such signs can be observed on the figure on the right.

Case 3

Male, 49, AIDS. All the dental elements are rather well preserved, with just one case of destructive caries (23) and 2 cases of filling (17 and 27) (Fig. 6.5). However, 31, 21 and all the teeth of the lower arch display consistent toothwear; tartar and periodontitis are evident on almost all the dental elements.

Of interest is the presence, on the left side of the palate, of an area of bone resorption and bone proliferation of circular shape (diameter 1cm circa), probably due to a cyst.

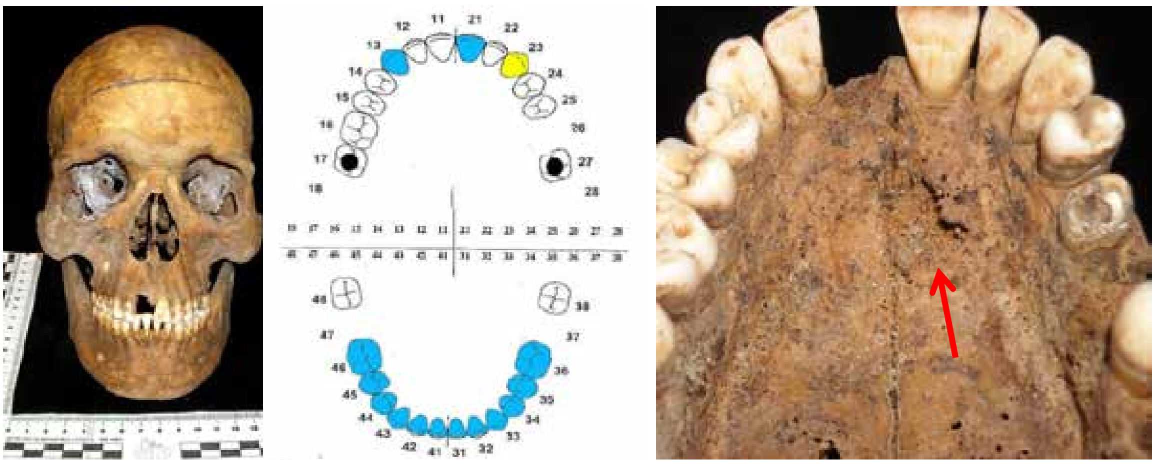


Fig. 6.5: on the left, cranium of Case 3. In the center, dental form for case 3 (yellow: caries; black: fillings; blue: toothwear). On the right, detail of the palate, and of the cyst on its left side (red arrow).

Case 4

Female, 41 years of age, affected by HIV for 10 years. Despite the young age, the subject displays complete loss of all dental elements. Although it is impossible to provide a range of time since the loss began, it is possible to assert that it is not of recent data.

The alveoli corresponding to teeth 21 and 22 display clear signs of lytic activity, probably consequent to an inflammatory process (abscess). In particular, the medial lesion is so developed that directly communicates with the piriform cavity (Fig. 6.6). Nothing relevant was noticed on the rest of the body, despite light periostitis on the right femur and on the external margin of the VII left rib.

Fig. 6.6: on the left, cranium of Case 4. On the right, detail of lytic areas (probably due to abscess).

Case 5

Male, 35 years of age, HIV positive. The upper dental arch displays a complete loss of all dental elements. On the contrary, the mandible still displays numerous teeth. Teeth 31-33 and 41-43 show consistent toothwear, whereas the posterior elements are all present as root remains, which were substituted by an orthodontic bridge (34-37 and 44-47). Periodontitis is also evident on all dental elements (Fig. 6.7).

Fig. 6.7: on the left, cranium and mandible of Case 5. On the right, dental form: in yellow the caries are highlighted, and in blue the toothwear.

Case 6

Female, 41 years of age. Only 1 tooth recovered (with the presence of tartar), and nothing relevant on the skeletal part.

Case 7

Male, 29 years of age, affected by HIV for 10 years. Teeth appear widely altered by pathologic interventions. Teeth 26 and 27 display antemortem fillings, probably as a caries treatment; nevertheless, caries are still evident on both such teeth and on almost all the other elements that were recovered. Teeth 35 and 36 are completely destroyed by the activity of such pathology. Similarly, toothwear is widely diffused, as well as periodontitis and tartar (Fig. 6.9).

Tooth 16 appears cut in a half, in the longitudinal direction of the tooth. It was supposed that such lesion was caused by postmortem modification instead of antemortem activity, due to the sharpness of the margins.

Of interest, on the cranium, is also the presence of bone loss and remodeling on the anterior left part of the nasal bone and vomer, probably antemortem. No clear explanation for such modification is however available.

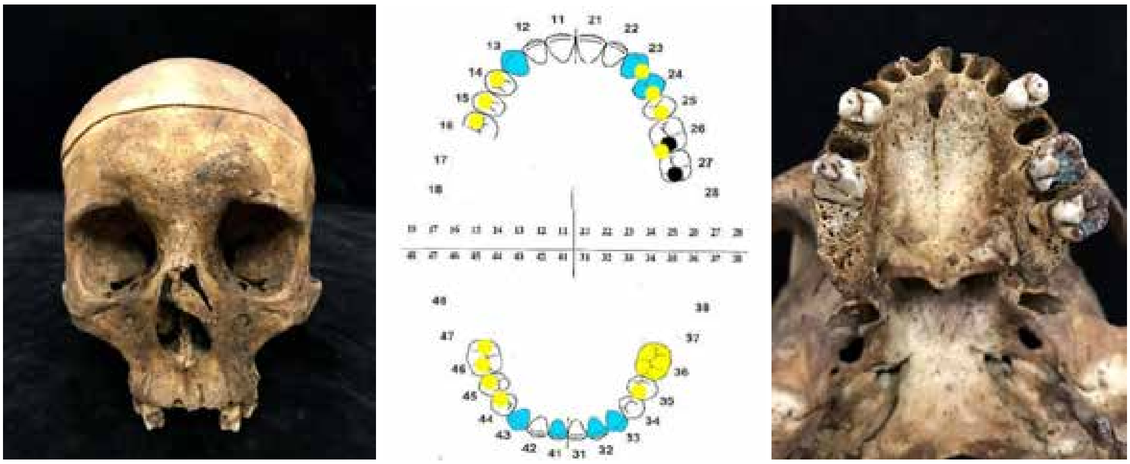


Fig. 6.9: On the left, dental form: in yellow the caries are highlighted, in blue the toothwear, and in black the fillings. On the right, details of the upper dental arch and of the cranium, anterior view.

Case 8

Male, 55 years of age, affected by HIV and pneumonia. Once again, toothwear and tartar are widespread on almost all the elements recovered. Tooth 27 displays an antemortem filling, and on almost all the anterior teeth (11-12, 21-23, 31, 33, 41-43) signs that can be recollected to hypoplasia can be noticed. Periodontitis is also visible, together with diffuse tartar. Nothing else of pathologic interest need to be highlighted from the cranium (Fig. 6.10).

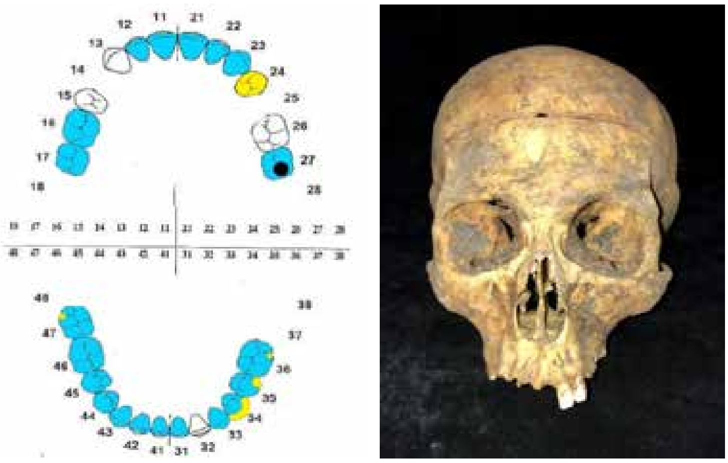


Fig. 6.10: On the left, dental form: in yellow the caries are highlighted, in blue the toothwear, and in black the fillings. On the right, the cranium, anterior view

Table 6.2 briefly summarizes all the observations collected in these first 8 cases investigated.

Case number	Caries	Abscess	Toothwear	Tartar	Hypoplasia	Periodontitis	Fillings
1	X					X	
2	X	X		X		X	
3	X		X	X		X	X
4		X					
5	X		X			X	X
6				X			
7	X		X	X		X	X
8	X		X	X	X	X	

Table 6.2: summary of the dental pathologies observed.

Caries, tartar and periodontitis seem to be the dental alteration most widespread in the sample analyzed. Fillings are a rare finding, although they were observed in 3 different subjects. Widespread is also the complete loss of dental elements, ranging from cases where just 2 or 3 teeth are missing, to subjects with all the 32 teeth lost. In no one case, however, all the teeth are recovered. No great oral modifications were observed, despite one case of suspected cyst on the left hard palate, and a severe case of lytic activity presumably due to caries and infection.

Discussion

Many are the information that the bare skeleton can provide. Among them, and as previously stated, even the chronic assumption of xenobiotics represents a mandatory data for the reconstruction of the biological profile. The knowledge of the addiction of a missing person to drugs and the possibility to recollect it to a recovered skeleton can increase the possibility of a positive identification or, at least, narrow the field of research when human remains is found. Many studies investigated the modifications caused by drug assumption on teeth and on the oral area (165, 167, 174). The availability of the Milano Cemetery Skeletal Collection provides a chance to directly observe such modifications. The sample in analysis is represented by individuals who died in the ‘90s of last century, an historical period known for the wide diffusion of drug consumption (heroin and cocaine, in particular), often connected to HIV.

Thanks to the literature, HIV is known to be almost invisible on the skeleton, as the only sign that it leaves is the weakening of the bone tissue, observed as osteopenia, osteomalacia and osteonecrosis. Considering the age of the individuals and the severity of the dental alterations observed, it is therefore plausible that such modifications were the consequence of a drug abuse. In fact, years of studies, mainly on living, demonstrated how severely such behavior can alter (among other systems) the dental health and structure. The need for sweet foods and beverages, together with a lack of oral care, often caused caries and infections, which in fact were observed in almost all of the cases considered. Such modifications are often so serious that the entire crowns of the teeth are eroded, thus leaving just part of the root (Fig. 6.11). Just in a few cases the presence of fillings was observed, which further demonstrates the paucity of care adopted.

Fig. 6.11: Example of severe modification of the teeth (Case 1)

One of the most "shocking" features is, however the complete loss of dental elements, in particular in subjects of such a young age. The most representative example is Case 1: the subject, a male individual of 31 years of age, displays a complete edentulia of the upper dental arch, and a serious involvement of the lower one (destructive caries, tartar and periodontitis). Another representative case is number 4: 41-year-old woman with complete upper and lower loss of the dental elements, and deep abscesses on the left maxillary bone, which communicated with the oral and the piriform cavities. Periodontitis and tooth loss therefore represents a common finding in such individuals. Gingivitis, the inflammation of the soft tissues (gums) that surround the tooth at the junction between the dental crown and the root, is indeed a common event in drug addiction cases. Such a situation often develops into the more severe periodontitis, which consequently leads to tooth loss. The main consequence of periodontitis is in fact the loss of alveolar bone along with the periodontal ligament, which undermines the support structure of the tooth (175). This specific manifestation was observed in almost all the cases investigated, thus demonstrating the relevance of such condition connected to supposed drug abuse. The importance of inflammatory and infective processes is demonstrated also in the diffuse presence of caries. Pindborg (176) defines dental caries as an infectious and transmissible disease in which progressive destruction of tooth structure, crown or root, is initiated by microbial activity on the tooth surface. Previous as well as present concepts of caries formation are in fact based on the chemical process of fermentation of carbohydrates (glucose, fructose, sucrose) by bacteria, which results in acid formation, thus lowering pH of the plaque, which, in turn, leads to demineralization of the hard dental tissues (177-179). Saliva has a protective effect on the health of hard and soft oral cavity tissues (180). Chronically reduced salivation is one of the most important factors that increase the risk of caries, as often is observed in drug addiction cases (181-182).

It is important to highlight that in the available ISTAT certificates the direct assumption of drugs was not reported. The subject were therefore selected due to the known HIV infection, as specified on such forms. The possibility to correlate such disease to the drug assumption is done thanks to the well-known link between them in the historical period in which the subjects in analysis lived; the authors are however aware of the bias of the analysis. Nevertheless, since HIV is known for not producing dental modifications, all the findings support the initial hypothesis that they are caused by the drug addiction. This assumption is further demonstrated by the gravity of such findings, which are not commonly found in not-addicted people of the same age in the same years. Nevertheless, just the description of teeth alteration does not provide sufficient information for assessing a positive diagnosis of drug dependence. This investigation was just an initial approach, aimed at finding possible reading-keys of the disease and habit from the skeleton. The findings first of all highlight the necessity to perform an accurate age estimation on such subjects. To an untrained eye, in fact, the hard alteration of teeth and the deep modifications of the oral area could be misleading and therefore it is important to assess the age in an accurate way. Subsequently, it would be of great help to be aware of the exact substance those individuals were addicted to. Of course the modification are caused by the consequences of the addiction (scarce production of saliva, huge intake of sweets and beverages, scarce oral hygiene), and therefore such signs are common among different cases; however, a more precise differentiation would improve the precision of the investigation. As many other aspects of the anthropological practice, the dental alteration observed are aspecific; nevertheless, the possibility to have a certain diagnosis to rely on (e.g. data collected in life or at death regarding the chronic assumption of drugs) would be useful to identify even small parameters useful for a more accurate diagnosis. More data, and more cases investigated, would be once more a valid instrument to be used.

In this context however, also other techniques, as the toxicological investigation of the remains, could be a road to follow. One of the main aims of postmortem toxicology is indeed, when a cadavers are found, to be able to assess whether he or she assumed drugs while alive. However, the bone tissue does not represent a typical sample for this kind of investigations due to the difficulties with dealing with such material. Nevertheless, since it is the most resilient part of the body and often the only proof available in postmortem investigations, many research projects aimed at testing the usefulness of bone as means of toxicological investigation, demonstrating the possibility to detect xenobiotics from bones (183-188). In particular, Cattaneo *et al.* (188) developed a research project aimed at identifying the presence of morphine and codeine in teeth of subject known to have died of heroin overdose, of subject whose history of drug abuse was unknown, and in teeth from cases of burnt, putrefied and skeletonized remains found in conditions strongly suggestive of a drug-related death. Their results were strongly promising, proving that these tissues may be a reliable source for toxicological information concerning the history of the individual also in cases of badly preserved skeletal remains. The possibility to identify the assumption of specific substances by a subject indeed furnishes important information about who he was, therefore narrowing the possibilities of match in cases of missing persons. Like pathologic recognition, the knowledge of the specific assumption of medicines or drugs can help in add important information for the identification of the subject or the recognition of the cause of death.

In tight connection to the study concerning HIV and drug abuse, a research project aimed at testing the best method for extracting and identifying drugs from the skeleton. However, the possibility of examining in depth specific research areas is often limited by the specificity and by the scientific context of each laboratory. Therefore, only the cooperation among different laboratories allows for overcoming such limits.

With this perspective, an agreement between the University of Milan (the LABANOF) and the University of Catania (the laboratory of forensic toxicology, directed by *Prof. Nunziata Barbera*) for carrying on this investigation was stipulated. The project aims at finding a protocol for the extraction of drugs from the bone tissue, sampled from subject for which the toxicological history is known (which drugs they used and their chronic assumption). The project would be structured in different phases:

1. Analyze an initial group of samples collected from autopsy cases, which are taken from subjects known for being drug addicted.
2. Analyze the same group of samples after different periods of time (6 months, 1 year or more), in order to see if the substances can still be rediscovered and, in case of positivity, in which ratio in respect to the initial investigation.
3. Apply the extraction method to samples of 20 years old (e.g. which remained in the burial for 20 years, as those that belong to the Milano Cemetery Skeletal Collection).

Forensic toxicology on bones (University of Catania)

In the month (06.02.17-03.03.17) spent in Catania, 8 cases were considered. For each case, beyond the common toxicological investigation requested on blood and organs, also rib and skull fragment were sampled and analyzed.

After the collection at autopsy, bone fragments were cleaned from the soft tissue by means of scissors and tweezers (no water was used) and dried. A piece of rib was kept intact; both from skull and ribs a total amount of 1g of bone powder was obtained (by using an electric polisher) and used for the analyses.

Being an experimental analysis, different procedures were performed before finding the most fitting one. In particular, two methods were applied in all cases. Both methods permit the simultaneous extraction of cocaine and of its metabolite (benzoylecgonine), of benzodiazepines, opioids and other basic substances.

After pulverizing bones, 5 µL of internal standard are added (a methanolic solution of bupivacaine 200 ng/µl, nalorphine 200 ng/µl, scopolamine 200 ng/µl, pinazepam 20 ng/µl), together with 5mL of methanol. The solution is left for 48h at room temperature (23°C), after which it is agitated and centrifuged at 4000 rpm for 5 minutes. Methanol is therefore collected and filtered in glass wool-filled pipettes (previously activated with methanol), and subsequently nitrogen evaporated.

Method 1: Solid Phase Extraction

4 mL of cyclohexane and 2 mL of HCl 0,1 M are then added; after vortex and centrifuge (4000 rpm for 5 minutes), the aqueous phase (the lower one) is collected and transferred in other test tubes after filtration in cotton-filled pipettes (activated with distilled water). pH is then adjusted (6-7) by adding phosphate buffer 0,1 M (pH 7).

Bond Elut Certify columns are activated with (in sequence) with 2 mL of methanol and 2 mL of phosphate buffer pH 6; after this phase, the sample is slowly filtered through the column and further eluted with 6 mL of distilled water and 3 mL of HCl 0,1 M. The column is then vacuum-dried for 10 minutes, washed with 9 mL of methanol and then dried for further 4 minutes.

In the rack inside the Vac Elut are prepared bacteriology tubes in order to recover the substances, diluted from the column with 2 mL of dichloromethane:isopropyl alcohol (80:20 v/v) solution at 3% ammonia.

The eluate is nitrogen-dried, recollected with 200 µL acetone and moved into 2mL vials; the eluent is dried once again and recollected with 50 µL acetone. The sample is then ready for being analyzed in GC-MS (SCAN mood).

Finally, the sample is derivatized with 50 µL BSTFA + 1% TMS, heated at 70°C for 20 minutes and analyzed in GC-MS.

Method 2: liquid-liquid

6 mL of ammonium sulfate saturated solution was added and the sample was agitated for almost 2 minutes (Vortex); 4 mL of concentrated HCl were subsequently added and agitated for 2 minutes.

Everything is then filtered through filter paper**.** 5mL of cyclohexane are added and, after agitating the sample, it is centrifuged for 4 minutes (3000 rpm).

The inferior phase (the aqueous one) is sampled and transferred into 22 mL tubes, after filtering it through cotton-activated pipettes; the sample is then alkalinized with ammonia until reaching pH 7,5-8. 4mL of ethyl acetate are added; everything is first agitated and then centrifuged. The organic phase (the upper one) is sampled and transferred in bacteriology tubes, after filtering it through glass wool-filled pipettes (first activated with acetate). The aqueous phase left in the tube is further alkalinized with ammonia until reaching pH 8,5-9. 4mL of a chloroform:isopropyl alcohol mixture (3:1 v/v) is added, and everything is agitated and centrifuged. The organic phase (the lower one) is now sampled and transferred in bacteriology tubes (with filtration through glass wool-filled pipettes activated with chloroform:isopropyl alcohol mixture).

The eluate is nitrogen-dried, recollected with 200 µL acetone and moved into 2mL vials; the eluent is dried once again and recollected with 50 µL acetone. The sample is then ready for being analyzed in GC-MS.

Finally, the sample is derivatized with 50 µL BSTFA + 1% TMS, heated at 70 °C for 20 minutes and analyzed in GC-MS. The GC-ECD analysis is instead used for searching for benzodiazepines and halogenated organic substances.

Results and discussion

The initial analyses performed permitted the identification of xenobiotics in many of the investigated cases, in concordance with the information obtained also by the body fluids that were simultaneously analyzed. The optimization of the method required multiple trials: different fragmentation of the bone, different times of incubation and different procedures for the extraction.

From the graphs obtained by the final analyses in GC-MS it was possible to identify the presence of substances like cocaine, metylecgonine, benzoylecgnonine, codeine, 6-MAM, morphine, phenobarbital but also of caffeine and cotinine (the main metabolite of nicotine).

A significant case is represented by a drug-addicted man found dead after an overdose. When the body was found, also a syringe was collected and analyzed. Inside the syringe caffeine, cocaine, 6-MAM and heroin were found. The analysis on the blood permitted to obtain the following findings:

Cocaine – 135,5 ng/g

Benzoylecgonine – 726,05 ng/g

Codeine – 2,12 ng/g

Morphine – 99,43 ng/g

6-MAM – 1,6 ng/g

The two methods on bones revealed the following findings:

Liquid-liquid Method:

Cocaine – 21,9 ng/g

Benzoylecgonine – 12,9 ng/g

Codeine – 12,2 ng/g

Morphine – 250,5 ng/g

SPE Method:

Nicotine

Cocaine – 30,3 ng/g

Benzoylecgonine – 80,8 ng/g

Codeine – 12,4 ng/g

Morphine – 326,4 ng/g

6-MAM – 0,2 ng/g

These results demonstrate the concordance between what it is found in bones and what it is found in blood, despite the differences in concentration. It is important to highlight that the bones in analysis are taken freshly from cadavers, cleaned and dried. However, despite the accurate process of cleaning, some bone marrow can still be present and somehow influence the analysis. Bone marrow, in fact, due to its strong irroration, is an optimal reservoir of all the substances that can be recovered and found in blood. Therefore, the aim of the analysis is to avoid this "contamination" and perform the investigation on the same samples after different months of time. Some samples were in fact left in the laboratory in some containers exposed at room air, in order to simulate a normal process of decomposition, with its common processes of degradation of soft and hard tissues, and washout of the substances attached to the bone. The investigation is still ongoing, and the analysis will be (initially) performed after 6, 12 and 18 months from the collection of the sample. The aim of such analysis is to investigate whether the substances recovered can be collected also after months of decomposition in soil, after the complete loss of the bone marrow and of the organic components of the bone tissue, and in which quantity.

This investigation would help in finding a method that could be used also in older samples for testing the addiction to drugs, as it could be done with the individuals of the Milano Cemetery Skeletal Collection. The possibility to apply this investigation also on such subjects would help both in developing analytical method that would clarify the aspects of drug addiction and modification of dentition previously exposed, and therefore in helping the observers in the forensic anthropological field in applying the method and collecting much more information on the person they want to identify.

Scientific articles regarding both these topics are going to be prepared.

Chapter 8

CONCLUSIONS

Skeletal pathology is widely known for being one of the most complicated aspects of the forensic anthropological and palaeopathological practice. The similar manifestation of different pathologies on the skeleton, the monotony of the reaction of the bone tissue to stimuli, and the still scarce knowledge of how bones and different disease interact is the cause of deep "black areas" in this topic.

The diagnosis of the pathology is critical to an accurate evaluation of its significance, relevance, and discriminatory capacity, and this is equally true whether examining material of archaeological or recent provenance (189). Visual observation has generally been considered the first method employed when examining skeletal remains for pathological lesions. In many cases it may be the only method required, while in some circumstances it may be the only method available (190). The methodology of analysis is therefore of fundamental importance. Precise recording of anatomical location and distribution of abnormal bone is integral to macroscopic analysis. This is because different pathogens, along with physiological changes in body functions and abilities, differentially affect areas and/or groups of bones (190). Providing a detailed and descriptive summary of the morphology of the abnormal bone is also key to macroscopic evaluation. The pace of formation varies, resulting in the development of different organizational structures (e.g. plaques of bone tissue deposited over well-organized compact bone, or rapidly formed spicules of bone) that can provide important information for a correct pathologic diagnosis (190).

When no information is available for the subject investigated, the differential diagnosis is an important component of macroscopic analysis. Creating an exhaustive list of potential causes of a lesion, considering also the mimic action of taphonomic processes, is a reasonable starting point. Adding demographic data, as well as the archaeological and environmental context of the specimen, provide further evaluative tools. The next step requires adopting clinically created and pathologically supported criteria for the presence of the disease. With only the bone tissue available for evaluation in most samples, the adoption of clinical criteria is often impossible. Pathologically supported criteria of disease seek to unite clinical and pathological research by finding common ground through histological evaluation, microscopy, and a range of imaging techniques (6, 144, 191). While incorporating these approaches can lead to a narrowing of possible aetiologies, they often leave the researcher with more than a single possible cause (190).

In this perspective the availability of known causes of death and related pathologies proves itself to be a turning point in the pathological investigation of human remains. Where only the union of background and historical information with statistical and modern clinical data on living served as material for the pathological investigation, the precise knowledge of the disease that a person suffered in life and the possibility to directly investigate the skeleton of interest provides new tools and methods of investigation. The importance of these data is also easily proved by the extremely limited in the number of ways in which bone can respond to changing stimuli: New bone can be added, existing bone can be removed, or a combination of both activities will most likely occurs. As a result of these alterations, the bone will change either in its size or in its shape and appearance. Although the patterns may be largely predictable and even definitively diagnostic, there can be considerable ambiguity in presentation (189). But when such patterns are tested and confirmed by clinical antemortem data, the scenario changes and can become clearer.

The study conducted in more than one case proved two different but fundamental aspects. First of all, as previously said, the importance of a positive control in the study of human remains. All the concepts previously described highlight the difficulties in interpreting how the bone tissue reacts to stimuli, due to a series of criticisms (the paucity of bone reactions, the ignorance of the original cause, the modifications caused by taphonomy). In this perspective, the precise knowledge (since the diagnosis of the pathology was done to the person in life) of the diseases suffered by each individual permits to investigate the skeleton with a more critical eye, and to make an evaluation which is more focused and valid.

Secondly, it is important to highlight that such ISTAT death certificates do not provide a 100% valid instrument, since they do not work as clinical forms. This concept produces a sort of bias. An example is provided by the cases of cancer that were displayed previously; on the ISTAT forms only the pathological condition was pointed out, without mention to the presence (except few cases) and to the location of the metastases, and to the involvement of the skeleton. This fact therefore left many difficult sides in the investigation. The pathology is known, documented and reported, but no information on what exactly should be sought and observed is available. Therefore much is still left to the interpretation of the observer. In particular, such aspect is further complicated by the intervention of environmental elements, that somehow always alter the aspect and the structure of buried human remain, sometimes changing the aspect of pathologic lesions on the skeleton, and sometimes mimicking them. The best positive control in such investigation would be therefore all the clinical documentation relative to the subject, correlated with TCs, X-rays and similar data; obviously, such kind of material is almost impossible to find and obtain, in particular relative to persons who died 20 years before. Nevertheless, despite the limits just described, the possibility of knowing the cause of the skeletal modifications observed on the remains in analysis proved itself to be an outstanding material. The investigator can address with a good percentage of probability a specific lesion observed to the reported pathology. It is useless to highlight that pathological data by far known need to be taken into consideration when conducing such analysis, but clinical data could probably be more useful for correctly addressing and correlating the pathology to the skeletal manifestation.

Another bias that could be pointed out regards the possibility that such ISTAT forms do not report all the pathological conditions that the person suffered. This is an assumption that was excluded as starting point of the analyses performed. This because otherwise the study would not have relevance and scientific reliability, and also because such forms are requested by the Italian law, and both for statistical and epidemiological purposes; therefore, the description can be more or less precise, but the accuracy of the information reported is mandatory.

It could be argued that pathologies are insufficient for personal identification. We agree with such assumption, as pathology, like many other personal features, can be shared among a great number of subjects. Nevertheless, the importance of improving the biological profile of a subject when human remains are found is of great importance, in particular in the forensic anthropological practice. A more precise description of a possible person whose remains are found, with detailed information also on possible habits and pathologies, would of course be of great help. Similarly, in the archaeological and palaeopathological practice, many information regarding the diffusion of specific diseases in particular centuries or areas, could improve the knowledge of our past.

The macroscopic approach is of course the starting one, and the “easier”. All the work presented here is based on the knowledge of the pathology that was sought. After this first approach, in particular in those cases where the macroscopic observation proved itself to be insufficient, it would be of great help to try also other approaches, as it would be using X-rays (which could provide important information regarding the presence of pathological signs not visible on the outer bone, or help in better diagnosing the pathogenesis of a specific disease), TCs, but also microbiology, histology (6, 191-192) and biochemistry. Many studies already demonstrated the possibility to find remains of viruses and bacteria in old bones, as well as specific proteins absorbed to hydroxyapatite that prove biochemical inquiries to be a valuable instrument for confirming the diagnosis of diseases by specific markers (75, 193). This instrument would be of great help also for identifying all those skeletal lesions of uncertain origin observed on a skeleton, thus inquiring also the taphonomic modification encountered by skeletal lesions or, on the contrary, exclude the pathological origin of the taphonomic ones. All these data demonstrate how fundamental a multidisciplinary approach (involving histology, biochemistry and Scanning Electron Microscopy, as examples) would be, also considering the comparisons with modern documented cases, providing a better comprehension of the development and a more accurate diagnosis of pathologies from bones. A starting project about the introduction of the toxicological method to the investigation of HIV and drug addicted case was for example performed. Nevertheless, further cases and additional time of decomposition need to be considered, in order to obtain a more realistic reconstruction of the events.

The anthropological practice, due to all the topics still not clarified displays many uncertainties in almost all of its diagnosis. Positive controls and certain data need therefore to be improved in the material available and considered as a fundamental element in all the investigation performed from now on. Each person is different from the other, and each organ, element or system reacts to stimuli in different ways in different people. Much still needs to be discovered and clarified in the pathological and anthropological field, but step by step, as this initial analyses wants to be, many issues can be explained.

Chapter 9

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