Legal Medicine 71 (2024) 102499

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Towards an integrative approach to the biological profile

Lucie Biehler-Gomez [a,b,1,*], Gaia Giordano [a,b,1], Francesco Sardanelli [a,c], Domenico Di Candia [a,d], Cristina Cattaneo [a,b]

[a] Department of Biomedical Science for Health, University of Milan, 20133 Milan, Italy
[b] LABANOF, Laboratorio Di Antropologia E Odontologia Forense, Department of Biomedical Science for Health, University of Milan, 20133 Milan, Italy
[c] Unit of Radiology, IRCCS Policlinico San Donato, Via Morandi 30, San Donato Milanese, 20097 Milan, Italy
[d] Bureau of Legal Medicine and Insurance, Department of Biomedical Science for Health, University of Milan, 20133 Milan, Italy

A R T I C L E I N F O

Keywords:
Biological profile
Forensic anthropology
Bone disease
Toxicology

A B S T R A C T

One of the most important tasks in forensic anthropology is the construction of the biological profile, classically defined as a set of four basic biological descriptors: biological sex, age-at-death, ancestry, and stature. Yet, our empirical and technological abilities in reconstructing the life experiences and health from skeletal remains far exceed these four parameters and forensic anthropology could benefit from further descriptors in the search for an identity. In this paper, we propose the inclusion of two other investigations to forensic anthropology practice to implement the already known biological profile: the interpretation of bone disease and lesions, and forensic toxicology on unconventional biological matrices. These analyses can provide information regarding health, habits, and disease burden, and by implementing them in our practice of forensic anthropology, they have the potential to improve the biological profile. We also propose a new term that can include not only the classical biological profile but also further descriptors, namely, the "biocultural profile".

1. The biological profile

Forensic anthropology consists in the application of physical anthropology methods to legal contexts, such as in the recovery and analysis of decomposed/skeletonized remains, in cases of mass disasters and violations of international law, and in investigations involving the living (e.g., age estimation on unaccompanied minors). When confronted to skeletal remains, one of the main tasks of the forensic anthropologist is the construction of the so-called "biological profile", which corresponds to a set of biological characteristics determinable after death from the remains of the body but describing the individual during life, and in particular, at their time of death. In forensic settings, the aim of the biological profile is to provide as many relevant biological descriptors as possible to narrow down the list of missing persons in the search of the identity to whom the skeletal remains belong.

All of the information that can be extrapolated from skeletal remains is determined by biological (i.e., physiological, pathological, biomechanical) processes. Christensen and colleagues, in their manual "Forensic Anthropology. Current Methods and Practice", a reference work in forensic anthropology, refer to the biological profile as a "summary of estimated biological parameters" [1], but more than a checklist, the biological profile is a puzzle where each piece provides one more information regarding the identity of an individual. Indeed, what is an identity if not a collection of characteristics that allow to recognize and name one unique individual? Classically, the biological profile has been defined by the estimations of biological sex, age-at-death, ancestry, and stature: four descriptors that are genetically determined (i.e., sex, ancestry, and stature – albeit with environmental components for stature) or estimated based on stable physiological growth and skeletal degeneration over time (i.e., age-at-death). However, progress in the discipline of anthropology, with new theoretical and technological advancements (e.g., medical imaging, biomolecular techniques) have allowed to further the analysis of skeletal remains and provide more information on the life experiences of the individuals under study which may assist with identification.

In this paper, we explore the potential of further descriptors and, in particular, the interpretation of bone lesions and diseases, and forensic toxicology on unconventional biological matrices.

* Corresponding author at: LABANOF, Laboratorio di Antropologia e Odontologia Forense, Sezione di Medicina Legale, Dipartimento di Scienze Biomediche per la Salute, Università degli Studi di Milano, Via Mangiagalli 37, 20133 Milan, Italy.

E-mail address: lucie.biehler@unimi.it (L. Biehler-Gomez).

[1] co-first authors.

https://doi.org/10.1016/j.legalmed.2024.102499
Received 5 December 2023; Received in revised form 10 June 2024; Accepted 21 July 2024
Available online 22 July 2024

2. The overlooked potential of bone disease

Non-traumatic bone pathology is rarely (if ever) considered in forensic skeletal cases, and generally seen as not pertinent to forensic scenarios. By not considering bone pathology, one risks losing precious information that may prove determinant to forensic identification. Given its potential in the discipline, we argue that the study of bone disease should be more systematically included in forensic anthropology.

2.1. The potential of bone disease in forensic anthropology

The applicability of bone disease in forensic anthropology is twofold: 1) to implement the biological profile and 2) as a valid identifier for personal identification.

Health is a determinant part of a person's life, and even sometimes their death, indicative of their habits and ways of life. Identifying bone lesions and disease can provide information related to way of life, potential symptoms, and disabilities. For example, rheumatoid arthritis is an extremely painful condition that causes joint stiffness and swelling as well as a set of extra-articular and systemic manifestations [2–4]. Skeletal metastases are associated with increased clinical morbidity and life-threatening complications including severe bone pain, pathologic fractures, impaired mobility, hypercalcemia, spinal cord compression, and other nerve compression syndromes [5–9]. Osteomyelitis, an infection of the bone, may induce local pain, swelling, ulcers, limping during walking if affecting the legs and overall reduced mobility of the limb affected [10]. Such information will give further descriptors regarding the individual during life and may therefore help in narrowing down the search. Similarly, the presence (and recognition) of medical devices (e.g., prostheses, implants, pacemakers) indicate a surgical intervention for which personal records may also exist in medical institutions [11]. Moreover, the morphological features of skeletal lesions can inform on the severity, extent, degree of healing and temporality of the lesion. For instance, woven bone indicates a recent and rapid periosteal new bone deposition, lamellar bone implicates a chronic and relatively slow process of deposition, and spiculated bone is the result of a very rapid and aggressive process of bone formation [12,13]. Similarly, smooth margins in an osteolytic lesion indicate a chronic and relatively slow process contrasting with the poorly defined margins of the aggressive and rapid process of other pathologies [12,13]. Consequently, the analysis of bone lesions, with the correct training, can not only provide clues as to their etiology and pathogenesis, but also contains useful details regarding the time of their occurrence as well as their clinical impact and implications.

Furthermore, bone disease can provide valid means for personal identification. Specifically, morphological features of pathological bone markers may be compared to antemortem imaging and used as factors of individualization that, in combination with other personal descriptors, may result in a positive identification [14,15]. In a recent paper, the members of the board of the Forensic Anthropology Society of Europe (FASE) described various anthropological identifiers that can be used as means of personal identification, particularly in situations with limited availability of traditional identification methods (i.e., dactyloscopy, odontology, and molecular genetic analysis), including pathological changes [16]. In a similar approach as frontal sinuses patterns [17], the morphological features of bone lesions may be superimposed to antemortem imaging in order to highlight points of correspondence in morphological characteristics. Importantly, antemortem images must have been taken close to the time of death to be relevant for forensic identification, so as to limit the potential of evolution over time of the aspect of bone lesions. This is essential because pathological bone lesions are not always stable over time: they may show signs of healing and resolve with medical treatments, or they may worsen if left untreated.

2.2. No stone left unturned: Concretions and calcifications

Biological concretions are products of disease [10,18–24] that may be found among skeletal remains in more insidious ways. Their calcified properties enable them to survive the decomposition processes and be recovered in forensic (but also archaeological) contexts. Because of their small size, they may easily move among the remains after decomposition and lay in the soil, mixed with roots and dirt, be trapped among clothing or rest in the cranial vault. These calcifications/concretions may cause clinical manifestations ranging from localized pain to life-threatening events. Their recognition and identification may be particularly challenging, especially if they are no longer *in situ* and/or if the decomposition of the body occurred in an outdoor environment where they may be easily missed and confused with soil, dirt, and geological stones. In fact, reports of such findings are exceedingly rare (except for biological stones in archaeological contexts), and this may be due to the lack of training on the importance of recovery of this material and its recognition [18,19,23,25–34]. One example is the forensic recovery presented by Subirana-Domènech et al [35]: the authors describe the finding of a "calcified cylindrical structure […] brown, partly fenestrated, rough and crumbly" among scattered skeletal remains found in a wooded area. The calcification was identified as an arteriosclerotic calcification after radiographic and histopathological examinations. The careful recovery and identification of this non-skeletal biological material can provide further information to the biological profile and may even be used as factors of individualization when recent antemortem imaging is available.

2.3. Embodied experiences: Bone disease as a biological adaptation to social, cultural, and environmental factors

Going even further, the understanding of the pathogenesis and etiology causing skeletal and extra-skeletal lesions can inform on ways of life. For instance, cribriotic lesions and enamel hypoplasia are skeletal responses to physiological stressors during growth. Although they may be related to various etiologies [36–40], they are most commonly indicators of malnutrition or infectious disease during growth, which can inform on living conditions and early life experiences. In fact, Beatrice and Soler [41] found that undocumented border crossers at the Mexico/USA border in South Arizona were 7.91 times more likely to exhibit porotic hyperostosis and 3.01 times more likely to present enamel hypoplasia than American-born individuals. Later, Biehler-Gomez et al [42] showed that these lesions were statistically more frequent and severe in their sample of deceased migrants from the Mediterranean Sea than in contemporary Italian individuals, which may be related to the general poor living conditions and systemic poverty of migrants during their early life. Beatrice and Soler [41] suggested that cranial stress markers can be used to distinguish undocumented border crossers (from Mexico and Central America) from American-born individuals. This is not limited to migration and may be applied to other social situations: in certain contexts, poor living conditions and general poverty can generate biological conditions (e.g., nutritional deprivation and insufficiencies and/or infections) which may embody on skeletal remains through bone lesions. Therefore, paleopathology can bring forward a new dimension to the biological profile by interpreting the biological effects of cultural and social living conditions and providing valuable information to forensic cases.

2.4. Case study

We propose to describe a specific forensic case to further illustrate the potential for contribution of bone disease to forensic anthropology practice [43].

In March 2016, the skeletal remains of a single individual were found in the woods a few hundred meters away from the university hospital of Nancy (France). The individual was identified through dental records.

Interestingly, various pathological lesions were found, in particular on the cranium, sacrum and thoracic vertebral body resulting in a kyphotic deformity, typical of "Pott's disease". By confrontation with the clinical and paleopathological literature, these lesions are highly consistent with a diagnosis of tuberculosis [36,44]. In France, as in most Western European countries, the infectious disease is uncommon. Although the diagnosis of tuberculosis may not allow to single out the missing individual, this information would have permitted to substantially narrow down the list if medical records existed. In fact, various computed tomography scans and magnetic resonance imaging were performed in late 2013 for diagnostic and therapeutic purposes, close to the time of disappearance of the individual. Comparisons and superimposition between antemortem and postmortem images evidenced several points of anatomical and pathological correspondence (i.e., anatomical details of the bones and pathological features of the lesions) which would have allowed for the personal identification of the individual [43].

3. Digging around one's past: Toxicological analysis on bones

Forensic toxicology is a branch of toxicology at the service of forensic sciences. It consists in an analytical investigation aimed at researching any type of exogenous substance that can be found in an organism. The analytical investigation should be performed with qualitative confirmation and quantitative analyses with the objective of interpreting the likely role of a xenobiotic in a forensic case [45].

In forensic anthropology, when an unknown individual is recovered, it becomes essential to reconstruct their biological profile for the purpose of identification, both for the subject themselves and for their relatives. But can other essential information be "extracted" from a dead body in addition to biological sex, age-at-death, stature, and population affinity? We suggested earlier in this paper that bone disease constitutes a relevant example, and we aim now to propose another: bone toxicology.

Forensic toxicological analyses are often involved in forensic sciences to perform investigation on conventional matrices as biological fluids or samples of viscera [45–47]. When conventional matrices are not available, usually due to extremely decomposed bodies or complete skeletonized corpses, alternative matrices can be used in forensic toxicology to perform investigations. Unconventional matrices such as bones, nails, paraffin-embedded blocks tissues, and teeth can be involved in toxicological analyses [46,48–56] to investigate the intake of drugs when conventional matrices are no longer available. Even other non-biological elements such as fabrics remains [57] have shown to be valid matrices in toxicological investigation to evaluate the acute administration of drugs, providing a time frame of drug-intake near the time of death of a subject.

Here, we explore the most difficult forensic toxicology scenario: when skeletal remains are found without the presence of any other matrix that can be used in forensic toxicological investigation.

Bone is an unconventional matrix, and little is known in forensic toxicology about how and how much drugs can remain entrapped inside it [58]. Since bone is a vascularized matrix, it can be possible to find substances administered after an acute drug intake. However, the prevailing theory in the literature is that drugs found in the bone matrix result from an occasional/chronic drug administration. Indeed, Rubin [58]explained that drugs are incorporated into the bone matrix as part of primary or secondary mineralization through bone remodeling. Even if the details regarding incorporation into the bone tissue remains unclear, we believe that storage is due to more than one administration of the substance, rather than a single intake.

In fact, the results obtained in our previous paper [48] support this hypothesis. In this study, 30 autopsy cases were selected and different biological samples from each cadaver were collected: one femoral blood sample, two cranial bone samples and two costal bone samples. From the results obtained, we noted that some molecules were only detected in bone samples and not in blood specimen. This suggests that the molecules absent in blood were not present at the time of death in the organism, whereas the presence of those molecules in bone specimens highlighted the accumulation of xenobiotics into the bone matrix. Analogously, some molecules were noted only in blood samples and not in bone specimens, confirming that the single administration of molecules is difficult to be detected in the bone matrix. Other studies obtained similar results: Horak and Jenkins [59] detected citalopram and olanzapine in blood samples, but the analysis of bone samples revealed citalopram only. In McGrath and Jenkins' study [60], most of the drugs detected were found in higher concentrations in bone than in blood. This result acts as further evidence of the accumulation of deposits of drugs in bone.

Therefore, the integration within bone tissue and the detection of substances after complete skeletonization of a body should refer to occasional or chronic drug administration. Nonetheless, this statement does not exclude the administration of a substance close to the time of death due to the high vascularization of the bone matrix, but rather includes the probability that this substance was also taken during life, and at least occasionally administered.

The literature shows various examples of substances detected in bone which may increase the quantity of data that can be obtained from a skeleton and act as good discriminants for forensic identification. Indeed, the literature reports the detection of amphetamines, anesthetic drugs, anticonvulsant drugs, antihistamine drugs, antihypertensive drugs, stimulants, cannabinoids, antidepressants, antipsychotics, benzodiazepines, and opioids in various bone samples [61]. Interestingly, some of the substances detected, such as cannabinoids, opioids, stimulants, anticonvulsant drugs, and antidepressant drugs, have been found to have an effect on bones and thus potentially impact the estimations of the various parameters of the biological profile [61]. Therefore, the importance of a toxicological profile from skeletal remains is clear: the presence or absence of xenobiotics of toxicological interest is essential to understand and eventually adjust methodologies for the construction of the biological profile, and/or to gather information related to behavior and lifestyle which may be used to narrow down the search for an identity.

In fact, in the hypothesis of a forensic scenario, the finding of peculiar substances can become a funnel for matching an individual's profile to a list of possible missing persons. Moreover, in certain situations, substances of toxicological interest can also discriminate one subject from another by matching postmortem and antemortem data, which is why precise information is essential for a complete profile.

In seven cases of our previous study [48], out of 19 individuals positive to toxicological analyses, it was not possible to confirm the antemortem information. It should not be forgotten that the antemortem information are often declared by the relatives of the deceased, so it is possible that they are not exact accounts. Indeed, they may not have known exactly which substances the deceased took during life. Alternatively, the deceased could have taken the substances reported in the antemortem data for a short period of time, not allowing the deposition of drugs in bones and rendering more difficult the detection of xenobiotics. For this reason, a broad spectrum of drugs should be investigated. Overall, the results of toxicological interest, even those not superimposable to antemortem data, provided further information to implement the biological profiles, thus acting as further descriptors in the search of their identity. Another source of antemortem data is clinical records which may provide more precise information which can be compared with toxicological results. In the remaining 12 cases, the toxicological findings confirmed what was described in the antemortem data, and provided further specification: e.g., a general "benzodiazepines administration", became "occasional or chronic administration of alprazolam". Thus, if we hypothetically think about a missing person, any toxicological finding can prove determinant in narrowing down the list of missing persons.

Consequently, forensic toxicology is a powerful instrument with a substantial potential in forensic anthropology as it can provide details regarding an individual's lifestyle and health which may implement the biological profile (biological sex, age-at-death, stature, and ancestry) but can also be used for person identification by comparing toxicological results with antemortem data obtained from the victim's relatives.

4. Towards a new definition of the biological profile?

Defining the biological profile as a sum of only four basic characteristics (biological sex, age-at-death, stature, and ancestry) has become reductive in view of the progresses in the study of skeletal remains. An identity cannot be reduced to these four descriptors and despite their fundamental value, they may not be enough to help investigators when narrowing down a list of missing persons, because of the quantity of people these four basic characteristics may apply to. In addition, the estimation of ancestry is one of the most challenging and controversial parameters of the biological profile. Just like sex, a biological characteristic distinct from the socially constructed "gender", ancestry is often confused with social race, leading to ethical debates on the scientific validity of the estimation of this parameter [62].

The aim of the biological profile is to assist with identification by providing a biological set of descriptors that may narrow down a list of missing persons for the identification of an unknown decedent. Yet, further information may be obtained from skeletal remains to fulfill this purpose. Indeed, the skeleton represents an archive of life experiences of an individual including living conditions, habits, health, and traumatic events. These are routinely analyzed and interpreted in an interdisciplinary approach in archaeological contexts [63–73], leading to true "osteobiographies" of the individual. The application of some of these techniques could change our anthropological practice while maintaining scientific reliability and accuracy necessary to forensic sciences. In this paper, we demonstrated the substantial potential of two further descriptors in particular: the interpretation of bone lesions and diseases, and forensic toxicology on unconventional biological matrices. The inclusion of the information that can be obtained from these analyses to the biological profile (including disease burden, diagnoses of disease, temporality of lesions, disabilities, drugs use and/or abuse, pharmacological therapies and, as a consequence, the long-standing habits and lifestyle of an individual) can implement the data we already present (sex, age-at-death, ancestry, and stature) and further assist for the search of an identity, if not provide usable factors of individualization. Moreover, exploring living conditions and life experiences on the skeleton through the analysis of bone disease and toxicology gives a new dimension to the study of skeletal remains, furthering anthropological analysis from the estimation of four basic biological characteristics to a biocultural profile, which integrates the effects of cultural and social influences on an individual's biology.

Given that the "biological profile" has been defined for decades as a set of four parameters, we propose the provocative idea that it may have become opportune to develop a new terminology that includes further descriptors. In all logic, the use of a new term that includes not only the anthropological study of biological sex, age-at-death, stature, and ancestry, but also the two additional descriptors mentioned above, seems the path forward in keeping track with the technological and empirical progress of the discipline. It is important to mention that the term is not intended to be limited to the addition of these two descriptors (i.e., the interpretation of bone lesions and diseases, and forensic toxicology on unconventional biological matrices), but also to accommodate others that bear relevance in forensic anthropology, thanks to continuous technological innovations.

Consequently, the authors propose to update the term "biological profile" to "biocultural profile" when further descriptors are involved in the forensic anthropological study: this new term derives from the union of the constant biological source of information that can be extrapolated from skeletal remains and the cultural and social aspects the new methodologies discussed in this paper can provide, relating to biological impacts of lifestyle and habits. This new term is not intended as a precision of an old terminology, but as an occasion to increase the capacity of the biological profile, which is to assist in the search of an identity and ultimately give back the identity to a skeleton, help alleviate the terrible weight of the "ambiguous loss", and safeguard human rights.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A.M. Christensen, N.V. Passalacqua, E.J. Bartelink, *Forensic Anthropology: Current methods and practice*, Elsevier, 2014. ISBN 0124172903.
[2] D.M. Lee, M.E. Weinblatt, Rheumatoid arthritis, Lancet 358 (2001) 903–911, https://doi.org/10.1016/s0140-6736(01)06075-5.
[3] L. Biehler-Gomez, C. Cattaneo, The diagnostic implications of two cases of known rheumatoid arthritis from the CAL milano cemetery skeletal collection, J Forensic Sci 63 (2018) 1880–1887, https://doi.org/10.1111/1556-4029.13799.
[4] J.S. Smolen, D. Aletaha, I.B. McInnes, Rheumatoid arthritis, Lancet 388 (2016) 2023–2038, https://doi.org/10.1016/S0140-6736(16)30173-8.
[5] V. Vassiliou, E. Chow, D. Kardamakis, Bone metastases: A translational and clinical approach, Springer Science & Business Media, 2013. ISBN 9400775695.
[6] H.A. Harvey, L. von Reyn Cream, Biology of bone metastases: Causes and consequences, Clin Breast Cancer 7 (2007) S7–S13, https://doi.org/10.3816/CBC.2007.s.001.
[7] L. Biehler-Gomez, G. Giordano, C. Cattaneo, The appearance of breast cancer metastases on dry bone: Implications for forensic anthropology, J Forensic Leg Med 61 (2019) 5–12, https://doi.org/10.1016/j.jflm.2018.10.007.
[8] L. Biehler-Gomez, G. Giordano, C. Cattaneo, The overlooked primary: bladder cancer metastases on dry bone. a study of the 20th century CAL milano cemetery skeletal collection, Int J Paleopathol 24 (2019) 130–140, https://doi.org/10.1016/j.ijpp.2018.10.005.
[9] R.E. Coleman, Clinical features of metastatic bone disease and risk of skeletal morbidity, Clin. Cancer Res. 12 (2006) 6243.
[10] L. Biehler-Gomez, C. Cattaneo, *Interpreting bone lesions and pathology for forensic practice*, Academic Press, 2021. ISBN 978-0-323-85162-6.
[11] A. Cappella, D. Gibelli, Z. Obertová, M. Cummaudo, E. Castoldi, D. De Angelis, C. Sforza, C. Cattaneo, The utility of skeletal and surgical features for the personal identification process: A pilot study, J Forensic Sci 64 (2019) 1796–1802.
[12] N.C. Lovell, Paleopathological description and diagnosis, in: S.R. Saunders, A. M. Katzenberg (Eds.), Biological Anthropology of the Human Skeleton, New York, USA, Wiley-Liss New York, 2000, pp. 217–248.
[13] B.D. Ragsdale, R.A. Campbell, C.L. Kirkpatrick, Neoplasm or Not? General Principles of Morphologic Analysis of Dry Bone Specimens, Int J Paleopathol 21 (2018) 27–40, https://doi.org/10.1016/j.ijpp.2017.02.002.
[14] E. Cunha, Pathology as a factor of personal identity in forensic anthropology, Springer, 2006, pp. 333–358.
[15] L. Biehler-Gomez, C. Cattaneo, Interpreting bone lesions and pathology for forensic practice, Elsevier Academic Press, 2020. ISBN 9780323851626.
[16] H.H. de Boer, Z. Obertová, E. Cunha, P. Adalian, E. Baccino, T. Fracasso, E. Kranioti, P. Lefévre, N. Lynnerup, A. Petaros, et al., Strengthening the role of forensic anthropology in personal identification: Position statement by the board of the forensic anthropology society of europe (FASE), Forensic Sci Int 315 (2020) 1–7, https://doi.org/10.1016/j.forsciint.2020.110456.
[17] G. Quatrehomme, P. Fronty, M. Sapanet, G. Grévin, P. Bailet, A. Ollier, Identification by frontal sinus pattern in forensic anthropology, Forensic Sci Int 83 (1996) 147–153, https://doi.org/10.1016/S0379-0738(96)02033-6.
[18] D.A. Komar, J.E. Buikstra, Differential diagnosis of a prehistoric biological object from the koster (Illinois) site, Int J Osteoarchaeol 13 (2003) 157–164, https://doi.org/10.1002/oa.670.
[19] C.-A. Baud, C. Kramar, Soft tissue calcifications in paleopathology, in: D.J. Ortner, A.C. Aufderheide (Eds.), Human Paleopathology: Current Syntheses and Future Options, Smithsonian Institution Press, Washington, DC, 1991, pp. 257–260.
[20] D.A. Towler, Vascular calcification: A perspective on an imminent disease epidemic, IBMS BoneKey 5 (2008) 41–58.
[21] R.T. Steinbock, Studies in ancient calcified soft tissues and organic concretions. III: Gallstones (Cholelithiasis), Journal of Paleopathology 3 (1990) 95–106.
[22] R.T. Steinbock, Studies in ancient calcified soft tissues and organic concretions. II: Urolithiasis (Renal and Urinary Bladder Stone Disease), Journal of Paleopathology 3 (1990) 39–59.
[23] L. Biehler-Gomez, E. Maderna, C. Cattaneo, The challenging diagnosis of cardiovascular disease in skeletal remains: Distinction and recognition of atherosclerotic calcifications from known cases, Cambridge University Press: In press, 2023, pp. 229–245. ISBN 9781108572224.
[24] B. Rothschild, L. Biehler-Gomez, Osteophytes: The product of convergent evolution, Anat Rec 305 (2022) 2113–2118.
[25] L. Biehler-Gomez, A. Cappella, E. Castoldi, L. Martrille, C. Cattaneo, Survival of atherosclerotic calcifications in skeletonized material: Forensic and pathological

implications, J Forensic Sci 63 (2018) 386–394, https://doi.org/10.1111/1556-4029.13592.
[26] L. Biehler-Gomez, E. Maderna, G. Brescia, A. Cappella, A. Rizzi, C. Cattaneo, "Aged" autopsy gallstones simulating dry bone context: A morphological, histological and SEM-EDS analysis, Int J Paleopathol 24 (2019) 60–65, https://doi.org/10.1016/j.ijpp.2018.09.004.
[27] C.-A. Baud, C. Kramar, Les calcifications biologiques en archéologie, Bull Mem Soc Anthropol Paris 2 (1990) 163–170, https://doi.org/10.1103/PhysRevLett.101.076402.
[28] G. Cole, C. Rando, L. Sibun, T. Waldron, Case report: A giant calcified uterus, likely due to benign leiomyoma, Int J Paleopathol 10 (2015) 51–57, https://doi.org/10.1016/j.ijpp.2015.05.003.
[29] A.L. Waters-Rist, K. Faccia, A. Lieverse, V.I. Bazaliiskii, M.A. Katzenberg, R. J. Losey, Multicomponent analyses of a hydatid cyst from an early neolithic hunter-fisher-gatherer from lake baikal, siberia, *J Archaeol Sci* 50 (2014) 51–62, https://doi.org/10.1016/j.jas.2014.06.015.
[30] N. Armentano, M. Subirana, A. Isidro, O. Escala, A. Malgosa, An ovarian teratoma of late roman age, Int J Paleopathol 2 (2012) 236–239, https://doi.org/10.1016/j.ijpp.2012.11.003.
[31] K. Özdemir, A.A. Akyol, Y.S. Erdal, A case of ancient bladder stones from oluz höyük, amasya, turkey, *Int J Osteoarchaeol* 25 (2015) 827–837, https://doi.org/10.1002/oa.2349.
[32] T.C. Jaskowiec, A.L. Grauer, M. Lee, S. Rajnic, No stone unturned: the presence of kidney stones in a skeleton from 19th century Peoria, Illinois, *Int J Paleopathol* 19 (2017) 18–23, https://doi.org/10.1016/j.ijpp.2017.08.004.
[33] J.M. Streitz, A.C. Aufderheide, M.EI. Najjar, D.J. Ortner, A 1,500-year-old bladder stone, J Urol 126 (1981) 452–453, https://doi.org/10.1016/S0022-5347(17)54571-1.
[34] V. Giuffra, L. Costantini, L. Costantini Biasini, D. Caramella, G. Fornaciari, Giant bladder stone in a natural mummy of the early 19th century, Urology 72 (2008) 780–781, https://doi.org/10.1016/j.urology.2008.04.031.
[35] M. Subirana-Domènech, J.C. Borondo-Alcázar, N. Armentano-Oller, G. Font Valsecchi, I. Galtés Vicente, J. Castellà-García, Arteriosclerosis in paleopathology. are macroscopic findings well known? Int J Paleopathol 2 (2012) 246–248, https://doi.org/10.1016/j.ijpp.2012.10.001.
[36] D.J. Ortner, Identification of pathological conditions in human skeletal remains, 3rd ed.;, Smithsonian Institution Press, New York, 2003.
[37] M. Brickley, R. Ives, *The bioarchaeology of metabolic bone disease*, Elsevier Academic Press, London, 2010.
[38] L. O'Donnell, E.C. Hill, A.S.A. Anderson, H.J.H. Edgar, Cribra orbitalia and porotic hyperostosis are associated with respiratory infections in a contemporary mortality sample from new mexico, Am J Phys Anthropol 173 (2020) 721–733, https://doi.org/10.1002/ajpa.24131.
[39] C.A. Roberts, K. Manchester, The archaeology of disease, Third edit, Cornell University Press, Ithaca, New York, 2007.
[40] A.H. Goodman, J.C. Rose, Assessment of systemic physiological perturbations from dental enamel hypoplasias and associated histological structures, Yearb Phys Anthropol 33 (1990) 59–110.
[41] J.S. Beatrice, A. Soler, Skeletal indicators of stress: A component of the biocultural profile of undocumented migrants in southern arizona, J Forensic Sci 61 (2016) 1164–1172, https://doi.org/10.1111/1556-4029.13131.
[42] L. Biehler-Gomez, A. Palamenghi, M. Baudu, G. Caccia, G.L. Attisano, D. Gibelli, D. Mazzarelli, C. Cattaneo, Skeletal markers of physiological stress as indicators of structural violence: A comparative study between the deceased migrants of the mediterranean sea and the CAL milano cemetery skeletal collection, Biology (basel) 335 (2023), https://doi.org/10.3390/biology12020335.
[43] L. Martrille, D. De Angelis, A. Blum, G. Gauchotte, C. Cattaneo, L. Biehler-Gomez, The potential of bone disease for personal identification: A case of tuberculosis, Int J Legal Med 134 (2020) 1957–1962, https://doi.org/10.1007/s00414-020-02348-3.
[44] T. Waldron, *Palaeopathology*, Cambridge University Press, Cambridge, United Kingdom, 2008. ISBN 9781139474009.
[45] R.J. Dinis-Oliveira, F. Carvalho, J.A. Duarte, F. Remião, A. Marques, A. Santos, T. Magalhães, Collection of biological samples in forensic toxicology, Toxicol Mech Methods 20 (2010) 363–414, https://doi.org/10.3109/15376516.2010.497976.
[46] G. Giordano, L. Biehler-Gomez, P. Seneci, C. Cattaneo, D. Di Candia, Detecting drugs in dry bone : A pilot study of skeletal remains with a post-mortem interval over 23 years, Int J Legal Med 135 (2021) 457–463, https://doi.org/10.1007/s00414-020-02494-8.
[47] F. Bévalot, N. Cartiser, C. Bottinelli, J. Guitton, L. Fanton, State of the art in bile analysis in forensic toxicology, Forensic Sci Int 259 (2016) 133–154, https://doi.org/10.1016/j.forsciint.2015.10.034.
[48] L. Franceschetti, D. Di Candia, G. Giordano, I. Carabelli, G. Vignali, C. Cattaneo, Drugs in bone: Detectability of substances of toxicological interest in different states of preservation, J Forensic Sci (2020) 1–10, https://doi.org/10.1111/1556-4029.14636.
[49] J.F. Wiart, F. Hakim, A. Andry, C. Eiden, G. Drevin, B. Lelièvre, C. Rougé-Maillart, M. Decourcelle, A.S. Lemaire-Hurtel, D. Allorge, et al., Pitfalls of toxicological investigations in hair, bones, and nails in extensively decomposed bodies: Illustration with two cases, Int J Legal Med 134 (2020) 1339–1344, https://doi.org/10.1007/S00414-020-02267-3/TABLES/4.
[50] C. Cattaneo, F. Gigli, F. Lodi, M. Grandi, The detection of morphine and codeine in human teeth: An aid in the identification and study of human skeletal remains, Journal of Forensic Odonto-Stomatology 21 (2003) 1–5.
[51] D.A. Engelhart, E.S. Lavins, C.A. Sutheimer, Detection of drugs of abuse in nails, J Anal Toxicol 22 (1998) 314–318, https://doi.org/10.1093/jat/22.4.314.
[52] T.P. Magalhães, S. Cravo, D. Dias da Silva, R.J. Dinis-Oliveira, C. Afonso, M. de Lourdes Bastos, H. Carmo, Quantification of methadone and main metabolites in nails, J Anal Toxicol (2018), https://doi.org/10.1093/jat/bkx099.
[53] A. Palmeri, S. Pichini, R. Pacifici, P. Zuccaro, A. Lopez, Drugs in nails. physiology, pharmacokinetics and forensic toxicology, Clin Pharmacokinet 38 (2000) 95–110, https://doi.org/10.2165/00003088-200038020-00001.
[54] G. Ottaviani, R. Cameriere, M. Cippitelli, R. Froldi, G. Tassoni, M. Zampi, M. Cingolani, Determination of drugs of abuse in a single sample of human teeth by a gas chromatography-mass spectrometry method, J Anal Toxicol 41 (2017) 32–36, https://doi.org/10.1093/jat/bkw105.
[55] M. Pellegrini, A. Casá, E. Marchei, R. Pacifici, R. Mayné, V. Barbero, O. Garcia-Algar, S. Pichini, Development and validation of a gas chromatography-mass spectrometry assay for opiates and cocaine in human teeth, J Pharm Biomed Anal 40 (2006) 662–668, https://doi.org/10.1016/j.jpba.2005.07.003.
[56] Di Candia Domenico, Michele Boracchi, Guendalina Gentile, Gaia Giordano, Riccardo Zoja, Histological paraffin-embedded block: A good alternative specimen to detect the use of opiates at least 20 years Ago, *Forensic Toxicol* 40 (2022) 302–311, https://doi.org/10.1007/s11419-022-00614-0.
[57] G. Giordano, G. Caccia, M. Boracchi, F. Sardanelli, C. Cattaneo, D. Di Candia, Water-related atmospheric agents and solubility: Two parameters of validation in toxicological screening on clothing worn by skeletal remains, Int J Legal Med 137 (2023) 1051–1057, https://doi.org/10.1007/s00414-023-02997-0.
[58] K.M. Rubin, The current state and future directions of skeletal toxicology: Forensic and humanitarian implications of a proposed model for the in vivo incorporation of drugs into the human skeleton, Forensic Sci Int 289 (2018) 419–428, https://doi.org/10.1016/j.forsciint.2018.06.024.
[59] E.L. Horak, A.J. Jenkins, Postmortem tissue distribution of olanzapine and citalopram in a drug intoxication, J Forensic Sci 50 (2005) 1–3, https://doi.org/10.1520/jfs2004067.
[60] K.K. McGrath, A.J. Jenkins, Detection of drugs of forensic importance in postmortem bone, Am. J. Forensic Med. Pathol. 30 (2009) 40–44, https://doi.org/10.1097/PAF.0b013e31818738c9.
[61] N. Márquez-Grant, E. Baldini, V. Jeynes, L. Biehler-Gomez, L. Aoukhiyad, N. V. Passalacqua, G. Giordano, D. Di Candia, C. Cattaneo, How do drugs affect the skeleton? implications for forensic anthropology, *Biology (basel)* 11 (2022) 524, https://doi.org/10.3390/BIOLOGY11040524.
[62] A.H. Ross, S.E. Williams, Ancestry studies in forensic anthropology: Back on the frontier of racism, Biology (basel) 10 (2021), https://doi.org/10.3390/biology10070602.
[63] G. Giordano, M. Mattia, M. Boracchi, L. Biehler-Gomez, M. Cummaudo, A. Porro, M. Caccianiga, F. Sardanelli, F. Slavazzi, P.M. Galimberti, et al., Forensic toxicological analyses reveal the use of cannabis in milano (Italy) in the 1600's, J Archaeol Sci 160 (2023), 10.1016/j.jas.2023.105873.
[64] G. Giordano, M. Mattia, L. Biehler-Gomez, M. Boracchi, S. Tritella, E. Maderna, A. Porro, M.M.C. Romanelli, A.F. Franchini, P.M. Galimberti, et al., Papaver somniferum in seventeenth century (Italy): Archaeotoxicological study on brain and bone samples in patients from a hospital in milan, Sci Rep 13 (2023) 3390, https://doi.org/10.1038/s41598-023-27953-1.
[65] L. Biehler-Gomez, M. Mattia, C. Sala, G. Giordano, D. Di Candia, C. Messina, L. M. Sconfienza, A.F. Franchini, A. Porro, P.M. Galimberti, mercury poisoning in two patients with tertiary syphilis from the ca'granda hospital (17th-century Milan), Archaeometry 64 (2021) 500–510.
[66] K.L. Rasmussen, J.L. Boldsen, H.K. Kristensen, L. Skytte, K.L. Hansen, L. Mølholm, P.M. Grootes, M.J. Nadeau, K.M. Flöche Eriksen, Mercury levels in danish medieval human bones, J Archaeol Sci 35 (2008) 2295–2306, https://doi.org/10.1016/j.jas.2008.03.003.
[67] M. Kepa, T. Kozłowski, K. Szostek, A. Drozd, S. Walas, H. Mrowiec, B. Stepańczak, H. Głab, M. Grupa, Analysis of mercury levels in historical bone material from syphilitic subjects - pilot studies (Short Report), Anthropol. Anz. 69 (2012) 367–377, https://doi.org/10.1127/0003-5548/2012/0163.
[68] G. Giordano, L. Biehler-Gomez, P. Seneci, C. Cattaneo, C.D. Di, Detecting drugs in dry bone: A pilot study of skeletal remains with a post-mortem interval over 23 years, Int J Legal Med (2021) 1–7, https://doi.org/10.1007/s00414-020-02494-8.
[69] K.L. Rasmussen, L. Skytte, A.J. Jensen, J.L. Boldsen, Comparison of mercury and lead levels in the bones of rural and urban populations in southern denmark and northern germany during the middle ages, J Archaeol Sci Rep 3 (2015) 358–370, https://doi.org/10.1016/j.jasrep.2015.06.021.
[70] O. López-Costas, M. Kylander, N. Mattielli, N. Álvarez-Fernández, M. Pérez-Rodríguez, T. Mighall, R. Bindler, A. Martínez Cortizas, Human bones tell the story of atmospheric mercury and lead exposure at the edge of roman world, Sci. Total Environ. 710 (2020) 136319, https://doi.org/10.1016/j.scitotenv.2019.136319.
[71] J. Yoshinaga, A. Hisada, M. Yoneda, H. Ishida, Lead content of bones excavated from archaeological sites in hokkaido, Nihon Eiseigaku Zasshi 68 (2013) 53–57, https://doi.org/10.1265/jjh.68.53.
[72] N. Álvarez-Fernández, A. Martínez Cortizas, O. López-Costas, Atmospheric mercury pollution deciphered through archaeological bones, J Archaeol Sci 119 (2020), https://doi.org/10.1016/j.jas.2020.105159.
[73] Moki Yamada, S. Tohno, Y. Tohno, T. Minami, M. Ichii, Y. Okazaki, Accumulation of mercury in excavated bones of two natives in japan, Sci. Total Environ. 162 (1995) 253–256, https://doi.org/10.1016/0048-9697(95)04435-4.