A possible case of acquired syphilis at the former Royal Hospital of All-Saints (RHAS) in Lisbon, Portugal (18th century): a comparative methodological approach to differential diagnosis

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Abstract
Between the years of 1999 and 2001, during the excavation of the Praça da Figueira (Lisbon, Portugal), several human osteological remains from various chronological periods were discovered. Amongst them several skeletons are known to be related with the *Hospital Real de Todos-os-Santos* (Royal Hospital of All Saints - RHAS), which had an important role . The hospital history begun in 1492 and ended in 1755 largely as a consequence of the Lisbon earthquake. Of the skeletons exhumed, one in particular, the adult female Sk. 1310 showed significant pathological changes. The bone lesions characterized by new bone deposition, with a symmetric and disseminate pattern, were found in the upper limbs, distal end of femurs and in tibia and fibula diaphyses. A bowing deformity with "sabre shape" morphology was also observed in the tibiae. The most striking lesions, characterized by healed nodular cavitations and similar to those of caries sicca, were recorded on the frontal bone. Considering the value of a complete description, as well as the application of multiple lines of enquiry for a reliable differential diagnosis, three distinct techniques were applied and compared: visual examination, imagiology and histology. The results showed that the macroscopic analysis coupled with conventional X-ray analysis were fundamental to obtain a possible diagnosis of acquired syphilis. In contrast, the CT-scan and the histological analyses were less informative. The application of a new scoring system also supports a diagnosis of acquired syphilis. This case-study constitutes the first evidence of syphilis associated with the RHAS, supporting historical data on the pivotal role that this hospital had on the treatment of several conditions, namely, syphilis.

Key-words: paleopathology, syphilis, archaeology, imagiologic and histologic techniques, Royal Hospital of All Saints (RHAS)

Introduction

The differential diagnosis in paleopathology depends on a careful evaluation of the type of bone abnormalities observed, their distribution, as well as some knowledge regarding the disease process and the physiological factors that affect the body's response to disease (Ortner, 2003 and 2011a). However it is well known from the paleopathological literature that the bone response to disease is limited to abnormalities of size, shape, density, bone formation and bone destruction (Ortner, 2003, 2011a and 2012; Roberts and Manchester, 2005). This fact means that disease diagnosis in paleopathology is not as straightforward as in medicine (Larsen, 2002). As a consequence, many conditions may be under-represented or unrecorded since they may not have produced visible bone changes (Zimmerman, 2004; Dutour, 2008; Ortner, 2011b). Even in those disorders that typically involve the skeleton, individuals are not equally affected (Ortner, 2011a). Moreover, a number of different bone conditions may coexist at the time of death, which makes differential diagnosis difficult (Ortner, 2011a). The reliability of paleopathological diagnosis also depends on the methods used. Although the importance of using multiple lines of inquiry in the analysis of skeletal remains is well recognized, the majority of studies are based on visual inspection (Grauer, 2008). This reality may lead to simplistic and misleading interpretations, since different conditions, acting solely or in synergistic interaction, may produce the same pattern of bone lesions (Wood et al., 1992; Grauer, 2008). In fact, only a few conditions that affect the skeleton leave pathognomonic traits that allow for a positive diagnosis (Waldron, 2007). The main solution advanced by several authors (e.g. Ortner, 1991 and 2003; Buikstra, 2010; Ortner, 2011a and 2011b; Ragsdale and Lehmer, 2012; Wanek et al., 2012) relies on the application of more accurate and diversified techniques for the description and diagnosis of pathological conditions. Ortner (2011a and 2011b) states that an effective collaboration between skeletal paleopathology and the medical knowledge derived from orthopaedic pathology and radiology is required to improve the disease description. Radiological imaging procedures contribute significantly to the diagnosis of certain paleopathological conditions (e.g., trauma, Paget disease), as it enables direct comparison of lesion morphology with that visualized in modern clinical practice (Mays, 2012; Wanek et al., 2012). Furthermore, the application of biomolecular and histological techniques may be very useful in clarifying the nature of bone changes (Wright and Yoder, 2003). For example, histological analysis may be especially important for examining the degrees of bone healing, as well as, to identify traces of disease in cases where little bone response occurred prior to death (Wright and Yoder, 2003), and/or to support inferences made from macroscopic analysis (Mays, 2012). Wanek and co-authors (2012) emphasize that for a good differential diagnosis one must have to use as many approaches as possible in order to carefully evaluate a particular bone lesion. In fact, the combination of visual examination and descriptive analysis with modern diagnostic techniques, such as radiology, histology, immunology, and more recently ancient DNA, is nowadays claimed as to be responsible for the contemporary scientific character of paleopathology (Aufderheide and Rodríguez-Martín, 1998; Mann and Hunt, 2005).

Considering these recommendations, this article introduces the case of a female skeleton with a pattern of bone lesions compatible with a diagnosis of acquired syphilis. The remains were exhumed from the former necropolis of the Royal Hospital of All Saints (RHAS) of Lisbon, Portugal. Through a combination of three distinct methods, this research aims: (1) to compare the role of different techniques in the description and differential diagnosis of bony lesions; and (2) to discuss the importance of the skeletal evidences of disease in the reconstruction of the historiography of the Royal Hospital of All-Saints, Lisbon (Portugal).

Material and Methods

The Royal Hospital of All Saints (RHAS) was founded in the year of 1504 by the Portuguese prince D. João II (1455-1495) and aimed to modernize the medical assistance provided to the population, replacing the fragile network of health assistance mostly guaranteed by small hospitals and other institutions of communal residential care, such as hostels (for homeless and pilgrims), leper houses and hospices (Ramos, 1993; Panarra, 1994). The RHAS was in function until 1755, date of one the most significant Lisbon earthquake (Ramos, 1993).

The former location of the RHAS, nowadays occupied by the Praça da Figueira square (downtown Lisbon), was last intervened between the years 1999 – 2001 so that an underground parking lot could be built. During the archaeological survey circa of 15 primary inhumations without coffin or clothing evidences were excavated. The location of the area where the material was exhumed, alongside the material evidences collected within the funerary spaces and the stratigraphy of the location support that the remains belong to the 18th century. This data was provided by the archaeologist responsible for the excavation of the site, which fell under the responsibility of the Museu da Cidade, of Lisbon.

Among the skeletons recovered there was a relatively well-preserved young to middle aged adult female individual (Sk. 1310). Apart from the intact frontal bone, the remaining skull vault and facial bones exhibited some sort of postmortem breakage. Only four teeth with no atypical color or morphological changes were observed *in situ*: the lower right incisors and the upper left canine and 1st premolar. Additional postmortem changes were observed in the ribs, vertebrae, upper extremity of the humerii and radii, lower extremity of the ulnae and right fibula, and upper and lower extremity of the left fibula. The sexual diagnose was performed based on the analysis of the coxae according to Bruzek (2002) proposed method. Complementary analyses were performed according to methodologies described in Buisktra and Ubelaker (1994) as well as in White and Folken (2005). Due to the growing discussion with regard to the reliability of methods for adults' age at death estimation (Hens *et al,* 2008; Mulhern & Jones, 2005) only a crude age at death estimation was performed, taking into account the lack of significant auricular surface changes (hipbone) (Lovejoy et al., 1985) and overall skeletal elements degeneration, with particular attention to the articular facets.

The skeleton under scrutiny showed significant osteological changes throughout the skeleton, all of which of pathological origin. The bone lesions were first observed by naked eye and then recorded in a visual recording form according to its side, location and bone portion affected, extent and type of lesions observed (proliferative, destructive or both) and degree of bone healing. Conventional X-ray and CT-scan techniques were applied to infer the extension, and severity of the lesions on the lower limb bones (i.e. femurs and tibiae) and on the skull (i.e. frontal bone). To evaluate and characterize the microstructure of the bone lesions, a distal left fibula bone sample showing periosteal lesions was collected and prepared for histological analysis. The criterion that guided the bone sampling was the presence of postmortem breakage. By means of a modified version of the protocol developed by Fitzgerald and Saunders (2007), the bone specimen was cleaned in multiple sonic baths, first in tap water and than in alcohol, embedded in epoxy resin and then cut with a slow speed saw. The bone section produced was then polished using a grinder-polisher device and analyzed by light and polarized microscopy.

The differential diagnosis of the bone lesions observed at macroscopic, imagiologic and histological level was conducted following the pathological features summarized in Table 1 and present in the literature. For the study of treponemal diseases, it was also employed the scoring criteria proposed by Harper et al. (2011), which is based on Hackett's (1976) standards for diagnosing treponematosis in dry bone remains (Table 2).

Results

Macroscopic, radiographic and CT-scan analysis

The macroscopic analysis of the Sk. 1310 showed the presence of multiple bone lesions characterized by periosteal new bone deposition with a bilateral and disseminate pattern localized in the frontal and right parietal bones, upper limbs, distal end of the femurae, tibiae and more slightly in the fibulae shaft (Figure 1). Apart from tibiae that exhibited a generalized involvement of the entirely diaphyses, major foci of bone lesions were noticed in the lower third of the shaft of the remaining long bones. Of the proliferative type, the bone lesions observed were characterized by dense and compact layers of new bone, pinpointed by scattered area exhibiting estriae, pitting and nodule. Bellow a complete description of the lesions observed by bone piece is provided:

1. Skull: multiple radial scars partially healed were observed in the outer surface of the frontal bone, mainly in its anterior central portion, and in the right parietal bone. The lesions were formed by irregular depressions of distinct sizes and shapes, and smooth contours, crisscrossed by stellated grooves. Small islands or nodules of bone were seen in-between grooves. Extensive porosity in the outer surface of the frontal bone was also visible (Figure 2 A and B). A score of 4 was obtained after the application of the diagnosing criteria proposed by Harper et al. (2011). The CT-scan analysis of the frontal bone revealed a marked irregular relief caused by punctuated grooves (Figure 3 A and B). A slight bone radiopacity extending from the coronal suture to the anterior portion of the frontal bone was observed during radiographic scrutiny.

2. Humeri: periosteal reactions in distinct stages of bone remodeling were observed in the posterior portion of the distal extremity of the humeri. In the distal end of left humerus, new bone deposition with a porous appearance was seen (Figure 4 A and B).

3. Radii: symmetrical new bone deposition was observed in the distal end of radii especially in its anterior surface. In the left radius, periosteal lesions exhibiting a porous surface and signs of bone healing were observed (Figure 5 A and B).

4. Femora: two-sided bone expansion was noticed on the lower portion of the midshaft, more conspicuous in the right bone. In the posterior surface of the femoral diaphysis, smooth periosteal irregularities were observed. Both femora showed evidences of periosteal new bone remodeling. In the left femur, slight parallel and longitudinal bone striae with scattered pitting were noticed (Figure 6 A, B and C). The radiographic analysis showed a massive bone thickness of the lower portion of femora with subsequent narrowing of the medullar cavity. An increase radiopacity of the cortical bone with a rough and irregular appearance was observed at endosteal level (Figure 7).

5. Tibiae and Fibulae: Extensive bone expansion with anterior bowing of the shaft was observed in both tibiae. This bending deformity conferred to tibiae a *sabre* shape appearance. At periosteal level, new bone deposition combining smooth areas with striae and scattered pitting was observed along the tibia antero-medial and lateral portions, respectively. In the left tibia, localized bony nodes and plaques were seen in the lateral portion of the diaphysis (Figure 8 A, B and C). Under radiograph analysis, a widened cortex with a narrowed medullar cavity was observed. Furthermore, a demarcating line between the older cortex and the new appositional bone was seen. On both fibulae, new bone deposition with a remodeled appearance was noticed along the bone shaft. The lesions observed conferred to the bone surface an irregular and striated appearance.

The application of Harper's and co-authors (2011) scoring system resulted in a score of 3 for the appendicular skeleton.

Histological study

Despite the apparent well-preservation at a macroscopic level, the magnification of the fibula bone section revealed massive diagenetic changes at microscopic level, and a lack of bone birefringence (Figure 9). The only microstructures preserved were Haversian canals, some of them showing large spaces of bone remodelling, and a few recognizable shadows of Haversian systems. No system of lamellae or osteocyte lacunae was seen. Instead, the cortical bone was filled with an indistinct mass of composites probably resulting from microbial and fungal activity. The periosteal outline showed a mosaic of patterns: a section had a wave-like and round morphology, whilst in another section the new bone deposits had a finger-like or thorny morphology as illustrated in Figure 9. In some areas a wavy-like pattern of new bone, packed between periosteal blood vessels and separated from the underlying cortex by resorption spaces, was also identified.

Discussion

In the last decades, paleopathologists have re-focused their research interests moving from a descriptive case-study approach toward one based on the analysis of patterns of health and disease at a population level (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Mann and Hunt, 2005; Grauer, 2008, Mays, 2012). In both trends, a rigorous differential diagnosis of the specimens under study is desirable. As pointed out by Mays (2012), a reliable differential diagnosis performed at the individual level is the foundation to assess the prevalence rate of a certain condition in past population groups. In paleopathology, it is relatively straightforward to classify a bone abnormality in at least one of the major categories of skeletal disorders, such as trauma, infection, tumor, among others (Ortner, 2012). The major challenge, and a potential source of error, resides in trying to achieve a more specific diagnosis (Ortner, 2012). Zuckerman and co-authors (2014) have recently alerted to the need of bridging methodological rigor based on standardize criteria to diagnose diseases, to the presentation and evaluation of data. When this endeavor is reached and a positive diagnosis is achieved, an enormous contribute to the history and evolution of diseases and how past humans have coped with it, arises.

In this case-study, the visual inspection and the application of imaging techniques, especially conventional X-ray, were pivotal to identify a group of bone features compatible with a chronic infectious process diagnosed as treponematosis (possibly acquired syphilis). The application of multiple approaches has also allowed discarding other pathologies such as Paget's disease and leprosy (Table 1). For example, none of the bones of the feet and hand showed concentric atrophy, nor truncation of the phalanges as expected in cases of leprosy (Ortner, 2003); also, no marked bone osteolysis (Ortner, 2003; Brickley and Ives, 2008; Mays, 2008; Chow, 2009) or extreme thickening of bones from the crania (Zimmerman and Kelley, 1982) that characterizes, respectively, the first and last stages, of Paget's disease were recorded. Other Pagetic features such as a true lateral and anterior long bones bowing, osteoporosis circumscripta and "cotton wool" skull bones were also absent (Parson, 1980; Zimmerman and Kelley, 1982; Ortner, 2003; Brickley and Ives, 2008; Mays, 2008). Furthermore, conditions as tuberculosis are improbable if we consider the skeletal lesions' distribution and morphology characterized by a predominance of erosive lesions over proliferative ones, especially in vertebrae and long bone joints (Aufderheide and Rodríguez-Martín, 1998; Ortner, 2003; Zimmerman and Kelley, 1982; Ortner, 2008). The widespread location of the bone lesions and the absence of cloacae also rules out osteomyelitis as a probable diagnosis (Ortner, 2008), despite the enlarged Sk. 1310 femurae and tibiae shafts. Within treponematosis, and although yaws and bejel may produce bone changes similar to the ones recorded, these may be exclude as probably cause due to their particular geographical distribution and endemic nature (Aufderheide and Rodríguez-Martín, 1998). The overall bone

lesions may also be observed in cases of congenital and acquired syphilis (Ortner, 2008). However, the absence of Hutchinson's teeth and of a true bending deformity in tibiae favors acquired syphilis (Waldron, 2009) as the most possible aetiology of the lesions observed.

Acquired syphilis is a chronic treponematosis characterized by three developmental stages (Hackett, 1976). Noticeable skeletal lesions, mostly in the tibiae and skull, are indicative of the tertiary stage of the disease. Cranial vault lesions (also termed caries sicca) similar to those observed in the skull of Sk. 1310 are normally considered pathognomonic of acquired syphilis. The application of the scoring criteria (acquired syphilis) developed by Harper and colleagues (2011) also supports the etiology of the changes: the cranial vault lesions were scored 4 in 5 (presence of lesions specific to treponemal disease), and the long bone lesions were scored 3 in 5 (presence of lesions suggestive to treponemal disease on multiple skeletal elements), since no superficial or metaphyseal cavitation were observed on the long bones. In the tertiary stage of the disease, long bones tend to exhibit both gummatous and nongummatous alterations. Althought, the *gumma* is the most characteristic lesion; nongummatous lesions that include periostitis, osteitis and osteoperiostitis are more frequent. Periosteal new bone formation (PNBF) may be composed of woven bone, but most frequently it consists of compact bone, reflecting the chronic nature of the condition (Ortner, 2008). A pattern consisting of reactive bone formation of lamellar type is commonly encountered in tibia (Ortner, 2008). Due to the massive bone deposition, tibiae may assume an abnormal shape - saber shin. Despite the absence of *gumma*, all of the abovementioned bone features, namely the presence of striate and rugose nodes, expansion and deformity were observed in the tibiae of the Sk. 1310.

Albeit not specific, the X-ray bone features supported a diagnosis of acquired syphilis. According to Chhem and Brothwell (2008), in acquired syphilis, the bony changes of osteities and osteoperiostitis appear in the form of radiolucent and sclerotic areas associated with some increase density at the periosteal zone. In fact, the application of conventional X-ray analysis was determinant to confirm the non-structural origin of the tibiae deformity, which seems instead to be the result from an abnormal accumulation of remodeled newly built bone. The same can be mentioned with regard to the diaphyseal enlargement observed in the lower portion of femurae, which lead to exclude Paget disease from the differential diagnosis. The use of conventional X-ray techniques was also valuable to exclude the presence of hidden osteolytic lesions linked with the case of syphilis or indicating the presence of co-morbilities.

In comparison with conventional radiology, only minor contributes regarding the surface relief and distribution of caries sicca were added by computed tomography (CT-scan). Identical conclusions can be drawn from the application of histological techniques in the study of a fibula sample. The postmortem damage observed at microscopic level had an enormous impact on the analysis and description of the bone lesions of Sk. 1310. Despite the intact contours of the bone segments under analysis, it was impossible to characterize their periosteal and intracortical microanatomy, and infer or corroborate the aetiology of the bone lesions. Nevertheless, it was possible to distinguish on the periosteal surface new bone deposits ranging from a round to a thorny morphology. Furthermore, a wavy-like pattern of new bone packed between spaces of former periosteal blood vessels and separated from the underlying cortex by resorption spaces was also identified. This last bone feature resembles "polster" structures described by Schultz (1994, 2001, 2003 and 2012) as being associated with syphilis. Unfortunately, only the contours of the periosteal bone were preserved, which made confirmation of the orientation of the collagen fibers impossible. Schultz has defined polsters as a "pillow-like" structure of new bone that develops at the external surface of the long bones (periphery of the cross-section) and only within some parts of the circumference. In tertiary syphilis, these histological features are caused by a relatively slow growing process (Schultz, 2012). Other microscopic features that have been associated with treponemal

conditions, and that were taken into consideration when performing the microscopically analysis of Sk.1310 bone segments, are the "grenzstreifen" and the "sinous lacunae". Grenzstreifen is defined as a boundary line of varying thickness located between the original cortical surface and the secondary pathological new bone deposition in a subperiosteal position (Schultz, 2001, 2003, and 2012). It is a remnant of the external circumferential lamellae that is preserved due to the relatively slow growth of the PNBF (Schultz, 2012). As a consequence, it may present a reduction in their size and shape (Schultz, 2012). The "sinous lacunae" resembles a resorption lacuna, and it is a structure normally found between the cortical surface and the new layer of PNBF (Schultz, 1994). Some of these microscopic changes were also observed by von Hunnius and co-authors (2006) in two Pre-Columbian England cases of acquired syphilis. In the current study, apart from the atypical outline of the periosteal reaction that resembles "polsters" and some resorption spaces, no further microscopical pathological bone features (e.g. grenzstreifen) were observed in the fibula segment of Sk. 1310, which has affected our ability to make statements about the disease progression. It should be stated that the specificity of these histological features have been recently challenged by several researchers (e.g. Weston, 2009; Van Der Merwe et al. 2010). Although the minor ads introduced through histological analysis, the visual examination and the X-ray analysis were fundamental to establish a possible diagnosis of acquired syphilis, which was greatly helped by the preservation of key-bone elements (i.e., skull and tibiae) for diagnosing treponematosis.

The paleopathological literature has many descriptive cases of possible treponematosis identified worldwide. Amongst them, and regarding congenital syphilis, one can highlight the works of Pàlfi and colleagues (1992); Mansilla and Pijoan (1995); Malgosa et al. (1996); Hillson et al. (1998); Jacobi and Cook (1992); Erdal (2006); and concerning the manifestations of acquired syphilis the research of Stirland (1991); Buzhilova (1999); Mays and colleagues (2003); Bouwman and Brown (2005); Lefort and Bennike (2007); von Hunnius and co-authors (2007) are some of the references to bear in mind. Numerous studies have also been published with regard to the geographic and chronological origin of syphilis, with emphasis to its possible association (or not) with the arrival of Columbus to the New World (e.g. Baker et al., 1988; Rothschild et al., 2000; Saunders et al., 2000; Powell and Cook, 2005; Rothschild, 2005; Harper et al., 2008; de Melo, 2010; Harper et al., 2011; Armelagos et al., 2012; Mays et al., 2012; Rissech et al., 2013; Schaffer and Carr, 2013; Zuckerman et al., 2014).

In the Portuguese paleopathological record, a treponematosis case study was firstly reported by Lopes and Cardoso (2000) with regard to the bone lesions observed in a right femur and fibula (eventually belonging to the same individual) identified in an ossuary from the Igreja do Convento do Carmo (C. 1500-1800 AD), in Lisbon. Also referring to the city of Lisbon, Codinha (2002) has described possible cases of venereal syphilis identified in two adult male and female skeletons exhumed from the necropolis located in the ruins of the Igreja do Convento do Carmo (16th-18th centuries). According to the author, both individuals exhibited remodeled osteolytic lesions in skull compatible with those of caries sicca, as well as an extensive involvement of the long bones from the upper and lower limbs (Codinha, 2002). Souza and co-authors (2006) also identified a possible case of congenital syphilis in the mummified remains of a young girl (~18 months) buried in the crypt at the church of Sacramento, Lisbon (18th century). The authors describe not only the dental and skeletal lesions compatible with congenital syphilis, but also some mercury-induced dental changes consistent with the treatment of the condition used at that time (Souza et al., 2006). Another case of a 19th century syphilitic individual was diagnosed by Lopes and colleagues (2010) in a female skull (number 282) from the Medical School Collection housed at the University of Coimbra, in Coimbra (Portugal). This female was an identified individual, with a known

cause of death listed as "hypertrophic cirrhosis of the liver". However, the extensive lytic lesions found in the skull, alongside the typical caries sicca pattern led the authors to discuss the association of these bone changes with a diagnosis of syphilis, and not necessarily with cause of death (Lopes et al., 2010).

The lower number of cases, associated with Portugal, contrasts with historical evidences that described syphilis as major social burden, and a significant source of health concerns in Portugal during several centuries (Sousa, 1996). From the 15th century onwards, and during the Portuguese maritime expansion (known as *Período dos Descobrimentos*), Portugal capitalized the development of Europe through the discovery and exploration of new continents and resources. The city of Lisbon was a pivotal reference for the main European trading routes. As a consequence, the Portuguese population grew, which in turn favored the emergence of epidemics (Sousa, 1996; Pacheco, 2008). In spite of the decreasing number of some conditions (e.g. leprae), others became more frequent such as tuberculosis, smallpox, malaria, as well as cases associated with dysentery and with the Black Death and last, but not least, was syphilis (Pacheco, 2008). Eighteenth-century documents state that syphilis, in addition with cholera and yellow fever, were common diseases and relevant health issues of the time in the city of Lisbon (Souza et al., 2006).

When studying a particular chronological period or funerary context, such as a hospital, the analysis of historic texts can help in the interpretation of uncovered bone conditions (Mitchell, 2012). In relation to the Royal Hospital of All-Saints (RHAS), it is known from historic sources that the hospital was organized in four major wards, which were sex specific: one was dedicated to the treatment of wounds and "fevers" in women, and two others addressed male illnesses (Pacheco, 2008). Additionally, the hospital had another sex-specific ward which was solely dedicated to the treatment of syphilis, at the time also called as the "*mal gálico*" (Sousa, 1996; Pacheco, 2008: 52). The social relevance of syphilis was such that in 1539 a treatise about syphilis was published by Ruy Dias D' Ysla, a Spanish physician who had worked at the RHAS (Sousa, 1996). According to his chronicles, between the years of 1511 and 1532 a total of 20,000 syphilitic patients were treated at the RHAS (Sousa, 1996). Nevertheless, and among the skeletons recovered, only the adult female Sk. 1310 possesses lesions compatible with acquired syphilis. The disease information that we obtain from historic texts was coined by Mitchell (2011: 82) as a "social diagnosis", in the sense that the diagnosis was a "label" given by people in the past, which may be different from the modern view of disease. Presently, it is known that the pandemic of syphilis was not only caused by syphilis but also by other venereal diseases misdiagnosed as syphilis (Forrai, 2011). In fact, only in the 19th century and in the beginning of the 20th century the causative agents of many sexually transmitted diseases, such as chancroid, gonorrhea and syphilis were isolated and treated as separate diseases (Forrai, 2011; Maatouk and Moutran, 2014). Before that, they were considered a same entity, which might have caused misunderstandings and mistakes in its identification, treatment and report (Forrai, 2011, Burg, 2012). If entangled with textual sources that allude to a particular condition, one adds biological evidences, the knowledge about the historic and social impact of that condition increases considerably. This ideal scenario was achieved with the present case-study. The osteological data retrieved from the analysis of the Sk. 1310 seems to be in agreement with the known historical data, adding a new dimension to the written historiography of the RHAS: it brings primary biological data to what were previously only written sources.

Conclusion

A complete description of the bone lesions and the application of multiple lines of enquiry are key-points for a consistent differential diagnosis. In the present case-study we have used and compared three distinct methods – visual inspection, imagiological and histological

techniques – in order to assess the etiology of the bone lesions observed in an adult female skeleton associated with the former RHAS. An original scoring system developed by Harper and co-authors (2011) was also used to differentiate among treponemal conditions. The macroscopic analysis, attending the distribution, side, extent and type of bone lesions, coupled with conventional X-ray analysis revealed to be fundamental to attain a possible diagnosis of acquired syphilis. In contrast, the CT-scan and the histological analyses were less informative. It should be mentioned that the histological study was severely conditioned by the presence of diagenetic changes, whose pervasive action is difficult to overcome or avoid in any paleopathological analysis. In addition with the possibility to perform a more precise diagnosis, a reality not always attainable in paleopathology, this case-study also represents the first evidence of syphilis associated with the Royal Hospital of All Saints (RHAS). It serves as the remaining biological testimony, not only of a major social burden that has plagued Lisbon during at least four centuries, but also corroborates the pivotal role that the RHAS had on the treatment of syphilis in Portugal; a function that until now was only known from written records.

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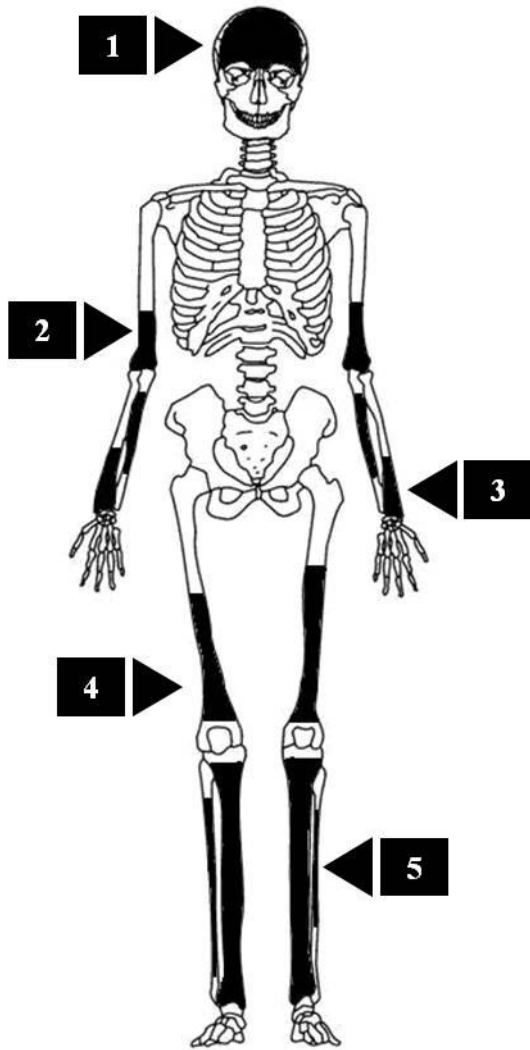
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644 Figure 1. Distribution of the most distinctive bone lesions observed in the individual Sk. 1310
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Figure 2. A: frontal bone of Sk. 1310 showing multiple bony scars with a radiate morphology and partially healed. B: Close-up of a small radial scar with a bone nodule and smooth contours.
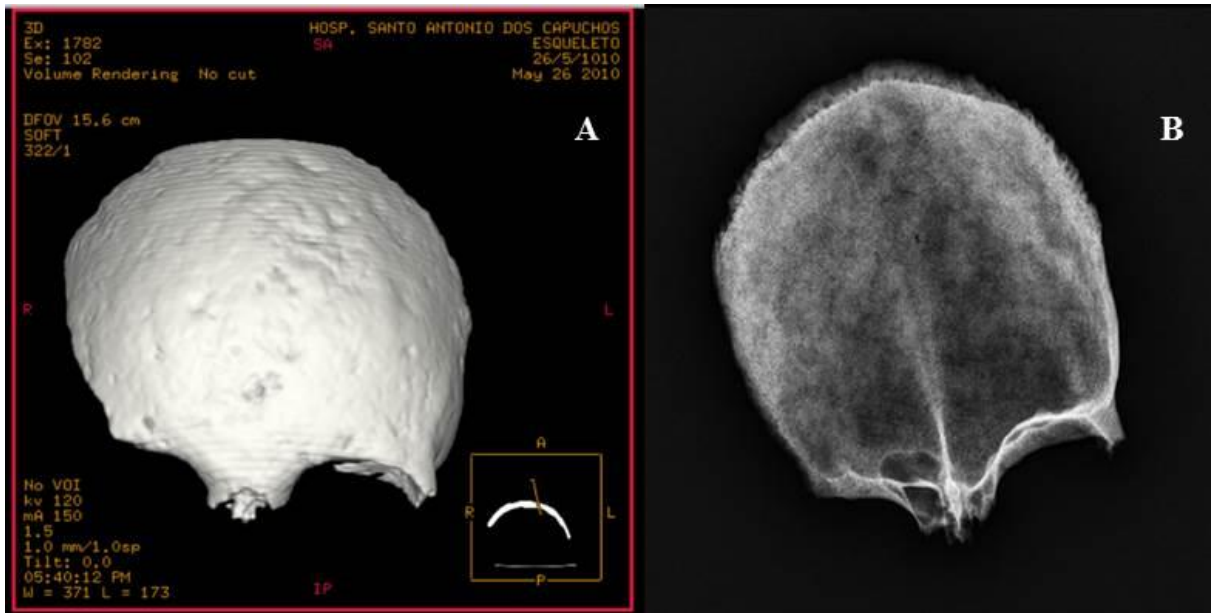
Figure 3. A: CT-scan of the lesions observed at the outer surface of the frontal bone of Sk. 1310. B: Radiograph of the frontal bone showing some degree of bone radioluscence.

Figure 4. A: Humerae showing periosteal new bone formation (PNBF) in different stages of bone remodeling at the distal end. B: Close-up of the periosteal reaction observed on the posterior-inferior portion of the left humerus shaft. Note the porous appearance of the lesions.

Figure 5. A: Bilateral PNBF observed at the distal end of radii. B: Detailed picture of the new bone foci exhibiting signs of bone remodeling observed in the left radius (anterior surface).

Figure 6. A: Bilateral expansion of the mid-lower portion of the femurae due to PNBF (anterior view). B: Posterior view of the affected femurae. Note the increased thickness of the shafts. C: Close-up of the left femur showing foci of new bone with a remodeling appearance and composed of fine parallel and longitudinal striae.
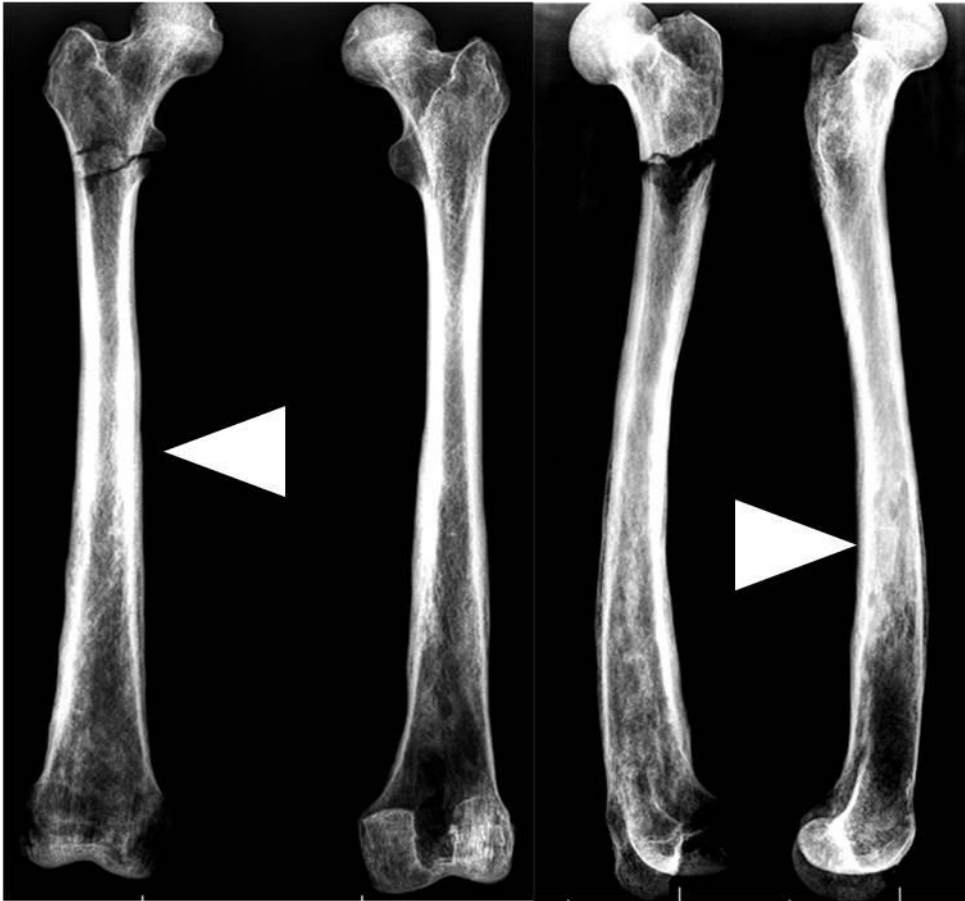
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810 Figure 7. Radiograph of femurae showing an increased radiopacity of the cortical bone. Note
811 the thickened cortex and the narrowing of the medullar cavity.
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Figure 8. A: Exuberant expansion of the tibiae due to periosteal new bone deposition with anterior bowing of the shaft (anteromedial and lateral view). B: Close-up of the left tibia showing multiple plaques and nodes of new bone in distinct stages of bone remodelling. C. Radiography of tibiae showing a widened cortex with a narrowed medullar cavity (anteriorposterior view).
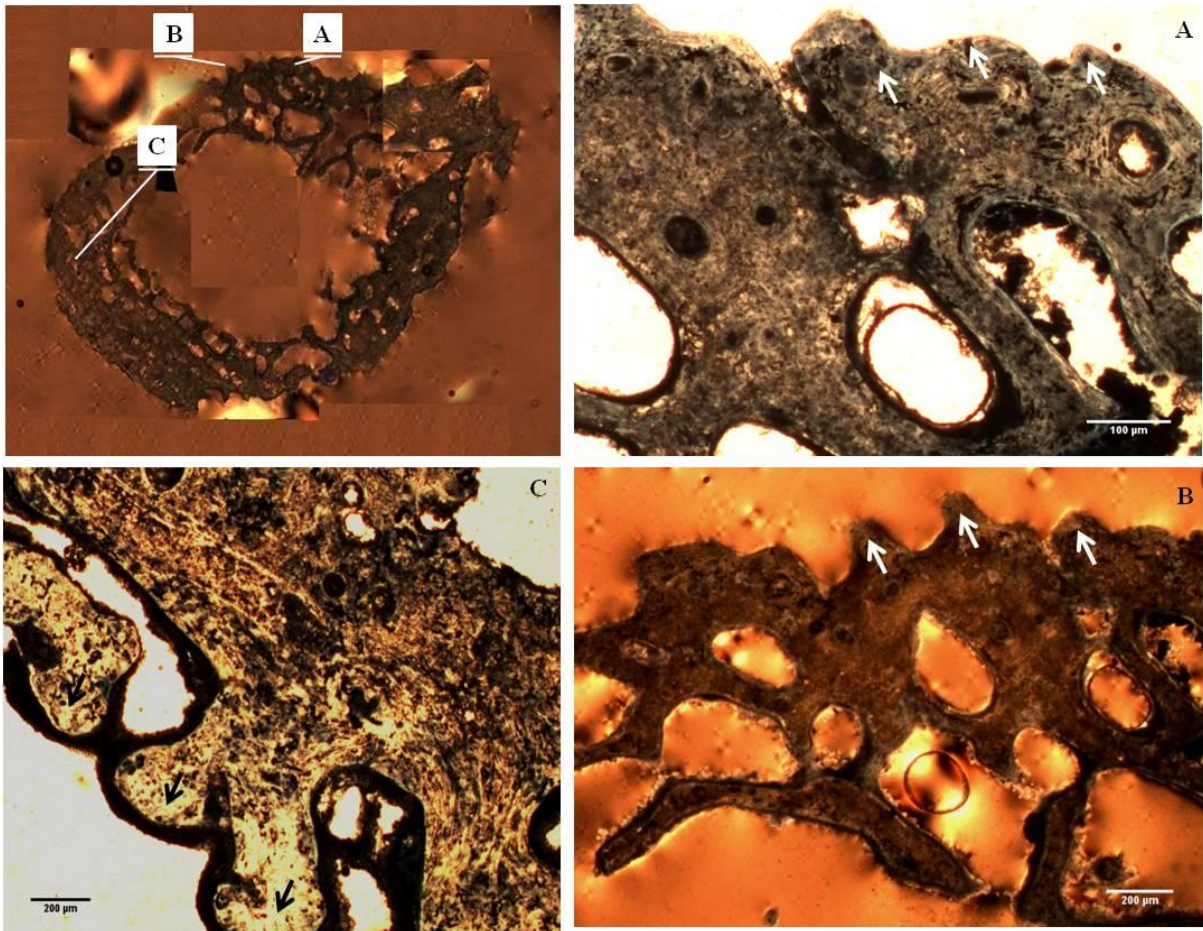
Figure 9. General microscopic view of the fibula thin section observed under polarized light. The bone contours and content seem to be intact however no birefringence is observed (Magnification 10x). A: Close-up of a segment of the fibula diaphysis observed under transmitted light exhibiting periosteal bone with a wave-like and round morphology (arrows). Note the preservation of enlarged Haversian canals and the lack of other histological features due to diagenetic changes (Magnification 40x). B: Magnification of another portion of the fibula diaphysis under polarized light. Observe the finger-like morphology of the periosteal new bone formation (arrows). Several Haversian canals some of them with enlarged spaces are preserved (Magnification 40x). C: Close-up of the fibula surface showing the new periosteal bone arranged in a wave-like pattern (arrows) that is separated from the underlying cortex by bone spaces. Almost no Haversian canals or other histological features are present. In contrast there is a proliferation of diagenetic changes (Magnification 40x).

Table 1. Macroscopic, radiologic and histological bone features considered in the differential diagnosis of Sk. 1310 adult female. (Macroscopic descriptions from: Aufderheide and Rodríguez-Martín (1998); Ortner (2003); Ortner (2008); Waldron (2009); and Zimmerman and Kelley (1992). Radiologic descriptions from: Adler (2000); Resnick and Kransdorf (2005); Burgener et al. (2006); Chhem and Brothwell (2008). Histological descriptions from: Schultz (1994; 2001; 2003; and 2012); Adler (2000); Weston (2004 and 2009); von Hunnius et al. (2006); and Brickley and Ives (2008). Legend: **Y** – yes, **N**-no, **No** – non-observable.

Pathology	Macroscopic features	Radiologic features	Histological features
Tuberculosis	• *Predilection upon the axial skeleton (spine) and joints* **(N)** • *Spine:* abscess formation (> anterior portion of the vertebral bodies); collapse of vertebrae - Pott's deformity **(NO)** • *Joints:* localized or diffuse lesions (regularly symmetrical), marginal erosion of the subchondral bone, massive joint destruction, subluxation and ankylosis (severe cases) **(N)** • *Skull lesions: inner table origin* **(N)** • *Predominance of destructive over formative lesions* **(N)**	• Large areas of translucency *(tuberculous cavities)* and pronounced osteopenia **(N)** • Periosteal reactions (PR) and osteosclerosis are uncommon. When present the foci are solitary (rarely multiple) and show a solid thin or thick appearance **(N)**	• Extensive destruction of spongiosa with few trabeculae preserved **(NO)** • Reduced or absent osteoclerotic response **(NO)**
Leprosy	• *Predilection upon facial bones and distal appendicular skeleton* **(N)** • *Primary bone involvement (skull):* rhinomaxillary syndrome or "facies leprosa" - atrophy of the anterior nasal spine; recession of the maxillary alveolar margin with possible antemortem loss of the incisors; inflammatory changes on the superior surface of the hard palate with thinning, pitting, or perforation **(NO)** • *Primary and secondary bone involvement (hand and foot):* bone resorption with little bone formation; pencilling of the metatarsals; arthritic changes; ankylosis (uncommon) and osteitis (rare) **(N)**	• Bone destruction **(N)** • Solitary or multiple foci of PR that may be solid thin or thick, or laminated **(N)**	• Presence of grenzstreifen and of several layers of new bone deposition at the same place **(NO)**

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	• *Primary and secondary bone involvement (tibia and fibula):* irregular deposits of subperiosteal new bone (>distal third of the diaphysis); prominent transverse striation and vascular grooves may cross the PR **(N)**		
Osteomyelitis	• *Presence of sequestra, massive bone apposition along the bone surface forming an involucrum, and several cloacae perforating the involucrum.* **(N)**	• Scattered radiolucent areas which may contain a dense bone sequestrum **(N)** • Solitary, rarely multiple foci of PR that may be solid thick, often undulating and cloaking **(N)**	• Extensive osteolytic bone loss and enormous osteoblastic bone reaction. **(NO)** • Presence of small foci of decayed bone matrix located within the region of the former original compact bone or in the area of the secondarily filled medullary cavity **(NO)** • Grenzstreifen and sinuous lacunae may be present **(NO)** • Presence of a clear line of separation between the periosteal new bone and the underlying cortical tissue **(NO)**
Acquired syphilis	• *Predilection (late stages): tibiae, cranial vault, perinasal skull bones, sternum, femur, fibula and osseous structures of the hand and foot* **(Y)**	• Presence of extended sclerosis and osteolytic defects (small and/or large),	• Presence of polsters: pillow-like newly built bone formations **(Y)**

Disease			
	• *Skull:* cranial vault gumma, especially in the frontal bone - stellate lesion called "caries sicca" **(Y)** • *Long bones*: proliferative periostitis (subperiosteal new bone formation limited to a part of the shaft or diffuse, leading to an increased thickening and bone deformation - *sabre* shaped tibiae); **(Y)** osteitis and osteoperiostitis (increased thickening with subsequent narrowing of the medullary cavity, especially in the tibia and femur) **(Y)**; and gummatous lesions formation **(N)**	sharply delineated and indenting the cortex **(Y)** • Extensive PR and cortical thickening (osteitis). Dense bony sclerosis with areas of destruction (gumma formation) **(Y)** • Localized or generalized foci of PR that may be solid thin or thick, often undulating and with squat spicules, or laminated **(Y)**	• Presence of grenzstreifen: a very fine or a narrow, band-like structure that represents the original external surface of the bone shaft **(NO)** • Presence of sinuous lacunae: located between the cortical bone and the pathological periosteal deposition **(NO)**
Congenital syphilis	• *Predilection (late stages - from infancy to adulthood - 5-20 y.o.): long bones (e.g. tibiae), skull and occasionally facial bones* **(N)** • *Skull:* destruction of the nasal bones ("saddle nose"), calvarial gumma, and Hutchinson's teeth **(N)** • *Long bones:* hyperplastic osteoperiostitis (fusiform new bone thickening that involves the middle third of the diaphyses. Endosteal bone proliferation with subsequent narrowing of the medullary cavity) **(Y)**; and gummatous osteomyelitis (destructive foci are normally localized at the sites of gummata) **(N)**	• The epiphyseal plates are faded, and seem to be detached from the brighter metaphyses **(N)** • Transverse striping of metaphyses and destructive lesions, initially involving the corners of the metaphyses **(N)** • Generalized and symmetric PR that may be solid thick or laminated **(Y)**	
Paget's Disease	• *Predilection: axial skeleton (e.g. lumbar spine), skull, pelvis and proximal femur* **(N)**	• *Skull:* osteoporosis circumscripta (early stages);	• Marked osteoclastic resorption and mosaic pattern of reversal

- *Skull:* patches of demineralization (early stages); cranial vault thickness (later stages) **(N)**
- *Vertebrae:* compression fractures **(N)**
- *Long bones:* True long bones bowing (anterior and/or lateral), coxa vara **(N)**

cotton wool – variable density (later stages) **(N)**
- *Vertebrae:* radiolucency or decreased density. Sclerosis of vertebral margins (vertebral frame) (early stages) **(N)**
- *Long bones:* "V-shaped" radiolucency (early stages). Wider and thickened bones (inactive stage) **(N)**

lines: bone resoprtion followed by bone formation (early stages) **(NO)**
- Increased amount of lamellar bone (intermediate stage) **(NO)**
- Sclerotic bone with decreased vascularity (inactive stage) **(NO)**

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901 **Table 2.** Score criteria applied in the diagnosis of treponemal bone lesions (adapted from Harper et al., 2011: 119).

Disease category	Score	Bone lesion criteria
Acquired treponemal disease	**0**	Lesions consistent with a nontreponemal process (e.g., taphonomic process, noninfectious etiology, etc.).
	1	Lesions consistent with treponemal disease on one or more skeletal elements (periostitis, tibial pseudo-bowing, polsters, grenzlinie).
	2	Lesions suggestive of treponemal disease on a single element [Hackett's (1976) on trial characteristics: finely striated nodes and expansions; coarsely striated and pitted expansions; and rugose nodes and expansions on long bones]; or stage 1–3 caries sicca lesions (clustered pits, confluent pits, focal superficial cavitation).
	3	Lesions suggestive of treponemal disease on multiple skeletal elements.
	4	Lesions specific to treponemal disease [Hackett's (1976) diagnostic criteria: stage 4–6 caries sicca lesions (serpiginous cavitation, nodular cavitation, and caries sicca) or nodes/expansions with superficial cavitations on long bones] on a single skeletal element.
	5	Lesions specific to treponemal disease found on multiple skeletal elements or in the presence of lesions suggestive of treponemal disease on other skeletal elements.
Congenital treponemal disease	**0**	Lesions consistent with a nontreponemal process (e.g., taphonomic process, noninfectious etiology, etc.)
	1	Lesions consistent with congenital syphilis (periostitis, high palatal arch, disproportionate maxillae and mandible, true tibial bowing).
	2	Lesions suggestive of congenital syphilis (Parrot's/Higoumenakia sign, flared scapulae, Fournier's/Mulberry molar).
	3	Lesions highly suggestive of congenital syphilis (Wimberger's sign, notched and tapering (Hutchinson's) incisors, Moon's molars).

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