RESEARCH ARTICLE

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Macromorphometric Analysis of Metastatic Lesions in Bones due to Breast Cancer: Contribution to Human Identification

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Abstract

Objective: This study aimed to report the metastatic lesions observed in identified skeletons whose deaths were caused by breast cancer complications to provide information and evidence that can be used in cases of human identification forensics. **Methods:** The research was conducted at the Centre for Forensic Anthropology Studies of the Faculty of Odontology of the University of Pernambuco (CEAF/FOP/UPE), Recife, Brazil. The data bank of the CEAF/FOP/UPE was searched for skeletons with the cause of death reported as due to breast cancer, resulting in five cases. The skeletons were arranged in anatomical positions and macroscopically inspected to register, describe and measure the lesions present to establish the macroscopic patterns of bone destruction caused by breast cancer. **Results:** Of the five skeletons, two presented metastatic lesions. In the first, lesions were observed in a disseminated form, affecting almost all bones. The lesions were predominantly osteolytic and ellipsoid-shaped; however, mixed and circular lesions were also found. The second skeleton presented four lesions of mixed characteristics. The finding of bone lesions in the macroscopic analysis of skeletons may reveal a more advanced stage of the neoplasm, as well as its dissemination in areas little rich in hematopoietic tissue, such as the diaphyses of long bones, a situation widely observed in the first reported case. **Conclusion:** Besides providing more excellent knowledge of their macroscopic presentation, bone metastatic lesions may act as an individualizing factor in human identification cases, narrowing the sample of possible victims.

Keywords: Breast cancer- neoplastic metastasis- bones- forensic anthropology

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Introduction

The skeleton is one of the most favorable sites for metastases from solid tumors (Akhtari et al., 2008). Despite being hidden and unapparent in the initial stages, metastasis develops devastatingly during the patient's care since, once the tumour cells have installed themselves in the bone, the disease usually becomes incurable (Biehler-Gomez et al., 2019). This event, frequent in patients with breast cancer, can be defined as the migration and growth of tumour cells to distant organs and is fundamental for the characterisation of malignancy.

There are three types of lesions in bone metastases: osteolytic, osteoblastic, and mixed. In osteolytic lesions, the tumoral tissue distorts the typical remodeling sequence, so there is osteolytic differentiation of hematopoietic cells and activation of mature osteoclasts, thus prevailing destructive lesions. On the other hand, osteoblastic lesions participate in the process of deregulated bone neoformation. Therefore, the respective destruction and deregulated bone formation findings will represent two ends of a spectrum, which may act concomitantly in mixed lesions (Akhtari et al., 2008; Biehler-Gomez et al., 2019).

In breast cancer, destructive lesions prevail, which are the most common causes of morbidity and mortality, considering that they can result in severe pain, pathological fractures, nerve compression syndromes, and hypercalcemia described as the most accelerated fatal complication. The mechanism of this bone loss occurs either by an increase in activated osteoclasts or by suppression of osteoblasts (Chen et al., 2010). Despite this prevalence, it is not uncommon to find patients who also present with osteoblastic and mixed lesions (Akhtari et al., 2008; Biehler-Gomez et al., 2019; Coleman & Rubens, 1987; Guise et al., 2005).

Hematogenous metastases of cancer cells result from a sequence of events favoring tumor cells' survival and proliferation. It is a complex, multistep process

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that involves the migration of cancer cells into the bloodstream from the primary site of the lesion, evasion of their immunological control, adherence to the vascular endothelium of distant organs, and extravasation in specific secondary sites (Guise et al., 2006).

Within the circulation, cancer cells from the primary tumour will encounter the vascular endothelium of the secondary tumour. In the trabecular bone, where breast cancer cells often migrate, there is a reduced rate of blood flow, facilitating the adhesion of cells to vascular surfaces. Once adhered, the cells can migrate towards chemotactic factors emanating from nearby trabecular bone cells. When cancer cells are closely juxtaposed to bone cells within the bone marrow compartment, which has a variety of cytokines and growth factors, tumour cells will provide a fertile environment by exchanging mutual biological information with bone tissue in order to establish bone metastasis (Akhtari et al., 2008; Mastro et al., 2003; Zhang et al., 2010).

Therefore, although the distribution of metastases to distant organs can be predicted by the anatomical distribution of blood flow from the primary site of the disease, specific properties of the tumour cells and the characteristics of the metastatic site determine where metastasis occurs in most cases. This theory was proposed by Stephan Paget in 1889 and confirmed by Liotta and Kohn in the following year (Liotta & Kohn, 1990).

The term “seed and soil” was used for this hypothesis to explain the phenomenon of tumour distribution to specific sites in the body: the bone environment has unique biological characteristics which allow cancer cells to survive, proliferate and destroy bone tissue. Then, cancer cells (seeds) successfully metastasize to bone tissue because this is a favourable environment (soil) for the growth of tumours (Zhang et al., 2010; Paget, 1889). Besides the steps mentioned above, some factors do not depend on the tumor, such as sex hormone deficiency. Still, they can influence bone resorption, contributing to this vicious cycle of tumor growth in the bone (Guise et al., 2006).

Tumour cells predominantly affect the highly vascularised areas of the skeleton, particularly the red bone marrow. This is feasible for haematological malignancies and all solid tumours. For this reason, the most affected sites in the skeleton are the pelvis, vertebrae, ribs, skull, and the extremities of long bones (Chen et al., 2010; Käkönen et al., 2003).

When there is no previous diagnosis, interpreting information on the human skeleton depends fundamentally on the precise differential diagnosis of diseases and how they behave in dry bones. However, it is also based on evaluating other variables contributing to understanding data about the condition in a skeletal sample. In situations in which an osteological collection has a previous diagnosis about the type of neoplasm that victimized the individual, the forensic anthropologist has the possibility of extracting as much information as possible, besides a detailed description of all the bone implications of the disease, to gather enough evidence that makes it possible to identify the substrates of the neoplasm based on the characteristics and, if possible, more accurate differential diagnoses (Ortner, 2003; Mays, 2018).

For this, it is fundamental to establish the biological profile of the individual, which comprises four parameters: sex, age, stature, and populational affinity, since knowledge of these data can have a substantial influence on the analysis of the various types of morbid conditions. For example, suppose it is possible to estimate the skeleton’s age, as a young adult, at the time of death. In that case, this data reduces the possibility that the possible lesions found are compatible with breast cancer since metastases affecting the skeleton as a preferential site are characteristics associated with individuals with a greater age range. Likewise, the analysis of a male skeleton with disseminated and destructive lesions leads to an almost unlikely, although possible, diagnosis of breast cancer. In addition, many diseases are specific to a population, further contributing to narrowing the identification process (Ortner, 2003; Mays, 2018).

Having previously known about the medical diagnosis of a disease affecting the skeleton, the focus will be on the analysis of the pathology, as well as on the detailed report of how the lesions behave in the bone, which sites are most affected, the epidemiological study in the population studied, which age range is most affected and, last but not least, which gender is most affected by the disease. Thus, it is possible to establish a differential diagnosis with other pathologies and individualize the skeletons analysed with similar biological profiles.

Therefore, this article aims to report and describe the bone lesions found in identified skeletons from northeastern Brazil, diagnosed during life with breast cancer, and whose deaths were caused by complications resulting from the neoplasm. It is intended, thus, to contribute to forensic anthropology for the differential diagnosis between skeletal neoplastic expressions and human identification forensics in the perspective of the observed condition acting as an individualization factor in forensic contexts.

Materials and Methods

Case Reports

From the database of the osteological collection of the Centro de Estudos em Antropologia Forense (CEAF/FOP/UPE), a multi-user laboratory linked to the Master’s Degree in Forensic Science at the Pernambuco School of Dentistry, Pernambuco University (FOP/UPE), the skeletons that presented as the cause of death the pathology outlined in this study were selected.

Initially, macroscopic analysis of the bones was performed to identify findings with characteristics compatible with metastatic bone lesions, which were established according to the description in the literature (Ortner, 2003). Those skeletons that presented the compatible lesions were separated for analysis. It was impossible to access the individuals’ medical data, as it would have been necessary to contact the respective families, information unavailable in the database.

The skeletons examined belong to the CEAF/FOP/UPE collection, located at the Faculty of Dentistry of the University of Pernambuco in Recife, Brazil. The entire collection is composed of skeletons from the Santo Amaro cemetery, located in Recife, inhumed from 2011 to 2016 and administratively exhumed from 2013 to 2018/2019 (Cunha et al., 2018). There are currently 427 skeletons, with inventory already completed within the collection, about which data regarding sex and age are available. In addition to this information, some present additional records, such as place of death, place of birth, cause of death, and profession (Carvalho et al., 2020).

The skeletons were arranged in anatomical positions and macroscopically inspected to register, describe and measure the lesions present to establish the macroscopic patterns of bone destruction caused by breast cancer. The affected bone regions were measured in their sagittal and transverse diameters, with a digital pachymeter, and later photographed.

Results

The database analysis selected five skeletons, as seen in Table 1. Two of the five skeletons found with breast cancer as the cause of death presented metastatic lesions on macroscopic examination. The description of these cases with their respective findings is as follows.

Case #1

The first case presented bone metastasis in large part of the skeleton. Some bones were incomplete or fragmented due to the disarrangement of the bone tissue during life, which was reflected in post-mortem fractures

Figure 1. Anterior View of the Skull of Case #1 Belonging to CEAF/FOP/UPE, Showing Metastatic Lesions due to Breast Cancer. On the right front-orbital region, an osteolytic lesion with surrounding osteolytic and osteoblastic foci is highlighted. Source: CEAF/FOP/UPE Archives.

and presenting taphonomic alterations.

Topographical distribution of the lesions
Cranial Injuries

The skull was intact, except for the bones comprising the left hemiface, which were absent due to post-mortem trauma. Although they were present in many skull bones, the lesions were more concentrated in the frontal bone and varied in size from 8.47 to 16.04 millimeters (mm) in their larger axes. When analyzing them, the presence of osteolytic and mixed lesions was verified.

a) Frontal bone

i) Right front-orbital region: osteolytic lesion with external and internal plate involvement, with maximum diameters equivalent to 11.72 mm x 15.80 mm. Around this bone expression, there were macroscopic foci compatible with osteolytic and osteoblastic bone reactions (Figure 1).

ii) Medial third: osteolytic macroscopic reaction, with a sieve-like aspect in which the holes converge, allowing its classification as a single lesion. The lesion has a more triangular shape, with sclerotic margins and an anfractuous aspect, with a maximum diameter of 6.40 mm x 5.07 mm (Figure 1).

iii) Mid-lateral third: the holes caused by the disease did not converge and, therefore, it was not possible to measure the lesion as a single one, but its aspect already demonstrated alterations in the healthy bone tissue and, for this reason, it was reported like the others. In the lesion conglomerate, scattered holes were observed in the periphery, becoming confluent in the centre (Figure 1).

iv) coronal suture: the lesion was medially located, reaching both bones (frontal and right parietal) of the respective suture. As a characteristic of the bone reaction present, it is noteworthy that the lesion has a predominantly mixed macroscopic aspect and, therefore, resulted from osteoblastic and osteolytic cellular activities, measuring about 11.33 x 16.04 millimeters in its largest diameters (Figure 2).

Parietal bones

Bone alterations caused by metastasis affected the sagittal suture and surrounding regions. One of the lesions, measuring approximately 11.80 mm x 13.52 mm in its largest diameter, perforated the external bone plate and presented irregular borders compatible with superimposed reactions of bone neoformation and destruction. Similarly, another adjacent lesioned site exhibited the same reactive characteristics, in which internal bone crests intertwined, filling spaces left by osteolytic destruction; however, this

Table 1. Information Regarding Sex, Age, Cause of Death, and Place of Death of the Skeletons Studied

Sex	Age	Cause of death	Place of death
Female	74	Metastatic breast cancer	Public hospital
Female	79	Sepsis, respiratory infection, and breast cancer	Private hospital
Female	73	Respiratory failure, bronchopneumonia, and breast cancer	Private hospital
Female	69	Advanced breast cancer	Public hospital
Female	51	Advanced breast cancer	Public hospital

Figure 2. Superior view of the Skull of Case 01 Belonging to CEAF/FOP/UPE with Metastatic Lesions Resulting from Breast Cancer. In the picture, a mixed lesion is located at the coronal suture affecting the right frontal and parietal bones. Source: CEAF/FOP/UPE Archives.

was smaller, measuring 9.03 mm by 8.56 mm in the largest axes (Figures 2 and 3).

Left mastoid process

An osteolytic lesion with irregular and anfractuous borders was observed, measuring 6.20 mm x 7.65 mm at its largest dimensions, located in the central portion of the left mastoid process (Figure 4).

Occipital bone

Two lesions with mixed characteristics, separated by the external occipital protuberance, were identified, which varied between 7.64mm and 15.24mm in their largest axes (Figure 5).

Mandible

The predominant bone reaction was osteolytic action

Figure 3. Posterior View of the Skull of Case #1 Showing a Lesion with Mixed Characteristics Observed at the Sagittal Suture. Source: CEAF/FOP/UPE Archives.

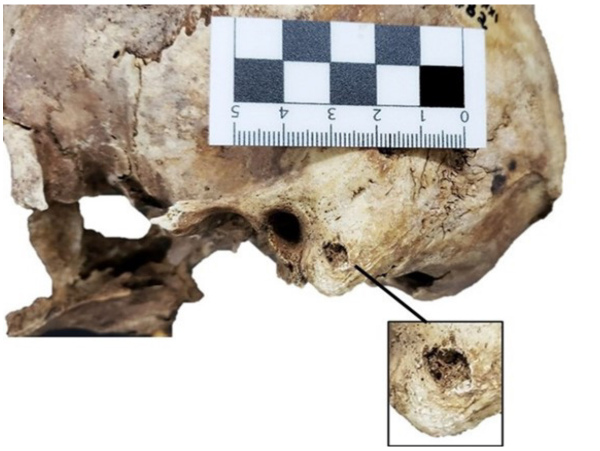

Figure 4. Left Lateral View of the Skull of Case #1 Showing an Osteolytic Lesion Located in the Left Mastoid Process. Source: CEAF/FOP/UPE Archives.

in the three regions, involving the outer cortical surface in the mentonian location, with irregular margins, measuring in its largest axes (6.20 mm x 3.80 mm) (Figure 6). The

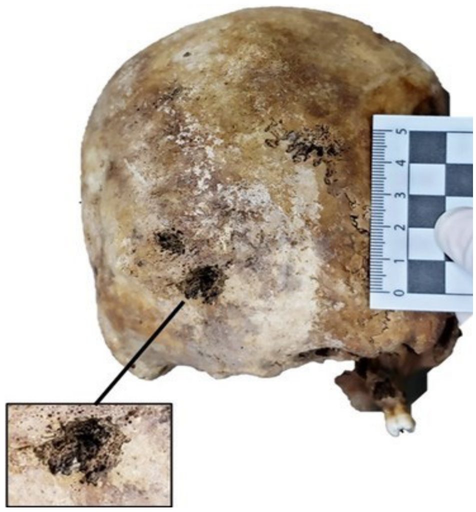

Figure 5. Posterior View of the Skull of Case #1 Showing Mixed Lesions Separated by the External Occipital Protuberance. Source: CEAF/FOP/UPE Archives.

Figure 6. Osteolytic Lesion Located in the Mentonian Region, Case #1, CEAF/FOP/UPE. Source: CEAF/FOP/UPE Archives.

Figure 7. Osteolytic Lesions Located on the Mandible Body. A) Osteolytic lesion located in the external cortical of the mandibular body. B) Osteolytic lesion in the internal cortical bone near the mylohyoid groove. Source: CEAF/FOP/UPE Archives.

mandible was affected in the region of the body (near the left ascending ramus) and the chin in the external and internal cortical surfaces. The two lesions present in the body region (near the ramus) had ellipsoid shapes, with the following maximum dimensions (external: 5.16 mm x 8.67 mm; internal: 3.11 mm x 5.81 mm) (Figure 7). The condyles also presented lesions similar to the aforementioned; however, because they showed taphonomic expressions, it was not possible to establish a macroscopic distinction between the taphonomic findings and the metastatic lesions.

Post-Cranial Injuries

a) Right clavicle

The right clavicle exhibited two metastatic alterations at the acromial extremity, of purely osteolytic appearance, with irregular and anfractuous margins, the largest measuring approximately 3.74mm x 5.20mm and 9.54mm x 15.82mm (Figure 8).

Figure 8. Osteolytic Lesions Located at the Acromial Extremity of the Right Clavicle. Source: CEAF/FOP/UPE Archives

b) Ribs

All the ribs presented taphonomic alterations. Therefore, it was not possible to specify whether the findings could result from an overlap with taphonomic elements or whether they were unequivocally due to metastatic alterations.

c) Vertebrae

Seven vertebrae were present in the skeleton, five cervical and two thoracics. Like the ribs, the vertebrae presented taphonomic alterations, so it was impossible to unequivocally confirm the metastatic lesions' presence.

d) Coxal bones

Due to taphonomic processes, the hip bones were incomplete, with the right ischium and pubis absent. The metastatic alterations observed in the iliac bones ranged from 4.54 mm to 21.03 mm, with a predominance of osteolytic reaction. Regarding the shape of the lesions, all of them were ellipsoidal, with irregular borders and no sclerotic reaction (Figure 9).

e) Humeri

In the right humerus, osteolytic lesions were located at proximal and distal ends, with measurements ranging from

Figure 9. Left Ilium Presenting Ellipsoid-Shaped Osteolytic Lesions. Source: CEAF/FOP/UPE Archives.

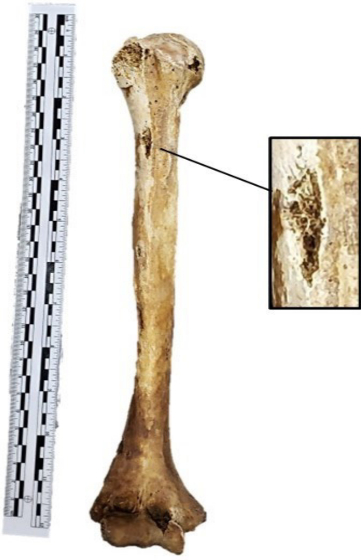

Figure 10. Anterior View of the Right Humerus Showing a Lesion with Mixed Features Located in the Proximal Third of the Diaphysis. Source: CEAF/FOP/UPE Archives.

3.46 mm to 14.26 mm at their largest axes (Figure 10). The left humeral head presented a loss of bone substance; therefore, it was impossible to register the possible alterations. The other four lesions were ellipsoid-shaped, with irregular margins and predominantly osteolytic macroscopic aspect, with maximum diameters ranging from 2.87 mm to 29.28 mm, and were sparsely located along the diaphyseal axis (Figure 11).

f) Radiuses

The right radius presented lesions in the middle third of the diaphysis, demonstrating osteolytic and osteoblastic

Figure 11. Anterior and Posterior Views of the Left Humerus, Case #1. On the left, a highlight of the osteolytic lesion is located in the middle third of the diaphysis; on the right, lytic lesions are located on the posterior surface of the bone diaphysis. Source: CEAF/FOP/UPE Archives.

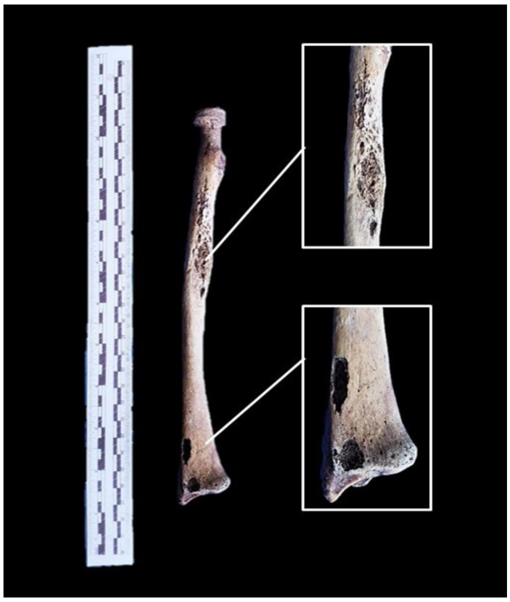

Figure 12. Anterior View of the Right Radius, Case #1. On the left, mixed lesion affects the proximal and middle thirds of the bone diaphysis; on the right, osteolytic lesions are located in the distal diaphyseal third and the distal epiphysis. Source: CEAF/FOP/UPE Archives.

reaction, with maximum axes measuring approximately 21.59 mm x 7.95 mm. On the other hand, the lower third was affected by a purely osteolytic lesion with an elliptical shape, in which length predominated over width, respectively: 14.46 mm x 5.01 mm (Figure 12). As for the distal epiphysis, two metastatic bone alterations were found, confluent in their lateral and medial margins, which did not prevent their separate measurement in their larger axes, respectively: 4.48 mm x 3.14 mm and 8.60 mm x 8.22 mm. All lesions presented irregular edges, similar to anfractuous and punched-out margins. In the left radius, lesions were observed at the proximal and distal ends and the middle third. The alteration visualized at the distal radius end also presented compatibility with taphonomy and, therefore, was not included in the list of metastatic lesions.

Figure 13. Right Tibia and Fbula, Case #1. In the highlight, an osteolytic lesion is located in the proximal third of the posterior face of the bone diaphysis. Source: CEAF/FOP/UPE Archives.

Figure 14. Anterior View of the Right and Left Femurs of Case #1. In the highlight, an osteolytic lesion is seen in the middle third of the diaphyseal bone. Source: CEAF/FOP/UPE Archives.

g) Tibiae and fibulae

In all four bones, it was possible to observe a predominance of lytic lesions; however, mixed lesions were also found. The right tibia and fibula presented a metallic shaft joining a fracture between the middle third of the diaphysis and the distal epiphysis. The fractures were not consolidated, as there were no signs of bone remodeling, justified by the space between the extremities comprised by the surgical rod (Figure 13 A).

The right tibia showed six lesions, the largest measuring 21.49 mm x 6.49 mm and 23.82 mm x 7.74mm, while the ipsilateral fibula showed three lesions measuring: 4.50 mm x 2.38 mm; 13.50 mm x 2.78 mm and 10.81 mm x 3.60 mm.

The left tibia exhibited ten lesions, the largest measuring about 28.90mm x 7.72mm and 24.03mm x 8.23mm. The fibulae (left and right) showed three lesions: 6.92mm x 1.70mm, 3.84mm x 2.92mm, and 23.97mm x 2.42mm) (Figure 13 B).

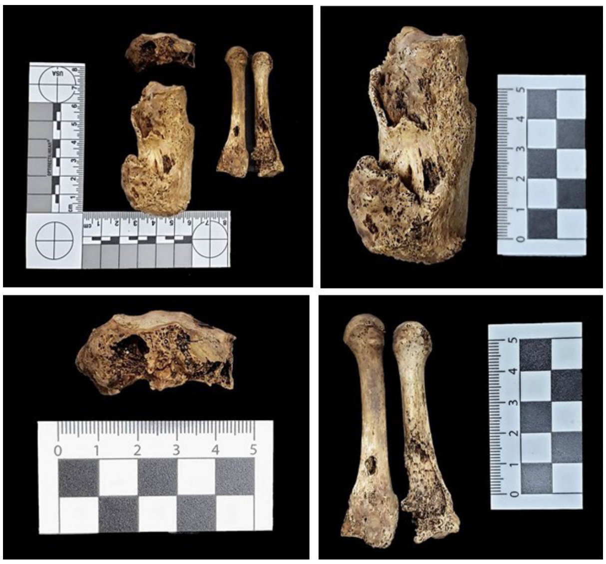

Figure 15. Left Calcaneus, Navicular, Third, and Fourth Metatarsal of Case #1. Source: CEAF/FOP/UPE Archives.

Figure 16. Left First and Third Metacarpals of Case #1. Source: CEAF/FOP/UPE Archives.

h) Femurs

As in the tibias and fibulas, purely osteolytic and mixed lesions were found in the femurs. The right femur showed nine lesions, the largest of which measured 19.69mm x 15.38mm in the largest axes (Figure 14 A). The left femur showed seventeen well-identified lesions, the three largest measuring 25.94 mm x 5.25 mm, 19.69 mm x 5.25 mm, and 27.35 mm x 14.07 mm (Figure 14 B).

i) Left foot and hand bones

Lesions corresponding to the three different spectra of metastatic reactions were observed in four bones of the left foot: calcaneus, navicular, third, and fourth metatarsal (Figure 15). On the upper surface of the calcaneus, an irregularly shaped osteolytic lesion is noted, measuring 8mm x 11mm in its longest axes. The navicular presents two contiguous lesions of distinct aspects. The first one shows perforation of the external and internal bone cortices

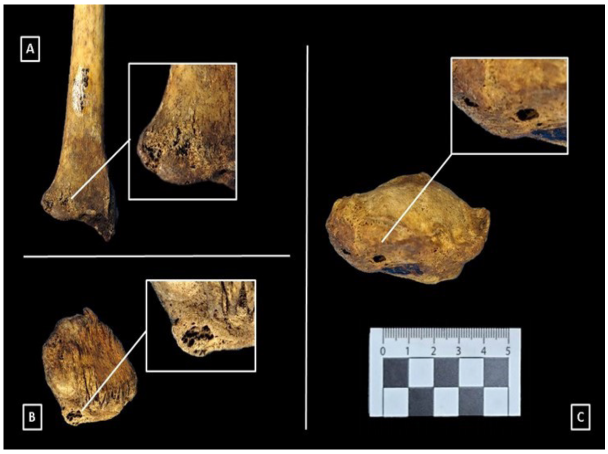

Figure 17. Anterior View of the Left Radius, Right Patella, and Right Navicular of Case #2. A) Mixed lesions in the distal epiphysis of the left radius. B) Mixed lesion in the right patella. C) Mixed lesions in the right navicular. Source: CEAF/FOP/UPE Archives.

due to an osteolytic reaction, with maximum diameters of 12mm x 14 mm. The second alteration shows osteoblastic characteristics, with bone tissue growth and thickening and measurements corresponding to 8mm x 12mm.

In the third metatarsal, an osteolytic lesion is observed, located on the distal third of the bone diaphysis and ellipsoid in shape, whose transverse diameter corresponds to 5 mm and sagittal diameter to 7 mm. The fourth metatarsal presents an extensive mixed lesion affecting the medial and distal thirds of the diaphysis and the distal epiphysis of the bone. There is both cortical and trabecular bone involvement, and the margins and shape are irregular. The lesion measures 18mm x 31mm at its longest axes.

The first metacarpal bone presents an ellipsoid-shaped osteolytic lesion at the middle portion of the diaphysis (Figure 16). It involves the external bone plate, measuring approximately 4 mm x 6 mm in larger diameters. At the distal epiphysis of the third metacarpal, there is a lesion with purely lytic characteristics, regular margins, and shape, with a maximum diameter of 2 mm.

Case #1

The second case presented mixed lesions in three bones: left radius, right patella, and right navicular. The lesions showed perforation of the external cortical surface, with trabecular bone neoformation inside. Two lesions were found in the left radius's distal epiphysis, measuring 3.00 mm x 2.00 mm and 4.50 mm x 3.00 mm, both with irregular borders (Figure 17). The lesion in the patella measured approximately 5.50mm x 5.00mm in the largest axes (Figure 17). The lesion measurement in the largest diameters in the right navicular was 4.00 mm x 3.00 mm (Figure 17).

Discussion

Most individuals affected by breast cancer develop bone metastases (Coleman, 2006; Kuchuk, Hutton, Moretto et al., 2013; Fang, Xu, 2015). For this phenomenon, there is the explanation of the "seed and soil" theory, justified by the "fertility" of the bone, which is a favorable environment for the "germination" of cancer cells, propitiating the proliferative cycle in the skeleton. These tumour cells can produce osteomimetic factors that facilitate their survival in the bone microenvironment and promote the development of metastases (Brook et al., 2018).

The focus of breast neoplasm research concentrates on the molecular study of the events and factors involved in the development and progression of bone metastases so that efficient therapies can be developed to combat or even delay the process (Coleman, 2006; Brook et al., 2018; Tahara et al., 2019; Salvador et al., 2019). However, questions related to macroscopic characteristics of the behaviour these events express in the skeleton still need to be explored. The availability of a clinically diagnosed skeleton in a good state of preservation constitutes a valuable tool for comparative interpretations and the diagnosis of unknown bone lesions (Marks and Hamilton, 2007).

Of the five cases from CEAF/FOP/UPE diagnosed in life with breast cancer, only two presented metastatic lesions (40%). The data are similar to those obtained in a study of skeletons diagnosed in life with breast cancer in an osteological collection in Milan, where 43% of the 14 bones analyzed presented bone metastases at macroscopic observation (Biehler-Gomez et al., 2019). The literature reports that some conditions, such as early diagnosis, staging, age, individualities, ethnic and socio-economic factors, influence the evolution of the neoplasm and can justify the presence or absence of bone metastases, as they cause different clinical manifestations and prognoses (Biehler-Gomez et al., 2019; Fayer, 2014; Spiegel et al., 1989; Sitlinger & Zafar, 2018; Koo et al., 2017; Woods et al., 2006; Diel et al., 1998; Devita & Chu, 2008). Although the youngest skeleton had the greatest metastatic involvement, age was an important factor in the study cases, as all the bones were older than 50 years at death, corroborating what the literature points out regarding the age range of the greatest occurrence of breast cancer.

The bone metastases found in this study are not similar to the percentage reported in studies such as that of Coleman and Rubens (1987) with patients in the terminal stage. However, as stated by Biehler-Gomez et al. (2019), this finding cannot be dismissed and affirmed as different from that found in other studies, considering the lower sensitivity to detection of bone metastases only by macroscopic analysis, which is limited to an unarmed morphological examination (with the naked eye), which may justify the absence of metastatic lesions in the other skeletons diagnosed in this collection. It is also noteworthy that the visualization of metastatic lesions is more sensitive to radiographic analysis, providing valuable information regarding initial lesions besides supplementing and enriching the characterization of macroscopic osteological analyses (Ragsdale et al., 2018). However, it can be inferred that the presence of metastatic lesions reveals the advanced stage of the disease, resulting from a possible late diagnosis and consequent inefficiency of treatment amid the aggressiveness of bone tissue damage caused by neoplastic cells (Guise et al. 2005; Mundy, 2002).

Based on the analysis of the two skeletons that presented bone metastases, the lesions were categorized according to quantity and location. The first skeleton showed numerous lesions, while no more than five were found in the second skeleton. When analyzing only the number of metastatic lesions, there are not many possibilities of inferences since medical information regarding the current stage of the disease and time of discovery since diagnosis is not available. Going further, the numbers reported for each skeleton were analysed macroscopically. Therefore, there is a high possibility of hidden initial lesions, in addition to those missed by fragmentation or absence of bone elements (Biehler-Gomez et al., 2019). However, there is a consensus in the literature that numerous metastatic bone lesions, as mentioned above, are characteristic of more advanced stages of the disease in resistance to treatment or even the absence of treatment (Marks and Hamilton, 2007), as well as the opposite is feasible, where the lack of macroscopic detection of bone metastases may indicate an earlier stage of the disease (Mundy, 2002).

Also, based only on the number of lesions, the literature reports that it is possible to narrow the pathway for a differential diagnosis with other types of bone metastases, as in the case of multiple myeloma, which is referred to in the literature as a neoplasm presenting bone tissue as the primary site of involvement; however, differently from metastatic carcinomas, which show more isolated lesions, myeloma presents numerous lesions disseminated throughout the skeleton. Although the literature describes the usual macroscopic behavior of several neoplasias, there will always be a reserved space that allows exceptions. In this sense, the lesions of metastatic breast carcinoma observed in the first skeleton presented in a disseminated form, similar to those in multiple myeloma. The lesion's shape is also the object of study as a subsidy for the differential diagnosis. It is reported as circular - perforated aspect - in multiple myeloma, while in metastatic carcinoma, the lesions may present with irregular geographic and ellipsoid shapes (Rothschild et al., 1998). In fact, in the skeletons of this research, the lesions confirm the formats analysed in other studies (Biehler-Gomez et al., 2019; Rothschild et al., 1998) for metastatic carcinoma, which despite presenting disseminated, showed irregular formats, varying from circular good irregular edges to ellipsoid. Therefore, dissemination may not be fundamental for establishing the differential diagnosis between neoplasias. However, the lesion shape, together with other characteristics which will be discussed below, may aid in the diagnosis estimation.

In the present study, the lesions were mostly located in the skull, pelvis, extremities, and diaphysis of long bones. According to Ortner (2003) and Biehler-Gomez (2019), the most affected sites in metastatic carcinoma are the spine, femur, ribs, sternum, skull, pelvis, humerus, and scapular waist. Therefore, the results, to a great extent, are consistent with literature data, which may be justified by the great vascularization to which these bones are submitted. The diaphysis of long bones, affected mainly in one of the skeletons, should be emphasized, as it is not usually reported in other studies as an affected site. Besides these, the metacarpals were also affected; likewise, they are not sites of common occurrence, justified by the absence of metastatic lesions in the literature records. Despite not having information on the time since diagnosis of the disease, the advanced stage may be a plausible justification for this event, as there was sufficient aggression to advance beyond the proximal epiphyses, which are, in fact, regions rich in haematopoietic tissue, and therefore primarily affected (Strouhal, 1991). These data can consequently enrich the precision of information regarding the state of the neoplasm at the time of death when medical data, as is the case, are unavailable.

It was not possible to state whether, in fact, the vertebrae are the most affected bones due to the absence and/or fragmentation of the remaining ones. It is valid to stress that, as they coincide with the hematopoietic bone marrow, the sites affected by bone metastases from metastatic carcinoma and multiple myeloma will be similar. Therefore, it is necessary to emphasize the need to analyse the other criteria to point out a possible diagnosis.

In breast cancer, osteolytic lesions are prevalent; however, purely osteoblastic and mixed reactions are not rare (Biehler-Gomez et al., 2019; Ortner, 2003; Tulotta and Ottewell, 2018; Kozlow and Guise, 2005). The justification for osteolytic bone reactions lies in the secretion of parathyroid hormone-related protein (PTHrP), secreted in several malignancies. Osteoblastic lesions result from the secretion of osteoblast-derived growth factors, which stimulate tumour cell growth. Mixed lesions, on the other hand, have combined aspects that still need to be studied in preclinical models. Therefore, the response of the bone at the metastatic site is unpredictable. It was possible to establish a correlation between the study and literature data since all three bone reactions were found in the diagnosed skeleton.

One of the skeletons in the study (case #1) presented two surgical metal shafts in the right tibia and fibula, each one joining the middle third of the diaphysis to the distal epiphysis of the respective bones. The fracture was not yet consolidated, justified by the absence of signs of complete bone remodeling, which indicates that there was not much time between the fracture and death. This event corroborates the literature since bone fractures resulting from the fragility of the skeleton, not infrequently, are consequences of destructive lesions - which are the predominant ones in metastatic breast carcinoma and prevailed in the study - which contribute to the morbidity and mortality of the individual (Akhtari et al., 2008; Mundy, 2002; Käkönen & Mundy, 2003).

Marks and Hamilton (2007), based on the descriptive classification of pathologies by Ortner (1991), analyzed a female skeleton diagnosed during life with the neoplasm explained in this study, with no history of therapeutic intervention. Most lesions affected the skull, ranging from small (less than 20 mm) to extra-wide lesions, up to 98 mm, with involvement of both cortical surfaces (plates) of bone, exhibiting osteolytic and osteoblastic bone reactions. They were specifically located in the left parietal bone and frontal bone, in the region of the coronal suture, as well as in the ventral portion of the right parietal bone, adjacent to the same suture. There was also the involvement of the occipital bone as well as the left greater wing of the sphenoid. The latter showed osteolytic lesions. All lesions showed anfractuous margins. The author's data partly corroborate those found in the present study, where the skull lesions were also located in the parietal, frontal, and coronal sutures, compromising both bones.

Furthermore, most cranial lesions presented with anfractuous borders and sclerotic margins. Another common finding in the studies was the presence of purely osteolytic lesions in the same anatomical site (greater wing of the sphenoid). When analyzing the lesions' size, there was a significant difference among them. This fact may be justified by the possible absence of treatment for the pathology, which contributed to a rapid progression of bone destruction. The data also agree with the literature, ratifying that the expressive lesions in breast cancer act in an osteolytic or mixed shape.

Biehler-Gomez et al. (2019) described metastatic lesions found in six skeletons diagnosed in life with breast cancer. The lesions were located in the ribs, pelvis, vertebrae, skull, scapula, and the proximal end of the femur and humerus. The absence of the lesions in more than half of the skeletons resembles the data of this study, reinforcing, therefore, that not all cases of the neoplasm will present macroscopic expression in bone tissue and may, however, exhibit metastatic alterations at imaging examination, as demonstrated by Marks and Hamilton (2007). The locations of the lesions are also similar to those in this study and corroborate the data in the literature (Biehler-Gomez et al., 2019; Ortner, 2003), reinforcing the affinity for highly vascularized regions close to the hematopoietic medulla.

To support a differential diagnosis with other neoplasias that have bone tissue as the primary site for metastases, it is necessary to compile information regarding all the points mentioned above: number, size, and location of the lesions, the spectrum of the bone reaction, and the biological profile itself, which works as a two-way street, since the knowledge of the sex and age of the individual can help in the narrowing for the differential diagnosis among the neoplasias that affect the bone tissue, as well as, the establishment of the diagnosis of the disease contributes with information for the construction of some elements of the biological profile, besides being able to serve as an additional factor of individualization, in the forensic anthropological procedures for human identification purposes.

In conclusion, the finding of bone lesions in the disarmed macroscopic analysis of skeletons diagnosed with breast cancer during life may reveal a more advanced stage of the neoplasm since the metastasis is hidden from the evaluation with the naked eye when in its initial phase, being better visualized only by imaging examinations. The dissemination of the lesions and their presence in regions with lower blood supply, such as the diaphysis of long bones, a situation widely observed in one of the cases at CEAF/FOP/UPE, may also reflect the advanced stage of the disease.

Lytic lesions represent the most frequent pattern found in metastatic breast cancer. However, mixed or osteoblastic reactions are not rarely observed. These lesions are particularly ellipsoid-shaped, which may constitute an aspect to be evaluated in the differential diagnosis with other types of cancer, such as multiple myeloma. However, examining other characteristics is necessary to reach a definitive diagnosis.

Finally, we conclude that the recognition of breast cancer may constitute a relevant auxiliary component in the identification process in cases of anthropological forensics and may act as an individualizing factor.

Author Contribution Statement

EP: Conceptualization, Methodology, Investigation, Writing –original draft preparation. MC: Conceptualization, Writing - Review and Editing. EN and RQ: Conceptualization, Methodology. AA, AA, and GP: Writing - Reviewing and Editing. ES: Conceptualization, Supervision, Writing - Reviewing and Editing..

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Ethical Approval

The procedures of this research respected the guidelines and norms that regulate research involving human beings (Resolution number 466 of 12 December 2012 of the Brazilian National Health Council) and were duly approved by the Research Ethics Committee under Opinion number 4780547 (CAAE: 46598821.6.0000.5207).

Availability of Data

The data of the current study is available upon request to the corresponding author.

Conflict of Interest

The authors declare that they have no known conflicts of interest that could have appeared to influence the work reported in this paper.

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