Absence of evidence or evidence of absence? A discussion on paleoepidemiology of neoplasms with contributions from two Portuguese human skeletal reference collections (19th-20th century)

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Abstract

Biological, sociocultural, demographic and environmental factors are major contributors to the contemporary burden of oncological diseases. Although cancer's current epidemiological landscape is fairly well known, its past occurrence and history seem more obscure. In order to test the hypothesis that paleopathological diagnosis is an adequate measure of the prevalence of malignant neoplasms in human remains, 131 skeletons (78 females, 53 males, age-at-death range: 15-93 years) from Coimbra and Lisbon Identified Skeletal Collections, $19^{th}/20^{th}$ century (Portugal), were examined. The cause of death for all of the selected skeletons was a malignant neoplasm, as recorded in the collection's documental files. Through the application of standard paleopathological protocols, it was determined that 17.6% (n=23) of the skeletons had unequivocal osseous signs of metastatic and/or neoplastic lesions. Forty-five percent (n=59) had manifest osseous lesions, however the lesional patterns were not clearly pathognomonic. Although all of the analyzed individuals were documented as having succumbed to malignant neoplastic disease, a total of 37.4% (n=49) of the individuals did not exhibit osseous abnormalities. Individuals with breast cancer often exhibited lesions. This study presents a quantitative estimate of the accuracy of paleopathological diagnosis; as well as a theoretical reflection on the burden of cancer in the past. We emphasize the need for a paradigm shift while thinking about the future of paleo-oncology.

Keywords: tumors; cancer; neoplastic disease; metastases; paleo-oncology; quantification; accuracy

1. Introduction

Oncological conditions became the epitome of modern diseases, with malignant neoplasms figuring among the leading causes of death worldwide, ranking second in high-income countries and third in low and middle income ones (Ferlay et al., 2013; World Health Organization, 2015). As contributor to 15% of total deaths globally—with an estimation of 8.8 million for 2015—, cancer becomes a global scale problem, and ubiquitous in human societies (Forman and Ferlay, 2014; Kumar et al., 2015; World Health Organization, 2015). The growing burden of cancer attained recognition by the turn of the 20th century, as it became the "new scourge" of mankind (Hoffman, 1915; Hayter, 2003; Pinell, 2004). As Hayter (2003: 258) writes, this period brought a "new view of an old illness"; an antiquity testified through the non-human and human paleopathological record (Capasso, 2005). Malignant neoplasms affected human populations from the Neolithic Period to modern times (Capasso, 2005; Strouhal and Němečková, 2009; Hunt, 2013), with sporadic evidence noted in the paleoanthropological record (Odes et al. 2016). Although the historiography of cancer provides written depictions of these conditions from antiquity to the early Middle Ages, the documentary information for these periods remains fairly incomplete and often raises interpretative doubts (Retief and Cilliers, 2006; Strouhal and Němečková, 2009; Olszewski, 2010). Nevertheless, the impact, fear, and social stigma of cancer was already appreciable in early periods of our history, as illustrated by Pinell (2004: x) describing how "at the end of the fourteenth century, Catherine of Sienna herself tested her charitable heroism by collecting in a bowl the pus from the breast of a cancerous women to drink it like Christ's blood". Post-mortem examination reports from 15th century anatomists also clearly depict the presence of cancer (Hajdu, 2011). Still, the question of how ubiquitous and prevalent it was in past populations remains unanswered. Paleopathology is exceptionally positioned to assist in such a quest for knowledge (Waldron, 1996; Brothwell, 2012; Zuckerman et al., 2015).

In the last decade, paleopathological reports of malignant neoplasms have become more

frequent, with recent evidence recovered from diverse geographic and chronological settings (e.g. Prates et al., 2011; Binder et al., 2014; Lieverse et al., 2014; Luna et al., 2015). Strouhal and Němečková (2009) listed 250 cancer cases published up to 2007, and Hunt's (2013) survey points to similar values (n=263). The majority of these publications are case-reports, whereas paleoepidemiological approaches are less frequent. Some population-based archaeological approaches suggest that the prevalence of cancer in the past ranged from ca. 0.5% to ca. 3.1% (Nerlich et al., 2006; Baxarias et al., 2009). Waldron (1996) and Nerlich et al. (2006) provided estimates of cancer burden in archaeological samples. In these works, the obtained prevalence in the skeletal samples was not very different from the expected values based on a reference model from the 1900-1905 period in England, provided by Waldron (1996). Despite these figures, malignant neoplasms are still traditionally deemed rare in paleopathology, leading to the assumption that cancer burden was negligible in past populations (Capasso, 2005; David and Zimmerman, 2010). Advocates of this viewpoint argue that shorter life expectancy, low exposure to carcinogens, different lifestyle and dietary habits, or higher mortality due to infectious diseases, trauma or famine were responsible for a low prevalence of cancer in the past. In addition, several scholars have also argued that small sample sizes, an expected low prevalence, taphonomic effects, or methodological and diagnostic limits can bias the interpretation of past cancer rates (Waldron, 1996; Capasso, 2005; Nerlich et al., 2006; Strouhal and Němečková, 2009; Brothwell, 2012; Zuckerman et al., 2015).

It is well known that the limits in paleopathological diagnostic accuracy preclude reliable estimations of disease prevalence in the past (Wood et al., 1992; Armelagos and van Gerven, 2003; Ortner, 2003; Waldron, 2007). Quantifying these limits for the case of malignant neoplasms is germane to the debate on its antiquity. Non-systematic use of radiological survey (neoplasms located in the medullary cavity without cortical breakthrough will be undetected by gross examination), presence of unspecific bony lesions in primary or secondary malignant neoplasms

(especially in early stages of disease), difficulties in differential diagnosis (particularly with infectious diseases), absence of standardized diagnostic criteria, or the small proportion of soft tissue neoplasms that produce osseous metastases, are well-known problems in the recognition of neoplasms in archaeological settings. Thus, a key question is whether the diagnostic difficulties can justify the scarce paleopathological evidence, or if, as David and Zimmerman (2010: 731) argue, "the minimal diagnostic evidence for cancer in ancient remains indicates the rarity of the disease in antiquity". It seems reasonable to emphasize the need to conduct paleopathological research that quantifies the diagnostic accuracy of cancer in skeletal remains, before reaching conclusions on their rarity in the past (Brothwell, 2012).

Analysis of identified human skeletal collections with documented and known cause of death has the potential to clarify the aforementioned question, as demonstrated by several studies concerning other diseases (e.g. Matos and Santos, 2006). In the present paper we selected two Portuguese identified human skeletal collections (Coimbra and *Museu Bocage* Identified Skeletal Collections), from the $19^{th}/20^{th}$ century, as the starting point to address this problem.

Since this paper heavily relies on the biographic data contained within the collection records that exist for each individual of the Coimbra and *Museu Bocage* Identified Skeletal Collections, some brief considerations on the accuracy of the cause of death on these records are needed. The cause of death that is registered for each individual in the collection was transcribed from the respective cemetery records that are based on the certified cause of death. The transcription of the cause of death was carried out by the researchers that amassed the collections (Santos, 2000; Cardoso, 2006). One should expect some inaccuracies in death certification for this period, and caution on interpretations is necessary. This constraint is not unique to paleopathological research in identified collections, but is common in historical demographic research (Alter and Carmichael, 1996; Morais, 2002), retrospective epidemiological studies, and contemporary epidemiological health research that is also based on data obtained from death certificates (Alter and Carmichael,

1996). In Portugal, by the late 19th century, certification of the cause of death was a legal obligation of the attending physician, the municipal doctor, public health medical examiner, or administrative authority (Nunes, 1923; Bicho, 1926). As such, some degree of accuracy is expected. For the particular case of cancer, Nunes (1923) noted that malignant neoplasms were underrepresented in death certificates and in the overall Portuguese mortality statistics of that period. Raposo (1950: 621) also claims that "when the word cancer is registered as cause of death it is because there is confidence in the diagnosis. On the other hand, other diagnoses are frequently obscuring the true oncological cause of death. Many internal cancers are undetected by the physician […], and from the innumerous ignored causes of death a significant portion must belong to cancer". Through these testimonies we can infer that, in the majority of cases, when cancer was registered in the death certificates of this period there was a fairly high degree of certainty of diagnosis. Cancer diagnosis in clinical contexts was also significantly improving during the first decades of the 20th century, with improvement in complementary diagnostic techniques, such as histology, biochemical analysis, and radiology (Costa, 2010). Autopsies were also a common practice within hospital contexts, to validate diagnosis of doubtful cancer cases (e.g. Morais and Melo, 1943). Hence, we have no reasons to consider that a great majority of the records of neoplasms in the reference collections herein analyzed were misdiagnosed. Conversely, more cases may exist without documentation of the true malignant cause of death, as described by Santos (2000) and Marques et al. (2013a).

1.1. Objectives

This study aims to present new data on the paleopathology and paleo-epidemiology of neoplasms, based on the analysis of human remains and their associated documentary records from Coimbra and *Museu Bocage* Identified Skeletal Collections.

Using paleopathological approaches, we quantified the frequency of detectable neoplasms as

determined through visual inspection, in a cohort of individuals that died due to a neoplastic disease (as recorded in the collection's cause of death registries). Subsequently, we explored the role of various demographic and biological factors, namely: sex, age-at-death, degree of skeletal completeness/preservation, year of death, and the primary site of the neoplasm (primary organ). Comparison of lesion frequencies obtained in our sample with coeval biomedical data was performed, in order to test our hypothesis: paleopathological diagnosis is an adequate measure of the prevalence of neoplasms in human skeletal remains. In doing this, we attempted to answer the question: how feasible and accurate is the diagnosis of neoplastic disease, based solely on the visual inspection of human skeletal remains?

2. Materials and Methods

2.1. Sample

Two Portuguese skeletal reference collections, from the 19th/20th century, were surveyed: Coimbra Identified Skeletal Collection (CISC), housed at the Department of Life Sciences, University of Coimbra, and *Museu Bocage* Identified Skeletal Collection (MBISC), presently curated at the *Museu Bocage*, National Museum of Natural History, Lisbon (Santos, 2000; Cardoso, 2006). The human remains from these collections are unclaimed bodies from Lisbon and Coimbra modern cemeteries, and biographic data is available for each individual (e.g. name, sex, birth place, date of death and birth, cause of death, marital status, occupation). Coimbra collection is composed of 505 individuals, deceased between 1904 and 1936 (Table 1). *Museu Bocage* collection had 769 skeletons with data on sex, age at death, and cause of death, at the time of the present study. Dates of death range from 1880 to 1974, yet the majority (88.7% [682/769]) died after the 1930's. MBISC has much wider, but also later, chronological boundaries than the CISC. Of note, also, is a quite distinctive age at death pattern. While MBISC has a clearly negatively skewed age at death distribution, the CISC has a predominance of young adults (in 20-29 and 30-

39 age classes) (Table 1). Overview of the collection's biodemographic profile is described in Table 1 (see also Supplementary Material Fig. S1).

In order to select the skeletal sample for the paleopathological study (from the 1274 individuals available [CISC n=505 and MBISC n=769]), a convenience sampling strategy was applied, as follows: inclusion of all definitive cases of malignant neoplasms reported in the records (n=105), as well as, a minority of cases (n=26) for which it was not clearly stated in the records if the neoplasm was benign or malignant. These cases without reference to malignancy are presumed malignant (and considered as such in the present work) because the neoplasm was recorded as the cause of death. Individuals with documentary records of benign neoplasms were excluded (n=3), as well as two skeletons not observable due to post-depositional processes. A total of 131 individuals (10.3% [131/1274]) with neoplasm recorded as the cause of death were selected (MBISC=11.6% [89/769] and CISC= 8.3% [42/505]).

[Table 1 here]

2.2. Methods

Observation of the skeletal sample [by the first author, C.M.] was made according to standard paleopathological protocol, with all anatomical areas examined visually. Distinct patterns of bone formation and destruction, lesion topography, extension and severity were recorded, following paleopathological and biomedical guidelines (Ragsdale, 1993; Buikstra and Ubelaker, 1994; Ortner, 2003; Resnick and Kransdorf, 2005; Matos and Santos, 2006; Costelloe and Madewell, 2016; Greenspan and Borys, 2016). Abnormal bone destruction was evaluated according to the following features: *i*) type of bone destruction and margin (coalescent porosity, osteolytic foci [geographic or moth-eaten/permeative patterns], and absence of bony parts); *ii*) location, extension, number, and size of lesions. Abnormal bone proliferation was recorded, accounting for: *i*) their distinct visual characteristics (woven/lamellar/mixed, spiculated [hair-on-end, sunburst or

coarse patterns], and widening of bone contour); *ii*) location, extension, number and size. Determination of these macroscopic features can aid in the differentiation between indolent or aggressive lesions, and can also inform the rate of progression (Ragsdale, 1993; Burgener et al., 2008; Schirrmeister and Arslandemir, 2010; Costelloe and Madewell, 2016; Greenspan and Borys, 2016).

Taking into account differences in the lesion typology, topography, extension and severity, we created a scoring system that allowed the allocation of each individual into three distinct groups: *score 1*, *score 2*, and *score 3*. The implementation of the scoring system was merely operational, as it allowed for the measurement of diagnostic ability in the present study. By pooling the data into large analytic groups we could properly quantify the number of skeletons which could easily be recognized as having a malignant neoplastic condition (mostly metastases) compatible with the stated cause of death, the ones with intermediate (non-specific) features, and the ones not showing any evidence of osseous alterations in association with the neoplasm recorded as the cause of death. We adopted the terminology for "degree of certainty of diagnosis in paleopathology" proposed by Appleby et al. (2015: 20), yet with modifications. The stratifying scheme was created as follows:

Score 1. Not consistent with a malignant neoplasm: absence of destructive or proliferative lesions that could have been caused by a neoplastic/metastatic condition.

Score 2. Moderately consistent with a malignant neoplasm: evidence of destructive and/or proliferative processes that could have been caused by a malignant neoplasm, but it is non-specific and there are other causes that could be evoked. It does not allow a confident diagnosis. Inclusion criteria:

> *i*) presence of discreet areas of new bone formation— woven/lamellar, small spicules (excluding exuberant radiant, parallel or coarse spicules), and/or expanded bone contour— located in at least one anatomical area other than lower limb long bones;

or

ii) presence of discreet, small and well delimited (geographic) osteolytic lesion affecting three or less bones.

Score 3. Highly consistent with a malignant neoplasm: presence of destructive and/or proliferative processes that are typically observed in malignant neoplasms. High degree of severity and specificity of the lesions. Inclusion criteria (based on paleopathological and biomedical literature):

i) diffuse areas of new bone formation (radiant, parallel or coarse spicules);

or

ii) diffuse areas of bone destruction (multiple geographic foci, moth-eaten, absence of parts);

and

iii) multifocal distribution (unifocal excluded) in the skeleton and preferential location in the axial region, hip bones and proximal portions of long bones.

Inter-observer error for scoring of the skeletons was evaluated in 10% of the sample, resulting in 100% concordance of observations between the first [CM] and second [VM] authors.

An analytical category of more "osteotropic" neoplasms, i.e. the ones that most often metastasize to bone, was created. The category of osteotropic neoplasms was defined based on biomedical literature (Resnick and Kransdorf, 2005; Reith, 2016). As such, it included the combination of all cases of breast, prostate, lung and kidney neoplasms, with primary bone neoplasms also included.

Categories for age at death classes were established with ten-year intervals. Each skeleton was also evaluated for the degree of completeness/preservation according to the method proposed by Bello et al. (2003), with computation of the *Anatomical Preservation Index* (API=100 x Σ P

[1,N]/N).

Statistical analysis was performed with SPSS Statistics (v. 21, IBM SPSS, Chicago, IL). Fisher chi-square (χ^2) (Monte Carlo method), t-Student test, ANOVA *one way* (*post-hoc* Tukey HSD), logistic and multinomial regression models were the statistical tests applied (significance accepted for $p \leq 0.05$). The use of regression models is particularly important for the present study, since it can take into account composition effects or confounding effects of multiple independent variables (also called factors), as for example, sex, age at death, chronology of death or preservation index.

3. Results

3.1 Documental records

A total of 131 (10.3% [131/1274]) individuals with a malignant neoplasm documented as the cause of death were selected (pooled from MBISC [11.6% = 89/769] and CISC [8.3%=42/505]) [Table 1]). In the documentary records (Table 1) it was noted that more females than males had neoplasm registered as the cause of death ($n_{females}$=78, n_{males}=53, χ^2=4.428, df=1, p=0.035). Mean age at death did not differ between sexes ($Mean_{males}$=59.5, SD=14.6; $Mean_{females}$=59.6, SD=16.3, t=-0.031, df=129, p=0.975, CI]-5.595-5.421[, n=131). Age at death of the individuals ranged from 15 to 93 years, with most cases allocated in the 50-59 and 60-69 age at death classes (Fig. 1a). These individuals died between 1904 and 1969, with the highest proportion of cases recorded in latter decades of the 20th century (Fig. 1b).

In order to test which biodemographic factors were influential in the prevalence of malignant neoplasms recorded in the identified collections, a logistic regression analysis was performed. In the model, neoplasm (n=131) *vs* other causes of death (n=1143) was the dependent variable, and sex, age-at-death, and year of death were the independent variables. The model was significant (χ^2=26.357, df=3, p≤0.001, n_{total}=1274, $n_{neoplasms}$=131, $n_{other\ causes\ death}$ =1143) with 89.4% of overall correct predictions. A significant increase in the frequency of neoplasms with increasing age at

death ($p \leq 0.001$, 95% CI: 1.007—1.025), and chronology (years) of death ($p=0.025$; 95% CI: 1.002-1.029) was noticed (Supplementary Material Table S1). That is, neoplasms were recorded mostly in older individuals and also were more frequently recorded in later time periods.

Neoplasms of digestive (45.8%, n=60) and reproductive (27.5%, n=36) systems were the most commonly recorded, especially gastric (n=32), uterine (n=21), colorectal (n=18), prostatic (n=9), breast (n=6) and liver (n=6) cancers. Primary bone neoplasms were recorded in 2.3% (n=3) of the sample. Thus, the results reported in section 3.2 largely refer to alterations due to metastatic carcinoma (Figure 2).

Distribution of the sample by primary organ and sex subset is illustrated in Figure 2. A similar proportion of males (26.4%) and females (23.1%) died from gastric neoplasms, while colorectal neoplasms were more frequent in males (18.9%).

Only one case was registered in the 10-19 age at death class, which corresponded to a myeloid leukemia. The six cases allocated to the 20-29 age at death class included cases of: leukemia, sarcoma of the carotid (it should be noted that this cause of death might have terminological inaccuracies), brain, mediastinal, and colorectal neoplasms. Gastric neoplasm predominated in the remaining age at death classes, except for the age at death class of 40-49 years, in which uterine neoplasms (35.3%, six out of 17 neoplasm cases in the age class) were predominant, and in the 80-89 age at death class with a predominance of prostate neoplasms (37.5%, three out of eight neoplasm cases in the age class).

[Figures 1a,b and Figure 2 here]

3.2. Paleopathological analysis: scoring groups

Overall, the skeletal sample under study (n=131) was well preserved, since 97.7% (n=128) of the skeletons exhibited *Anatomical Preservation Index* (API) scores higher than 50%, with a mean API value of 90.4 (SD=12.7) (Table 2). The results of the scoring system analysis showed that

37.4% of the skeletons were allocated to *score 1*, 45.0% to *score 2*, and 17.6% to *score 3* (Table 2).

Under *score 1,* the individuals that did not show osseous lesions attributable to a neoplastic condition were listed (37.4% [n=49] of the analyzed skeletons). This score included all skeletons with absence of proliferative/destructive lesions, the ones with lesions clearly associated with a non-neoplastic etiology (e.g. trauma, rheumatic conditions, entheseal changes), and a few cases with very superficial and small appositional patches of woven/lamellar bone (periosteal reactions) located only in the long bone diaphysis.

A wide range of osteoclastic and/or osteoblastic osseous alterations were noticed on the remaining individuals, allocated to *scores 2* and *3* (62.6%, n=82). The majority (45.0%, n=59) of the analyzed skeletons met the criteria for *score 2:* moderately consistent with a malignant neoplasm but other conditions could not be clearly ruled out by visual inspection alone. The patterns of lesions could have been caused by a metastatic process, because there was some evidence of bone formation and/or bone destruction, yet the lesions were mostly unifocal, non-specific, and with a low degree of severity. As a result, despite a known neoplastic cause of death of these individuals, the diagnosis was uncertain when based only on the visual observation of the osseous abnormalities. Twenty-one skeletons had small areas of bone formation — woven or mixed, small spicules, or widening of the bone contour. Thirty-eight skeletons showed at least one occurrence of osteolytic activity (with variable degrees of associated bone formation), albeit in a delimited, tenuous, and mostly unifocal distribution. For these cases, since the cause of death is known one can tentatively infer a neoplastic cause for the lesions, with a caveat that the lesions may be co-morbidity. A good example of individuals included in *score 2* is depicted in Figure 3, where the only lesions observed in a 46 year old female with uterine cancer, were two minor areas (20 x 12 mm and 26 x 17 mm) of woven bone formation– one of those with a small osteolytic focus– in the ventral surface of the pubic bones. A discreet patch of woven bone (6 mm) on the

right scapula was also noticed. Although these alterations may be a consequence of the uterine cancer reported in the cause of death, the non-specific morphology of lesions does not allow a confident diagnosis. Another example refers to the case of a 45 year old male with a rectal cancer. The skeleton shows signs of osteolytic activity (geographic, well remodeled, with sclerotic borders) and new bone formation in the sacrum (max. 60 mm) (Fig. 4). No other significant bony alteration was noticed in the remaining bones. A 38 year old female had a "lymphosarcoma of neck and mediastinum" reported as the cause of death, yet the skeleton only showed multiple areas of woven bone located in the visceral and costal surfaces of the 1st to 12th ribs (bilaterally), and dispersed throughout the vertebral, shaft and sternal areas (Fig. 5). In the right clavicle several areas of woven bone formation (max. 17 x 9 mm) was also evident. None of the above-mentioned cases would be securely reported as a metastatic process in skeletons from an archaeological context, nevertheless this is the most likely etiological possibility considering the cause of death.

[Figure 3, Figure 4 and Figure 5 here]

Score 3 encompasses 17.6% (n=23) of the individuals (Table 2), comprising cases with a reliable diagnosis of malignant neoplasia. The lesion pattern, severity and extension correlate strongly with the expected patterns as described in the paleopathological and biomedical literature. Distribution of lesions was multifocal in the skeleton and/or bone, and were characterized by the presence of multiple osteolytic foci (geographic or moth-eaten/permeative) (Fig. 6), and/or exuberant osteoblastic activity, as exemplified in Figure 7.

Scoring differences between MBISC and CISC were observed. The CISC have a greater proportion of skeletons allocated to *score 2* (64.3%, n=27) than the MBISC (36.0%, n=32). The results were inverse for *score 3* (MBISC: 24.7% [n= 22], CISC: 2.4% [n=1], χ^2=16.136, df=2, $p \leq 0.001$). No significant differences between the collections were noticed for *score 1* (MBISC: 39.3% [n=35] and CISC: 33.3% [n=14]).

[Figures 6 and Figure 7 here]

3.2.1. Lesion patterns and biodemographic profile

No sex differences were noticed between scoring groups (χ^2=2.518, df=2, *p*=0.284) (Table 2). Mean age at death was also similar for the three scores (F=2.053, df=2, *p*=0.133) (Table 2, Supplementary Material Fig. S2). Thus, sex and age at death do not seem to be determining factors for the presence or severity of lesions. On the other hand, the chronology of death (years) seems to be influential (Table 2). While cases given *scores 1* and *2* were distributed along all decades of death, individuals given *score 3* died later in time and were recorded only in the decade of 1921-1930 and afterwards (Supplementary Material Fig. S3). The mean values of year of death were statistically significant between *score 3* and *score 2* ($Mean_{score\ 1}$=1940, SD=13.8, $Mean_{score\ 2}$=1938, SD=13.1, $Mean_{score\ 3}$=1948, SD=10.9, F=4.853, df=2, *p=0.009,* n=127, *post-hoc* Tukey HSD: *p*=0.006).

The degree of skeletal completeness/preservation did not influence the previous results, since mean values of API (Table 2) did not show significant differences between scores (F=0.485, df=2, *p=0.618,* n=131). In order to account for the effect of all variables conjointly, a multinomial regression analysis was performed (Supplementary Material Table S2). The dependent variables were the scoring categories (*score 2* as a reference group), and sex, age at death, year of death, and API as independent variables. The overall model was significant (χ^2=16.429, df=8, *p*=0.037), producing 48.8% of correct predictions (accuracy *score 2* =68.4%, *score 3*=18.2%). The year of death was significant in predicting a *score 3* classification: an increase of one year in the date of death was associated with an increase in the odds of *score 3* classification by a factor of 1.07 (*p*=0.009; 95% CI: 1.02—1.12). Meaning that individuals with more widespread and diagnostic lesions died in latter time periods, even when the effect of sex, age at death, and API were taken into account.

3.2.2. Lesion patterns and documented primary organ

Most of the individuals allocated to *score 1* had gastric (34.7% [17/49]), colorectal (16.3% [8/49]), and uterine (12.2% [6/49]) neoplasms recorded as the cause of death. All remaining neoplasms contributed less than 4.1% (two or less individuals) to *score 1*. Individuals under *score 2* had a higher frequency of documented neoplasms of the stomach (20.3% [12/59]), uterus (20.3% [12/59]), colorectum (15.3% [9/59]), liver (8.5% [5/59]), prostate (8.5% [5/59]), multiple organs (6.8% [4/59), and the skeleton (5.1% [3/59]). Other primary neoplasm types contributed less than two cases (<3.4%) for this score. Individuals under *score 3* were most frequently affected by breast neoplasms (21.7% [5/23]), followed by neoplasms of the prostate, stomach, and uterus, each representing 13% (3/23). Leukemia/lymphoma represented 8.7% (2/23) of documented diagnoses allocated to *score 3,* and all remaining diagnoses were represented in one or no cases (<4.3%). In Figure 8, an illustration of the distribution of scores and types of primary neoplasms shows the computed percentages of scores within each primary neoplasm location.

[Figure 8 here]

Analysis of the 82 skeletons that had at least one osseous lesion (pooled *scores 2* and *3*) showed that in all cases of liver neoplasm (n=6) the skeletons had at least one lesion, however this value was highly influenced by the small sample size for this neoplasm. Prostate (88.9% [8/9]), breast (83.3% [5/6]), and uterine (71.4% [15/21] neoplasms also frequently presented with one or more lesion in the skeleton. Moreover, all cases of primary bone neoplasms showed bony lesions. Overall, there was a significant difference in the manifestation of one or more observable osseous lesions for neoplasms of the reproductive system and those of the digestive system (77.8% [28/36] and 55.0% [33/60], respectively, χ^2=5.238, df=1, p=0.022).

It seems relevant to analyze differences between the group of neoplasms operationalized as more osteotropic (n=22) and the remaining ones (n=101). Individuals with osteotropic neoplasms were more frequently classified with *score 3* (40.9%) and *score 2* (40.9%) than *score 1* (18.2%,

χ^2=9.843, df=2, p=0.012). A logistic regression model, taking into account age at death, sex, year of death, and classification as an osteotropic neoplasm [yes *vs*. no] (independent variables), significantly predicted the classification within scoring groups [*score 1 + score 2 vs score 3*] (χ^2=16.571, df=4, p=0.002). Overall, 83.2% of the predictions were accurate. Cases with osteotropic neoplasms have increased odds of being classified as *score 3* lesions by a factor of 4.912 (p=0.010; 95% CI: 1.463—16.501) (Supplementary Material Table S3). These results support the idea that, in fact, osteotropic neoplasms have higher odds of manifesting skeletal lesions than the less osteotropic neoplasms.

4. Discussion

4.1 Documental records and inferences to paleoepidemiology

Malignant neoplasms accounted for a considerable number of the documented causes of death in the two reference collections surveyed (10.3% [131/1274]). This was an unsurprising outcome, considering that it results from cumulative deaths of nearly one century. Neoplasms ranked third among the major groups of causes of death registered in the MBISC— only surpassed by diseases of the circulatory system and infectious and parasitic diseases. Neoplasms ranked fourth in the CISC, with infectious and parasitic diseases, and diseases of the circulatory and respiratory systems being predominant. This representation of malignant neoplasms (see section 2.1 and Fig. 1b) broadly echoes the epidemiological trends for this period in Portugal, with a progressive rise of non-communicable diseases and decline of infectious and parasitic ones in overall mortality, as well as a second epidemiological transition, that in Portugal consolidated after the mid-20th century (Ferrão, 1996; Morais, 2002). If in the Portuguese mortality statistics cancer-related deaths represented only 1.2% of all deaths during the first years of the century (crude mortality rate: 22.00 per 100 000 in 1904), they gradually arose in the following decades. For instance, cancer represented 4.8% of deaths in the 1950s (crude mortality rate: 82.7 for males and 87.3 for females

per 100 000 in 1955) and 11.7% in the 1970s (crude mortality rate: 135.9 for males and 115.5 for females per 100 000 in 1970) (Neves, 1906; Morais, 2002; WHO, 2014). A similar trend was noticeable in the collections studied, with the frequency of documented neoplasms statistically increasing along the chronology of death (see Fig. 1b).

The profile of individuals with malignant neoplasms in the collections (section 2.1) showed a preponderance of females, and of individuals in the 50-59 and 60-69 age at death classes. Coeval mortality statistics revealed similar patterns, with higher mortality of females up to the 1970's in Portugal, and a reverse trend thereafter. Similarly, most cancer-related deaths happened in individuals aged 50-65 years, with age-at-death incrementing steeply only after the mid-20th century (Neves, 1906; Nunes, 1923; Raposo, 1950; Morais, 2002; Instituto Nacional de Estatística-INE, 1920-1960; WHO, 2014). Cancers of the stomach, uterus and breast in females, and stomach, intestines, liver and lip/skin in males, were major contributors to mortality up to the 1970's in Portugal (Neves, 1906; INE, 1920; Nunes, 1923; WHO, 2014), with a similar trend observed in the collections. These parallels reinforce the idea that these reference collections are a good repository of information for the analysis of malignant neoplastic conditions, since they broadly fit the panorama of the mortality data for this period.

One aspect that requires further reflection is the relationship between age and cancer-related mortality. Undeniably, age is a risk factor for both oncogenesis and mortality due to malignant neoplasms in human societies, as an ageing population is one of the major drivers of the epidemiological landscape of cancer nowadays (Coleman and Rubinas, 2009; Forman and Ferlay, 2014; Kumar et al., 2015). A longer life span increases the exposure to exogenous and endogenous carcinogens and allows time for a stepwise acquisition of genetic/epigenetic changes leading to malignant transformation. Reduction of immune competence and efficacy of DNA repair mechanisms also occurs with increasing age (Coleman and Rubinas, 2009; Kumar et al., 2015). As such, it seems plausible to argue that the demographic profile of ancient populations, composed

predominantly of younger individuals, contributed to a lower cancer prevalence in the past. However, if we take a look into the early Portuguese mortality statistics and the collections data (which are not extremely aged demographically, in particular the CISC, see Fig. 1), most of the cases were documented in middle adulthood and not exclusively in the elderly. This data supports the idea that in archaeological contexts one might still encounter some cancer cases among middle-aged groups and in individuals with longer life spans. Lieverse et al. (2014) and Chamberlain (2006: 53) note that there is "no clear historical evidence that maximum lifespan was reduced in earlier historical times". Moreover, research in modern hunter-gatherers suggests that "longevity has a deep human history" (Osborne and Hames, 2014). As such, one might expect to encounter cancer cases in this group of individuals that, not so rarely, had longer longevity. Moreover, one should not disregard the fact that in paleopathological studies other age-related conditions are often found. Conditions such as arteriosclerosis (e.g. Thompson et al., 2013), osteoarthritis, which is a common finding in paleopathology (Waldron, 2007), or osteoporosis, that "although typically acknowledged as a modern disease, OP [osteoporosis] has a vast diachronic depth" (Curate, 2014: 120), serve as testimonies that diseases prevalent in older age categories were often present in past societies. Thus, although the demographic argument is very important to understand cancer landscape, it does not fully justify the absence of cancer in the past. Other factors must surely have an impact on the low frequency of cancer detected in paleopathology, as will be discussed in the following sections.

One other aspect to be considered while pondering the relationship between demography and malignant neoplasms in archaeological contexts, is the problem of computation of crude prevalence. Calculation of crude prevalence of cancer, instead of age-adjusted prevalence, in archaeological settings represents a source of interpretative bias. Since cancer is an age-related condition, the age structure of a skeletal assemblage has an impact on the comparison of archaeological samples. Lack of systematic use of sex- and age-standardization methods in

bioarchaeological research has been emphasized by several scholars (e.g. Nerlich et al., 2006; Waldron, 2007; Pinhasi and Bourbou, 2008; Faltas, 2011; Marques et al., 2013b; Zuckerman et al., 2015), and the increased consideration of these factors is particularly important for paleo-oncology. Regrettably, these approaches have not been regularly practiced, which limits the kind of inferences we are able to make. Our data serves to illustrate this point. The difference in the prevalence of malignant neoplasms between the MBISC and CISC collections decreases from 3.2% to 1.2%, when crude prevalence (MBISC: 11.6%, CISC: 8.4%) is compared with age-standardized prevalence (MBISC: 7.3%, CISC: 6.1%), using a direct method for age standardization (standard population: year of 1980, Portugal). In other words, the difference in cancer prevalence between these collections was not as high as indicated by crude prevalence values, when the different age at death distribution patterns of the samples were taken into consideration. Thus, reports of the prevalence of malignant neoplasms in the archaeological record must take into account the effects of the demographic structure of the sample under study.

Case-studies represent the majority of publications on ancient malignant neoplasms up to now. Their usefulness could be enhanced if they could function as a source for data compilation, useful for broader meta-analytical and paleoepidemiological approaches. This purpose can only be achieved if detailed information on the "study-base" (total sample under study), sex proportions, and age at death structures, are consistently reported in case-study reports.

4.2 Paleopathological diagnosis

Limits and constraints of disease identification in human remains have been widely debated by several scholars (e.g. Wood et al., 1992; Armelagos and van Gerven, 2003; Ortner, 2003; Waldron, 2007). Measuring the limits of paleopathological diagnosis of neoplastic conditions is germane to advances in paleo-oncological research. The present work addresses the following question: can the visual inspection of osseous lesions be an approximate measure of the true

prevalence of malignant neoplasms?

Our results show that the recognition of evident skeletal metastasis is not straightforward, when based uniquely on the visual inspection of skeletal remains. Only 17.6% (n=23) of the skeletons exhibited the expected lesional pattern (*score 3*), on which a diagnosis of malignant neoplasia could be made with confidence. Most skeletons in our sample 45.0% (n=59), had lesions that could not be clearly attributable to a neoplastic/metastatic condition (*score 2*). If encountered in archaeological settings, it is unlikely that such cases would be accurately diagnosed. The sum of *score 1* and *score 2* represents all skeletons exhibiting some kind of osseous alteration, totaling 62.6% (n=82). Thus, the percent of cases with possible neoplastic bone lesions in our cohort ranges between 17.6% and 62.6%, with the lower limit corresponding to the diagnosis made with confidence and the upper limit including all skeletons with lesions. The lower limit represents a gross estimation of *diagnostic sensitivity* (defined in clinical epidemiology as the statistical measure of the proportion of positives—individuals with the disease— that are correctly identified among all individuals with disease). It becomes clear that a *diagnostic sensitivity* of 17.6% is fairly low, demonstrating that by visual inspection alone we were able to identify a relatively small percentage of the malignant neoplasms present in our cohort. The lower limit values obtained in our study are within the range found in other reference collections. Rothschild and Rothschild (1995) noted that among the individuals with cancer in the Hamann-Todd Collection (Cleveland, USA), only 9.0% showed signs of bone metastases through visual examination. A similar study from Maijanen and Steadman (2013) reported 27.4% of individuals with evidence of neoplastic disease in the W. M. Bass Donated Skeletal Collection (Tennessee, USA).

One cannot confidently evaluate the paleopathological diagnostic limits without taking into consideration the composition of the sample in terms of the site of primary neoplasm. The skeletal system is one of the preferential sites for metastatic lesions (Greenspan and Borys, 2016; Reith, 2016). Clinically, osseous metastases are generally detected in nearly 25-30% of the patients at

initial diagnosis and in ca. 50% for advanced cases (Coleman and Rubinas, 2009; Schirrmeister and Arslandemir, 2010; Kumar et al., 2015). Not all primary neoplastic cell types have equal osseous metastatic propensity. The most osteotropic primary cancers, i.e. breast and prostate, tend to metastasize to bone in ca. 60-80% of the patients, followed by lung, kidney and thyroid cancers (ca. 30-65%). Gastrointestinal cancers have a far lesser predilection for bone metastasis (<18%) (Resnick and Kransdorf, 2005; Kumar et al., 2015; Greenspan and Borys, 2016; Reith, 2016). Based on metastasizing propensity reference values, one can estimate the theoretical frequency of metastatic cases expected in our skeletal sample ($F_{skeleton}$). As follows:

$F_{skeleton}=P_{ns}$ X P_{mtt}

Where P_{ns}= sample cases per organ, and P_{mtt}= proportion of bone metastasis per organ.

[Table 3 here]

Table 3 shows our calculation of expected values based on clinical studies published between 1902 and 1985, thus suitable for the chronology of our sample. The sum of the expected frequency of metastases ranged between 19% and 41%, varying in accordance with the distribution of the different types of primary malignant neoplasms. As it can be seen on Table 3, the values of identification of metastases in our study are in close proximity with the lower limit of the theoretical frequencies based on clinical studies.

4.2.1. *Interpretation of the score 2 results*

A brief discussion regarding the group of skeletons classified as *score 2* (examples described in section 3.1) must take into account three scenarios. Firstly, it is plausible that some of these osseous lesions represent markers of a metastatic process in the early stages of development. For the cases with new bone formation (n=22), a neoplastic pathogenesis cannot be excluded. As

Greenspan and Borys (2016:7) note, “no single periosteal response is hallmark to a particular neoplasm”, even if interrupted and rapidly produced bone is more likely to be seen in aggressive neoplasms (Ragsdale, 1993; Burgener et al., 2008; Schirrmeister and Arslandemir, 2010; Costelloe and Madewell, 2016; Greenspan and Borys, 2016). Thus, these discreet areas of bone formation or enlargement of the bone contour could be a response to the neoplastic disease recorded in the cause of death; however their typology and pattern are non-specific. Detection of unifocal osteolytic lesions (n=37) can also suggest initial stages of disease progression or solitary metastases (Resnick and Kransdorf, 2005; Reith, 2016). However, without previous knowledge of the cause of death it would not be possible to diagnose such cases with confidence, because osteolytic lesions also occur in non-neoplastic diseases. Paleopathologists “observe one stage of an often-complex sequence of skeletal involvement that is arrested at the time of death”, and not necessarily their prototypical manifestations (Ortner, 2011: 6). The high percentage of *score 2* patterns in this cohort also underscores the idea that there is potential to improve diagnostic criteria in paleopathology.

Secondly, we could hypothesize that *score 2* cases could be a consequence of para-neoplastic syndromes (occurring in ca. 8-15% of patients in clinical settings) (Coleman and Rubinas, 2009; Pelosof et al., 2010), some of which may have the potential to induce osseous changes. One such example is the well-established relationship between hypertrophic osteoarthropathy and thoracic cancers (Resnick and Kransdorf, 2005; Pelosof et al., 2010). Pelosof et al. (2010) notes that in current clinical practice, 90% of cases of hypertrophic osteoarthropathy are para-neoplastic syndromes. These events are difficult to account for in paleopathology, and are rarely attributed to neoplastic manifestations.

Lastly, individuals under *score 2* could also represent cases of co-morbidities (Schaik et al., 2014). Malignant neoplasms not only result in dysfunction of the affected organ, but also frequently lead to a decline in nutritional status and an associated rise in susceptibility to systemic

or local infectious diseases (Monteiro, 1921, Palmore et al., 2014). Thus, some non-specific lesions observed in skeletons may result from other disease processes associated with the underlying neoplasm.

4.2.2. *Scoring groups and biodemographic factors*

The most important biodemographic parameter that seemed to influence the scoring group allocation was the year of death. For later chronologies of death, the proportion of individuals with *score 3* augmented substantially. This result was not influenced by a differential proportion of more osteotropic malignancies in later decades of death (p=0.852). In order to interpret these time-related results, first we must consider the possibility of chance. We may also hypothesize that improvements in therapeutic measures and palliative care, in later decades, produced a longer survival time of these individuals, leading to a longer period living with the metastatic disease, and increasing skeletal dissemination (Mundy, 2002; Sundermeyer et al., 2005; Santini et al., 2012). In a clinical study of a cohort of individuals with colorectal cancers (1993-2002), Sundermeyer et al. (2005) showed that the incidence of bone metastases, as well as survival, increased significantly with the number of systemic therapies administered. Thus, it seems conceivable that in past populations, with less effective therapeutic options, individuals would have been less likely to develop skeletal lesions due to a shorter survival time with cancer. This might have been particularly true for rapidly progressing cancers. Conversely, we may also consider that in the past, the absence of therapies that target and reduce the burden of bone metastases may have caused the development of severe lesions, particularly in cancers that progress more slowly. Thus, although our study suggests that there is a relationship between the period when the individuals died and the presence of more severe lesions, the interpretation of this correlation is difficult. The presence and progression of bone metastases depend on multiple factors like the primary cancer cell-type, cancer grade, presence of extraosseous metastatic foci,

therapeutic procedure, and other factors (Coleman, 2006; Santini et al., 2012).

As expected, individuals with breast neoplasms were often allocated to *score 3* (21.7%); conversely, gastric neoplasms (34%) accounted for the highest percentage of individuals in *score 1* and *score 2*. The category of osteotropic (n=22) neoplasms had a higher frequency of cases in *score 3*. These results are compatible with clinical, radiographic, and autopsy findings, as previously mentioned (Galasko, 1986; Resnick and Kransdorf, 2005; Coleman, 2006; Rieth, 2016), and serve to emphasize the significance that the type of primary neoplasm has on the likelihood of developing bone metastasis. We argue that generalizations of the apparent paucity of cancer, as a homogenous entity, in ancient times, is misleading. Assuming that all types of cancer were rare in the past and applying current epidemiological trends to establish such inferences can lead to erroneous conclusions regarding these diseases in past societies. Naturally, paleopathologists will most likely diagnose osteotropic malignant neoplasms and also the ones in which the individuals have longer survival span (allowing time for the development of osseous lesions). Were those types of cancer the most prevalent in the past? Besides breast and skin cancers, infection-related cancers (e.g. stomach, uterus/cervix, liver) led the mortality statistics in the Western world up to the mid-20th century. After this period, a sharp downward trend occurred for uterine/cervical and stomach cancers, with a rise of prostate and lung cancers (Hoffman, 1915; Kumar et al., 2015; WHO, 2014). Infection-related cancers are still highly prevalent in low-income countries nowadays (Foreman and Ferlay, 2014). Estimates for 2008 indicate that 16.1% of new cancer cases were attributable to infections, with 22.9% recorded in low-income countries in sub-Saharan Africa (Ohshima et al., 2014). The burden of liver, stomach, and cervical cancers is still notorious in this region. The relative prevalence of cancers by primary site in ancient populations might more closely resemble the early 20th century trends (Waldron, 1996) or the ones observed today in low-income countries. Broadly, most of these infection-related cancers have less osteotropic propensity and generally a poorer survival span. If these were the prevailing types

of malignant neoplasms in past populations, then it would be expected that skeletal evidence of cancer in the past would be less likely to be detected.

This idea, allied with a relatively low *sensitivity* of paleopathological diagnosis, and the fragmentary nature of archaeological remains, can hinder the detection of a highly significant portion of cases in the past (Waldron, 1996; Capasso, 2005; Brothwell, 2012). As shown in our study, a major proportion of cases either did not manifest osseous lesions, or presented a set of skeletal alterations that were not severe, widespread, or specific enough to suggest a preferred diagnosis of cancer. Lack of observation of inner bone structures (deliberately, no imaging techniques were considered for scoring allocation) also limited our diagnostic ability. Radiology is a key technique to identify osseous metastasis and is not systematically applied while studying skeletons from archaeological sites.

To place major emphasis on a lesser carcinogenic environment in ancient populations for their extreme rarity (David and Zimmerman, 2010), underestimates the effect of a wide range of other limits, proposed in the present work. Furthermore, one cannot be oblivious to the impact of endogenous (e.g. hormones, products of metabolism), and exogenous carcinogens (e.g. sunlight, ionizing radiation, infectious vectors), some of them man-made (e.g. exposure to lead, copper or arsenic [Ozdemir et al., 2010]) in pre-contemporary societies (Halperin, 2004).

We argue against generalizations of past scarcity of cancer, as well as the notion that cancer can be addressed as one single disease, with one cause, etiology, or a similar epidemiological trend over time (Aktipis and Nesse, 2013). This nosologic entity encompasses heterogeneous diseases with a crucial interplay of multiple factors (Aktipis and Nesse, 2013). Naturally, "cancer risk is underpinned by intrinsic fallibility, increasing in expression age and greatly exacerbated by some aspects of human activity" (Greaves, 2008: 278). Thus biological, sociocultural, and demographic shifts, as well as better clinical diagnostic methods and epidemiological surveys, all contributed to the currently known epidemiological landscape of cancer (Coleman and Rubinas,

2009; Forman and Ferlay, 2014; Kumar et al., 2015).

As a final note, we would like to emphasize that although neoplasms are often part of the differential diagnosis of many conditions in paleopathology, the theoretical assumption of their rarity often serves as the main argument to rule them out as a diagnostic possibility, which by its turn feeds the idea of their paucity in the past, as a self-fulfilling prophecy of sorts. Thus, this theoretical "circular reasoning" can be harmful to advances in paleo-oncology.

5. Conclusion

Biological, societal, cultural, demographic, and environmental parameters are amongst the promoters of temporal increase on cancer burden. Despite the extreme complexity of oncological disorders, the study of their past epidemiology is a worthwhile and justifiable endeavor. Evaluation of diachronic shifts in cancer, and the search for explanatory models for its variability, is a crucial, and surely promising, future goal of paleo-oncological research. However, it cannot be fully reached without reformulating some current paradigms and implementing tailored approaches to diagnosis and paleoepidemiological methods. As shown by the present study, the impact of the limits of paleopathological diagnosis is by no means negligible. The problem of false-negatives is a real one, and it must be accounted for while debating the scarcity of cancer in the past. We agree with Faltas (2011) and other scholars that argue that diverse methodological constraints hinder the appraisal of oncological disorders in past populations. Our study indicates that there is a relatively low capacity for cancer detection based only in the visual inspection of human skeletal remains. A lack of systematic radiological surveys of skeletons from archaeological sites and a lack of paleoepidemiological approaches are two major obstacles to the study of cancer in the past.

We also emphasize that one cannot make generalizations about the overall absence of malignant neoplasms without taking into account that cancers are not a single pathogenetic entity

(Aktipis and Nesse, 2013). The question should be readdressed to which types of cancers were, in fact, rare, or if the most frequent cancers in the past were also the ones that show less osteotropic potential. Despite the undeniable fact that the burden of cancer increased dramatically in the last century due to societal and lifestyle changes, as well as growing and ageing populations, paleopathology still has an enormous potential to uncover more clues to its past prevalence.

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Tables

Table 1. Biodemographic profile of Coimbra (CISC) and *Museu Bocage* (MBISC) reference collections (1.), and of the neoplasms cohort under study (2.).

	1. Reference Collections			**2. Neoplasm Cohort**		
	MBISC	**CISC**	**Total[a]**	**MBISC**	**CISC**	**Total[a]**
N [%]	769 [60.4]	505 [39.6]	1274 [100]	89 [67.9] [11.6]	42 [32.1] [8.3]	131 [100] [10.3]
Sex (n, [%])						
Males	360 [46.8]	266 [52.7]	626 [49.1]	36 [40.4]	17 [40.5]	53 [40.5]
Females	409 [53.2]	239 [47.3]	648 [50.9]	53 [59.6]	25 [59.5]	78 [59.5]
Age at death						
Mean ± SD	53.2 ±26.1[b]	44.9 ±20.2[b]	49.9 ±24.2	62.9 ±14.5	52.6 ±15.6	59.6 ±15.6
Interval	0-98	7-96	0-98	15-93	21-79	15-93
Birth (yr.)						
Mean ± SD	1889 ±27.2	1880 ±22.0	1885 ±25.5	1884 ±19.4	1874 ±16.5	1880±19.1
Interval	1805-1974	1822-1921	1805-1974	1821-1949	1843-1906	1821-1949
Death (yr)						
Mean ± SD	1945±13.2[c]	1925 ±7.1[c]	1937 ±14.6	1947 ±11.3	1927 ±4.6	1941±13.4
Interval	1880-1974	1904-1936	1880-1974	1904-1969	1910-1936	1904-1969

n= number of individuals; %= crude prevalence, yr= years, SD= standard deviation (years), [a]= pooled data from CISC and MBISC, [b]= statistical differences in the mean age at death between MBISC and CISC (t=6.378, df=1235.6, p<0.001, 95% CI]5.736-10.833[, n=1274), [c]= statistical differences in the mean years of death between MBISC and CISC (t=30.023, df=1196, p<0.001, 95% CI]18.084-20.613[, n=1274).

Table 2. Biodemographic profile by score for the neoplasm cohort.

	Lesional pattern scoring groups			
	Score 1	**Score 2**	**Score 3**	**Sign.**
N [%]	49 [37.4]	59 [45.0]	23 [17.6]	
Sex (n, [%][a])				
Males	21 [39.6]	26 [49.1]	6 [11.3]	*p*=0.284
Females	28 [35.9]	33 [42.3]	17 [21.8]	
Age at death				
Mean ± SD	62.0 ±12.1	56.6 ±16.6	62.2 ±19.0	*p*=0.133
Interval	36-91	21-93	15-87	
Birth (yr.)				
Mean ± SD	1878 ±17.2	1881 ±18.1	1884 ±24.6	*p*=0.466
Interval	1821-1913	1843-1924	1852-1949	
Death (yr)				
Mean ± SD	1940 ±13.7	1938 ±13.1	1948 ±10.9	*p*=**0.009**
Interval	1904-1965	1910-1969	1926-1968	
API				
Mean ± SD	90.3 ±14.1	91.3 ±11.3	88.2 ±13.2	*p*=0.618

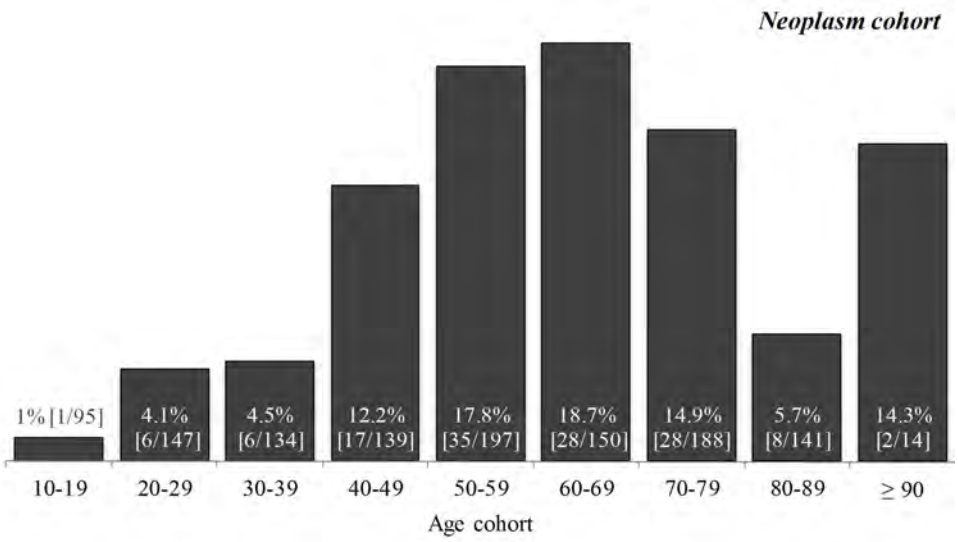
n= number of individuals, %= crude prevalence, yr= years, SD= standard deviation (years), [a]= percent value calculated within sex sub-set ($n_{females}$=78, n_{males}=53).

Table 3. Reference values for frequency of skeletal lesions in clinical settings and computation of expected number on the sample under study.

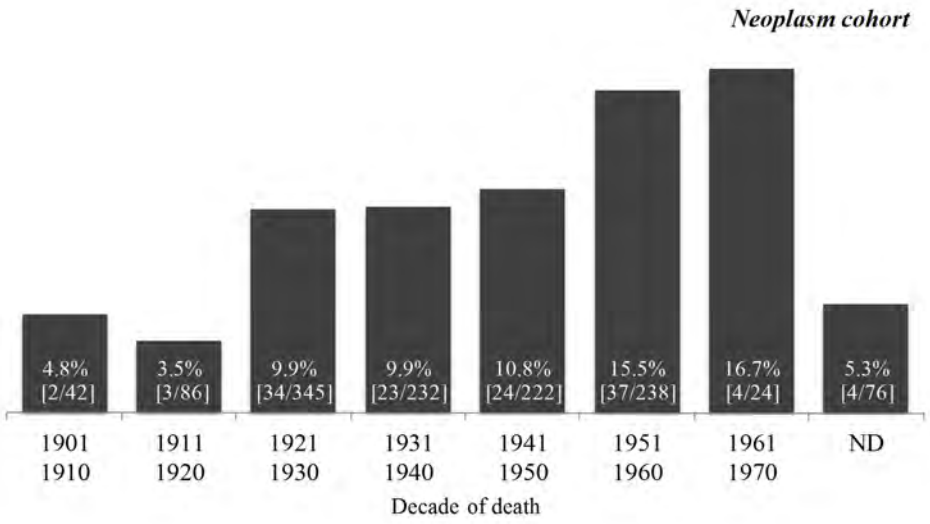
	Sample	**Clinical**		**Expected**	
	n	*Lower bound %*	*Upper bound %*	*Lower bound n*	*Upper bound n*
Prostate	9	33	85	3	8
Breast	6	47	85	3	5
Lung	1	30	64	0	1
Kidney	2	33	60	1	1
Colon/rectum	18	8	61	1	11
Uterus	21	50	56	11	12
Leuk./Lymp.	5	5	50	0	3
Bladder	2	13	42	0	1
Oral cavity/ larynx/pharynx	3	14	21	0	1
Stomach	32	2	18	1	6
Pancreas	2	1	4	0	0
Oesophagus	1	1	2	0	0
Liver	6	0	1	0	0
Brain	4	0	1	0	0
Skeleton	3	100	100	3	3
Multiple	7	-	-	0	0
Total n	**122**			**23**	**50**
Total %				**18.9%**	**41.0%**
Excluded					
No reference	8				
Carotid/aorta	1				
Total	***131***				

n= number of individuals, %= percent. Data obtained from autopsy and imagiologic studies compiled in Galasko (1986) and Resnick & Kransdorf (2005), based on diverse works published between 1902 and 1985.

Figures



[a]



[b]

Fig. 1. [a] Distribution of malignant neoplasm cohort (n=131) by age at death classes (sexes pooled). [b] Distribution of malignant neoplasm cohort (n=131) by decades of death (sexes pooled). Legend: %= percent, [n/N]= number of neoplasms/ number of the individuals in the collection per age class or decade of death, ND= no data.

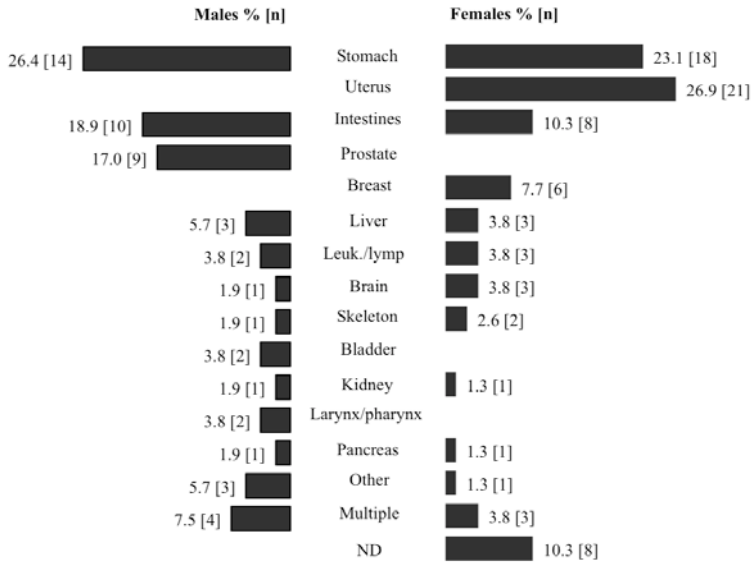


Fig. 2. Prevalence (%) and absolute frequency (n) of the malignant neoplasm cohort (n=131) by sex sub-set ($n_{females}$=78, n_{males}=53). Legend: n= number of individuals, Intestines=intestines, colon and rectum, Leuk./lymp= leukaemia, lymphoma and similar, Other=lung, carotid/aorta, mouth, oesophagus, Multiple= multiple organs, and diffuse in the abdomen, peritoneum, mediastine, ND= no data for primary site.

Fig. 3. Illustration of *score 2*: delimited area of new bone formation, with one osteolytic focus (geographic type), in the ventral surface of pubic bones (sk. 1609, female, 46 years old, uterine cancer).

Fig. 4. Illustration of *score 2*: a focus of osteolytic activity with remodeled borders, accompanied by new bone formation (max. 60 mm) in the sacrum (sk. 847, male, 45 years old, rectal cancer).

Fig. 5. Illustration of *score 2*: focal new bone formation (woven) in the shaft of the 8th right rib (visceral surface) (sk. 456, female, 38 years old, "lymphosarcoma of neck and mediastine").

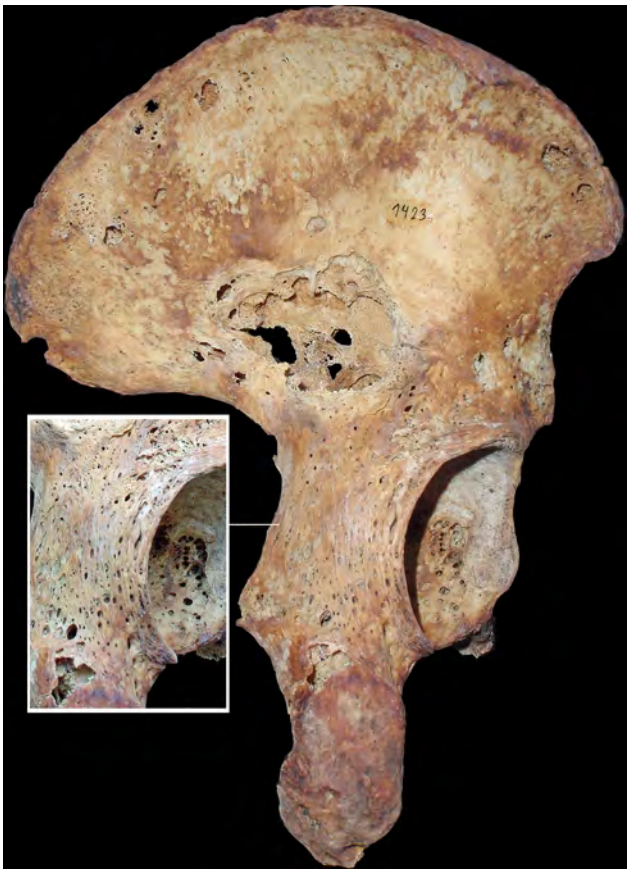


Fig. 6. Illustration of *score 3*: diffuse osteolytic activity (multiple geographic foci and moth-eaten patterns [white square]) in the posterior surface of the right ilium. The lesions were also located in the skull, mandible, scapulae, sternum, pelvic bones, femurs, and humeri. (sk. 1423, female, 32 years old, carcinoma of the stomach).

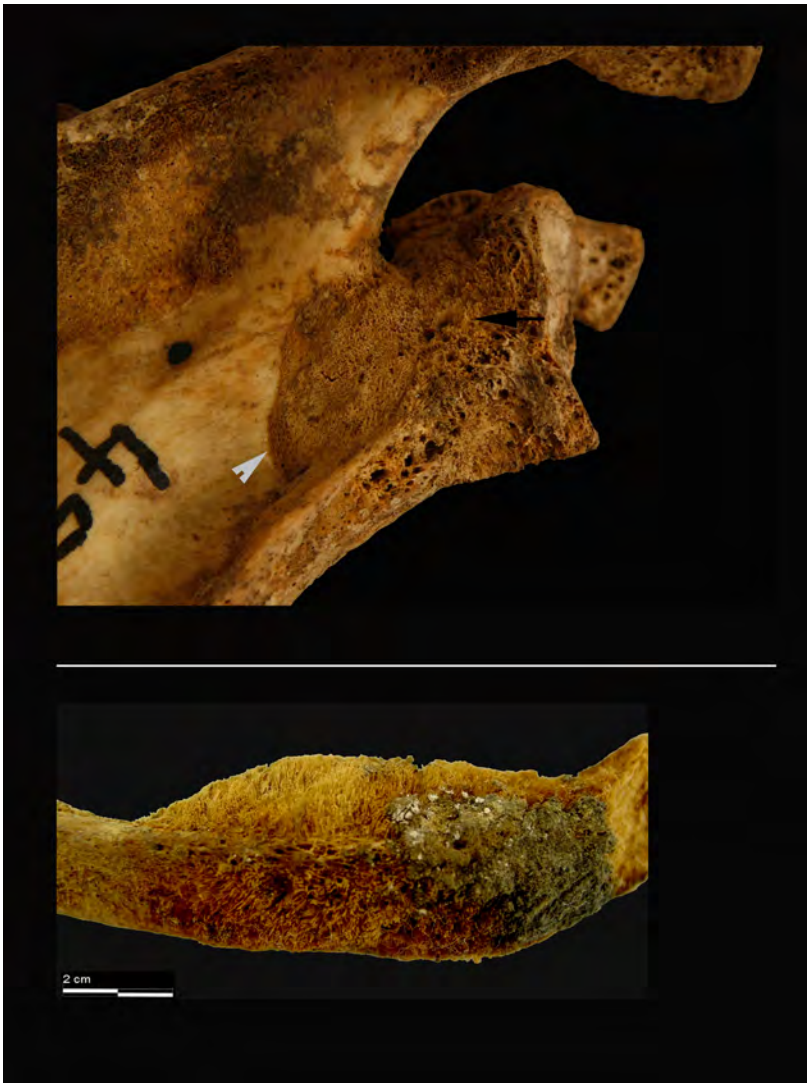


Fig. 7. Illustration of *score 3*: exuberant osteoblastic activity associated with osteoclastic activity in the right scapula, posteriorly (upper image), with radiant spicules ("sunburst" appearance) (black arrow), as well as areas with coarse and rounded spicules ("coral-like" appearance) (white arrow). Sternal area of the 6th right rib sternal (lower image) shows radiant spicules ("sunburst" appearance). Lesions were present in all anatomical areas except the forearm, patellae, fibulae and hands (sk. 457, male, 66 years old, rectal neoplasm).

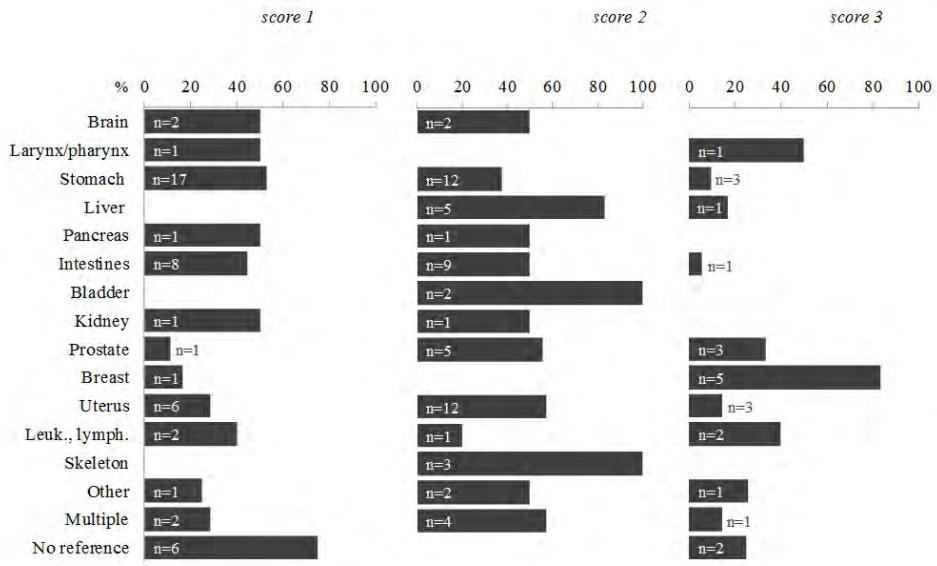


Fig. 8. Prevalence (%) and absolute frequency (n) of malignant neoplasms by primary organ and score groups.