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**dry bone histology**



## Chapter 4

### THE DIAGNOSTIC VALUE OF MICROSCOPY IN DRY BONE PALAEOPATHOLOGY: A REVIEW

From: *The diagnostic value of microscopy in dry bone palaeopathology: a review*. De Boer, H.H., Van der Merwe, A.E., Maat, G.J.R., 2013. *International Journal of Paleopathology* 3(2), 113-121.

**Abstract.** Over recent decades histology has increasingly been used as a diagnostic tool in human dry bone palaeopathology. Still, the use of histology in human dry bone is associated with various problems, including a lack of pathognomonic histomorphology and a need for more experimental data. Consequently, the value of histology as diagnostic tool in human dry bone remains a subject for debate.

Here we review all published palaeohistopathological research in human dry bone. A systematic search identified 3363 articles, with the 64 most relevant citations studied in depth. We specifically focused on the interpretation of histomorphological parameters and the use of comparative fresh bone tissue and/or experimental data.

Our literature review shows that only a few disorders demonstrate a 'specific' histomorphology: Paget's disease, osteoporosis, hyperparathyroidism and possibly osteomalacia. In all other cases, histology may aid during the differential diagnostic process, but alone it is unable to confirm a definitive diagnosis. The histological diagnostic process and consequential recommendations for the use of histology are discussed per following disease categories: metabolic disease, neoplasm, infectious disease and trauma.

### Introduction

Numerous papers have been published in which the palaeopathological diagnosis of disease was supported by histological investigation. However, no recent reviews regarding the impact of these efforts have been published. Here we discuss the limitations of palaeohistopathological investigation in general and systematically review palaeohistopathological diagnostic efforts and/or potential per disease category. Special emphasis will be placed on the difficulties associated with the

interpretation of palaeohistopathological images. This paper will also touch on the interplay between palaeopathological findings, fresh tissue pathology and experimental research. Our aim is to add depth to ongoing debates about the diagnostic value of microscopy in dry bone tissue.

## **Limitations of palaeohistopathology**

In palaeopathology, the identification of gross anatomical processes in human skeletal remains and their interpretation are considered challenging (Wood *et al.*, 1992; Aufderheide and Rodriguez-Martín, 1998; Ortner, 2003). The same challenges are relevant in palaeohistopathology. Dry bone remains are void of soft tissue and bone tissue cells, and it is just these components that provide fresh tissue pathologists with the pathognomonic features leading to a reliable diagnosis (e.g. Ross *et al.*, 1995; Vigorita, 2007; Rosai and Ackermans, 2011). This problem is further complicated by the nature of bone tissue, which only reacts to a stimulus (disease) in three ways visible on a microscopic level: resorption of bone tissue (an osteoclastic bone response), deposition of new bone tissue (an osteoblastic bone response) or a combination of the two (Frost, 1985). Only a small subset of diseases can be associated with such characteristic histological alterations that they can be regarded as pathognomonic. For all other diseases, microscopic changes appear to be similar, and as a result various authors have downplayed the value of palaeohistopathology as a diagnostic tool (Putschar, 1966; Stout and Simmons, 1979; Waldron, 2009).

Bianco and Ascenzi (1993) stated that the lack of pathognomonic histological information derived from skeletal remains, in combination with a lack of independent extra source information, such as medical data, posed a fundamental problem to the advance of palaeohistopathology. According to these authors, palaeohistopathologists risk 'making nonscientific statements', i.e. statements that cannot be proven false; a problem that could be minimized by new knowledge on the visibility of changes that specific diseases show in dry bone. They stressed the need for research in which palaeohistopathological diagnoses are supported by experimental research or comparative research with the use of current documented dry bone reference specimens.

## **Palaeohistopathological research in the past decades**

The popularity of histology as a diagnostic tool for archaeological remains has increased over recent decades. In 2001, pronouncements on the value of microscopy

as a diagnostic tool were generally optimistic (Schultz, 2001). Palaeohistopathology was said to be a dependable tool in the differentiation between tumors, metabolic disorders and infectious diseases since specific histoarchitectural characteristics could be linked to specific diseases. This latter statement contrasted sharply with earlier consensus, which was generally more skeptical (Putschar, 1966; Stout and Simmons, 1979; Bianco and Ascenzi, 1993). Several examples were presented to prove the specificity of morphological features, such as those for the diagnosis of syphilis in dry bone tissue (Schultz, 2001; Schultz, 2003; Von Hunnius *et al.*, 2006).

However, the diagnostic power of these features has been challenged. For instance, Weston (2009) and Van der Merwe *et al.* (2010) showed that microarchitectural features alone were by no means diagnostic for specific diseases such as treponematosi, and Schutkowski and Fernandez-Gil (2010) came to the same conclusions for tuberculosis and leprosy. All in all, more recent publications challenged the diagnostic value of microscopy, a debate that is pressing due to the questioned acceptance of destructive sampling.

## Methods

To avoid bias from expert-based reviewing, we used a systematic and sequential inclusion-exclusion strategy to select relevant articles. Ten keynote articles were selected prior to key word article retrieval to test the adequacy of the search (Table 1). Since palaeohistopathological papers are distributed widely throughout the medical, physical anthropological and archaeological literatures, a general search was executed for both Pubmed and Web of Science references. The search strategy consisted of the key word combination of 'physical anthropology AND histology'. The search details can be found in Table 2. In order to ensure that no relevant articles were missed, publications from the American Journal of Physical Anthropology, Journal of Archaeological Science, International Journal of Osteoarchaeology and International Journal of Paleopathology with the terms 'histolog\*' or 'microscop\*' in the title were also included. A total of 4155 articles were automatically retrieved of which 792 duplicates could be excluded.

To continue in the study sample, the remaining articles had to comply with the following: 1) the article was written in English, 2) histological investigation was performed on human dry bone, 3) microscopic investigation (light microscopy, microradiography or scanning electron microscopy) was used as a palaeopathological diagnostic tool, 4) the article was an original research paper. An exception for the latter criterion was made for the review article by Schultz (2001), since it added a substantial amount of key data from outside the English literature.

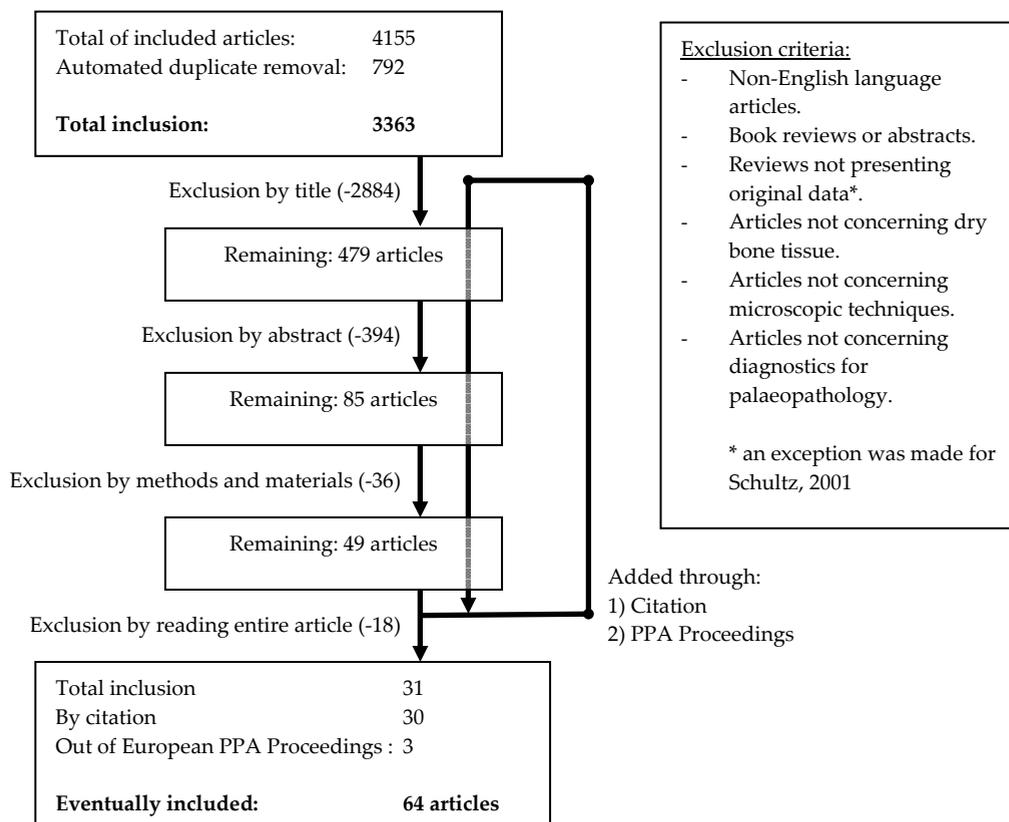
**Table 1. Key-note articles used for testing the sensitivity of the inclusion criteria.**

<b>Authors</b>	<b>Year</b>	<b>Title</b>	<b>Journal</b>
<i>Aaron et al.</i>	1992	Paleohistology of Pagets-Disease in 2 Medieval Skeletons.	Int. J. of Osteoarchaeology
<i>Blondiaux et al.</i>	1994	Microradiographs of leprosy from an osteoarchaeological context.	Int. J. of Osteoarchaeology
<i>Cuijpers</i>	2009	Distinguishing between the bone fragments of medium-sized mammals and children. A histological identification method for archaeology.	Anthropol. Anzeiger
<i>Guarino et al.</i>	2006	Bone preservation in human remains from the Terme del Sarno at Pompeii using light microscopy and scanning electron microscopy.	J. of Archaeological Science
<i>Von Hunnius et al.</i>	2006	Histological identification of syphilis in pre-Columbian England.	Am. J. of Phys. Anthrop.
<i>Hackett</i>	1981	Development of caries sicca in a dry calvaria.	Virchows Archiv
<i>Hanson &amp; Chester</i>	2007	Examining histology to identify burned bone.	J. of Archaeological Science
<i>Maat et al.</i>	2001	Manual preparation of ground sections for the microscopy of natural bone tissue: update and modification of Frost's 'rapid manual method'.	Int. J. of Osteoarchaeology
<i>Schultz</i>	2001	Paleohistopathology of bone: a new approach to the study of ancient diseases.	Yearb. of Phys. Anthrop.
<i>Stout &amp; Teitelbaum</i>	1976	Histomorphometric determination of formation rates of archaeological bone.	Calcif. Tiss. Res.

**Table 2. Search strategies per database.**

<b>Database</b>	<b>Strategy</b>
<b>PubMed</b>	((('Anthropology, Physical'[mesh] NOT 'Fossils'[mesh]) OR paleopathology OR 'forensic anthropology' OR 'physical anthropology' OR paleopatholog* OR 'biologic anthropologic' OR archaeology [mesh] OR archaeology [tw] OR archaeological [tw]) AND ('histology'[tw] OR 'histology'[mesh] OR 'microscopy'[mesh] OR 'microscopy'[tw] OR 'histological techniques'[mesh])).
<b>Web of Science</b>	TS=((paleopathology OR forensic anthropology OR physical anthropology OR paleopatholog* OR biologic anthropologic OR biologic anthropology OR forensic anthropologic OR physical anthropologic OR archaeologic*) AND (histology OR histologic OR histolog* OR Histological OR Histological Techniques OR Autoradiography OR Bone Demineralization Technique OR Decalcification Technique OR Histocytochemistry OR Immunohistochemistry OR Histocytochemical OR Immunohistochemical OR Histocytological Preparation Techniques OR Microdissection OR Microtomy OR Replica Techniques OR Staining and Labeling OR Tissue Embedding OR Tissue Preservation OR microscopy OR Diagnostic Imaging OR Photomicrography OR Age Determination by Skeleton OR age determination OR Fluoroscopy OR Microradiography OR Sex Determination by Skeleton OR sex determination OR Radionuclide Imaging OR Spectroscopy)).

**Table 3. Total count of references in- and exclusion.**



Other relevant citations were also included, for instance those connected to the Proceedings of the European Meetings of the Palaeopathological Associations beginning in 1982. The procedures for article selection are illustrated in Table 3.

The histological diagnostic features used in the articles were grouped by diagnosis. Their value was assessed by evaluating whether the features were rooted in experimental data, fresh tissue specimen of known cases or a rigorous biomedical discussion of pathogenesis.

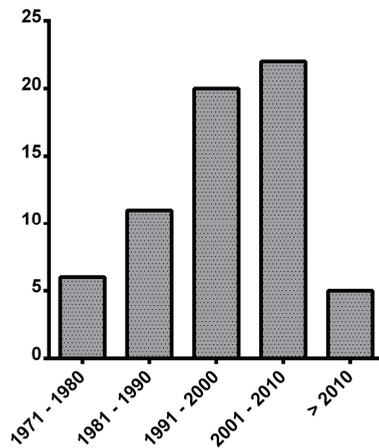
## Results and discussion

The seemingly small number of remaining articles, when compared to the large body of literature that was initially identified (see Table 3), was in line with statements by Schultz (2001) and Weston (2009) that the histology of dry bones had

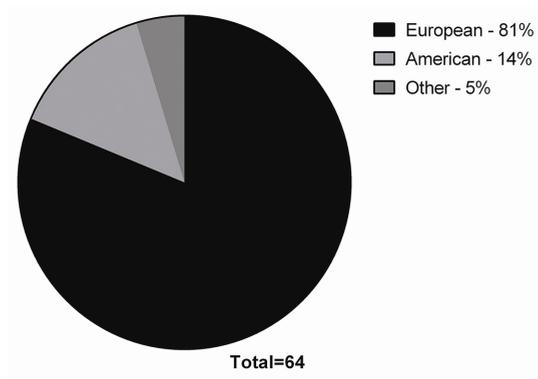
not been frequently used for palaeopathological diagnoses. Nevertheless, the growing popularity of palaeohistopathology was illustrated by a steady increase in the number of journal articles per decade (see Table 4), with the majority being published within the last twenty years, 14% with American first authors and 81% with European first authors (see Figure 1).

This literature included metabolic, neoplastic, infectious and traumatic pathological conditions. These conditions will be discussed in the following sections with respect to the diagnostic value of their microscopic features.

**Table 4. Published articles per decennium.**



**Figure 1.**



Nationality of first authors.

## Possible and potential diagnostic features

### Metabolic disorders

#### *Osteoporosis and osteopenia*

Osteoporosis is not a disease in itself but a symptom, which may be associated with various conditions such as malnutrition, hormonal imbalance or disuse. It is defined as a decrease in bone tissue volume and primarily leaves the gross anatomical bone outline intact. It is routinely assessed clinically by radiographic measurement of the bone mineral density (BMD) (Resnick, 1996; Brown, 2006). Histomorphometric parameters such as trabeculae number, cortical thickness and mean trabecular width may also be used during the diagnostic process (Vigorita, 2007; Barger-Lux and Recker, 2009). Due to soil contamination, radiology may not be the method of choice for the assessment of osteoporosis in archaeological skeletal remains, and therefore palaeopathologists tend to use histomorphological parameters such as cortical thickness, cortical area and trabecular bone mass as diagnostic measures (Richmann and Ortner, 1979; Martin and Armegalos, 1979, 1985; Gonzales-Reimers and Arnay-de-la-Rosa, 1992; Velasco-Vazquez *et al.*, 1999; Paine and Brenton, 2006; Cho and Stout, 2011). The utility of these diagnostic features was corroborated by related clinical reports and fresh tissue pathology, which led researchers to conclude that dry bone microscopy allowed for differentiation between various causes of osteoporosis (Richmann and Ortner, 1979; Martin and Armegalos, 1979, 1985) and that the occurrence of osteoporosis could be linked to social structure, life style and dietary customs (Gonzales-Reimers and Arnay-de-la-Rosa, 1992; Velasco-Vazquez *et al.*, 1999; Paine and Brenton, 2006).

In examples where bone is imperfectly preserved, a lack of intact tissue may hamper a histomorphological assessment. Therefore, some researchers resorted to (subjective) qualitative assessment of osteoporotic changes. Roberts and Wakely (1992) used scanning electron microscopy (SEM) to detect microfractures in fragile osteoporotic bone. The authors suggested that SEM may detect osteoporosis at an earlier stage than radiography. However, they admitted that the sole presence of microfractures was a non-specific change, since there was no information available on its relationship with the severity of osteoporosis. Schultz (1999) diagnosed osteoporosis in a fractured Neanderthal ulna by virtue of its enlarged Haversian canals and the replacement of cortical shaft tissue by cancellous bone. The unilateral presence suggested disuse as the cause. While these results are tantalizing, we argue that the diagnosis of osteoporosis is most convincing when using a histomorphometrical approach, since osteoporosis is by definition, a quantitative change (Raisz, 2005).

## ***Scurvy***

Since scurvy leaves histomorphological traces in the skeletal record (Resnick, 1996; Cotran *et al.*, 1999; Ortner, 2003; Fain, 2005), histology has been used to corroborate gross anatomical palaeopathological diagnosis (Maat and Uytterschaut, 1984; Schultz, 2001; Maat, 2004; Van der Merwe *et al.*, 2010; Mays *et al.*, 2012). Maat (2004) described several histological features which may be indicative of active and healed scurvy, i.e. denaturalized hemoglobin in black maculas around metaphyseal endofractures and longstanding ossified hematomas (Maat and Uytterschaut, 1984; Maat, 2004). In contrast to periosteal reactions of an infectious or neoplastic nature, ossified hematomas do not affect the periosteal surface (also see Schultz, 2001). Stratification of such ossified hematomas indicates recurrent episodes of scurvy.

The criteria of Maat (2004) and Schultz (2001) were built on earlier studies of soft tissue pathology and experimental data (Murray and Kodicek, 1949; Van Wersch, 1953) and were recently confirmed by Van der Merwe *et al.* (2010), who added that gradual ossification and remodeling of a hematoma occurred in three phases in which the radiating structure of the appositional bone stayed visible for a considerable period of time. Maat (2004), Schultz (2001) and Van der Merwe *et al.* (2010) did not report increased resorption parameters and defective osteoid formation while others describe these (non-pathognomonic) features to be associated with scurvy (Fain, 2005; Waldron, 2009).

Isolated ossified hematomas are not pathognomonic for scurvy (Schultz, 2001; Van der Merwe *et al.*, 2010). Their appearance in true scorbutic cases is dominated by gross anatomical symmetry, especially in the lower extremities. Therefore, it can be concluded that although histology aids in the identification of ossified hematomas, additional gross anatomical features are needed for a definitive scurvy diagnosis.

## ***Rickets and osteomalacia***

Fresh bone from active rickets and osteomalacia has a distinct histomorphology showing the accumulation of osteoid and increased resorptive activity (Mankin, 1974; Vigorita, 2007). Nevertheless, the identification of these features in dry bone is difficult. Stout and Teitelbaum (1976) suggested that increased resorption in suspected cases might indicate osteomalacia. Yet, they admitted that increased osteoclastic activity is by no means specific. Schultz (2001) stated that rickets can cause porotic hyperostosis of the vault and can be differentiated from other causes by microscopic features, such as restricted thickening of the vault, splintered internal and external lamina and a 'totally changed microstructure'. However, the vague description of these features and a lack of fresh bone tissue diagnoses and experimental data made these criteria of little practical value.

In an attempt to describe identifiable diagnostic features, Schamall *et al.* (2003) used histomorphometry and qualitative histology on documented museum specimens of rickets and osteomalacia. In both, diminished bone tissue volume (osteopenia) was noted, together with resorptive features such as fields of Howship's lacunae and enlarged osteocyte lacunae. Backscattered electron microscopy and microradiographs showed hypomineralization. These results corresponded to those found by Brickley *et al.* (2007), who studied osteomalacic skeletons by means of regular scanning electron microscopy. In addition, Brickley *et al.* described distinct ring-like structures, so-called 'defective cement lines', which most likely resulted from the postmortem loss of osteoid. It was suggested that 'defective cement lines' were pathognomonic for osteomalacia. All results of Schamall *et al.* (2003) and Brickley *et al.* (2007) were thoroughly supported by clinical case studies in which osteomalacia was diagnosed based on fresh bone tissue specimens.

Osteopenia and resorptive features can be seen in various diseases, such as infection or hyperparathyroidism. Hypomineralisation can be caused by any process that induces rapid growth and remodeling (Gryn timer, 1993). We therefore conclude that Schamall *et al.*'s (2003) sole finding of described features in rachitic and osteomalacic specimens cannot be regarded as pathognomonic. Yet, in cases where these histological features can be associated with the characteristic pattern of gross anatomical changes observed in cases of the two diseases (e.g. bowed long bones in case of rickets and Looser zones in case of osteomalacia), they will strongly support the diagnosis.

The 'defective cement lines' described by Brickley *et al.* (2007) might be a pathognomonic finding, but it has not been reported by other workers. Yet, it is thoroughly supported by comparative research and biomedical literature (e.g. Boyde *et al.*, 1986; Parfitt, 1998). Future research is required to establish its usefulness.

### ***Hyperparathyroidism***

Histomorphological features of hyperparathyroidism develop when the increased activity of the parathyroid glands stimulates profuse bone resorption, which is indicated by an increased number of Howship's lacunae and the enlargement of Haversian canals. The disease may be primary, secondary to hypocalcaemia, or even tertiary (in the case of long-lasting secondary hyperparathyroidism). Resorption is particularly noticeable in (sub)periosteal bone tissue and cancellous bone, which in the latter causes 'dissecting osteitis' or 'tunneling resorption' (Cotran, 1999; Vigorita, 2007; Fraser, 2009; McCarthy, 2010). These features, especially 'tunneling resorption', remain detectable in well-preserved palaeopathological material (Weinstein *et al.*, 1981; Cook *et al.*, 1988; Zink *et al.*, 2005; Mays *et al.*, 2007; Waldron, 2009;).

Although osteoporosis and hypovitaminosis D may mimic hyperparathyroidism histologically (Weinstein *et al.*, 1981; Cook *et al.*, 1988; Mays *et al.*, 2007;), tunneling resorption is widely accepted as pathognomonic (Vigorita, 2007; McCarthy, 2010). Therefore, we can conclude that this disease can be diagnosed palaeohistopathologically in well-preserved cases. However, as this rare affliction appears without any gross anatomical changes of the skeleton, it will often remain undiagnosed in palaeopathology.

### ***Paget's disease***

On a microscopic level, Pagetic bone lesions develop in three phases: an initial osteolytic stage, followed by a mixed osteoclastic-osteoblastic stage, and ultimately evolving into a quiescent osteosclerotic stage (Cotran, 1999), which is characterized by a distinctive 'mosaic' pattern of woven and lamellar tissue, demarcated by numerous convoluted cement lines in trabecular and cortical bone tissue - a pathognomonic 'patchwork' architecture (Ralston, 2008). Stout and Teitelbaum (1976) hypothesized that this distinct histomorphology would remain visible in archaeological remains. Bell and Jones (1991) and Aaron *et al.* (1992) confirmed this, when they analyzed pagetic bone with SEM and light microscopy, respectively. Their findings concurred with those seen in a macerated specimen of a reported case of Paget's disease. Additionally, diagnosis was proved possible in poorly preserved archaeological bone material (Bell and Jones, 1991; Roches *et al.*, 2002).

As noted by Aaron *et al.* (1992) and Roches *et al.* (2002), some types of neoplasm may mimic pagetic histomorphology. Osteogenic sarcoma, which can be a complication of Paget's disease (Ortner, 2003), and osteoblastic carcinoma (e.g. metastases of prostate or mamma carcinoma) should be considered in the differential diagnoses. However, these do not present the same gross anatomical and radiological changes (Wells and Woodhouse 1975; Ortner, 2003). Consequently, histology is an appropriate method to confirm Paget's disease in dry bone material.

### ***Hereditary and acquired hemolytic anemia***

Anemia is not a disease, but like osteoporosis it is a symptom resulting from pathological conditions such as malaria, iron deficiency, sickle cell disease or thalassaemia to name a few. In some chronic cases (hereditary and acquired haemolytic types), an attempt to increase the production of red blood cells results in bone marrow hyperplasia. As a result, radial enlargement of adjacent cancellous bone occurs at the cost of (porotic) thinning of the external cortical lamina (Middlemiss and Raper, 1966; Stuart-MacAdam, 1987; Resnick, 1996; Tyler, 2006; Ejindu, 2007). Consequently, marrow hyperplasia may cause cranial porotic hyperostosis, which

was studied via SEM by Marcsik *et al.* (1984), Maat and Baig (1990) and Maat (1991). The expansion of the marrow cavity at the cost of the outer table has shown to be visible in archaeological remains. SEM has even allowed for the establishment of a conclusive diagnosis when malformed erythrocytes, pathognomonic for sickle cell disease, can be identified (Maat and Baig, 1990; Maat, 1997). However, such a finding is rare. Schultz (2001) and Wapler *et al.* (2004) confirmed the detectability of marrow hyperplasia in cranial porotic hyperostosis and cribra orbitalia by means of light microscopy. Furthermore, Schultz (2001) suggested six developmental phases, ranging from minor changes to the exuberant hair-on-end appearance.

When differentially diagnosing cranial porotic hyperostosis and cribra orbitalia, infectious disease, rickets or scurvy should be considered (Marcsik *et al.*, 1984; Schultz, 2001; Wapler *et al.*, 2004). Schultz (2001) and Wapler *et al.* (2004) state that infectious lesions usually present a relatively more destructive (lytic) and irregular microarchitecture, indicated by numerous Howship's lacunae and irregular new bone formation (Schultz, 2001; Wapler *et al.*, 2004). However, these statements were not corroborated by documented cases or experimental data. Marcsik and colleagues (1984) theorized that in principle, rickets and scurvy primarily affect the outer surface of the external bone table and do not cause an expansion of the diploë (Marcsik *et al.*, 1984). Their theory was in line with more recent clinical findings (Resnick, 1996; Tyler, 2006; Vigorita, 2007).

It can be concluded that the histomorphology of marrow hyperplasia allows for an accurate histological identification. Histology can be a useful tool to narrow the differential diagnosis associated with porotic hyperostosis and cribra orbitalia.

## Neoplasms

### *Primary tumors*

In a clinical context, fresh tissue pathology is the basis for the diagnosis of bone tissue tumors. As a result, a large amount of literature exists on their histomorphology (e.g. Cotran, 1999; Vigorita, 2007; Rosai and Ackermans, 2011). In contrast, only a small number of primary bone tumors have been studied palaeohistopathologically.

In the majority of cases, histology is used to identify whether the tumorous process is benign or malignant. In general, the differentiation of bone tumor tissue is inversely correlated to its malignancy grade. Benign lesions can be recognized by their overall regular lamellar tissue architecture. They do not grow invasively and therefore rarely show osteolytic activity (Strouhal *et al.*, 1996; Hershkovitz *et al.*, 1999; Vyhánek *et al.*, 1999; Eshed *et al.*, 2002). Conversely, malignant tumors are invasive by

definition. Malignant tissue is characteristically amorphous, often demonstrating both abundant osteolytic and osteoblastic activity (Suzuki, 1987; Schultz, 1991; Strouhal *et al.*, 1997). The erosive invasive areas are characterized by numerous Howship's lacunae, and any new bone is deposited as an atypical 'patchwork' of lamellar and woven bone with little or no remodeling.

The differentiation between benign and malignant tissue is only the first step in the differential diagnostic process. Eventually the conclusive, most likely diagnosis will be based on data such as the age of the individual and macroscopic and radiological observations, e.g. anatomical distribution pattern, size, contour, density etc. In many cases, the additional value of histology is often limited. For example, although Suzuki's (1987) histological observations on a tumor were compatible with a diagnosis of osteosarcoma, the individual's age at death and the tumor's location and radiological appearance had already been strong diagnostic indicators of the diagnosis. Similar situations occurred in the case of a paranasal carcinoma (Schultz, 1991), the case of an osteosarcoma of the skull described by Strouhal and colleagues (1997), and a case of a multiple myeloma reported by Wakely *et al.* (1998). All these examples had a high *a priori* likelihood of diagnosis, which reduced the additional value of histology. In the case of a suspected meningioma, histology is considered completely redundant (Campillo, 1991).

Nevertheless, in some specific cases, histology may very well have additional value, particularly when dry bone histomorphology is compared to fresh tissue specimens. For example, Schamall *et al.* (1999) used a comparative histological approach to differentiate between a possible osteosarcoma, meningioma or hemangioma in a cranial lesion observed in a young female. Hershkovitz *et al.* (1999) and Eshed *et al.* (2002) showed that histology may aid in the differentiation between similar looking benign lesions of the skull. If a tumor contains preserved soft tissue, histology may provide a conclusive diagnosis (Strouhal, 1976; Strouhal and Němečková, 2004). However, such findings are rare.

In conclusion, the histological analysis of primary tumors is useful for differentiation between benign and malignant cases. It can, however, corroborate suspected diagnoses in only a few specific examples.

### ***Secondary tumors (bone metastases)***

In the description of metastases, palaeopathologists adopted the fresh tissue pathology approach for categorizing metastases as being either osteolytic, osteoblastic or a combination of both (for fresh tissue pathology see Vigorita, 2007; Roodman, 2011, for palaeopathology cases see Anderson *et al.*, 1992; Campillo and Mari-Balcells, 1984; De La Rúa *et al.*, 1995; Grupe, 1988; Molnar *et al.*, 2009; Šefčáková

*et al.*, 2001; Schultz, 1993; Schultz *et al.*, 2007; Tkocz and Bierring, 1984; Wakely *et al.*, 1995). Since metastases are by definition malignant, the histomorphology of osteoblastic and osteolytic lesions is similar to those found in malignant primary bone tumors (see above). The hematogenous dissemination of the original tumor may be sometimes disclosed by osteoblastic apposition within Haversian canals (Anderson *et al.*, 1992; Wakely *et al.*, 1995).

Palaeohistopathologists use metastatic tissue type to differentiate between various original tumors. However, this approach has limitations. Firstly, the vast majority of metastases are osteolytic (Vigorita, 2007). Secondly, medical practice shows that both osteolytic and osteoblastic lesions are seen in the majority of patients with bone metastases (Roodman, 2011). Thirdly, there is no fixed relationship between the original tumor and the histological nature of its metastases (Roodman, 2011). This has resulted in conflicting statements by palaeopathologists, for instance when Tockz and Bierring (1984) stated that lung, kidney and thyroid gland metastases were osteoblastic, whereas Grupe (1988) suggested that they were osteolytic.

Histologically, osteoblastic lesions may mimic Paget's disease, fluorosis and osteopetrosis (Anderson *et al.*, 1992; Schultz, 2001; Schultz *et al.*, 2007; Tkocz and Bierring, 1984). Osteoclastic and mixed-type lesions can mimic lytic infectious lesions, although tumors should have a 'more regular trabecular microarchitecture' (Schultz, 2001). However, the relevance of this feature is limited, due to its subjective nature.

We conclude that histology is an apt tool to identify the three different types of metastatic tissue (osteolytic/osteoblastic/mixed). Yet, if used in isolation, histology is unable to provide a conclusive diagnosis. For identification of the origin of the malignant process, the demographic profile of the individual and epidemiological knowledge on the different types of bone metastases is pivotal.

## **Infectious diseases**

### ***General characteristics of non-specific infections.***

In fresh tissue, acute bone infection is histologically characterized by hypervascularisation, influx of acute inflammatory cells, small vessel thrombosis and edema, which eventually results in ischaemia and necrosis (Kahn and Pritzker, 1973; Lew and Waldvogel, 1997; Vigorita, 2007; Calhoun, 2012). If not eradicated, the infection changes into a chronic state, with necrotic bone (sequestrae) and reactive new bone (involucrum) as hallmarks (Kenan *et al.*, 1993; Vigorita, 2007; Calhoun, 2012). The deposition of reactive bone tissue by osteoblasts is triggered by periosteal irritation and the presence of growth factors (Kenan *et al.*, 1993; Vigorita, 2007). Throughout the clinical and pathological literature, authors emphasized that the

degree of bone reaction is defined by many variables such as age and immune status of the host, bone type and site of infection.

In palaeohistopathological investigations, the diagnosis of infectious lesions has been based primarily on the osteolytic destruction of original bone and the reactive formation of new bone tissue (Hackett, 1981a; Blondiaux *et al.*, 1994; Schultz, 2001; Schultz and Roberts, 2002; Wapler *et al.*, 2004; Von Hunnius *et al.*, 2006; Flohr and Schultz, 2009a, 2009b; Weston, 2009; Van der Merwe *et al.*, 2010; Nicklisch *et al.*, 2012). The osteolytic component of the infection demonstrates omnipresent Howship's lacunae and 'lytical' resorption cavities (Hackett, 1981a; Blondiaux *et al.*, 1994; Schultz, 2001; Wapler *et al.*, 2004; Flohr and Schultz, 2009a; Van der Merwe *et al.*, 2010;). The anatomical boundaries/site of bone tissue destruction defines whether an infection should be called periostitis, osteitis or osteomyelitis. Appositional reactive bone may vary from chaotic and speculated (e.g. Blondiaux *et al.*, 1994; Schultz, 2001; Wapler *et al.*, 2004) to regular, sclerotic and dense (e.g. Hackett, 1981a; Schultz and Roberts, 2002; Flohr and Schultz, 2009b;). Often, vestiges of hypervascularisation (i.e. vascular channels) are visible at the periosteal surface (Hackett, 1981a; Blondiaux *et al.*, 1994; Schultz, 2001; Von Hunnius *et al.*, 2006). Extensive remodeling may hamper the differentiation between appositional and original bone (Schultz, 2001; Van der Merwe *et al.*, 2010).

Throughout the palaeohistopathological literature, authors appear to have no difficulty differentiating between infected lesions and taphonomical processes (Schultz, 2001; Wapler *et al.*, 2004; Flohr and Schultz, 2009a) or ossified hematomas (Schultz, 2001; Maat, 2004; Van der Merwe *et al.*, 2010). However, differentiation from primary bone tumors or (osteolytic) metastases is challenging (Schultz, 2001). Gross anatomical and radiological analysis is needed in those cases.

### ***The identification of specific infectious diseases***

In addition to non-specific infections, the histomorphology of specific infectious diseases has also been studied, with many workers focused upon syphilis (Hackett, 1981a; Schultz, 2001; Von Hunnius *et al.*, 2006; Weston, 2009; Van der Merwe *et al.*, 2010) and leprosy (Blondiaux *et al.*, 1994; Schultz, 2001; Schultz and Roberts, 2002). The findings in some studies led to the assertion that these diseases may present distinct, pathognomonic histomorphological features (Schultz, 2001; Schultz and Roberts, 2002). However, recent developments have added nuance or even refuted these assertions (Von Hunnius *et al.*, 2006; Weston, 2009; Van der Merwe *et al.*, 2010). If the pathogenesis of infectious bone lesions is considered, the inability to identify pathognomonic histomorphology can be understood.

Both the osteolytic and osteoblastic component of the infected bone tissue is nonspecific. Osteolytic bone destruction is caused by the nonspecific recruitment and activation of osteoclasts by inflammatory cytokines (Lew and Waldvogel, 1997; Phan, 2004). Reactive new bone formation is caused by the nonspecific excretion of growth factors and the lifting and irritation of the periosteum, both of which result in osteoblastic apposition of new bone tissue (Calhoun, 2012; Kenan *et al.*, 1993).

Therefore, the eventual histomorphology of an infectious process will be an index of the biological activity and anatomical location of the infectious process, the patient's age and metabolic state, and the thickness and firmness of attachment of the periosteum (Kahn, 1973; Kenan, 1993). As a result, the infectious microorganism does not correlate with one typical histomorphology, and one microorganism can create a wide range of bone lesion phenotypes. Thus, those features once thought to be pathognomonic for syphilis and leprosy are not specific for infection with *treponema pallidum* or *mycobacterium leprae*, respectively. If anything, the features indicate a slowly developing and recurrent infection. The same holds for *mycobacterium tuberculosis* infections.

## **Mechanical trauma**

The identification of traumatic lesions in dry bone material is usually based on gross anatomical and radiological investigation. As a result, microscopy has seldom been used for trauma analysis. This general lack of palaeohistopathological investigation of traumatic lesions contrasts with recent suggestions that it may aid in the assessment of posttraumatic survival time or could aid in the differentiation between peri- and antemortem lesions (Blondiaux, 2000; Maat, 2006a, 2008; Cattaneo *et al.*, 2010; De Boer *et al.*, 2012a, 2013a.). Lagier and Baud (1980) analyzed a juxtacortical osteoblastic lesion on a femur, secondary to mechanical trauma. Light microscopy showed an intact femoral cortex, which excluded a diagnosis of malignant tumor or infection. Their comparison with fresh tissue histology of known cases established a diagnosis of myositis ossificans.

## **Conclusion**

Histology has become an essential and integral part in the investigation of human dry bone. It may aid in the differentiation between human and animal bones (Owsley *et al.*, 1985; Cuijpers *et al.*, 2009; Hincak *et al.*, 2009), the study of taphonomy (Hackett, 1981b; Garland, 1989; Hedges *et al.*, 1995; Jans *et al.*, 2004), age determination (Kerley,

1965; Stout, 1976; Maat *et al.*, 2006b;) or can usefully supplement analyses of ancient DNA (Guarino *et al.*, 2000, 2006) and ancient proteins (Haynes *et al.*, 2002; Schmidt-Schultz *et al.*, 2004). As our review shows, histology is also useful for a wide range of diagnoses in palaeopathology.

In our analysis of the palaeohistopathological literature, we found that histological features were often described in a vague and ambiguous manner. Moreover, supporting biomedical literature was rarely cited. This restricts the rigor of palaeohistopathological papers, may discourage investigators to pursue histological methods and may hamper the evaluation of results.

Because of its value, histological investigative methods should be accessible to all palaeopathological investigators. For decades, the preparation of sections of undecalcified bone tissue was restricted to well-equipped histological laboratories. Fortunately, it is now possible for high-standard histological sections to be made with relative ease by means of accessible and inexpensive equipment (Maat *et al.*, 2001; Beauchesne and Saunders, 2006; De Boer *et al.*, 2012b, 2013b).

Thus, we see that microscopy is used for the palaeopathological diagnosis of a wide variety of diseases and conditions. In almost all cases, the histological analysis of bone tissue aids in differential diagnosis, but in only a few diseases can a definitive pathognomonic histomorphology be identified. In general, histological analysis is of value if used in comparison to fresh tissue specimens of 'known cases' and if combined with gross anatomical and radiological results.

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