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MICROSCOPIC METHODS FOR THE DIFFERENTIATION OF LYTIC METASTATIC CARCINOMA AND MULTIPLE MYELOMA

ABSTRACT: Today, we have the possibility to use the following examination methods for palaeopathologic specimens: scanning electron microscopy (SEM), classic light histology using semi-thin Epon sections, stained by toluidine blue (LH), and laser confocal scanning microscopy (LCSM). Samples from two cases are described to demonstrate the microscopic distinction between osteolytic metastases of carcinoma and multiple myeloma.

KEY WORDS: Scanning electron microscopy – Light histology using semi-thin sections – Laser confocal electron microscopy – Osteolytic metastases of carcinoma – Multiple myeloma

MATERIAL

Anthropological material from archaeological excavations No.1
Origin: Borovce, Piešťany (Slovakia)
Dating: end of the 8th century – early 9th century
Age: between 50 and 60 years old
Sex: male
Diagnosis: osteolytic metastatic carcinoma

This skeleton was studied by the second author of this paper together with Alena Šefčáková, the Head of the Anthropological Department of the Slovak National Museum, Bratislava (Slovakia) in 2000 (Šefčáková *et al.* 2001).

Anthropological material from archaeological excavations No.2
Origin: Mauer, 23rd District of Vienna (Austria)
Dating: Neolithic Age, Lengyel culture, beginning of the 4th millennium B.C.
Age: between 30 and 40 years old
Sex: female
Diagnosis: multiple myeloma

This skeleton was studied by the second author of this paper during his working stay in Vienna in cooperation with Johann Jungwirth, the former Director of the Anthropological Department of the Museum of Natural History in Vienna (Austria), in 1969–70 (Strouhal, Jungwirth 1970). It was re-examined by Strouhal and Kritscher (1989, 1990).

METHODS

We applied scanning electron microscopy (SEM), light histology with semi-thin sections (LM) and laser confocal scanning microscopy (LCSM). The purpose of this study is to present the usefulness of the semi-thin sections along side the application of the two more recent methods in diagnostic palaeopathology.

For imaging in the SEM, specimens should be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. All samples are coated with an ultra-thin coating of electrically conducting material, commonly gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation. Conductive materials in current

use for specimen coating include gold, gold with palladium, platinum and osmium. The metal coat has a thickness of a few nanometers. Coating prevents the accumulation of static electric charge on the specimen during electron irradiation. For SEM a specimen is normally required to be completely dry since the specimen chamber is at high vacuum. Hard dry materials such as bone and tooth can be examined with little further treatment. A dry specimen is usually mounted on a specimen holder using electrically conductive double-sided adhesive tape and sputter coated with gold before examination in the microscope.

All bone samples were analysed using a SEM – JSM 6300 Scanning Microscope in the Czech Academy of Sciences, České Budějovice.

The light microscope LM still remains the supreme tool for the diagnosis of the majority of tumours. Various steps in specimen preparation are required in diagnostic palaeopathology studies of tissues. It is well known, that sectioning is one of the most tedious and exacting steps in preparing tissue for structural study. Ultra-microtome, specialized instrument using glass or diamond knives has been developed to cut semi-thin 1–2 μm sections. Semi-thin sections are used to study an all-over picture of the tissue.

All blocks are resin-embedded in the Epon. The semi-thin sections are cut on ultramicrotome. Sections cut at 2 μm are floated in a water trough attached to the glass knife. The sections are transferred to a drop of water on a clean microscope slide. The slide with floating sections is placed on a hot plate pre-warmed to 60 °C. It is left for few minutes, as the liquid warms, the sections spread and adhere to the slide. Without removing the slide from a hot plate, filtered toluidine blue is dropped on the sections. After staining, the slide is carefully rinsed with distilled water from a wash bottle. The slide is drained and blotted dry and mounted in solacrylate. The semi-thin sections are examined with light microscope (Bancroft,Gambale 2008).

Specimens were sectioned using a ultramicrotome LKB 2088 UTROTOME V in the Institute of Histology and Embryology, Faculty of Medicine in Pilsen, Charles University Prague.

LCSM is a technique for obtaining high resolution optical images with depth selectivity. The key feature of confocal microscopy is its ability to acquire in-focus images from selected depths, a process known as optical sectioning. Images are acquired point-by-point and reconstructed with a computer. The image represents a thin cross-section (4 μm) of the specimen.

Bone pathologists have always faced the difficulty of physically cutting osseous tissues for morphological analysis. The application of confocal microscopy for the palaeo-histological assessment of bone may be of value because the relatively easy "optical" sections replaces conventional physical cutting. Imaging by confocal microscopy minimizes artifacts of bone specimens. Moreover, it allows us three-dimensional (3D) reconstruction of areas of interest. In this study, we have used staining with acridine orange of 1 to 1.5 cm thick un-embedded bone to enable the assessment of histological details of bone by confocal microscopy. In short this simple rapid acridine staining of thick bone samples together with detailed imaging by confocal microscopy, may have its value in the palaeohistological assessment of various bone diseases.

All bone samples were analysed using a LCSM Olympus Fluoview AX70 Provis in the Institute of Biology, Faculty of Medicine in Pilsen.

RESULTS

The employment of histological methods aims to enlarge current knowledge about bone tumours from palaeopathological finds. Various circumstances influence the aspect and structure of the tissues of ancient people, in the first place the kind of tumour and biological activity of the tumorous process.

The first sample (osteolytic metastases of carcinoma) for histological processing was taken from the broken-off dorsal edge of the right ala ossis ilii. The compact layer and the cancellous (spongy) layer with lytic metastases of the tumour was documented in SEM (*Figure 1a*). The interaction between metastatic tumour and bone almost always involves both osteoblastic and osteoclastic activity. Malignant cells secrete many factors that stimulate osteoclasts including several growth factors. The release of these substances from metastatic cells probably accounts for the total numbers and activity of osteoclasts and large tumour cells inducing bone resorption of the trabeculae.

In the lytic foci, the cancellous (spongy) trabeculae are covered by many lacunae (*Figure 1b*).

Resorption of bone trabecula of the osteolytic foci is performed by large cells called osteoclasts which are apparent as depressions resorbed at the bone surface called Howship's lacunae (*Figure 1c*).

Osteoclastic resorption contributes to bone remodelling and also can cause a pathologic fracture of the bone in response to it.

In LH, spongy bone is composed of a network of bony trabeculae. Spongy bone does not usually contain Haversian systems. The trabeculae are lined by a delicate layer of tissue – endosteum (dark soft tissue) which contains osteoblasts and osteoclasts. On the trabecular surface there is very active resorption of bone by osteoclasts of the trabeculae (*Figure 2a, b, c* – arrows). The spongy trabeculae being reduced in size (*Figure 2a, b*) are composed of irregular lamellae of bone (*Figure 2a*).

In LCSM, the cancellous (spongy) trabeculae afflicted by the osteolytic process show, on their surface, destructive Howship's lacunae that are hollowed by osteoclastic activity (*Figure 3a*).

The figure shows 15 combined sections of the left ilium scanned at a distance of 5 μm. The fourth section from the surface of the tissue sample shows marked lacunae on the surface and inside the trabeculae (*Figure 3b*).

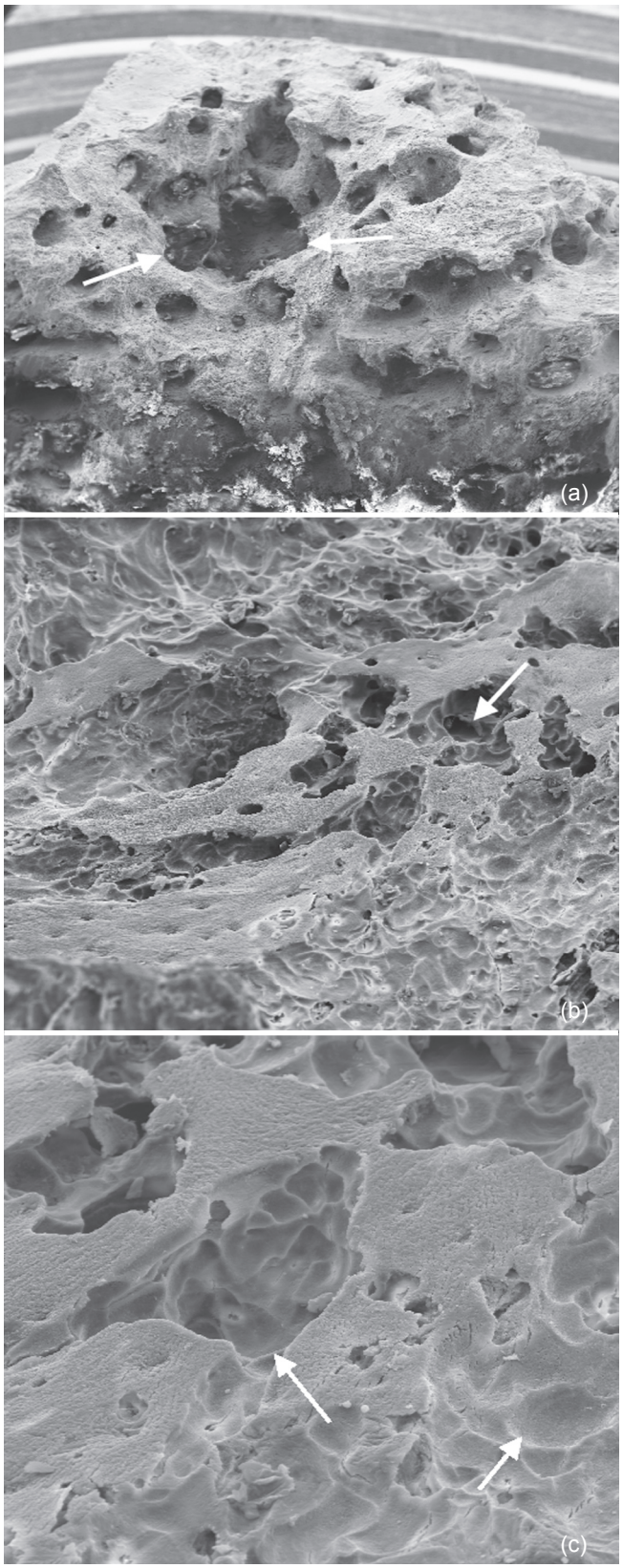

FIGURE 1. (a) Cavities of various shape and size can be found among the spongy trabeculae (SEM – Scanning Electron Microscopy, ×18); (b) Smooth (normal) surface of a spongy trabecula is covered by irregularly spaced lacunae (SEM, ×300); (c) Detail of a spongyotic trabecula from center of a pathological focus. It is covered on its whole surface by Howship's lacunae of various dimensions (SEM, ×700).

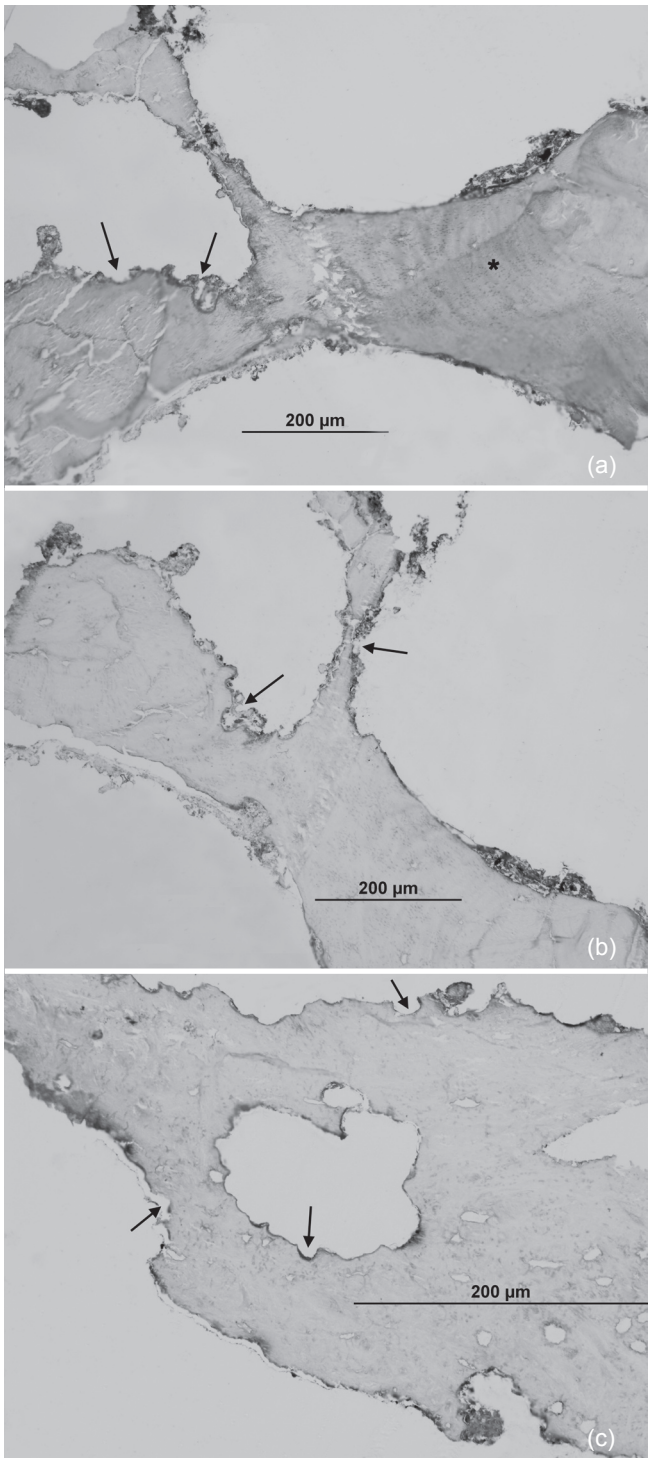

FIGURE 2. (a), (b), (c) Light histology – Howship's lacunas as well as rests of dark soft tissues can be seen on the spongy trabeculae (arrowheads). The internal structure of the trabeculae is lamellar.

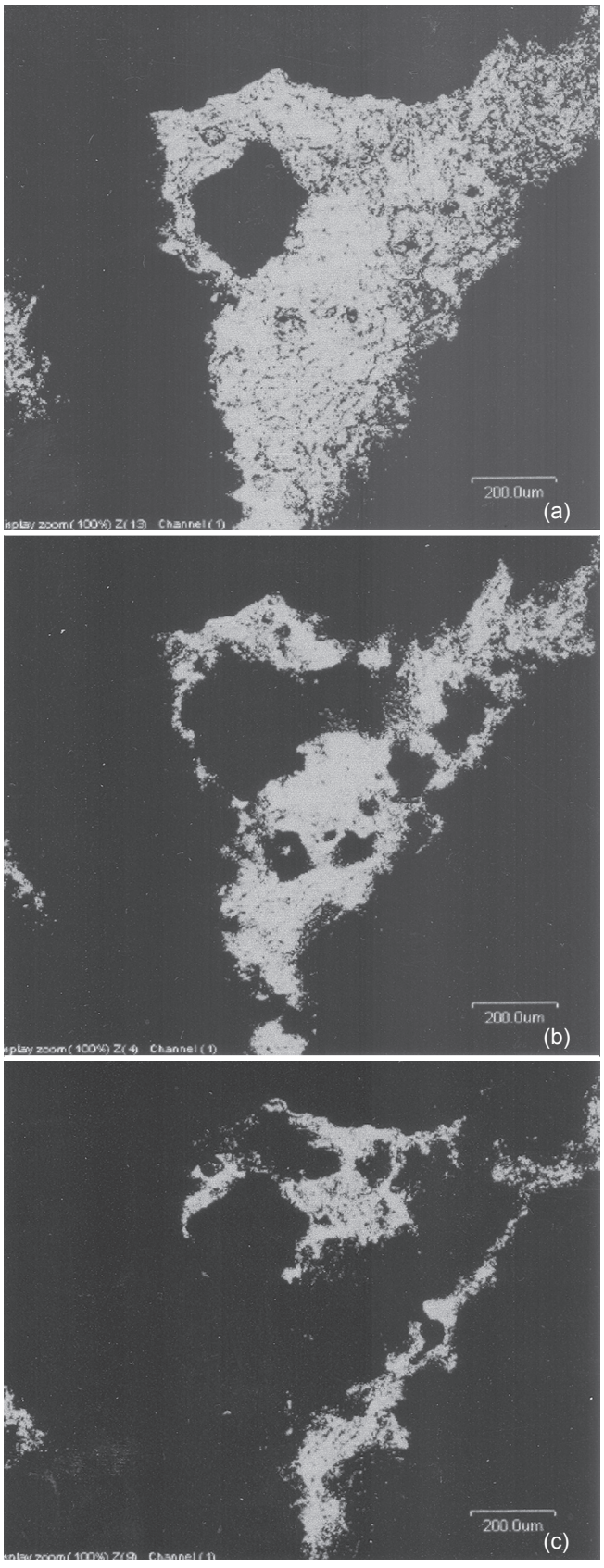

FIGURE 3. LCSM (Laser confocal scanning microscope); (a) sum of 15 sections, 5 µm distant; (b) fourth section from surface of trabeculae with marked Howship's lacunae; (c) seventh section, with confluenting lacunae and decrease of osseous tissue.

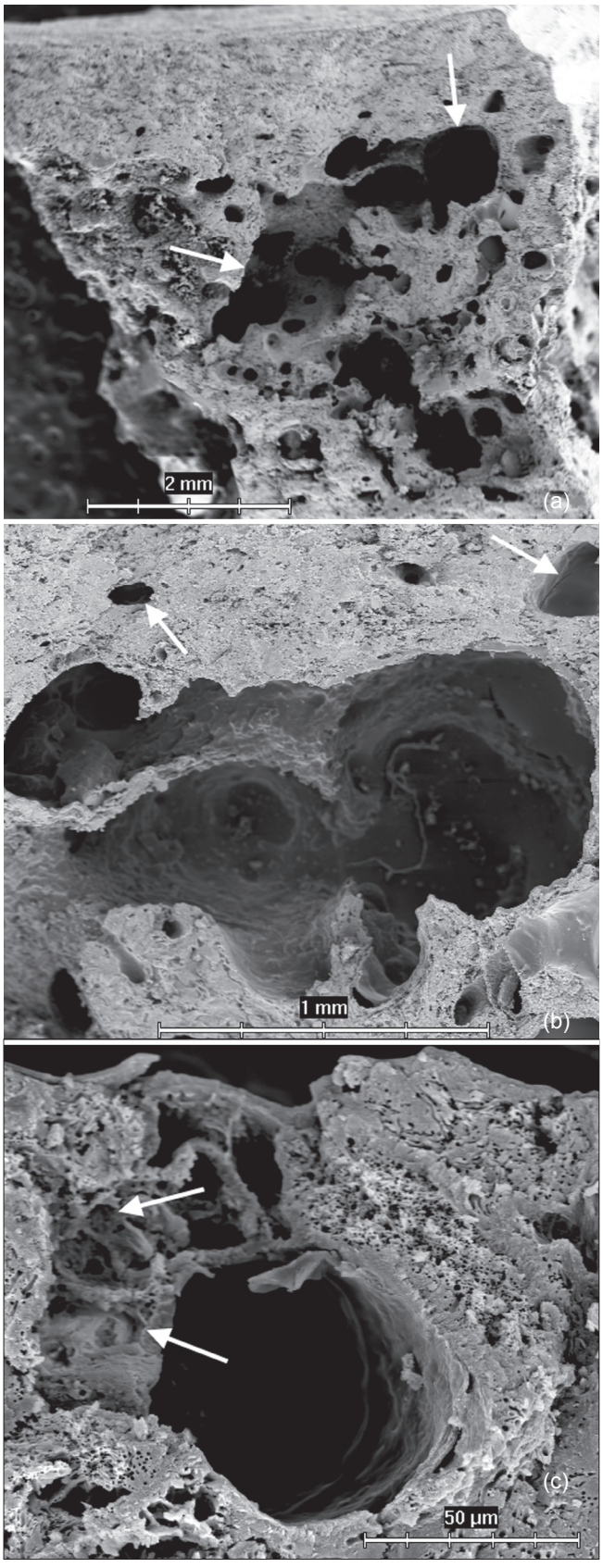

FIGURE 4. (a) The cavities of various shape and size can be found among the spongy trabeculae (SEM, ×18); (b) It is evident in some larger cavities how the process of destruction was getting into the spongy layer (SEM, ×100); (c) The nodules of soft tissue are osteolytic, destroying the bone lamellae (SEM, ×270).

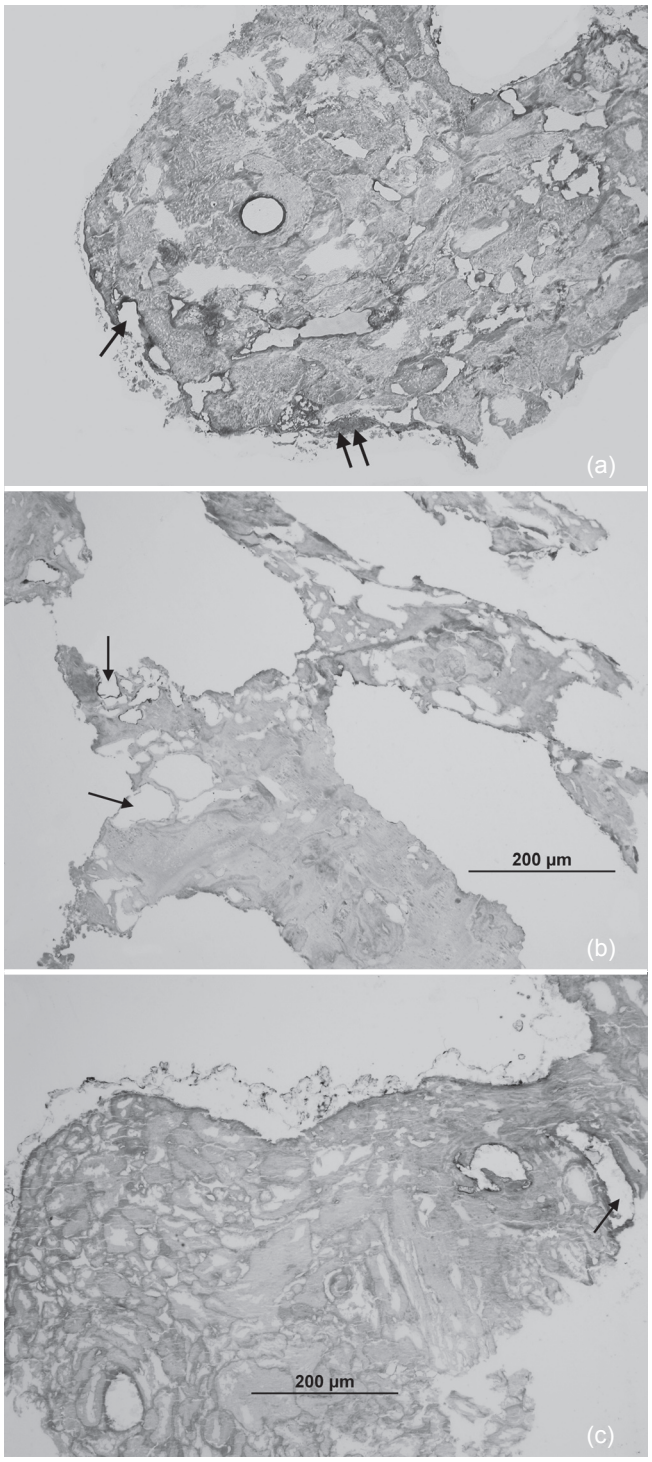

FIGURE 5. (a), (b), (c) Light histology – lacunas of the cells which eroded the surface of the spongyotic trabeculae can be seen in semi-thick sections, dyed with toluidine blue (arrowheads). The remnants of the cells are darker (a).

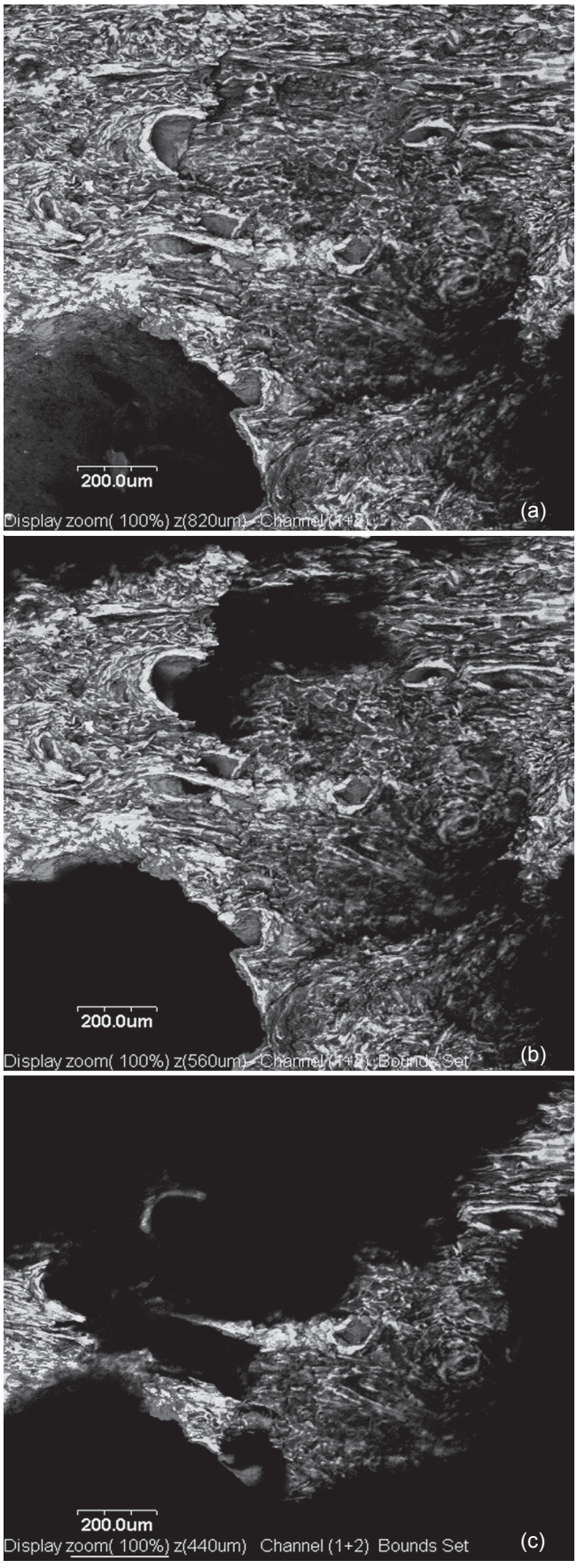

FIGURE 6. (a), (b), (c) The lytic process destroying the bone in the direction from the spongy layer to the compact layer (LCSM).

The seventh section displays confluent lacunae and a loss of the remaining tissue, which in places has disappeared completely (*Figure 3c*). Because the deep layers of spongy trabeculae are more affected by osteolysis than the surface layers, it appears that the destruction proceeded from the spongy layer towards the compact layer, sometimes perforating the bone completely.

The second sample (multiple myeloma) taken from the flat cranial bone shows damage of the compact and spongy layers. In SEM mostly rounded cavities of various shape and size can be found within the spongy trabeculae (*Figure 4a*). The holes in the lamina interna of the skull are formed in round to oval shapes and their size varies from starting dot-like ones up to 2 mm in diameter (*Figure 4a*).

It is evident in some larger cavities how the process of destruction penetrated into the compact layer (*Figure 4b*).

Diffuse multiple myeloma is characterized by extensive plasma cell infiltration of bone marrow and formation of distinct tumour nodules. The nodules of soft tissue are osteolytic, destroying the bone lamellae (*Figure 4c*). The cells which had eroded the surface of the trabeculae can be seen in semi-thin sections, dyed with toluidine blue. In LM, a section of bone trabeculae with characteristic atypical lacunae of cells are present (*Figure 5a ,b, c*). The bone trabeculae contains thin-walled blood vessels, and the surface of the trabeculae contains remnants of the cells (*Figure 5a* – double arrows).

In LCSM, by means of laser sections through the compact layer we can observe, without mechanically damaging the tissue the direction of the lytic process destroying the bone from the spongy to the compact layer (*Figure 6a, b, c*). *Figure 6a* shows 25 sections of the compact layer at a distance of 10µm. *Figure 6b* shows 19 to 22 sections and *Figure 6c* shows the fourth section from the compact layer.

DISCUSSION

It is not easy to distinguish myeloma from osteolytic metastases of carcinoma macroscopically or radiologically (Strouhal, Kritscher 1989, 1990, Rothschild *et al.* 1998). Both processes occur in the spongy bone from which they spread into the corticalis.

Osteolytic metastases – this is in cases of a systematic process creating multiple foci of osteolysis in a rather high number can vary widely from quite small ones to a hole of 100mm in diameter. The holes have round shape and irregular edges. They have uneven, from finely serrated to zigzag edges.

We can distinguish them by the structure of the spongy trabeculae influenced by the tumour. In osteolytic metastases the spongy trabeculae have their entire surface covered with many lacunae. These trabecules are absorbed by the activity of multinucleated cells of macrophage type-osteoclasts, which secrete proteo-lytic enzymes responsible for the bone resorption. The resorbed cavity is called Howship's lacunae. The internal structure of the trabeculae is lamellar in the metastases of carcinoma.

The case of myeloma shows damage of the compact and spongy layers. Mostly rounded cavities of various shape and size can be found. It is evident in some larger cavities how the process of destruction was getting into the compact layer. The places with an undamaged surface of the spongy trabeculae alternate with clusters of deep lacunae left by the cells causing decomposition of the spongy trabeculae. In the case of myeloma we can find fragile trabeculae with the unequally, atypical large foci of lacunae (Schajowicz 1994, Strouhal, Němečková 2008).

From our results we conclude that SEM, semi-thin sections and LCSM are methods applicable to archaeological and historical specimens of adequate but also poor preservation.

CONCLUSION

Our contribution was intended to find features distinguishing between lytic metastases of carcinoma and myeloma multiplex by three different histological methods, using samples from two archaeological cases from Slovakia and Austria. Their differences proved to be minute, concentrated in the changes of the osseous trabeculae, their morphological structure (semi-thick sections) and the number and form of the lytic foci afflicting them. There are evidently different processes of destruction which can be found in the spongy trabeculae and compact layers (SEM, LCSM).

REFERENCES

BANCROFT J., GAMBALE M., 2008: *Theory and Practice of Histological Techniques*. Churchill Livingstone, Elsevier. 796 pp.

ROTHSCHILD B. M., HERSHKOVITZ I., DUTOUR O., 1998: Clues potentionally distinguishing lytic lesions of multiple myeloma from those of metastatic carcinoma. *Amer. J. Phys. Anthropol.* 105, 2: 241–250.

SCHAJOWICZ F., 1994: *Tumors and Tumorlike Lesions of Bone. Patology, Radiology and Treatment*. Berlin, Springer. 649 pp.

ŠEFČÁKOVÁ A., STROUHAL E., NĚMEČKOVÁ A., THURZO M., ŠTUKOVSKÁ D., 2001: Case of metastatic carcinoma from end of the 8th – Early 9th century Slovakia. *Amer. J. Phys. Anthropol.* 116: 216–229.

STROUHAL E., JUNGWIRTH J., 1970: Die menschlichen Skelette aus dem neolitischen Hornsteinbergwerk von Mauer bei Wien. *Mitteil. d. Anthropol. Gesellsch.* 100: 85–100.

STROUHAL E., KRITSCHER H., 1989: Paleopathological differentiation of myelomatosis and osteolytic metastases in a Neolithic skeleton. *Rivista di Antropologia* 67: 265–272 (name of first author was omitted, see Erratum in *Rivista di Antropologia* 71: 343, 1993).

STROUHAL E., KRITSCHER H., 1990: Neolithic case of a multiple myeloma from Mauer (Vienna, Austria). *Anthropologie* 28, 1: 79–87.

STROUHAL E., NĚMEČKOVÁ A., 2008: *Trpěli i dávní lidé nádory?* Charles University – Karolinum, Prague. 194 pp.

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