BONE HISTOMORPHOMETRY AS A DIAGNOSTIC TOOL. A REVIEW ARTICLE

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ABSTRACT

INTRODUCTION: Various diagnostic modalities are used in the study of bone structure and metabolism. These include radiological examinations, laboratory and biochemical testing, histological and histomorphometric assessments, immunohistochemistry, and some non-invasive techniques. Bone histomorphometry is regarded as the gold standard in the diagnostics of bone-related conditions. It is a reliable method for detailed quantitative assessment of bone microarchitecture and physiology. It allows observation of cell types and their activity and provides essential information on bone healing, modeling, and remodeling.

AIM: The present review aims to summarize the applications and limitations of bone histomorphometry and define its role in the diagnostics, monitoring, and treatment of various bone-related conditions.

MATERIALS AND METHODS: An electronic search using Google Scholar, PubMed, Scopus, and ScienceDirect was conducted up to July 2022. The article is based on the existing scientific database and includes 198 studies. It summarizes the current knowledge on bone histomorphometry, highlights its advantages and limitations, and gives some recommendations for further research.

RESULTS: Bone histomorphometry is a key diagnostic tool in the field of bone research. It is used for the detection and monitoring of metabolic bone diseases, for establishing the safety of the pharmaceutical agents that affect bone metabolism, and for the effects of different biomaterials, used for guided bone regeneration and implant treatment.

CONCLUSION: Bone histomorphometry is applied in various scientific fields. Although some innovative non-invasive techniques have been suggested, the method remains a significant component in the study of bone structure and physiology.

Keywords: *bone histomorphometry, histologic sections, bone diseases*

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INTRODUCTION

Bone tissue has unique abilities for modeling and remodeling, turnover, metabolic processes, and cellular activity, which change with age and under different circumstances. Its phases and features represent bone development, its current state, and its history. A great number of modalities are generally used to fully understand its local condition and the whole skeletal health. These include laboratory and

radiological assessment, biochemical testing, histomorphometric evaluation, immunohistochemistry, and some non-invasive tools.

Bone histomorphometry (BH) was introduced in the 1950s as a method for the assessment of metabolic bone disorders (1–3) and their impact on bone structure and the processes of bone modeling and remodeling. The microscopic observation was performed on two-dimensional (2D) sections and measurements were carried by point and line counting.

The technique is an essential investigative tool for the assessment of bone density and cellular activity. It detects biochemical markers of bone turnover and allows for detailed characterization of various pathological conditions and the response to different pharmaceutical agents. The method is also beneficial in establishing drug safety.

AIM

The present study aims to evaluate the use of bone histomorphometry in various scientific fields and summarize its advantages and limitations. The article provides a concise, unbiased literature review of the current knowledge and suggests some further research to compare the present techniques for bone assessment and give recommendations on their application.

MATERIALS AND METHODS

An electronic search using Google Scholar, PubMed, Scopus, and ScienceDirect was conducted up to July 2022. The articles that matched our keywords were compared, analyzed, and carefully summarized. Some recommendations for further assessment were suggested.

RESULTS

Before the 1950s the histologic examination of bone necessitated prior removal of its mineral components. The introduction of plastic-embedding technology allowed for more precise microscopic observation of undecalcified bone sections and, thus, for a better understanding of bone physiology (4).

A pioneer in the study of bone mass architecture and metabolism was the American orthopedic surgeon Harold Frost who boosted histomorphometry as a method back in 1960 (5). He published a study on bone assessment following tetracycline administration, which highlighted the need for more thorough bone research (6).

The specific terminology used by the practitioners of BH could be hard to understand for those outside the field.

In 1983 Recker (7) summarized the histomorphometric methodology and principles. The need for standardization (8) resulted in the development of a unified nomenclature system, published by Parfitt et al. in 1987 (9). It became widely adopted by researchers in the field. In 2012 the system was revisited and updated by Dempster et al. (10).

Histologic sections display 2D information, while the actual view of three-dimensional (3D) structures could be achieved by the applications of stereological principles. Parfitt et al. have suggested that histomorphometric data should be reported either by 2D primary measurements or preferably by the corresponding 3D terms. If choosing the second option, the authors should explain how the data was derived from the 2D measurements. A mixture of both groups of terms is not recommended (9).

Bone biopsy measurements are performed in undecalcified specimens, which allow for quantitative and qualitative analysis of bone microstructure and metabolism (11).

The quantum concept of Frost (1964) (12) explains bone mass alterations with an imbalance between the processes of bone formation and resorption at the remodeling site. Therefore, remodeling is regarded as a key determinant of bone volume and microarchitecture. Changes in bone mass occur not only with age but also as a sequence of metabolic disorders.

Metabolic bone diseases are characterized by changes in the volume of total bone and osteoid, as well as the cellular activity at trabecular surfaces. All important features could be detected after hematoxylin-eosin (HE) staining. To highlight some specific details, however, the use of other staining methods is necessary. For instance, the contrast between osteoid and mineralized bone could be achieved by combining von Kossa with van Gieson, safranin, eosin, etc. as a counterstain. Goldner trichrome (GT) and other trichrome stainings are appropriate for the differentiation of mineralized from unmineralized bone (13).

Similar results of osteoid amount have been observed following von Kossa and solochrome staining (14,15). In comparison trichrome has been reported to underestimate osteoid volume (14,16). HE and GT are suitable for the evaluation of trabecular surfaces. Toluidine blue and thionin could be used for detailed cellular assessment.

The mineralized front can be demonstrated by several methods, such as Sudan black, cobalt salts, solochrome cyanin, and toluidine blue (17–21). When the latter is used, the mineralized front is presented by a purple granular line at the mineralized bone-osteoid junction. Nevertheless, the most reliable method for visualization of the mineralization front is tetracycline labeling. Its fluorescence was first described by Milch et al. (22) in 1958 and became widely adopted soon afterward (23,24). Single-labelling represents defects in calcification, while double labels allow for assessment of the mineralization rate.

Results in BH could be expressed either by percentages or by ratios. Quantification is performed using one of the following: eye-piece graticules, semi-automatic system, or fully automatic equipment (13).

In recent years anti-remodeling agents, prescribed to osteoporotic patients, have caused some difficulties in bone labeling and assessment. Recker et al. evaluated the possible causes for incorrect and biased bone analysis in such patients and gave recommendations on how to results should be correctly interpreted in situations of missing or scarce data (4).

Bone histomorphometry provides a quantitative evaluation of bone microstructure and the effect that physiological and pathophysiological mechanisms have on it (25). Its complicated and sensitive algorithms make the method difficult for everyday practice. Therefore, various automated protocols have been suggested to optimize the process (25–29).

APPLICATIONS

Bone histomorphometry provides a cellular insight into the other methods for bone examination (25).

It is grouped into static BH, which represents bone parameters in an exact time point, and dynamic BH, which observes their changes during a period (30).

In addition, static BH evaluates the activity of osteoblasts and osteoclasts, while dynamic BH observes the mineralization by fluorochrome labeling.

Bone histomorphometry offers a wide variety of applications. It is an important diagnostic tool for numerous conditions that affect bone and provides a reliable assessment of their development and treatment.

The method is a key component in the evaluation of metabolic bone diseases, such as osteoporosis (31–35), osteomalacia (36–38), hyperparathyroidism (39–41), hypoparathyroidism (42,43) hypothyroidism (44–49), etc. These conditions have different histomorphometric profiles which enable their classification (50). The mineralization index, suggested by Parfitt et al. (36), is a useful tool for differentiation and diagnostics of bone disorders.

The utilization of BH in the study of Paget's disease (51,52), and cystic fibrosis (53–56) has been reported, as well. In addition, static and dynamic histomorphometry has been used in the evaluation of some genetic conditions, such as osteogenesis imperfecta (57,58).

Furthermore, the method has been utilized in the study and monitoring of dialysis patients (59–62), patients with renal osteodystrophy (including osteitis fibrosa) (63–72), and cholestatic liver disease (73–76). The technique has also been found beneficial in the evaluation of various endocrine disorders, such as diabetes mellitus (77–80) and acromegaly (81–83). Some authors use BH to assess bone features, metabolism, and abnormalities in patients with thalassemia (84,85).

Moreover, BH has been adopted as a sufficient diagnostic tool in the study of pharmaceutical agents that affect bone metabolism (86–89). These include hormonal therapy (90–93), anabolic steroids (94–98), corticosteroids (99–102), monoclonal antibodies (86,88,103,104), bisphosphonates (105–110), etc. The method has been used in the research of some vitamin deficiency and supplementation (112–115), as well.

Bone histomorphometry is a well-established technique for determining the age at death for forensic medicine and anthropology (116–118).

In addition, BH provides reliable diagnostic properties for the fields of oral surgery and dental im-

plantology. It has been used successfully for the qualitative assessment of different bone grafting materials (119–128) and the evaluation of peri-implant bone healing and remodeling (129–138). The utilization of BH has contributed sufficiently to the understanding and improvement of dental implant therapy.

Numerous parameters should be evaluated to understand the mechanisms of bone diseases and different pharmaceutical agents. Melsen F and Steiniche T (139) have summarized the characteristics of hyperthyroidism, primary hyperparathyroidism, estrogen, and etidronate treatment for one year. It is evident that the understanding of drugs' mechanism of action, which is a major factor in choosing an accurate treatment modality, requires profound knowledge and experience.

LIMITATIONS

The major drawback of BH is the invasive technique for obtaining bone biopsy samples (140) and the possible complications, such as pain, infection, hematoma, and, sometimes, neuropathy (141). In addition, it represents a two-dimensional picture of an exact moment for the study of three-dimensional structures and their dynamics (142).

The biopsy site is usually restricted to the iliac crest, which does not always represent the changes in the whole skeletal system. For instance, in cases of renal osteodystrophy and metabolic bone disorders, there is a general skeletal involvement and iliac specimens are representative. However, in patients with osteoporosis, the iliac crest biopsy is not a sufficient indicator of the severity of the condition (143). In addition, bone turnover is not similar at different sites (144).

Most histomorphometric research is usually confined to cancellous rather than cortical bone as the first shows greater remodeling ability. Frost (145) was the first to make a histomorphometric analysis of cortical bone from the rib. Recently, some authors have evaluated the cortical structure and remodeling activity in the femoral neck and the iliac crest (146–150).

The application of the stereological principles requires unbiased sampling of isotropic tissues. In bone assessment, this is rarely attainable since bone microarchitecture is oriented according to the direction of the mechanical forces. As for unbiased sampling, the use of vertical bone sections has been suggested (151,152).

Another limitation is the need for more reliable markers of bone resorption and remodeling. Currently, they are evaluated by bone formation rates and are based only on assumptions for the correlation between them (153).

Some limitations of BH concern the correct evaluation of bone formation and mineralization. Dynamic indices of bone formation and resorption are measured after tetracycline labeling. Several administration regimens have been suggested, most of which involve two time-spaced intakes (154). It should be noted that different tetracyclines show some dissimilarities. Parfitt et al. (155) demonstrated that demeclocycline labeling presented a larger surface of fluorescence compared to oxytetracycline. This resulted in different values of bone formation indices. Such discrepancies should be considered before comparing results from different investigators or when applying the quadruple labeling protocol (156–159).

Osteoid measurement could also represent a difficulty. If not measured directly, osteoid could be calculated from its area and perimeter. However, the latter is not applicable when the amounts of osteoid are insufficient (160). In addition, the values of osteoid surface extent depend on the magnification. The staining technique also influences the osteoid measurements and their accuracy (161).

Another challenging task is the assessment of bone turnover. Bone formation within remodeling units is expressed by wall width (10). It shows a great variation in healthy and osteoporotic individuals (153), which suggests differences in sampling and sometimes difficulty identifying the cement line.

Furthermore, it is hard to determine the effect of a disease or a treatment on wall width since the number of bone structural units could be scarce (162). As for bone resorption, it is also difficult and to some extent subjective to identify the cavities. The investigator has to recognize the cut-off lamellae at the edges (163) and the osteoclast-like cells inside the cavities. The latter requires histochemical identification of titrate-resistant acid phosphatase. It is not specific to osteoclasts but suggests their presence (164,165).

Courpron et al. (166) have suggested a method for indirect measurement of erosion depth, assuming that it is inversely proportional to the interstitial width. Nevertheless, other authors have claimed that the association between those parameters is not just inverse but depends on the concomitant changes of wall and trabecular widths (167,168).

Eriksen et al. (169) have suggested a direct assessment of erosion depths. However, the approach requires great accuracy and precision in calculating the number of eroded lamellae and identifying different cell types, associated with the resorptive phase. It is challenging even for skilled investigators. The authors excluded 24% of the resorption cavities due to the difficult identification of the cell types and the number of eroded lamellae. This approach has not been widely adopted. Although some simplified methods have been suggested (170), they failed to evaluate the final resorption depth.

Garraluan et al. (171) have introduced a computerized technique, which allows for the assessment of mean and maximum erosion depth, area surface, and amount of cavities.

The above-mentioned approaches could either underestimate the complete erosion depth or overestimate its value (as is likely to happen with the one suggested by Eriksen) (172). Therefore, further research is necessary to develop a more accurate and reliable protocol.

It is questionable whether structural determinants of bone strength could be evaluated precisely by the conventional histological methods (173). Although there is some evidence that 2D sections are representative of the 3D architecture (174,175), some additional assessments are required to determine whether all the necessary information for those 3D structures could be extrapolated that way (162).

Variability

The variability involves the following aspects: different sites in the bone, a bone from the same individual, the human factor (investigators), laboratories, methodological differences (staining methods, magnification, assessment, etc.) (13).

Various authors have compared specimens from adjacent sites of the iliac crest and reported insignificant differences (17,176–178). However, when the biopsy is obtained from more than 2 cm behind or below the standard site, differences have been detected (176). Comparison between biopsy samples from both left and right crest has shown similar results (176, 179–182). Furthermore, the assessment is influenced by the staining technique and the labeling—its timing of administration and the interval between two labels. If the interval is longer than 3 weeks, a decrease in the osteoid bearing two labels will be observed (13). The role of microscopic resolution in the analysis of bone specimens has been also demonstrated (180,183). Magnification of 25–400 times has not changed the volume density. However, an increase in surface density has been reported (183).

Delling et al. (184) have observed significant variability in results from volume and surface measurements. The authors have also demonstrated that the assessment of cellular activity depended on the experience of the observers, unlike surface and volume density. The assessments of 10 specimens, carried out by 4 different investigation groups, have shown a significant variation considering the values of osteoid surface.

DISCUSSION

Bone histomorphometry provides in vivo evaluation of bone microstructure and thus contributes to the diagnostics of bone-related conditions. It is beneficial for the detection of metabolic bone diseases such as osteoporosis (185), osteomalacia (36), renal osteodystrophy (50,64–69), etc. Furthermore, it provides information on the mechanism of action of some drugs and how they influence bone physiology.

Bone histomorphometry is a challenging method. Obtaining credible information is difficult, considering the limited biopsy site, disease heterogeneity, variations in sampling techniques, calculations, measurements, and other methodological factors (161,186,187). In addition, BH is a costly and time-consuming technique. It requires great knowledge, attention, and experience.

Innovative non-invasive methods such as micro-computed tomography (μCT) provide 3D reconstruction. However, this technique is applicable for the assessment of osteoporosis but not for other metabolic conditions.

In 2005 Chappard et al. (188) compared measurements obtained by histomorphometry and μCT

and found a significant correlation between both methods.

Müller R et al. (189) have also reported a sufficient correlation between BH and microtomographic bone analysis.

Three-dimensional bone imaging and quantification have been rapidly developing (190). Quantitative computed tomography (QCT) and high-resolution peripheral QCT, have been introduced recently. It has been demonstrated that data obtained by them corresponds to that from the histomorphometric analysis (162,191). However, the evaluation of cellular activity is only possible by the utilization of the latter.

Todisco M and Trisi P (192) have researched to evaluate the correlation between bone mineral density (BMD) obtained by both QCT and histomorphometric assessment. The authors evaluated BMD in 18 patients before implant placement. Specimens for BH were retrieved from the implant sites and the bone volume was assessed. The results showed that BMD and BH are statistically related and QCT could be used as a reliable method in implant therapy planning.

Gielkens et al. (193) have compared the results from μCT, microradiography (MR), and histomorphometry as methods for quantitative assessment of bone formation, graft modeling, and volume. They found that the differences between them were greater in defect width rather than graft width. Both μCT and MR were found reliable but the authors' advice was to be performed in combination. Graft width measurements obtained by μCT, MR, and histomorphometry were comparable. However, bone formation was overestimated by the MR analysis.

A present development in the field is the application of 18F-fluoride positron emission tomography for the evaluation of bone turnover, blood perfusion, osteoblast count, and activity. The advantages of the method include its noninvasiveness and the detailed information it provides on the effects of different bone disorders (140,194–196). Its major limitation is the high cost.

Further research is needed to compare the qualities and drawbacks of the different techniques for bone evaluation and summarize the recommendations for their application and combination when necessary.

CONCLUSION

Bone histomorphometry provides a quantitative assessment of bone microstructure, formation, remodeling, and resorption. The method allows observation of cell types and their activity. Histomorphometry is a powerful tool in the study of systemic skeletal disorders and other conditions affecting bone structure and physiology. It gives a profound understanding of bone healing and the mechanism of action of different drugs and biomaterials. A major drawback of the method is that it is expensive and time-consuming.

Diagnostics of the conditions, affecting bone tissue, are usually based on the following methods: histology, radiology, serum analysis, immunohistochemistry, and gene expression (25). Histologic evaluation has been regarded as the method of choice in the diagnostics of infectious diseases (197), while the radiological examination is the gold standard for diagnostics of bone loss or fractures. Moreover, bone histology and histomorphometry are essential tools in the study of skeletal diseases, such as osteoporosis and osteomalacia (25,198).

Bone histomorphometry provides useful information for mineralization and biomarkers activity. That is why Malhan et al. refer to BH as a "building block" in the bone study (25).

We have summarized the current knowledge on the applications, advantages, and limitations, of bone histomorphometry, as well as the latest developments in the field of bone research. Although some new non-invasive methods have been suggested, BH remains the gold standard for bone evaluation and diagnostics. It provides sufficient information on bone structure, physiology, and pathophysiology.

Further research is necessary to estimate the advantages and drawbacks of the methods of bone evaluation, compare the standard with the innovative non-invasive techniques and give recommendations for their application and possible complications.

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