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LETTER TO THE EDITOR

Studying the role of microcracks in the pathophysiology of BRONJ

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There has been a speculation about the potential role of microdamage in the pathophysiology of bisphosphonate-related osteonecrosis of the jaw (BRONJ) since the condition was first described five years ago. Much of the speculation is fueled by studies showing that treatment with bisphosphonates causes significant reductions in bone turnover and is associated with significant accumulation of microdamage at multiple bone sites [1–6]. In a recent paper, Hoefert et al. [7] report that microdamage exists in just over 50% of bone tissue samples collected from patients with BRONJ and conclude that microdamage "could be a first step in the pathogenesis of bisphosphonate-related ONJ." Given the importance of trying to understand the pathogenesis of BRONJ, Hoefert and colleagues are commended for attempting to study the role of microdamage accumulation in this condition. However, there are several aspects of this study that are important to consider when evaluating the conclusions.

Hoefert and colleagues studied bone tissue samples from patients who had been diagnosed with BRONJ, as well as from patients who had various other conditions (radiation-induced osteonecrosis, osteomyelitis, bisphosphonate-treated patients without osteonecrosis of the jaw, and individuals with osteoporosis who were untreated). Bone samples were collected during routine oral surgeries such as extractions, resections, or removal of the sequestra. The evaluation of the tissue was focused on microdamage, which the authors assessed using scanning electron microscopy. They found that 54% of the samples from patients with confirmed BRONJ had microcracks.

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The gold standard method for assessing microdamage in bone is basic fuchsin staining. In this technique the tissue is stained en bloc with basic fuchsin, embedded in plastic, sectioned, and then microcracks are assessed using light or fluorescence microscopy [8]. Other agents, such as fluorochromes or heavy metals (e.g., lead acetate), can also be used to stain microdamage en bloc, the latter being useful for assessment of microcracks using electron microscopy [9]. The importance of staining specimens prior to processing is that it allows separation of microcracks that existed prior to processing and those that are due to specimen preparation [10]. Without such staining, it is impossible to know whether any damage is due to processing or not.

Hoefert et al. assessed microcracks using scanning confocal microscopy without staining the tissue prior to analysis. This complicates the interpretation of the results as it is not clear whether the damage that was observed was true biological damage or whether it was generated during specimen preparation. Instead, the authors chose to use the presence of cells/debris in the cracks as confirmation that the damage existed prior to operation or preparation of the bone sample, arguing that the cells could only have entered the crack in vivo. This method of differentiating biological from artificial cracks has not been validated and seems morphologically improbable given the size of an average human cell (7–20 microns) relative to the width of a typical biological microcrack (1–2 microns).

It is also important to consider the limitations that come with obtaining specimens during surgery for microdamage assessment. Based on iliac crest biopsy studies, it is clear that the technique used to obtain the tissue specimen is of paramount importance, as any number of factors can lead to specimen damage during the retrieval process, rendering them unsuitable for analysis [11]. Even in specimens that are deemed appropriate for analysis, it is customary to exclude the

boundaries of the tissue from analysis because they typically have damage generated by the trephine needle.

The microcrack measures by Hoefert et al. were made only on the surface of the whole specimen (it was not embedded and sectioned); there were no deeper measures within the matrix. These surfaces that were assessed are analogous to the boundaries of a sample obtained using a trephine needle, the exact region that would be expected to have damage due to surgery and would be excluded in a more traditional analysis. Although the authors state that all samples were harvested using "similar mechanical stress," this cannot be assured. The induction of cracks during specimen removal is consistent with both cells being located within the cracks as well as the size of these cracks being substantially longer than is typically seen in vivo. Furthermore, it is possible, in fact probable, that cracks were more common in bisphosphonate-treated tissue (both those with and without BRONJ) compared to the others due to changes in both mineralization and collagen cross-linking that occur with treatment to make tissue more brittle [12].

The work of Hoefert and colleagues is applauded for attempting to collect important data on microdamage accumulation in patients with BRONJ. However, given the issues related to microdamage assessment, the conclusions of the study should be cautiously interpreted.

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