Peritrabecular clefting in fibrous dysplasia of the jaws: an important histopathologic feature for differentiating fibrous dysplasia from central ossifying fibroma

Ana Carolina Prado Ribeiro, DDS, PhD, Roman Carlos, DDS, Paul M. Speight, DDS, PhD, Keith D. Hunter, DDS, PhD, Alan Roger Santos-Silva, DDS, PhD, Oslei Paes de Almeida, DDS, PhD, and Pablo Agustin Vargas, DDS, PhD

Piracicaba, São Paulo, Brasil; Guatemala City, Guatemala; and Sheffield, United Kingdom

Objective. The aim of this multicenter study was to perform a histomorphometric analysis of peritrabecular clefting in fibrous dysplasia (FD) in an attempt to obtain data that could be useful for distinguishing between FD and ossifying fibroma (OF).

Study Design. A clinicopathologic analysis was performed in 68 patients diagnosed with FD and 37 patients diagnosed with OF. Histologic sections were scanned using an Aperio ScanScope CS. A histomorphometric analysis was performed with the aid of an image analyzer (UTHSCSA Image Tool 3.0 version) on 37 randomly selected samples of FD, and the results were compared with the 37 OF specimens.

Results. The presence of peritrabecular clefting was observed in 32 (86.5%) cases of FD, whereas no case of OF presented peritrabecular clefting.

Conclusions. Peritrabecular clefting may be a hallmark of the lesions in patients with FD, and it may be a valuable microscopic feature for distinguishing it from OF. (Oral Surg Oral Med Oral Pathol Oral Radiol 2012;114:503-508)

Benign fibro-osseous lesions (FOLs) are a group of lesions that affect the jaws and craniofacial bones in which normal bone is replaced by cellular fibrous tissue with different degrees of mineralized material. This group of bone diseases encompasses fibrous dysplasia (FD), central ossifying fibroma (OF), and osseous dysplasias.

The World Health Organization currently defines FD as a genetically based sporadic disease of the bone that may affect single or multiple bones (monostotic or polyostotic types, respectively). When it occurs in different craniofacial bones, it is regarded as craniofacial FD. Central OF is a benign neoplasm that often presents well-demarcated borders and is histologically composed of fibrocellular stroma and variable amounts of mineralized material.

Fibrous dysplasia and OF often present clinical, histopathologic, and imaging similarities, and a definitive diagnosis requires careful clinicopathologic correlations. These lesions must be distinguished from each other because they have distinct outcomes and require different forms of treatment.

Peritrabecular clefting is a histopathologic event characterized by empty spaces partially or completely encircling lesional trabecular bone. Remarkably, this phenomenon has been previously illustrated in a number of publications, but no studies have been undertaken to determine whether this feature is specific to FD, or if it may be a useful diagnostic marker. Therefore, the aim of the present study was to perform a descriptive analysis of peritrabecular clefting in FD and to further analyze this clefting phenomena through a histomorphometric study. In addition, the prevalence and extent of clefting in FD was compared with lesions of OF.

MATERIAL AND METHODS
A retrospective, multicenter, international, collaborative study was performed in 3 oral pathology centers.
ters: University of Campinas, Piracicaba Dental School, Brazil; Centro Clínico de Cabeza y Cuello, Guatemala; and The School of Clinical Dentistry, University of Sheffield, United Kingdom. Demo-
graphic data (age and gender) and site of the tumors were collected from patient charts. Tissue specimens were retrieved from all patients diagnosed with FD and OF of the jaws. The cases were evaluated and diagnosed by correlation of clinical, radiologic, and histopathologic features, and the lesions were clas-
sified according to the criteria of the World Health Organization. This study was approved by the Ethics Committee for Human Studies, Piracicaba Dental School, University of Campinas (58/2008), and the South Sheffield Research Ethics Committee, University of Sheffield (STH 15699).

**Histopathologic analysis**

Selected samples originating from Brazil and Guate-
mala were decalcified with 5% nitric acid. Samples

Fig. 1. Histomorphometric analysis of area of peritrabecular clefting, performed using an image analyzer—UTHSCSA Image Tool version 3.0.

Fig. 2. Fibrous dysplasia. A, Panoramic radiograph. Radiopaque image with poorly defined margins affecting posterior right maxilla. B, Reconstruction overview of cone beam computed tomography showing extensive lesion involving the posterior maxilla, maxillary sinus and the zygomatic bone. C, Axial computed tomography cone beam showing hyperdense homogeneous aspect, described as “ground glass.” Furthermore, there was expansion of buccal and palatal cortical bone.
from the United Kingdom were decalcified using 10% formic acid. In all cases, decalcification was performed at room temperature, and the volume of decalcifying solution used was the equivalent of 10 times the volume of each lesion piece. Solutions were changed daily, and decalcification was considered to be complete when samples could be easily cut with a razor. After decalcification, samples were washed in running water for 5 minutes and processed for embedding in paraffin. Five-micrometer thick sections were cut from the paraffin blocks, stained with hematoxylin and eosin (H&E), and re-examined under light microscopy for diagnostic confirmation.

All slides were scanned using an Aperio ScanScope CS (20× magnification; Aperio Technologies Inc., Vista, CA), and images of representative areas of the lesions were taken from each slide using the ImageScopeTM software (200× magnification; Aperio Technologies Inc.).

Histomorphometric analysis was performed with the aid of an image analyzer UTHSCSA Image Tool version 3.0 (University of Texas Health Science Center, San Antonio, TX). The parameters analyzed included the area of negative space between the trabecular bone and the stroma of the lesions (50× magnification). This was obtained by manually measuring the contours of the negative area with the adjustable line of the image analyzer. After the system was calibrated, measurements were performed true to scale in the free-hand mode (Fig. 1). All measurements were analyzed in 5 different randomly selected microscopic fields, and these data were used to calculate the mean value for each sample.

RESULTS
Clinical features
A group of 68 patients diagnosed with FD and 37 patients diagnosed with OF were studied. Their clinical, radiographic, and histopathologic features are summarized in Table I and the radiological features in Table II (Fig. 2A-C; Fig. 3A and B). From the 68 samples of FD, 37 cases were randomly selected for histomorphometric analysis and compared with all 37 cases of OF.

Histomorphometric analysis
Peritrabecular clefting was observed in 32 (86.5%) cases of FD; however, this feature was not observed in any of the OF cases (Fig. 4A and B). These clefts were characterized by a negative space between the trabecular bone and stroma of the lesions (Fig. 5A-D). The size, shape, and area of the clefts varied between the FD lesions, and the mean area obtained by manual measurement was 5,888.64 µm², ranging from 732.37 to 37,292.79 µm², with an amplitude of 36,560.42 µm².

Due to the overlapping clinical, radiographic, and histopathologic features of FD and OF, a definitive diagnosis requires a complete correlation between clinical, histopathologic, and imaging findings. Several diagnostic criteria have been proposed to distinguish between FOLs, but only a few of these features are truly specific and used during routine oral pathology. Waldron (1993), Speight and Carlos (2006), and Eversole et al. (2008) described the clinical, radiographic, and histopathologic features of FD and OF in an attempt to differentiate these lesions and, more recently, other authors have tried to find immunohisto-
imaging features of the samples

<table>
<thead>
<tr>
<th>Features</th>
<th>FD cases (n = 68)</th>
<th>OF cases (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiopaque image</td>
<td>38 (55.6)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>Poorly defined margins</td>
<td>31 (45.5)</td>
<td>0</td>
</tr>
<tr>
<td>Well-defined margins</td>
<td>4 (5.8)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>Information not available</td>
<td>3 (4.3)</td>
<td>0</td>
</tr>
<tr>
<td>Mixed image (radiopacity/radiolucent)</td>
<td>9 (13.2)</td>
<td>15 (40.2)</td>
</tr>
<tr>
<td>Poorly defined margins</td>
<td>1 (1.5)</td>
<td>4 (10.5)</td>
</tr>
<tr>
<td>Well-defined margins</td>
<td>5 (7.4)</td>
<td>11 (29.7)</td>
</tr>
<tr>
<td>Information not available</td>
<td>3 (4.3)</td>
<td>0</td>
</tr>
<tr>
<td>Radiolucent image</td>
<td>4 (6)</td>
<td>15 (40.4)</td>
</tr>
<tr>
<td>Poorly defined margins</td>
<td>2 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Well-defined margins</td>
<td>2 (3)</td>
<td>12 (32.5)</td>
</tr>
<tr>
<td>Information not available</td>
<td>0</td>
<td>3 (7.9)</td>
</tr>
<tr>
<td>Unknown</td>
<td>17 (25.3)</td>
<td>5 (13.5)</td>
</tr>
</tbody>
</table>

Values are n (%).

In the current study, a histomorphometric analysis was performed in an attempt to quantify the extent of peritrabecular clefting in FD, which was observed in 86.5% of the samples. The mean area of peritrabecular clefting was 5,888.64 μm², and a large variation in the area of the clefts and consequently a large amplitude were observed, which could be explained by the high variability in the trabecular bone size and the shape around the negative spaces (clefts). The main purpose of using histomorphometry in the current analysis was to provide a detailed characterization of the microscopic appearance of the peritrabecular clefts in FD. More importantly, the measurement of these peritrabecular clefts may contribute to the scientific reproducibility of the presented data.

This clefting phenomena was absent in only 5 (13.5%) of 37 FD cases. Interestingly, 3 of these 5 cases in which clefting was not observed was noted in very young patients and their lesions presented unusual features on radiology. These lesions were histologically characterized by an immature FD pattern. Such features may suggest that peritrabecular clefting in FDs is related to bone maturation. However, it is important to emphasize that many other very young patients enrolled in this study presented mature FDs; thus, patient age should not be regarded as an independent predictor of the grade of bone maturation in fibrous-osseous lesions of the jaws. Hence, clefting phenomenon in FDs of the jaws could not be directly associated with the patient’s age.

Retraction artifact is a widely known phenomenon in histopathology but has received very little attention in routine practice. Pathologists tend to see it merely as an artificially produced tissue alteration that interferes with the ability to make an appropriate diagnosis. Conversely, in several recent studies authors have demonstrated the diagnostic and prognostic significance of peritumoral clefts separating tumor cells from the adjacent stroma in several different tumors such as basal cell carcinoma, prostatic adenocarcinoma, breast carcinoma, and squamous cell carcinoma of the esophagus.13-18

Although frequently encountered in daily pathology practice, the origin or the biological mechanisms responsible for peritumoral microscopic clefting is largely unknown, but it may be regarded as an artifact resulting from tumor retraction occurring during routine tissue processing for the preparation of light-microscopy sections.14,16,17 It may be associated with an abnormality in the expression of basement membrane proteins, collagenases, or other enzymes.15 Interestingly, it is frequently stated that retraction of the stroma from tumor cells is absent on frozen section material, suggesting that retraction artifact is a biologically insignificant artifactual phenomenon simply brought about by the acts of tissue fixation and processing. However, Acs et al. (2009)17 recently observed retraction artifact in frozen sections of breast carcinomas, suggesting that they may in fact represent real spaces around the nests of tumor cells, and supporting the theory that retraction is a phenomenon intrinsically related to the biological features of certain tumors rather than simply representing unwanted side-effects of fixation and processing. Peritrabecular clefting in FDs may also be regarded as an artifact resulting from tissue retraction occurring during tissue fixation, decalcification, preparation, or sectioning. However, even if peritrabecular clefting merely represents a retraction artifact in FD of the jaws, the international multicenter approach of the present study was able to demonstrate that clefting does not depend on how the tissue was prepared. This observation is based on the different decalcification and processing protocols performed at the oral pathology biopsy services enrolled in this study. Most importantly, if peritrabecular clefting is a tissue processing artifact, it was still a distinctive feature of FD that has not been described in detail before and which was not identified in OF cases.

Remarkably, while reviewing the literature review, we observed that peritrabecular clefting was illustrated in many of the papers describing the histopathologic aspects of FDs, but such findings were not recognized as important microscopic features by the authors. Representative examples of peritrabecular clefting in FD images can be found in the papers by Speight and Carlos (Figs. 1 and 3),1 Eversole et al (Fig. 3),3 Alawi (Fig. 2),6 and Slootweg (Fig. 21).7 To the best of our knowledge, this is the first report to undertake a detailed analysis of peritrabecular clefting in FDs, and the origin of this phenomenon remains uncertain. However, because of the large amount of clefting detected in
the current FD specimens, this phenomenon cannot be ignored.

In conclusion, several diagnostic criteria have previously been proposed to diagnose and differentiate FD from OF, but none of these criteria alone have been shown to be sufficient for distinguishing these lesions. The presence of peritrabecular clefting may be an important microscopic diagnostic feature in FD, and we would like to propose this as an additional diagnostic criterion.

REFERENCES


Reprint requests:
Pablo Agustin Vargas, DDS, PhD
Piracicaba Dental School
Campinas State University (UNICAMP)
Oral Diagnosis Department (Oral Pathology Area)
Avenida Limeira, 901
Caixa Postal 52
Piracicaba, São Paulo, Brazil. CEP: 13414-903
pabloagustinvargasbr@yahoo.com