Clinical and histopathological studies using fibrin-rich plasma in the treatment of bisphosphonate-related osteonecrosis of the jaw

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Abstract
The authors report their experience using platelet-rich fibrin (PRF) therapy for the treatment of ten patients presenting bisphosphonate-related osteonecrosis of the jaw (BRONJ). The aim of our study was to evaluate the effect of this therapy on recurrent BRONJ and to describe the clinical and histopathological/immunohistochemical staining features of PRF treatment. As such, we describe the method we used and report the results observed in the areas treated as well as side effects. The reported results recommend the safety and efficacy of PRF in treatment of BRONJ.

Keywords: platelet-rich fibrin (PRF), bisphosphonate-related osteonecrosis of the jaw (BRONJ), maxillofacial.

Introduction
Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a challenging complication resulting from the long-term therapy with bisphosphonates [1]. Currently, not only the pathogenesis is still not completely understood, but the management of BRONJ remains challenging because there is no definitive treatment other than palliative methods [2]. Current recommendations contraindicate aggressive surgery because its results are unpredictable and may trigger disease progression [3]. The application of platelet-rich fibrin (PRF) in the surgical site may constitute a new alternative method of BRONJ treatment [4]. Dohan et al. (2006) developed the production protocol of PRF attempts to accumulate platelets and released cytokines in a fibrin clot. The platelets and leukocyte cytokines are important part in role-play of this bio-material, but the fibrin matrix supporting them is very helpful in constituting the determining elements responsible for therapeutic potential of PRF [5].

The aims of our study were to describe the clinical and histopathological/immunohistological staining features and to evaluate the PRF treatment outcomes of patients with BRONJ.

Patients, Methods and Results
We conducted a retrospective analysis of 10 consecutive patients who presented with bisphosphonate-related osteonecrosis of the jaw (BRONJ) stage II (Marx classification), diagnosed and treated between August 2013 and February 2014. The precipitating event leading to BRONJ was dental extraction in all cases. The diagnosis was performed basing on clinical and radiographic features.

After the BRONJ diagnosis, all patients discontinued the use of bisphosphonate. All patients included in study were treated with i.v. bisphosphonates for oncological reasons and meet the following criteria: absence of diagnosed malignancies, lack of maxillofacial radiotherapy medical history and recurrent BRONJ after application of the protocol proposed by American Association of Oral and Maxillofacial Surgeons (AAOMS): Chlorhexidine 0.12% mouth rinse, local irrigation with Povidone-Iodine and oral antibiotics – Amoxicillin/Clavulanic Acid (4 g/day) for 10 days. Patients with immunologic diseases, diabetes mellitus, low blood concentration of thrombocytes, chemotherapy or radiotherapy were excluded.

Presurgical standard blood analyses showed normal variables, particularly platelet and leukocyte concentrations. With the patient’s consent, treatment consisted in superficial removal of bone sequestra/superficial curettage and Choukroun’s fibrin-rich plasma (PRF) therapy. A single team (the authors) performed all the surgeries and applied the PRF in these patients. Every patient was operated with local anesthesia and conscious sedation.

PRF clots were prepared as described by Dohan et al. (2006) [5]. Blood was obtained several minutes before starting surgery and prior to anesthesia administration. Blood was drawn into four to eight A-PRF tubes without anticoagulant and was immediately centrifuged at 1300 rpm for 14 minutes using a centrifuge specifically designed for this application.
Post-surgical antibiotherapy was prescribed to all patients with oral Clindamycin 0.9 g daily in divided doses, for 10 days. The sutures were removed 10 days postoperatively.

Samples of PRF masses were fixed in 10% buffered formalin and routinely processed using Thermo Scientific STP 420D Tissue Processor. After embedding the tissue fragments, the resulting paraffin blocks were cut into 3 μm thick sections with a semi-automated Rotary Microtome Leica RM2245. The slides were routinely stained with Hematoxylin–Eosin (HE); immunohistochemical stains were performed for CD61 (Novocastra-Leica Biosystems, Newcastle Upon Tyne, U.K., 1:100, clone 2F2, heat induced epitope retrieval pH 6). As detection system, we used Novolink Polymer (Leica/Novocastra) and DAB (3,3'-diaminobenzidine) chromogen. Slides were counterstained with Mayer’s Hematoxylin, rehydrated and mounted.

In all cases, we performed removal of the bone sequestrations and curettage in the bone tissue until clear bleeding appeared from the subjacent bone. After that, bone cavities were filled with PRF clots. PRF membranes were used to protect the filled bone defect from muco-invagination. Mobilization of a mucoperi-osteal flap was made to obtain a hermetic closure at the wound margins. No postoperative complications were observed and all 10 patients were treated successfully. All the patients in our case series improved after PRF treatment, with mucosal healing. These patients continued with follow-up visits, without evidence of exposed bone after 30 days (Figure 1).

HE staining was used for histological examination by light microscopy. The results showed in all cases necrotic lamellar bone fragments with acute and chronic inflammatory cells well bacterial colonies (Figure 2). PRF clots consisted of a matrix of fibrin embedding numerous platelets (Figure 3A). Platelets, CD61-positive, were diffusely distributed in PRF clot (Figure 3B).

Table 1 – A summary of findings for each case

<table>
<thead>
<tr>
<th>No.</th>
<th>Gender</th>
<th>Age [years]</th>
<th>Bisphosphonate and dosage</th>
<th>Medical condition</th>
<th>Localization</th>
<th>Wound healing (30 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>F</td>
<td>42</td>
<td>Zoledronic Acid</td>
<td>breast cancer</td>
<td>mandible</td>
<td>healed</td>
</tr>
<tr>
<td>2.</td>
<td>F</td>
<td>68</td>
<td>Zoledronic Acid</td>
<td>breast cancer</td>
<td>mandible</td>
<td>healed</td>
</tr>
<tr>
<td>3.</td>
<td>M</td>
<td>69</td>
<td>Zoledronic Acid</td>
<td>prostate cancer</td>
<td>maxilla</td>
<td>healed</td>
</tr>
<tr>
<td>4.</td>
<td>M</td>
<td>52</td>
<td>Ibandronate</td>
<td>bowel cancer</td>
<td>mandible</td>
<td>healed</td>
</tr>
<tr>
<td>5.</td>
<td>F</td>
<td>59</td>
<td>Zoledronic Acid</td>
<td>breast cancer</td>
<td>maxilla</td>
<td>healed</td>
</tr>
<tr>
<td>6.</td>
<td>F</td>
<td>58</td>
<td>Ibandronate</td>
<td>kidney cancer</td>
<td>mandible</td>
<td>healed</td>
</tr>
<tr>
<td>7.</td>
<td>M</td>
<td>63</td>
<td>Zoledronic Acid</td>
<td>prostate cancer</td>
<td>mandible</td>
<td>healed</td>
</tr>
<tr>
<td>8.</td>
<td>F</td>
<td>30</td>
<td>Ibandronate</td>
<td>multiple myeloma</td>
<td>mandible</td>
<td>healed</td>
</tr>
<tr>
<td>9.</td>
<td>M</td>
<td>79</td>
<td>Zoledronic Acid</td>
<td>prostate cancer</td>
<td>maxilla</td>
<td>healed</td>
</tr>
<tr>
<td>10.</td>
<td>F</td>
<td>75</td>
<td>Zoledronic Acid</td>
<td>multiple myeloma</td>
<td>mandible</td>
<td>healed</td>
</tr>
</tbody>
</table>

F – Female; M – Male.

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Discussion

Some perspective is needed regarding what we are showing with our case series. Use of PRF in oral and maxillofacial surgery has been implicated in different procedures such as socket preservation, sinus lift and bone augmentation, root coverage procedures, and healing in donor site with good results [7].

To date, no universally accepted management protocol is available to treat BRONJ, because the etiopathogenic mechanism remains unclear [8]. The response to surgical debridement is unpredictable and there is a risk of worsening bone exposure [9].

Bisphosphonate toxicity to epithelial cells has been well documented [10, 11]. Bisphosphonates inhibits cell proliferation and the capacity for wound healing in murine oral keratinocytes [12]. However, the reports of autologous PRF use for BRONJ treatment are limited [13]. Therefore, what the benefit brought about by PRF should be studied, which is the purpose of the present study.

Additional biological effects of PRF may contribute to the improvement of clinical outcome in recurrent BRONJ. To our knowledge, PRF works via the degranulation of the alpha granules in platelets, which contain the synthesized and prepackaged growth factors [14]. The secreted growth factors bind to their transmembrane receptors on adult mesenchymal stem cells, osteoblasts and endothelial cells and then cause matrix formation and osteoid production trough cellular message transforming [15].

Besides, PRF also contains three proteins in blood known to act as cell adhesion molecules for osteoconduction and as a matrix for bone and connective tissue [16]. PRF creates the conditions for the development of microvascularization, guides epithelial cell migration to its surface [17, 18] and facilitates cellular migration, particularly for endothelial cells necessary for the neoangiogenesis [19]. Thus, PRF acts not just like a fibrin matrix too accelerate the healing of wound [20], but also by its high concentration of high amounts of growth factors such as TGBβ or PDGF (platelet-derived growth factor) released by the platelets [19].

Although the sample size was small, results from the present investigation showed that PRF was effective in significantly improving clinical parameters after surgery in patients with recurrent BRONJ. Although a standardized method has been proposed, the presently available series of patients do not provide strong evidence, as they are heterogeneous in terms of localization of lesions and age of patients. Further studies are necessary to assess the long-term effectiveness of PRF, and a larger sample size is recommended.

Conclusions

Within the limits of this study, PRF led to favorable clinical improvement in recurrent BRONJ. However, the long-term outcome needs better assessments, and further researches should address this issue.

Author contribution

All authors have equally contributed to this study.

References


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