The role of Leukocyte and Platelet-rich fibrin in the prevention of medication-related osteonecrosis of the jaw, in patients requiring dental extractions: An observational study

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Research Article

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Abstract

Purpose

Medication-related osteonecrosis of the jaw (MRONJ) is a significant complication which can present following a dental extraction in patients receiving anti-resorptive and anti-angiogenic medications. The purpose of this study was to investigate the possible beneficial effect of L-PRF in the prevention of MRONJ in patients receiving these medications and requiring dental extractions.

Methods

Thirty-nine patients were included and divided in two groups, depending on whether L-PRF was used after the required dental extraction or not. Subsequently, the patients were categorised into low and high-risk for developing MRONJ, as recommended by the SDCEP guidance.

Results

None of the patients in the L-PRF group returned with established MRONJ. Five high-risk patients in the control group presented with established MRONJ in the follow-up appointment. Significant statistical difference (p = 0.04) was observed following comparison of the high-risk patients of the two groups.

Conclusion

These encouraging results suggest that L-PRF may be useful in the prevention of MRONJ following a dental extraction especially in patients of the higher risk category. A protocol for the management of this type of patients is also introduced.

Introduction

Medication-related osteonecrosis of the jaw (MRONJ) is an adverse effect of treatment with anti-resorptive and anti-angiogenic medications for a wide range of conditions. It has been first reported 20 years ago by Marx and has been described and acknowledged further on the following years [1, 3–5]. The Scottish Dental Clinical Effectiveness Programme (SDCEP) published guidance has described it as ‘exposed bone, or bone that can be probed through an intraoral or extraoral fistula, in the maxillofacial region that has persisted for more than eight weeks in patients with a history of treatment with anti-resorptive or anti-angiogenic drugs, and where there has been no history of radiation therapy to the jaw or no obvious metastatic disease to the jaws’ [5].

Initially, bisphosphonates were the main medications responsible for the occurrence of MRONJ; hence the initial description of the disease as bisphosphonate-related osteonecrosis of jaw (BRONJ) [2]. Patients
were considered to have BRONJ if they fulfilled all three of the following characteristics: concurrent or previous treatment with bisphosphonates, exposed bone in the maxillofacial region persisting for more than 8 weeks (presence of fistula not mentioned) and no previous history of radiation of the jaw [3]. The mechanism of action of bisphosphonates is not well understood but it is believed that one of their actions is to inhibit osteoclastic activity. On the years to follow and as further treatment modalities were introduced, further medications such as biologic agents (i.e. denosumab) were found to result in MRONJ [4, 6]. Anti-resorptive medications are used to treat various conditions ranging from osteoporosis and prevention of pathological fractures to prevention or management of metastatic bone disease in cancer patients [1, 5].

Dentoalveolar surgery i.e. dental extraction is considered the main risk factor predisposing to the development of MRONJ. Anatomic factors have been associated significantly with the occurrence of MRONJ as it is more likely to appear in the mandible [1, 5, 7, 8]. Other risk factors are the medical condition for which the patient is treated, cumulative drug dose and duration and concurrent treatment with steroids. Demographic and genetic factors as well as the smoking status may also play a role [1, 5].

MRONJ is a significant complication following dental extractions in patients receiving anti-resorptive or anti-angiogenic medications. It may present with localised pain, swelling, pus discharge with or without exposed bone or with large areas of exposed bone extending to involve adjacent anatomical locations and neurovascular structures and even resulting in pathological fractures or extraoral fistulas [1, 4].

Autologous platelet concentrates (APC) have been previously used to promote healing following surgical procedures. The use of APCs in the maxillofacial region has been first described by Marx in 1998 [9]. They have been previously used as an adjunct for the management of patients with established MRONJ and have shown promising results by promoting soft tissue healing and bone regeneration as well as preventing recurrence [10, 11].

Leukocyte and platelet rich fibrin (L-PRF) has been first described by Dohan et al and Choukroun et al in 2006 as a new generation APC [12, 13]. Since then it had been increasingly gaining popularity for use in oral surgical procedures as an adjunct for accelerating soft and hard tissue healing [14].

The aim of this study is to evaluate whether L-PRF is effective on promoting healing in patients receiving treatment with anti-resorptive or anti-angiogenic medications and requiring dental extractions and whether its use can be considered a preventive measure.

**Materials and Methods**

This observational study was conducted in the Oral Surgery department, Edinburgh Dental Institute, over a period of 3 years, from 2019 to 2022. Approval was obtained by the Oral Health Service, Quality Improvement Team of NHS Lothian. The study included patients that required dental extractions and have been receiving or previously received bisphosphonates or biologic agents for the treatment of various conditions. The patients were divided into L-PRF group and a control group. All patients were
given the option of having L-PRF placement or not. The patients that opted to proceed without L-PRF placement were added on the control group. The surgical treatment and follow-up appointment were the same for both groups. Age, gender, condition for which medication was taken, type and duration of medication, high or low risk category, time of follow-up and healing result were recorded as seen in Table 1.
Table 1
Main characteristics of patients included in the study.

<table>
<thead>
<tr>
<th></th>
<th>L-PRF group (n = 15)</th>
<th>Control group (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High-risk (n = 11)</td>
<td>Low-risk (n = 4)</td>
</tr>
<tr>
<td>Gender</td>
<td>Males = 4</td>
<td>Females = 7</td>
</tr>
<tr>
<td></td>
<td>Females = 7</td>
<td></td>
</tr>
<tr>
<td>Age (mean, years)</td>
<td>71</td>
<td>79</td>
</tr>
<tr>
<td>Systemic disease</td>
<td>Osteoporosis = 7</td>
<td>Osteoporosis = 3</td>
</tr>
<tr>
<td></td>
<td>Myasthenia</td>
<td>Polymyalgia Rheumatica = 1</td>
</tr>
<tr>
<td></td>
<td>Graves = 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Breast Cancer = 2</td>
<td></td>
</tr>
<tr>
<td>Type of Drug</td>
<td>Alendronic acid = 5</td>
<td>Alendronic acid = 2</td>
</tr>
<tr>
<td></td>
<td>Risendronate = 1</td>
<td>Zolendronic acid = 2</td>
</tr>
<tr>
<td></td>
<td>Zolendronic acid = 4</td>
<td>Risendronate = 1</td>
</tr>
<tr>
<td></td>
<td>Denosumab = 1</td>
<td></td>
</tr>
<tr>
<td>Steroids (n)</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Duration (median, years)</td>
<td>5</td>
<td>1.6</td>
</tr>
<tr>
<td>Extractions (n), site</td>
<td>Mandible = 11</td>
<td>Mandible = 2</td>
</tr>
<tr>
<td></td>
<td>Maxilla = 7</td>
<td>Maxilla = 2</td>
</tr>
<tr>
<td></td>
<td>Total = 23</td>
<td>Total = 41</td>
</tr>
<tr>
<td>Review (weeks)</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

*Statistical significant difference is seen in healing outcome between the two high-risk groups.
<table>
<thead>
<tr>
<th>L-PRF group (n = 15)</th>
<th>Control group (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>High-risk (n = 11)</strong></td>
<td><strong>Low-risk (n = 4)</strong></td>
</tr>
<tr>
<td>Outcome</td>
<td>All healed-</td>
</tr>
<tr>
<td></td>
<td>No bone exposure/</td>
</tr>
<tr>
<td></td>
<td>Full mucosal coverage</td>
</tr>
</tbody>
</table>

*Statistical significant difference is seen in healing outcome between the two high-risk groups.

**L-PRF preparation**

L-PRF membranes were prepared by drawing 10–40 ml of blood, depending on the number of teeth to be removed. Blood was collected in 9ml tubes with serum clot activator and centrifuged at 2700rpm for 12–14 minutes. Once prepared, the clot created was placed on a special tray for 10 minutes and membranes and/or plugs were created. (Fig. 1)

**Surgical procedure**

The procedures in both groups were conducted by experienced Oral Surgeons. Following administration of local anaesthetic with adrenaline, atraumatic extraction was performed. Following dental extraction, the L-PRF products (2–3 membranes and/or plug) were placed in and over the socket and sutured in place with PTFE (polytetrafluoroethylene) sutures or Vicryl™ coated sutures. In the control group, following atraumatic extraction the same type of sutures was used for closure if required. (Fig. 2)

**Follow-up**

Patients were reviewed at least once at 6–8 weeks following the procedures. Established MRONJ was defined as the presence of exposed bone, bone sequestrum or bone that can be probed through fistula with or without pus discharge, on the follow-up appointment.

**Statistical analysis**

Data was transferred onto a spreadsheet and statistical analysis was conducted (Excel 2017; Microsoft). Basic descriptive statistics were used to analyse variables such as age, gender, indication for medication, medication received and duration, high or low risk for MRONJ, extraction site and treatment outcome. Differences were tested for statistical significance by using a chi-square test. Statistical significance was set to p < 0.05.

**Results**
A total of 39 patients were included in the study. Fifteen patients were included in the L-PRF group and 24 patients were included in the control group. A total of 63 extractions were performed in between the two groups. The patients were divided into low-risk and high-risk categories based on the guidance from SDCEP [5]. (Table 2)

Table 2
High-risk and low-risk categories of patients receiving antiresorptive or antiangiogenic medication, as recommended by the SDCEP guidance [5].

<table>
<thead>
<tr>
<th>Low-risk (If any of the following is present)</th>
<th>High-risk (If any of the following is present)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients being treated for osteoporosis or other non-malignant diseases of bone (e.g. Paget’s disease) with oral bisphosphonates for less than 5 years who are not concurrently being treated with systemic glucocorticoids.</td>
<td>Patients being treated for osteoporosis or other non-malignant diseases of bone (e.g. Paget’s disease) with quarterly or yearly infusions of intravenous bisphosphonates for more than 5 years.</td>
</tr>
<tr>
<td>Patients being treated for osteoporosis or other non-malignant diseases of bone with quarterly or yearly infusions of intravenous bisphosphonates for less than 5 years who are not concurrently being treated with systemic glucocorticoids.</td>
<td>Patients being treated for osteoporosis or other non-malignant diseases of bone with bisphosphonates or denosumab for any length of time who are being concurrently treated with systemic glucocorticoids.</td>
</tr>
<tr>
<td>Patients being treated for osteoporosis or other non-malignant diseases of bone with denosumab who are not being treated with systemic glucocorticoids.</td>
<td>Patients being treated with anti-resorptive or anti-angiogenic drugs (or both) as part of the management of cancer.</td>
</tr>
</tbody>
</table>

L-PRF group

Out of the 15 patients, 11 were females and 4 males. The mean age of patients was 73 years. A total of 22 extractions were performed, 13 in mandible and 9 in maxilla. Eleven patients were considered high-risk and 4 patients low-risk for developing MRONJ. The mean average of follow-up was 12 weeks. All the patients included presented with full mucosal coverage of the sockets, no exposed bone or bone that can be probed through fistula and reported no symptoms. (Fig. 3)

Control group
In the control group, out of the 24 patients, 18 were females and 6 were males. The mean age of patients was 70 years of age. A total of 41 extractions were performed, 20 in mandible and 21 in maxilla. Thirteen patients were considered high-risk and 11 patients low-risk for developing MRONJ. The mean average follow-up time was 10 weeks. Five patients (20% in total and 38% of the high-risk group) – 4 females and 1 male-all included in the high-risk category, presented with signs of MRONJ on their follow-up. One of them was being treated for osteoporosis with oral bisphosphonates for 5 years and concomitant steroids. Another one was being treated with oral and intravenous (IV) bisphosphonates for osteoporosis for a period of 6 years. The other 3 were being treated for cancer (breast cancer, multiple myeloma) with IV bisphosphonates. Three of them had extractions in the mandible and two of them in the maxilla. Three of these patients developed stage 1 MRONJ, one stage 2 MRONJ and one stage 3 MRONJ [1]. Two of these patients were treated with simple superficial sequestrum removal (stage 1), one had to undergo a second procedure for surgical exploration and debridement (stage 1) and the last one had to be referred to Oral and Maxillofacial surgery (OMFS) for an extensive procedure involving the maxillary antrum (stage 3). The Stage 2 case had a second procedure carried out for surgical exploration and debridement with placement of L-PRF membranes. All five patients demonstrated good healing on their next follow-up appointment although they had to undergo a second procedure. Interestingly, following statistical analysis and comparison of the high-risk groups significant statistical difference was found (p = 0.04). Analysis of patients in the low-risk groups showed no statistical difference as healing was seen in all patients.

Discussion

The results of this study show that the use of L-PRF membranes following a dental extraction may be beneficial for the prevention of MRONJ in patients receiving anti-resorptive or anti-angiogenic medications. In this study a treatment protocol is introduced with the use of L-PRF. (Fig. 4)

Anti-resorptive and anti-angiogenic medications, including bisphosphonates and the more recently introduced biological agents (i.e. denosumab), are increasingly used nowadays for the treatment of various bone conditions. They are commonly used for the management of osteoporosis and for the prevention or alleviation of symptoms of bone metastasis [4]. In this study, patients suffering from myasthenia gravis, rheumatoid arthritis and ankylosing spondylitis have also been receiving these medications.

Bisphosphonates reduce bone resorption by inhibiting osteoclastic activity. It has been also suggested that by inducing apoptosis they may inhibit angiogenesis and may decrease oral mucosal cell migration [16]. There is evidence suggesting that they may remain in the skeletal system for a period of 10 years [17]. As a consequence of this, it has been suggested that duration of taking the medication and the higher cumulative dose may increase the risk of developing MRONJ. This also explains the higher risk in patients receiving higher doses for cancer treatment [5, 18]. The chronic use of concurrent medications, such as glucocorticoids (steroids) has also shown to increase the risk for MRONJ [7, 19]. In this study one of the patients who developed MRONJ was being treated with a concurrent steroid medication and
another was receiving IV bisphosphonates for osteoporosis for a period of 6 years. The other 3 have been receiving high doses of antiresorptive medications as part of their cancer treatment. As mentioned previously, the patients in this study have been categorised into high and low-risk groups based on the classification introduced by the SDCEP guidance. All 5 patients which eventually developed MRONJ belonged to the higher risk category.

There is no high level of evidence in the literature to support drug holidays, except in the case of denosumab, a human monoclonal antibody, as its effect on bone turnover may diminish after 9 months of treatment completion [1, 5, 20]. In the recently published position paper by the American Association of Oral and Maxillofacial Surgeons (AAOMS), the members of the working group were unable to reach definitive conclusions regarding drug holidays although some of the members suggested drug holiday can be offered on a case-by-case basis [1]. In this study, one patient has been receiving denosumab injection for the management of osteoporosis every 6 months. We aimed to do the extractions towards the end of the 6-month period with a 2–3 weeks delay of their next dose in agreement with the osteoporosis clinic.

APCs are widely used as adjuncts in surgical procedures in different fields of medicine and dentistry and they have been gaining increasing popularity nowadays. The rationale behind the use of APCs is the fact that they are rich in platelets and leukocytes as well as mitogenic growth factors which may promote wound healing such as platelet-derived growth factor (PDGF), transforming growth factor-beta, epidermal growth factor, vascular endothelial growth factor (VEGF), procoagulant factor, cytokines, chemokines and antimicrobial proteins [21]. Since the introduction of platelet-rich plasma (PRP) in the maxillofacial region by Marx (1998) different systems have been promoted featuring different APCs such as plasma-rich in growth factors (PRGF), concentrated growth factors (CGF) and platelet-rich fibrin (PRF) [9].

In this study, L-PRF has been utilised; a second generation APC that regulates inflammation and stimulates chemotaxis in immune response. In contrast to PRP and PRGF, the preparation technique of L-PRF is simple, less costly and does not require multiple pipetting for separation and any biochemical modification of the blood by addition of anticoagulants or other adjuncts is unnecessary. [12, 13]. Beside platelet and leukocytes, it contains a significant amount of fibrins that create dense fibrin networks and provide a scaffold for attachment of tissue cells and stimulate angiogenesis [12, 22]. Activated platelets enmeshed in the fibrin matrix provide and sustain the release of many growth factors and also increase cell proliferation in osteoblasts, periodontal ligament fibroblasts and pulp fibroblasts by upregulating osteoprotegerin and alkaline phosphatase [23]. Moreover, the important growth factors are released for at least 1 week and up to 28 days during wound healing [24]. In the study by Asaka et al early mucosal epithelisation within 4 weeks was reported in all (30) L-PRF cases following dental extraction suggesting that L-PRF may assist and accelerate wound healing [25].

In the present study, extractions in both groups were performed by experienced clinicians and atraumatically were possible. Any sharp bony edges were smoothed to prevent MRONJ as suggested by previous studies [4, 26]. Following placement of a plug and a membrane adequate to cover the tooth
socket, securing of the membranes was performed. Simple approximation of tissues was deemed adequate in cases where primary closure was not possible. This is based on the results of previous studies that show satisfactory wound healing without primary closure [25, 27]. Chlorhexidine mouthwash use was advised and no antibiotic prophylaxis was prescribed as suggested by the SDCEP guidance [5].

The results of this study are comparable with the findings of other studies that show complete wound healing in patients with MRONJ risk, high or low, which had extractions with L-PRF application [25, 28]. Conversely, a significant percentage of high risk patients (38%, p = 0.04) in the control group presented with established MRONJ on the follow-up appointment suggesting that the use of L-PRF might have been beneficial for this type of patients. The majority of these patients were females and developed MRONJ in mandibular sites, a finding in agreement with results of previous studies [29, 30].

A systematic review published by Gaudin et al in 2015, suggested that the risk of developing MRONJ following a dental extraction in oncology patients was 3.2% [31]. This risk was found to decrease significantly to 0.9% with the use of biologic membranes i.e L-PRF, an outcome which comes in agreement with the results of the present study reporting no occurrence of MRONJ in this type of patients.

Limitations of study

The number of patients included in this study in both groups was small and the patient number allocated in each group was uneven. A larger number of patients would be required in order to reach definitive statistically significant results. Also, no randomisation of patients was possible as L-PRF was offered to all patients and not all opted for it. The use of L-PRF was not possible for some cases due to COVID-19 and the fact that only one centrifuge machine was available at that time. One patient also refused treatment with L-PRF due to religious reasons. Moreover, as in every other Trust, COVID-19 pandemic caused interruptions in the service and although the follow-up appointment was set to 8 weeks, this was not always possible resulting to some patients being reviewed weeks after. However, over the period of the three years the study was running, all patients included in the L-PRF group showed good healing and following discharge to their general dental practitioner, none of them returned with established MRONJ. No radiographic assessment was performed in all patients and this was reserved for patients demonstrating signs and symptoms of the disease.

However, the findings of this study demonstrate the usefulness of L-PRF in patients at risk of developing MRONJ following a dental extraction. Patients receiving treatment for osteoporosis as well as cancer treatment were included, showing favourable results for the prevention of MRONJ particularly in higher-risk patients. Although studies with larger number of patients and randomised controlled trial design are suggested, hopefully the results of this study will encourage the use of this useful adjunct in this type of patients.

L-PRF is a safe, minimally invasive, low-cost adjunct which demonstrates promising results when used for the prevention of MRONJ following a dental extraction. In this study, encouraging results were seen
especially with patients on the higher risk tier. A suggested protocol is introduced for the management of this type of patients requiring dental extractions. To conclude, L-PRF may be useful in preventing MRONJ following a dental extraction in patients treated with anti-resorptive or anti-angiogenic medications.

**Declarations**

**Author contribution**

All authors contributed to the conception and design of study. EB and PP completed the data collection, analysis, and drafting of the manuscript. PP completed the statistical analysis of the data. All authors contributed to the final review. All authors read and approved the final manuscript.

**Data availability**

Data are available from the authors upon reasonable request.

**Consent to participate**

Informed consent has been obtained from all patients prior to procedures.

**Consent for publication**

*The authors affirm that human participants provided informed consent for publication of the images in Figure(s) 1 (a, b, c, d, e, f), 2 (a, b, c, d) and 3 (a, b, c, d).*

**Conflict of interest**

*The authors have no relevant financial or non-financial interests to disclose.*

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**Ethics statement**

This is an observational study. The study has received approval by the Oral Health Service, Quality Improvement Team of NHS Lothian. No further ethical approval is required.

**References**


Figures
Figure 1

Preparation of L-PRF membranes and plug following the protocol published by Pinto et al [15].

A. Venipuncture and collection of blood in 9ml tubes with serum clot activator.

B. Cetrifugation at 2700rpm with centrifuge machine for 12 minutes (14 minutes if patient on antiplatelet/anticoagulants).

C+D. L-PRF clots, are removed from the tube and separated from red blood cells and platelet poor plasma. They are then placed in specially designed kit for 10 minutes.
E+F. After compression, membranes and plug are formed.

**Figure 2**

Operation protocol.

A. Atraumatic extraction of tooth and smoothening of sharp bony edges.

B+C. Placement of plug and membranes in and over the tooth socket.

D. Closure of the socket (primary closure if possible) with non-resorbable polytetrafluoroethylene (PTFE) sutures.
Figure 3

Procedure and outcome 8 weeks after.

A. Atraumatic extraction of tooth and smoothening of sharp bony edges.
B. Placement of plug and membranes in and over the tooth socket.
C. Closure of the socket (primary closure if possible) with resorbable Vicryl™ coated sutures.
D. Healing outcome at 8 weeks following procedure. Full mucosal coverage achieved.
- Venipuncture and collection of blood in 9ml tubes with serum clot activator.
- Centrifugation at 2700rpm with centrifuge machine for 12 minutes (14 minutes if patients on anticoagulants).
- L-PRF clots, are removed from the tube and separated from red blood cells and platelet poor plasma. They are then placed in specially designed kit for 10 minutes.
- After compression, membranes and plug are formed.
- Atraumatic extraction of tooth and smoothening of sharp bony edges.
- Placement of plug and membranes in and over the tooth socket.
- Closure of the socket (primary closure if possible) with non-resorbable polytetrafluoroethylene (PTFE) sutures or Vicryl™ coated sutures.
- Soft diet, chlorhexidine mouthwash 0.2%, no antibiotics.
- Follow-up in 8 weeks.

**Figure 4**

Recommended protocol for patients at risk of developing MRONJ following dental extraction.