Tohoku J. Exp. Med., 2018, **246**, 257-264

Risk of Delayed Healing of Tooth Extraction Wounds and Osteonecrosis of the Jaw among Patients Treated with Potential Immunosuppressive Drugs: A Retrospective Cohort Study

Megumi Hayashi,[1,2,*] Yoshinari Morimoto,[1,2,3] Takatoshi Iida,[1,*] Yohei Tanaka[1] and Shuntaro Sugiyama[1]

[1]Department of Critical Care Medicine and Dentistry, Graduate School of Dentistry, Kanagawa Dental University, Yokosuka, Kanagawa, Japan
[2]Special Patient Oral Care Unit, Kyushu University Hospital, Fukuoka, Fukuoka, Japan
[3]Department of Dentistry, National Cerebral and Cardiovascular Center, Suita, Osaka, Japan

Bone-modifying or antiresorptive agents that target osteoclasts, such as bisphosphonates, are known to cause delayed wound healing and osteonecrosis of the jaw (ONJ) following tooth extraction. However, there are no data on whether such adverse events are also caused by drugs that may suppress the immune system, including corticosteroids, immunosuppressants, biological agents, and disease-modifying anti-rheumatic drugs (DMARDs). The aim of this retrospective study was to examine the incidence of delayed post-extraction wound healing and identify risk factors among patients treated with potential immunosuppressive drugs undergoing tooth extraction. We performed a retrospective cohort study involving 101 patients by reviewing their medical records. The underlying diseases of the enrolled patients included dilated cardiomyopathy, hematological malignancy, sarcoidosis, rheumatoid arthritis, and systemic lupus erythematosus. The sample comprised 131 cases of tooth extraction among the 101 patients; delayed post-extraction wound healing occurred in 10 patients (12 cases, 9.2%), including ONJ in three patients (3 cases, 2.3%). The surgical tooth extraction performed for impacted teeth or a residual root ($P = 0.009$), the number of surgical tooth extraction ($P = 0.012$), decreased lymphocyte counts ($P = 0.008$), and decreased eosinophil counts ($P = 0.009$) were significantly related to delayed wound healing. Thus, among patients taking corticosteroids, immunosuppressants, biological agents, and/or DMARDs, there is a risk of delayed wound healing and ONJ. Moreover, the significant risk factors are low lymphocyte counts, low eosinophil counts, and surgical extraction. It is of particular importance to prevent surgical site infection, when the high-risk patients undergo tooth extraction.

Keywords: delayed wound healing; eosinophil; immunosuppression; lymphocyte; surgical tooth extraction
Tohoku J. Exp. Med., 2018 December, **246** (4), 257-264.

Introduction

Osteonecrosis of the jaw related to drugs (called medication-related osteonecrosis of the jaw: MRONJ) has often been reported in patients taking bone-modifying agents (BMAs), such as bisphosphonates (BPs) and denosumab, a monoclonal antibody against receptor activator of nuclear factor *κ*-B ligand (anti-RANKL) (Hellstein et al. 2011; Troeltzsch et al. 2012; Epstein et al. 2013; Qi et al. 2014). BPs directly inhibit osteoclast activity, inactivate osteoclasts, and lead to apoptosis. Denosumab affects RANKL cytokine growth factors, inhibits differentiation of osteoclasts from monocytes and macrophages, and inhibits osteoclast activity (Troeltzsch et al. 2012; Epstein et al. 2013). In patients taking these drugs, delayed wound healing or osteonecrosis of the jaw (ONJ) following tooth extraction can be a major problem. The American Association of Oral and Maxillofacial Surgeons (AAOMS) issued a revised position paper on MRONJ for its prevention, diagnosis, and treatment in 2014 (Ruggiero et al. 2014).

On the other hand, corticosteroids and immunosuppressants are known to cause delayed postoperative wound healing in some cases (Petri et al. 1998; Guilbeau 2002; Weinstein 2012a, b; Cavalli et al. 2014). However, delayed wound healing and ONJ caused by these drugs following tooth extraction has not been reported.

Biological drugs such as cytokines and monoclonal

Received July 17, 2018; revised and accepted November 28, 2018. Published online December 18, 2018; doi: 10.1620/tjem.246.257.
*These authors contributed equally to this work.
Correspondence: Yoshinari Morimoto, D.D.S., Ph.D., Department of Critical Care Medicine and Dentistry, Graduate School of Dentistry, Kanagawa Dental University, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan.
e-mail: morimoto@kdu.ac.jp

antibodies are now being used in the treatment of cancer and autoimmune diseases such as rheumatoid arthritis, along with disease-modifying anti-rheumatic drugs (DMARDs) (Rosman et al. 2013; Radfar et al. 2015). Although these biological drugs are effective for many diseases, they can also cause adverse reactions, such as decreased immune function and opportunistic infections including tuberculosis, fungal infections, hepatitis B infection, and cytomegalovirus infection (Rosman et al. 2013; Radfar et al. 2015). In regards to wound healing, impaired wound healing has been reported with tumor necrosis factor-α (TNF-α) inhibitors (Mooney et al. 1990; Salomon et al. 1991; Repala et al. 1996; Marchal et al. 2004; den Broeder et al. 2007), but, on the other hand, no differences in postoperative wound healing and infections have been reported after knee, ankle and foot, and abdominal surgeries (Bibbo and Goldberg 2004; Colombel et al. 2004). DMARDs such as methotrexate can also adversely affect wound healing, as shown in in vitro and experimental animal studies, while clinical studies have shown that low-dose methotrexate is safe and does not affect the incidence of postoperative wound complications (Pountos and Giannoudis 2017). Therefore, there is no uniform consensus about these drugs.

The aim of this retrospective study was to examine the incidence of and identify factors related to delayed post-extraction wound healing in patients undergoing tooth extraction taking drugs that may suppress the immune system (corticosteroids, immunosuppressants, biological agents, and DMARDs).

Patients and Methods

Patients

This retrospective study followed the principles of the Declaration of Helsinki and was approved by the Institutional Research Boards and Ethics Committees of Kyushu University Hospital and the National Cerebral and Cardiovascular Center.

This study included 131 cases of tooth extraction in 101 patients who were taking drugs that may suppress the immune system (target drugs). Tooth extractions were performed between April 2002 and January 2015 at the Special Patient Oral Care Unit of Kyushu University Hospital and the Department of Dentistry of the National Cerebral and Cardiovascular Center.

Evaluation parameters

In this study, patients' medical records were retrospectively reviewed to examine the items listed below. Evaluation parameters included patients' characteristics (age, sex), underlying disease, dental disease (marginal periodontitis, periapical periodontitis, or impacted tooth), number of tooth extractions, site of the extracted tooth, number of surgical tooth extractions, number of delayed post-extraction wound healing events, types and doses of drugs being taken at the time of tooth extraction, duration of target drug use, and laboratory blood test values on the day of each tooth extraction or a few days before each extraction.

The duration of target drug use was categorized as < 3 months, ≥ 3 but < 6 months, ≥ 6 but < 12 months, ≥ 1 but < 2 years, ≥ 2 but < 3 years, ≥ 3 but < 5 years, and ≥ 5 years, because some of the medical records for some patients were destroyed after 5 years, and patients' whose information was older than 5 years could not be fully collected. When the patients took many kinds of target drugs, the longest duration of drug administered was counted as the duration of medication. The site of the extracted tooth was categorized as upper incisor or canine, upper molar, lower incisor or canine, lower molar, or deciduous.

Tooth extraction was performed in a minimally invasive manner using dental elevators and forceps, and inflamed granulation tissue was completely curetted; horizontal mattress sutures using 4-0 silk were then placed if needed. This was defined as tooth extraction. Surgical extraction was defined as impacted teeth or a residual root was extracted with a concomitant gingival incision, reflecting the periodontal flaps, and/or removal of bone, and the extraction wound was closed using 4-0 silk sutures. Sutures were removed after 1 week.

The AAOMS position paper states that patients may be considered to have drug-induced ONJ if all the following conditions are present (Ruggiero et al. 2014): current or previous treatment with antiresorptive or antiangiogenic drugs; exposed bone or bone that can be probed through an intraoral or extraoral fistula in the maxillofacial region that has persisted for longer than 8 weeks; and no history of radiation therapy to the jaws or obvious metastatic disease to the jaws. In the present study, ONJ was defined with reference to the AAOMS statement.

The target drugs in the present study included corticosteroids, immunosuppressants, biological agents, and DMARDs. Corticosteroid dosages were converted to prednisolone equivalents for comparison. Since the guideline recommends that long-term use of corticosteroid should be combined with BMAs, many patients on corticosteroids were taking BMAs (Lekamwasam et al. 2012). The AAOMS position paper on MRONJ states that a 2-month drug-free period should be adequate before an invasive dental procedure (Ruggiero et al. 2014). In this study, BMAs were discontinued at least 3 months before tooth extraction with reference to the AAOMS position paper (Ruggiero et al. 2014), and the effects of these drugs on wound healing may have been minimized.

Laboratory blood tests (on the day of tooth extraction) included white blood cell (WBC) counts (neutrophils, lymphocytes, monocytes, and eosinophils), red blood cell (RBC) counts, hemoglobin, hematocrit, total protein, and albumin. Whether the patient had diabetes mellitus or was on hemodialysis was also reviewed.

Based on the review results, patients with and without delayed wound healing and/or ONJ were compared to identify factors related to delayed post-extraction wound healing.

Some drugs such as immunosuppressants and biological drugs can suppress an increase in the C-reactive protein (CRP) value with inflammation, and CRP can be increased due to autoimmune diseases; thus, CRP values may not be useful as indices of inflammation. Therefore, for extraction of teeth with acute inflammation in this study, antibiotics were given for 5-7 days, and tooth extraction was performed after acute symptoms had resolved. Tooth extraction was performed only in patients deemed by their physicians to be systemically stable with no acute conditions.

Patients at risk for infective endocarditis were treated with antibiotics in accordance with the "Guidelines for the prevention and treatment of infective endocarditis" by the Japanese Circulation Society (2008). In addition, because all patients were using drugs

that might suppress the immune system, amoxicillin (1-2 g once before and 750-1,000 mg/day for 3 days after tooth extraction) was given. Loxoprofen sodium or acetaminophen was used as needed for pain relief.

Evaluation of delayed post-extraction wound healing

Following tooth extraction, the wound is filled with a blood clot, and the clot is normally replaced by granulation tissue, followed by epithelialization over 7 days. Tissue organization progresses to complete epithelialization in a few weeks. Therefore, delayed wound healing was defined in this study as any area of bone exposure in the extraction socket due to less epithelialization that could be visually confirmed ≥ 10 days after tooth extraction or the development of ONJ at any time after tooth extraction.

Patients were examined by a dentist the day after and 1 week after tooth extraction to examine wound status and suture removal. Patients without wound healing after 1 week continued to be followed until there was epithelialization and no areas of bone exposure. Since there were no cases of complete impacted tooth extraction with complete wound closure, wound healing could be observed in all cases. When ONJ developed after several months, the patients came to our clinic with some spontaneous symptoms, such as pain, swelling, or pus discharge around the jaw. They were diagnosed as having MRONJ as defined in the AAOMS position paper (Ruggiero et al. 2014).

Statistical analysis

Statistical analysis was performed using SPSS version 16.0 software (SPSS Japan, Tokyo, Japan). The data are expressed as median values (interquartile range: IQR). For statistical analysis, patients' sex, use of medications, dental disease, site of the extracted tooth, duration of target drug use, diabetes, and hemodialysis were examined by the chi-squared test. The site of the extracted tooth was compared among the permanent teeth. Other data were examined by the Mann-Whitney U test. The level of significance was $P < 0.05$.

Results

Patients' characteristics

The 101 patients included 58 men and 43 women, ranging in age from 9 to 82 years (median 51 years, IQR 31-65 years). The underlying diseases of the patients are listed in Table 1.

A total of 230 teeth were extracted during the 131 cases of tooth extraction (median 1 tooth/case, IQR 1-2 teeth/case). Marginal periodontitis was seen in 18 cases, with periapical periodontitis in 80 cases and an impacted tooth including pericoronitis of a wisdom tooth in 33 cases. The type of tooth extraction was a simple extraction in 85 cases and surgical extraction in 46 cases. Multiple cases of tooth extraction included twice in 9 patients, 3 times in 7 patients, 4 times in 1 patient, and 5 times in 1 patient. The interval between each tooth extraction was at least 1 month, so that all of these cases were included in the study. None of the patients had an apparent bleeding diathesis, and all had a platelet count $\geq 8 \times 10^4$ /mm^3 at the time of tooth extraction. None of the patients was taking warfarin, but some were taking aspirin. When a bleeding tendency with a platelet count $< 10 \times 10^4$ /mm^3 or antithrombotic therapy is found, oxidized cellulose or gelatin sponges are generally

Table 1. Patients' underlying diseases.

Disease	Number of patients
Dilated cardiomyopathy (post heart transplantation)	34
Heart disease (ACS, AR, DCM, CHF)	5
Primary pulmonary hypertension	4
Rheumatoid arthritis	9
Hematological malignancy/ disease (AML, ALL, ML, MDS, MM) (post hematopoietic stem cell transplantation)	7
Hematological malignancy/ disease (AML, ATL, ML, ITP)	4
Sarcoidosis	6
Systemic lupus erythematosus	6
Sjögren's syndrome	4
Carcinoma (stomach, lung, pancreas, prostate, anus)	5
Post kidney or liver transplantation	5
Chronic kidney disease	4
Arteritis	2
Scleroderma	2
Others	8

Some patients had multiple diseases.
ACS, acute coronary syndrome; AR, aortic regurgitation; DCM, dilated cardiomyopathy; CHF, chronic heart failure; AML, acute myelogenous leukemia; ALL, acute lymphatic leukemia; ML, malignant lymphoma; MDS, myelodysplastic syndrome; MM, multiple myeloma; ATL, adult T cell leukemia; ITP, idiopathic thrombocytopenic purpura.

inserted in the wound, followed by biting gauze for 30 minutes for local hemostasis. In this study, no oxidized cellulose or gelatin sponges, which can impair wound healing, were used. The wound was sutured, and only biting gauze was used for compression hemostasis. There was no postoperative hemorrhage.

Immunosuppressants included tacrolimus in 38, mycophenolate mofetil in 26, cyclosporine in 8, everolimus in 8, azathioprine in 4, and mizoribine in 3 patients. Corticosteroids included prednisolone in 79 and dexamethasone in 2 patients. DMARDs and biological agents included methotrexate in 4, infliximab in 1, tocilizumab in 1, adalimumab in 1, and golimumab in 1 patient.

Among the 131 cases of tooth extraction, delayed post-extraction wound healing occurred in 10 patients (12 cases, 9.2%), including ONJ in three patients (3 cases, 2.3%).

Analysis of factors related to delayed post-extraction wound healing

Table 2 compares the results of the groups. Age, sex, dental disease, number of extracted teeth, and type of drugs did not differ significantly between the groups, but the number of surgical tooth extractions (P = 0.012) and the performance of surgical tooth extraction (P = 0.009) were significantly higher in the delayed wound healing group. In addition, a comparison of laboratory blood test values showed that the lymphocyte count (P = 0.008) and the eosinophil count (P = 0.009) were significantly decreased in the delayed wound healing group.

The duration of target drug use and the site of the extracted permanent tooth did not differ significantly between the groups, but duration of medication use ≥ 5 years tended to be associated with a higher incidence of delayed wound healing than that < 5 years (P = 0.067) (Tables 3 and 4).

Table 2. Comparison of factors related to delayed healing of tooth extraction wounds.

	Delayed healing of extraction wounds		Statistical analysis	
	Negative (n = 119)	Positive (n = 12)	P value	χ^2 value
Age (y)	51 (31-65)	57.5 (37-66.8)	0.470	0.522
Sex (male/female)	67 / 52	7 / 5	0.893	0.018
Dental disease (marginal periodontitis/periapical periodontitis/impacted tooth)	17/72/30	1/8/3	0.854	0.034
Median number of extracted teeth	1 (1-2)	1 (1-2.8)	0.961	0.002
Median number of surgical tooth extractions	0 (0-1)	1 (0-1)	0.012	6.300
Performance of surgical tooth extraction (+/–)	35 / 84	8/ 4	0.009	6.809
Drugs				
Immunosuppressants (+/–)	64 / 55	6 / 6	0.823	0.050
Tacrolimus (mg/day)[number of cases]	2.4 (1.5-3.9) [40]	2.1 (1.0-4.5) [6]	0.725	0.124
Mycophenolate mofetil (mg/day)[number of cases]	1,125 (875-2,000) [34]	1,500 (750-1,750) [3]	0.736	0.114
Cyclosporine (mg/day)[number of cases]	230 (163-433) [12]	[0]		
Everolimus (mg/day)[number of cases]	1.6 (1.5-2.5) [7]	1.5 [1]	0.542	0.172
Corticosteroids (+/–)	96 / 23	10 / 2	0.698	0.151
Corticosteroid (conversion to prednisolone potency)(mg/day)	7.5 (4.8-20.0)	8.5 (5.0-13.8)	0.553	0.351
Anti-rheumatic drugs (+/–)	9 /110	2 / 10	0.232	1.430
Laboratory tests				
White blood cells (/mm^3)	6,000 (4,450-7,503)	5,700 (2,775-6,680)	0.263	1.250
Neutrophils (/mm^3)	3,733 (2,409-4,940)	3,933 (1,793-5,023)	0.812	0.056
Lymphocytes (/mm^3)	1,265 (862-1,861)	862.5 (537-1,278.8)	0.008	6.982
Monocytes (/mm^3)	397 (209-513)	336 (228.5-526.5)	0.328	0.955
Eosinophils (/mm^3)	70 (32-143)	41 (11-54.5)	0.009	6.731
Red blood cells	405 (362-439)	418 (370-438.8)	0.712	0.136
Hemoglobin (g/dL)	12.0 (11.1-13.4)	12.3 (11.0-12.7)	0.916	0.011
Hematocrit (%)	37.5 (33.5-40.9)	38.6 (34.4-39.2)	0.590	0.291
Total protein (g/dL)	6.7 (6.1-7.1)	5.8 (5.8-6.9)	0.054	3.874
Albumin (g/dL)	3.9 (3.5-4.3)	3.8 (3.6-4.3)	0.995	0
Diabetes mellitus (+/–)	8 / 111	2 / 10	1.0	0
Hemodialysis (+/–)	4 / 115	0 / 12	1.0	0

The number of surgical tooth extractions and the performance of surgical tooth extraction were significantly higher in the delayed wound healing group. In addition, a comparison of laboratory blood test values showed that the lymphocyte count and the eosinophil count were significantly decreased in the delayed wound healing group.

Table 3. Correlation between delayed healing of tooth extraction wounds and duration of medication use.

Duration of medication use	Delayed healing	
	Positive	Negative
< 3 months	0	5
3-6 months	2	4
6-12 months	1	16
1-2 years	1	25
2-3 years	1	16
3-5 years	0	16
≥ 5 years	7	37

The duration of medication of the target drugs did not differ significantly between the groups, but duration of medication use ≥ 5 years tended to suggest a higher incidence of delayed wound healing than that < 5 years (P = 0.067, χ^2 = 3.343).

Table 4. Correlation between delayed healing of tooth extraction wounds and tooth extraction site.

Tooth extraction site	Delayed healing	
	Positive	Negative
Deciduous tooth	0	2
Upper incisor or canine	1	18
Upper molar	5	54
Lower incisor or canine	0	13
Lower molar	6	66

The comparison was performed among the permanent teeth. Several tooth extractions were performed on one occasion in some patients. The site of the extracted permanent tooth did not differ significantly between the groups (P = 0.714, χ^2 = 1.363).

Analysis of cases with delayed post-extraction wound healing

Table 5 shows the characteristics of the 10 patients (12 cases) with delayed post-extraction wound healing. Four of the 12 cases involved post-cardiac transplant patients. Nine cases involved only delayed wound healing, and 3 cases (Case 2, Case 5, and Case 10) involved ONJ. In Case 2, ONJ was probably related to the use of tacrolimus, and intravenous zoledronic acid and denosumab were suspected. ONJ was likely related to the use of immunosuppressants and a corticosteroid in Case 5, and the use of methotrexate and a corticosteroid in Case 10.

Discussion

In this study, among the 131 cases of tooth extraction in patients using the target drugs, there were 12 cases (9.2%) of delayed post-extraction wound healing, including 3 cases (2.3%) of ONJ. A comparison showed that the number of surgical tooth extractions (P = 0.012), the performance of surgical tooth extraction (P = 0.009), and decreased lymphocyte (P = 0.008) and eosinophil (P = 0.009) counts were significantly related to delayed wound healing.

BP-related osteomyelitis compared to other types of non-drug-related osteomyelitis is often associated with proliferation of *Actinomyces* at the surface of BP-related necrotic bone of the jaw (Kos et al. 2010). Therefore, MRONJ is not only due to osteoclast suppression, but it is probably also due to localized suppression of the immune system, primarily based on BP effects on monocytes and macrophages (Katsarelis et al. 2015). In the present study, BMAs were discontinued at least 3 months before tooth extraction with reference to the AAOMS position paper (Ruggiero et al. 2014), and the effects of the drugs on wound healing were minimized.

The present study found that, of the WBCs, decreased lymphocyte and eosinophil counts were related to delayed wound healing. On the other hand, there was no difference in the number of neutrophils or monocytes that differentiate into macrophages and phagocytize invading microorganisms.

Lymphocytes include NK cells, B cells (B lymphocytes), and T cells (T lymphocytes). Lymphocytes are found in all wounds several days following injury. Lymphokines, the soluble proteins produced by antigen-produced lymphocytes (such as interleukin-2: IL-2) affect fibroblast activity and collagen synthesis. The substances that suppress lymphocyte function, such as steroids and doxorubicin, have an adverse effect on wound healing; they decrease wound strength and collagen deposition (Keen 2008). In addition, systemic treatment with cyclosporine A and tacrolimus, both immunosuppressive drugs, inhibits T cell proliferation and IL-2 synthesis leading to impaired wound healing in rats (Schaffer and Barbul 1998). Furthermore, bacterial infection of an oral wound can occur and wound healing may be suppressed after surgery in the oral cavity. Moreover, surgical tooth extraction requires a large, deep procedure, so that there is an increased risk of microbial infection with decreased immunity during the dental wound healing process.

Tissue remodeling and repair are initiated by the release of growth factors, cytokines, chemokines, enzymes, lipid mediators, and reactive oxygen species from the tissue or infiltrating inflammatory cells. Eosinophils have been demonstrated to express and release both mediators of the epithelial-mesenchymal transition (such as TGF-β, basic fibroblast growth factors, etc.) and other repair/remodeling factors (nerve growth factors, neuropeptides, and cytokines such as IL-1β and IL-6) (Jacobsen et al. 2012). Eosinophils also express cytokines (such as IL-4, IL-5, etc.), as well as receptors for many of these cytokines (Jacobsen et al. 2012). These findings suggest that a decrease of eosinophils could play a significant role in delayed wound healing. Thus, the functions of both blood cells may affect

Table 5. Characteristics of the patients with delayed healing of tooth extraction wounds.

Case	Age (y)	Sex	Systemic disease	Medications		Number of tooth extractions	Number of surgical tooth extractions	Wound healing	Diabetes mellitus
				Drug	Dose				
1-1	77	male	chronic ITP	prednisolone	8 mg/day	1	1	delayed	–
1-2	78	male	acute ITP	prednisolone	50 mg/day	1	1	delayed	–
2	56	male	multiple myeloma	tacrolimus	1.0 mg/day	1	1	osteo-necrosis	–
				zoledronic acid (discontinued prior to 9 months)	4 mg/month				
				denosumab (discontinued prior to 4 months)	120 mg/month				
3	68	female	giant cell arteritis	prednisolone	25 mg/day	3	0	delayed	+
4	59	female	SLE	tacrolimus	1.0 mg/day	1	0	delayed	–
				mizoribine	150 mg/day				
				prednisolone	9 mg/day				
5	22	male	dilated cardiomyopathy (heart transplantation)	tacrolimus	3.7 mg/day	1	1	osteo-necrosis	–
				mycophenolate mofetil	1,750 mg/day				
				prednisolone	2.5 mg/day				
6	32	female	dilated cardiomyopathy (heart transplantation)	tacrolimus	2.2 mg/day	1	1	delayed	+
				mycophenolate mofetil	750 mg/day				
				prednisolone	7.5 mg/day				
7	31	female	dilated cardiomyopathy (heart transplantation)	tacrolimus	7 mg/day	3	1	delayed	–
				mycophenolate mofetil	1,500 mg/day				
				prednisolone	10 mg/day				
				alendronate (discontinued prior to 4 months)	35 mg/week				
8	53	male	dilated cardiomyopathy (heart transplantation)	everolimus	1.5 mg/day	2	0	delayed	–
9	52	female	PPH	prednisolone	10 mg/day	1	0	delayed	–
10-1	63	male	RA	prednisolone	5 mg/day	8	4	osteo-necrosis	–
				methotrexate	2 mg/day				
10-2	63	male	RA	prednisolone	5 mg/day	1	1		
				methotrexate	2 mg/day			delayed	–

The characteristics of the 10 patients (12 cases) with delayed post-extraction wound healing are shown. In case 1, the patient who had chronic ITP developed acute ITP after the first tooth extraction and was treated with high-dose prednisolone. The bone-modifying agents (BMAs) were discontinued prior to tooth extraction in two patients (Cases 2 and 7).
ITP, idiopathic thrombocytopenic purpura; SLE, systemic lupus erythematosus; PPH, primary pulmonary hypertension; RA, rheumatoid arthritis.

wound healing when immunosuppressed patients are treated.

Corticosteroids directly inhibit the production and activity of osteoclasts, osteoblasts, and osteocytes (Weinstein 2012a, b). In particular, stimulation of osteocyte apoptosis leads to osteonecrosis. Osteonecrosis of the femur and vertebra has often been reported, but ONJ caused by corticosteroids alone has not been reported (Weinstein 2012a, b). Likewise, in the present study, no delayed post-extraction wound healing occurred in any patient treated with prednisolone alone.

Immunosuppressants such as tacrolimus, mycophenolate mofetil, cyclosporine, everolimus, and mizoribine inhibit T lymphocyte and B lymphocyte activities, whereas azathioprine blocks the production of WBCs. Delayed wound healing has been reported with sirolimus, cyclosporine A, and tacrolimus (Petri et al. 1998; Guilbeau 2002; Cavalli et al. 2014). One patient in the present study on

tacrolimus, mycophenolate mofetil, and prednisolone had ONJ (case 5). Although ONJ due to immunosuppressants alone has not been reported, inhibition of lymphocyte activity has often been described. This was also related to the present findings of a risk of delayed post-extraction wound healing.

Biological drugs now used for treatment of rheumatoid arthritis can affect wound healing. For example, animal studies have shown that wound healing is inhibited by TNF-α inhibitors (Mooney et al. 1990; Salomon et al. 1991; Repala et al. 1996). On the other hand, no differences in wound healing or infection rates after knee, ankle and foot, or abdominal surgery in patients taking etanercept, adalimumab, and infliximab have been reported (Bibbo and Goldberg 2004; Colombel et al. 2004; den Broeder et al. 2007). In patients on corticosteroids, immunosuppressants, and infliximab who undergo bowel resection, slightly higher rates of early postoperative infection have also been reported (Marchal et al. 2004). Thus, no uniform consensus has been reached between animal studies and clinical studies or among the clinical studies themselves. The present study found no delayed post-extraction wound healing in patients using biological drugs, but further investigation in a larger number of patients is necessary.

In the present study, the incidence of delayed wound healing was 9.2%, with that of ONJ being 2.3%. In this context, no differences in ONJ rates between intravenous BPs (1.3-1.4%) and denosumab (1-2%) have been reported (Troeltzsch et al. 2012; Saad et al. 2012; Epstein et al. 2013), but the rates as high as 8.4-9.6% with intravenous BPs and denosumab have been reported (Kos 2015; Kajizono et al. 2015). Tooth extraction is the most important risk factor for MRONJ, but diabetes, osteoporosis, anemia, concomitant corticosteroids, and wound drainage are also risk factors (Kajizono et al. 2015; Tardast et al. 2015; Huang et al. 2015; Kos et al. 2010).

The limitations of this study include its retrospective, observational design and the small number, only 12 cases, of delayed post-extraction wound healing. In addition, some drugs can suppress the increase in the CRP values associated with inflammation, and CRP can increase due to autoimmune disease; thus, CRP values may not be useful as indices of inflammation. This study included patients who clinically had no acute systemic or other oral symptoms. However, the possibility of inflammation cannot be completely excluded.

In conclusion, when tooth extractions are performed in patients taking corticosteroids, immunosuppressants, biological drugs, and/or DMARDs, there is a risk of delayed wound healing and ONJ. Moreover, the significant risk factors for delayed wound healing and ONJ are low lymphocyte counts, low eosinophil counts, and surgical extraction. It is vitally important to prevent surgical site infection and observe wound healing for at least several weeks after tooth extraction in these high-risk patients.

Acknowledgments

This study was supported by our departmental funding.

Conflicts of Interest

The authors declare no conflict of interest.

References

Bibbo, C. & Goldberg, J.W. (2004) Infectious and healing complications after elective orthopaedic foot and ankle surgery during tumor necrosis factor-alpha inhibition therapy. *Foot Ankle Int.*, **25**, 331-335.

Cavalli, R.C., Tambara Filho, R., Gomes Rde, P., Veronez, D.A., Slongo, J. & Fraga, R. (2014) Analysis of the histology of the scar bladder and biochemical parameters of rats with a solitary kidney undergoing immunosuppression with tacrolimus. *Acta Cir. Bras.*, **29**, 508-514.

Colombel, J.F., Loftus, E.V. Jr., Tremaine, W.J., Pemberton, J.H., Wolff, B.G., Young-Fadok, T., Harmsen, W.S., Schleck, C.D. & Sandborn, W.J. (2004) Early postoperative complications are not increased in patients with Crohn's disease treated perioperatively with infliximab or immunosuppressive therapy. *Am. J. Gastroenterol.*, **99**, 878-883.

den Broeder, A.A., Creemers, M.C., Fransen, J., de Jong, E., de Rooij, D.J., Wymenga, A., de Waal-Malefijt, M. & van den Hoogen, F.H. (2007) Risk factors for surgical site infections and other complications in elective surgery in patients with rheumatoid arthritis with special attention for anti-tumor necrosis factor: a large retrospective study. *J. Rheumatol.*, **34**, 689-695.

Epstein, M.S., Ephros, H.D. & Epstein, J.B. (2013) Review of current literature and implications of RANKL inhibitors for oral health care providers. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.*, **116**, e437-442.

Guilbeau, J.M. (2002) Delayed wound healing with sirolimus after liver transplant. *Ann. Pharmacother.*, **36**, 1391-1395.

Hellstein, J.W., Adler, R.A., Edwards, B., Jacobsen, P.L., Kalmar, J.R., Koka, S., Migliorati, C.A. & Ristic, H.; American Dental Association Council on Scientific Affairs Expert Panel on Antiresorptive Agents (2011) Managing the care of patients receiving antiresorptive therapy for prevention and treatment of osteoporosis: executive summary of recommendations from the American Dental Association Council on Scientific Affairs. *J. Am. Dent. Assoc.*, **142**, 1243-1251.

Huang, Y.F., Chang, C.T., Muo, C.H., Tsai, C.H., Shen, Y.F. & Wu, C.Z. (2015) Impact of bisphosphonate-related osteonecrosis of the jaw on osteoporotic patients after dental extraction: a population-based cohort study. *PLoS One*, **10**, e0120756.

Jacobsen, E.A., Helmers, R.A., Lee, J.J. & Lee, N.A. (2012) The expanding role(s) of eosinophils in health and disease. *Blood*, **120**, 3882-3890.

Kajizono, M., Sada, H., Sugiura, Y., Soga, Y., Kitamura, Y., Matsuoka, J. & Sendo, T. (2015) Incidence and risk factors of osteonecrosis of the jaw in advanced cancer patients after treatment with zoledronic acid or denosumab: a retrospective cohort study. *Biol. Pharm. Bull.*, **38**, 1850-1855.

Katsarelis, H., Shah, N.P., Dhariwal, D.K. & Pazianas, M. (2015) Infection and medication-related osteonecrosis of the jaw. *J. Dent. Res.*, **94**, 534-539.

Keen, D. (2008) A review of research examining the regulatory role of lymphocytes in normal wound healing. *J. Wound Care*, **17**, 218-220, 222.

Kos, M. (2015) Incidence and risk predictors for osteonecrosis of the jaw in cancer patients treated with intravenous bisphosphonates. *Arch. Med. Sci.*, **11**, 319-324.

Kos, M., Brusco, D., Kuebler, J. & Engelke, W. (2010) Clinical comparison of patients with osteonecrosis of the jaws, with

and without a history of bisphosphonates administration. *Int. J. Oral Maxillofac. Surg.*, **39**, 1097-1102.

Lekamwasam, S., Adachi, J.D., Agnusdei, D., Bilezikian, J., Boonen, S., Borgstrom, F., Cooper, C., Diez Perez, A., Eastell, R., Hofbauer, L.C., Kanis, J.A., Langdahl, B.L., Lesnyak, O., Lorenc, R., McCloskey, E., et al. (2012) A framework for the development of guidelines for the management of glucocorticoid-induced osteoporosis. *Osteoporos. Int.*, **23**, 2257-2276.

Marchal, L., D'Haens, G., Van Assche, G., Vermeire, S., Noman, M., Ferrante, M., Hiele, M., Bueno De Mesquita, M., D'Hoore, A., Penninckx, F. & Rutgeerts, P. (2004) The risk of post-operative complications associated with infliximab therapy for Crohn's disease: a controlled cohort study. *Aliment. Pharmacol. Ther.*, **19**, 749-754.

Mooney, D.P., O'Reilly, M. & Gamelli, R.L. (1990) Tumor necrosis factor and wound healing. *Ann. Surg.*, **211**, 124-129.

Petri, J.B., Schurk, S., Gebauer, S. & Haustein, U.F. (1998) Cyclosporine A delays wound healing and apoptosis and suppresses activin beta-A expression in rats. *Eur. J. Dermatol.*, **8**, 104-113.

Pountos, I. & Giannoudis, P.V. (2017) Effect of methotrexate on bone and wound healing. *Expert Opin. Drug Saf.*, **16**, 535-545.

Qi, W.X., Tang, L.N., He, A.N., Yao, Y. & Shen, Z. (2014) Risk of osteonecrosis of the jaw in cancer patients receiving denosumab: a meta-analysis of seven randomized controlled trials. *Int. J. Clin. Oncol.*, **19**, 403-410.

Radfar, L., Ahmadabadi, R.E., Masood, F. & Scofield, R.H. (2015) Biological therapy and dentistry: a review paper. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.*, **120**, 594-601.

Repala, K.T., Vaha-Kreula, M.O., Heino, J.J., Vuorio, E.I. & Laato, M.K. (1996) Tumor necrosis factor-α inhibits collagen synthesis in human and rat granulation tissue fibroblasts. *Experientia*, **16**, 70-74.

Rosman, Z., Shoenfeld, Y. & Zandman-Goddard, G. (2013) Biologic therapy for autoimmune diseases: an update. *BMC Med.*, **11**, 88.

Ruggiero, S.L., Dodson, T.B., Fantasia, J., Goodday, R., Aghaloo, T., Mehrotra, B. & O'Ryan, F.; American Association of Oral and Maxillofacial Surgeons (2014) American Association of Oral and Maxillofacial Surgeons position paper on medication-related osteonecrosis of the jaw: 2014 update. *J. Oral Maxillofac. Surg.*, **72**, 1938-1956.

Saad, F., Brown, J.E., Van Poznak, C., Ibrahim, T., Stemmer, S.M., Stopeck, A.T., Diel, I.J., Takahashi, S., Shore, N., Henry, D.H., Barrios, C.H., Facon, T., Senecal, F., Fizazi, K., Zhou, L., et al. (2012) Incidence, risk factors, and outcomes of osteonecrosis of the jaw: integrated analysis from three blinded active-controlled phase III trials in cancer patients with bone metastases. *Ann. Oncol.*, **23**, 1341-1347.

Salomon, G.D., Kasid, A., Cromack, D.T., Director, E., Talbot, T.L., Sank, A. & Norton, J.A. (1991) The local effects of cachectin/tumor necrosis factor on wound healing. *Ann. Surg.*, **214**, 175-180.

Schaffer, M. & Barbul, A. (1998) Lymphocyte function in wound healing and following injury. *Br. J. Surg.*, **85**, 444-460.

Tardast, A., Sjoman, R., Loes, S. & Abtahi, J. (2015) Bisphosphonate associated osteomyelitis of the jaw in patients with bony exposure: prevention, a new way of thinking. *J. Appl. Oral Sci.*, **23**, 310-314.

The Japanese Circulation Society (2008) Guidelines for the prevention and treatment of infective endocarditis (JCS2008). http://www.j-circ.or.jp/guideline/pdf/JCS2008_miyatake_h.pdf
[*Accessed*: January 8, 2018].

Troeltzsch, M., Woodlock, T., Kriegelstein, S., Steiner, T., Messlinger, K. & Troeltzsch, M. (2012) Physiology and pharmacology of nonbisphosphonate drugs implicated in osteonecrosis of the jaw. *J. Can. Dent. Assoc.*, **78**, c85.

Weinstein, R.S. (2012a) Glucocorticoid-induced osteonecrosis. *Endocrine*, **41**, 183-190.

Weinstein, R.S. (2012b) Glucocorticoid-induced osteoporosis and osteonecrosis. *Endocrinol. Metab. Clin. North Am.*, **41**, 595-611.