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OZONE_EXO: Comparative Analysis of Protocols for Dental Exactions in Patients at Risk of MRONJ: Case-control Study

Study Protocol

This study, entitled the OZOPROMAF protocol, was reviewed and approved by the Ethics Committee of University Hospital of Palermo, Policlinico P. Giaccone (approval number 01/2018).
Patients were consecutively enrolled from February 2018 to March 2020.
The study protocol conformed with the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. All participants gave their written informed consent.

The aim of this study was to carry out a case-control study in order to compare two different protocols of dental extractions in patients at risk of MRONJ, with and without infiltration of a mixture of oxygen-ozone.

According to the considered criteria of inclusion and exclusion (Table 1), this study recruited 117 patients, 27 male and 90 female. Out of these patients, 54 had osteometabolic conditions, 57 were cancer patients, and 6 had both osteometabolic disease and cancer.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	· age ≥ 18 yrs · patients at risk of developing MRONJ for previous or current administration of drugs related · poor prognosis (for caries and/or periodontitis) of one or more teeth suitable for dental extraction
Exclusion criteria	· previous radiation in the head and neck area · neoplastic involvement of the jaw · previous MRONJ diagnosis

The patients were randomly assigned to two groups using a simple randomization method. Specifically, a software program was used to arbitrarily select 38 patients (group Test) to receive ozone therapy by insufflation to the post-extraction site. The remaining 79 patients (group Control) did not receive this treatment.
During first consultation, medical, pharmacological, and dental history of patients were recorded, including age, sex, indications for use of MRONJ-related drugs, type and duration of MRONJ-related medication use, medical history of chemotherapy, concurrent use of other medications, other concomitant diseases, and smoking habits.
The patients recruited for the study (Table 2) were: "*onc1*" (cancer patients undergoing antiresorptive and/or anti-angiogenic therapies for medical indications), "*onc2*" (cancer patients taking antiresorptive treatments to manage osteometabolic disorders), and "*ost*" (non-oncologic patients taking antiresorptives for the treatment of osteometabolic conditions). So, based on type of drugs, route of administration and cumulative dose two risk categories were identified, namely low and high risk: *onc1* patients were generally considered to be at high risk; *onc 2* and *ost* patients belonged to low-risk category.

Table 2. Descriptive data of enrolled patients.

	Test (N=38)	Controls (N=79)
Age		
Mean (SD)	66.7 (12.1)	69.6 (9.82)
Median [Q1, Q3]	71.00 [56.00, 74.75]	70.00 [63.00, 77.00]
Sex		
F	27 (71.1%)	63 (79.7%)
M	11 (28.9%)	16 (20.3%)
Patient risk category		
high	22 (57.9%)	35 (44.3%)
low	16 (42.1%)	44 (55.7%)
Patient type		
onc1	22 (57.9%)	35 (44.3%)
onc2	4 (10.5%)	2 (2.5%)
ost	12 (31.6%)	42 (53.2%)
Type of therapy		
antiresorptive medications	31 (81.6%)	74 (93.7%)
anti-angiogenetic medications	2 (5.3%)	1 (1.3%)
both	5 (13.2%)	4 (5.1%)

2.3 Protocol of intervention

To assess status of oral health, inspection and orthopantomography have carried out; if decay or periodontitis were suspected, an endoral radiograph was performed in order to confirm poor prognosis before extraction. Only when indicated (i.e., teeth near to inferior alveolar nerve and paranasal sinuses), a cone beam computed tomography (CBCT) of the maxillofacial region was performed. Extractions were performed by a resident in oral surgery.

Starting from the day before extraction, oral antibiotics (amoxicillin-clavulanate 1g and metronidazole 500mg, three times daily; in case of penicillin allergy, erythromycin 600mg, three times daily), and local antiseptics (0.2% chlorhexidine mouthwash) for a period of 7 days were prescribed (Table 3).

Written informed consents about off-label use of metronidazole (needed in Italy), oral surgery protocol and MRONJ risk were obtained from patients.

Table 3. Descriptive of profilaxis protocols to prevent MRONJ

	Group T	Group C
Prophylaxis	Amoxicillin-clavulanate 1g + Metronidazole 500 mg three times daily (in case of penicillin allergy: Erythromycin 600mg + Metronidazole 500 mg three times daily)	Amoxicillin-clavulanate 1g + Metronidazole 500 mg three times daily (in case of penicillin allergy: Erythromycin 600mg + Metronidazole 500 mg three times daily)
Adjuvant Therapy	Injections of a 15 mL mixture of Oxygen-Ozone (O_2O_3) with a 26Gx 1⁄2 - 0.45x13 mm needle	NA

GROUP T
On the day of the surgery (T0), the surgical protocol involved superficial local anesthesia by EMLA® cream, loco-regional anesthesia, incision and flap debridement, extraction of the tooth with poor prognosis, osteoplasty, intra-tissutal perialveolar injections of a 15-mL mixture of OxigenOzone (O_2O_3) with a 26Gx 1⁄2 - 0.45x13 mm needle and insufflation of the same mixture in the post-extraction site for at least 1 minute, hemostasis, and suture.
Additionally, each patient was scheduled for supplementary visits during which the OxigenOzone mixture was applied at T1 (3-5 days), T2 (14 days) and T3 (6 weeks) after the extraction.
When possible, depending on the patient's compliance and willingness, in 9 patients the mixture was applied twice a week within T2, until complete clinical healing or in case of ONJ sequestration formation.

GROUP C
On the day of the surgery (T0), the surgical protocol for Group B included the following steps: superficial local anesthesia using EMLA® cream, loco-regional anesthesia, incision and flap debridement, extraction of teeth with a poor prognosis, osteoplasty, achieving hemostasis, and suture.

Healing Evaluation and follow-up
In both groups, patients underwent additional follow-up visits at T1 (3-5 days), T2 (14 days), and T3 (6 weeks) post-extraction to meticulously monitor surgical wound healing by Inflammatory Proliferative Remodeling (IPR) Wound Healing Scale (Table 4) [20], and to record pain intensity by the NRS scale for enhanced ease of use, rather than the originally planned VAS scale [22].
The IPR scale provided a comprehensive assessment of wound healing through distinct subscales, each ranging from 0 to 1, resulting in a total score ranging from 0 to 16. These subscales evaluated the inflammatory response, proliferative response, and remodeling process. At the end of the follow-up period, the total IPR score was computed, with scores spanning from 0 to 16. Scores of 0-4 denoted poor healings, scores of 5-10 indicated acceptable healing, while scores of 11-16 suggested excellent healing.
Table 4. IPR (Inflammatory Proliferative Remodeling) Scale [21]

T/PHASE	Parameter	Score 0	Score 1	Total score
INFLAMMATORY T: 3−5 DAYS	Bleeding, spontaneously or on palpation	Yes	No	/8
	Granulation tissue	Yes	No	
	Haematoma	Yes	No	
	Tissue colour	Redder or whiter than opposite side tissue	Like the opposite side tissue	
	Incision margins	Incomplete flap closure/fibrin clot/partial necrosis/complete necrosis	Complete flap closure/fine fibrin line	

	Suppuration	Yes	No	
	Edema NRS (1−10)	NRS 6−10	NRS 1−5	
	Pain NRS (1−10)	NRS 6−10	NRS 1−5	
PROLIFERATIVE T: 14 DAYS	Re-epithelialisation	Partial	Complete	/5
	Tissue colour	Redder or whiter than opposite side tissue	Like the opposite side tissue	
	Scar	Scar wider than 2 mm/contour irregularity	No scar/scar less wide than 2 mm/contour regularity	
	Suppuration	Yes	No	
	Pain NRS (1−10)	NRS 6−10	NRS 1−5	
REMODELING T: 6 WEEKS	Scar	Scar wider than 2 mm/contour irregularity	No scar/scar less wide than 2 mm/contour regularity	/3
	Tissue colour	Redder or whiter than opposite side tissue	Like the opposite side tissue	
	Pain NRS (1−10)	NRS 6−10	NRS 1−5	
TOTAL PROCESS				/16

2.4 Statistical methods

The statistical analysis was performed using the R Statistical Software (v4.1.2; R Core Team 2021). Categorical variables were expressed as counts and percentages, quantitative variables as mean (standard deviation) or as median and interquartile range (the 25th and 75th percentiles), in case of skewed distributions. The Chi-square or the Fisher's exact test was used to compare groups A and B for categorical variables. Student's t test or the Wilcoxon-Mann-Whitney test was calculated to compare groups A and B for continuous variables. Statistically significant differences were assessed using two-sided P-values below 0.05.

At multivariable analysis, logistic regression was used to model binary responses (IPR>Median vs IPR≤Median) as related to demographic, pharmacological, systemic, and clinical covariates that have resulted significant at univariable analysis.

Table 4. IPR comparative statistical analysis

	Group T (ozone therapy)	Group C (controls)	*P-value*
	(N=38)	(N=79)	
Inflammatory T1 (3-5 Days) [§]			
Mean (SD)	2.00 (0.870)	2.49 (0.875)	0,006
Median [Q1, Q3]	2.00 [1.00, 2.75]	2.00 [2.00, 3.00]	
Proliferative T2 (14 Days) [§]			
Mean (SD)	4.24 (1.51)	3.70 (1.02)	<0.001
Median [Q1, Q3]	5.00 [4.00, 5.00]	4.00 [3.00, 4.00]	
Remodelling T3 (6 Weeks) [§]			
Mean (SD)	3.00 (0)	2.96 (0.192)	0,230
Median [Q1, Q3]	3.00 [3.00, 3.00]	3.00 [3.00, 3.00]	
IPR_Total Process[§]			
Mean (SD)	9.37 (1.79)	9.15 (1.38)	0,275
Median [Q1, Q3]	9.50 [9.00, 10.00]	9.00 [8.00, 10.00]	

[§]Wilcoxon signed-rang test