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Research Article

Efficacy and tolerability of medical ozone gas insufflations in patients with osteonecrosis of the jaw treated with bisphosphonates—Preliminary data ☆
Medical ozone gas insufflation in treating ONJ lesions

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ABSTRACT

Osteonecrosis of the Jaw (ONJ) is an adverse event reported especially in patients receiving cancer treatments regimen, bisphosphonates (BPs), and denosumab. We performed an open-label, prospective study in patients treated with zoledronic acid who developed ONJ lesions >2.5 cm, and had no benefit after the treatment with the standard therapy, to evaluate the efficacy and tolerability of medical ozone (O_3) treatment delivered as gas insufflations on each ONJ lesions.

Twenty-four patients (mean age 62.5, range 41–80; 12 female) with bone metastases due to breast (11), prostate (4)and lung (4)cancers, myeloma (2), or osteoporosis (3), previously treated with zoledronic acid and not underwent dental preventive measures and with ONJ lesions >2.5 cm, were observed and treated with topical O_3 gas insufflation every third day for a minimum of 10 for each pathological area or till necrotic bone sequestrum or surgery. We used a special insufflation bell-shaped device adjusted to the specific characteristics of the patient, capable of eliminating any residue of O_3 diffusion by degrading it and releasing O_2 into the air. Azithromicin 500 mg/day was administered for 10 days in all patients before the first three gas insufflation although they had previously received various cycles of antibiotics. Ten patients required more than 10 O_3 gas insufflations due to multiple lesions and/or purulent sovrainfections; one patient received two further O_3 insufflations while waiting the day of surgery. Six of 24 patients interrupted the O_3 gas therapy for oncological disease progression (five patients) and for fear of an experimental therapy (one patient). Six patients had the sequestrum and complete or partial (one patient) spontaneous expulsion of the necrotic bone followed by oral mucosa re-epithelization after a range of 4–27 of O_3 gas insufflations. No patient reported adverse events. In 12 patients with the largest and deeper ONJ lesions, O_3 gas therapy produced the sequestrum of the necrotic bone after 10 to 38 insufflations; surgery was necessary to remove it (11 patients). Of interest, removal was possible without the resection of healthy mandible edge because of the presence of bone sequestrum.

All together the response rate was 75.0% (95% CI, 53.3–90.2%) in ITT analysis and 100% (95% CI, 81.5–100%) in the PP analysis.

In all patients treated with O_3 gas $\pm$ surgery, no ONJ relapse appeared (follow-up mean 18 months, range 1–3 years). Medical O_3 gas insufflations is an effective and safe treatment for patients treated with BPs who developed ONJ lesions >2.5 cm.

Short abstract: ONJ is an adverse event reported in patients receiving cancer treatments regimen, bisphosphonates and denosumab. We performed an open-label, prospective study in 24 patients with solid tumours, myeloma or osteoporosis due to hormonal therapy, treated with zoledronic acid without previuos preventive dental screening, who developed ONJ lesions >2.5 cm, and had no benefit after standard therapy, to evaluate the efficacy and tolerability of medical ozone (O_3) treatment delivered as gas insufflations on each ONJ lesions.

The patients were treated with O_3 every third day for a minimum of 10 for each pathological area or till necrotic bone sequestrum or surgery. Eleven patients required more than ten O_3 gas insufflations.

☆The procedures followed in this study were reviewed and approved by the Ethics Committee of National Cancer Institute of Milan and are in accordance with the ethical standards of the Helsinki Declaration (1964, amended in 1975, 1983, 1989, 1996 and 2000) of the World Medical Association.

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All together the response rate was 75.0% (95% CI, 53.3–90.2%) in ITT analysis and 100% (95% CI, 81.5–100%) in the PP analysis.

In all patients treated with O_3 gas $\pm$ surgery, no ONJ relapse appeared (follow-up mean 18 months, range 1–3 years).

1. Introduction

Osteonecrosis of the jaw (ONJ) is an adverse event reported in patients receiving BPs and RANKL inhibitors such as denosumab [1–9].

ONJ is defined as the persistence of exposed bone in the oral cavity, despite an adequate treatment for six weeks, without local evidence of malignancy and no prior radiotherapy to the affected region [10]. However, ONJ may present with the non-exposed variant of ONJ.

The pooled risk estimated incidence of ONJ, in BPs users, is 2,4% [11–14]. In RCTs comparing zoledronic acid and denosumab in 5677 patients who underwent screening dental procedure, 89 ONJ cases were reported of which 52 in the denosumab group [8,11–13].

Factors adversely influencing bone remodelling are considered to be pivotal in the pathophysiology of the ONJ and preclinical data shows that the bone turnover is higher in the jaws with respect to other skeletal areas [10,15–17]. The presence of chronic periodontal pathologies, the duration and type of BP therapy, tooth extractions, the use of dental appliances, denture traumatisms, invasive dental surgery during the course of BP therapy, poor oral hygiene, concurrent disease (e.g. diabetes, peripheral vasculopathy) and the concomitant use of chemotherapy, antiretroviral therapies, thalidomide, and corticosteroids or the presence of anaemia are considered putative additional risk factors [1–5,18,19]. In a retrospective analysis of 567 cases Vescovi et al. [20] studied the differences between the non surgery-triggered vs surgery-triggered variants bisphosphonate-related osteonecrosis of the jaws. In 205 cases (36.2%) of ONJ no surgery was performed as against 362 cases (63.8%) of post-local invasive procedure forms including tooth extraction in 361 cases and implant placement in one case only.

Bisphosphonates are a well-established, standard-of care treatment option to reduce the frequency and severity and time of onset of the skeletal related events (SREs) in patients with bone metastases due to either solid tumours or multiple myeloma [21–33]. From many years, BPs have been incorporated into clinical practice recommendations for these patients [33–39]and denosumab has been approved in many countries for the delay of onset of SREs due to bone metastasis in breast or prostatic cancer patients.

Preventive dental measures, after dental screening examination [1,40–44], are advocated to reduce the ONJ incidence [14,45,46]due to their efficacy in patients with bone metastases but not in oncological patients with osteoporosis yet. Recent recommendations for ONJ, include a conservative approach with intermittent prophylactic antibiotic therapy, rinses with oral chlorhexidine and debridement [44]; moreover a careful sequestrum removal is recommended [1,17,40–47].

In a previous study [47]we evaluated the efficacy and tolerability of localised topical application of an oil suspension enriched with medical O_3 gas, as treatment for ONJ lesions ≤ 2.5 cm in another sample of patients who failed to respond to various cycles of antibiotics. Unexpectedly, total sequestration of the necrotic bone, with spontaneous expulsion in eight patients and new bone formation around the necrotic area in two patients was observed. No patient required surgical intervention. In two patients with pre- and post-treatment X-rays, no residual bone lesions were observed after treatment.

Ozone is a gas naturally produced by atmospheric air; medical ozone is produced from oxygen. Its role in treating bone lesions has been previously reported [47]. Ozone has antimicrobical and wound-healing properties. The role of O_3 produced by air to treat ONJ has been evaluated in some pre-clinical and clinical studies because it was thought that O_3 could induce the repair of tissues by cleansing the osteonecrotic lesions, which leads to mucosal healing [47–53]. Ozone therapy has previously shown to enhance the benefits of surgical and pharmacologic treatments of ONJ when administered before and after treatment procedures [47,51,53].

The aim of this open-label, prospective study, was to investigate the efficacy and tolerability of medical O_3 gas (produced from pure oxygen and not from air) topical insufflations, as the treatment for ONJ lesions > 2.5 cm in patients treated with BPs whose ONJ lesion did not heal with prior conservative therapy or relapsed after surgery performed before the patients arrived to our hospital for the specific consultation and cure with the Dental Team and the Supportive Care in Cancer Team.

2. Patients and methods

2.1. Eligibility criteria

All adult patients with solid tumours and multiple myeloma on stable disease or patients with osteoporosis due to hormonal therapy, who previously received nitrogen-containing BP treatment in the absence of preventive screening carried out by a dentist and a dental care team and who developed stage two ONJ lesions [10,44], and had no benefit after the treatment with the standard therapy, were included in the study. The patients with lesions > 2.5 cm were considered for O_3 gas therapy after they gave the consensus.

No patient took part in the previous published study [47]. No patient with metastatic disease of the jaw or osteoradionecrosis or treated with radiotherapy to the jaws were included.

2.2. Efficacy criteria

The level of clinical response was: (1) bone sequestrum followed by spontaneous expulsion of the necrotic bone with re-epithelization of oral mucosa and with regenerated epithelial tissue or (2) bone sequestrum followed by surgery to remove necrotic bone.

2.3. Safety criteria

The treatment area was assessed for the presence or absence of oral mucosa redness around the lesion area, pain, progressive

increasing of lesion, appearance of necrotic area, pethechiae and/or bleeding. Moreover odour intolerance, burning mucosa, coughing due to local irritation of respiratory tract, dysphagia post treatment, symptoms related to skin sore or mucosal lesion was assessed.

2.3.1. Procedure of O_3 gas insufflation

All the procedures are performed by two experienced dentists of the Dental Care Team (MM, EC).

Although antibiotic therapy had been received frequently by the patients prior to study entry, all the patients were pre-treated with azithromicin 500 mg/day for 10 days prior to the initiation of the medical O_3 gas treatment to reduce the local essudation/infection caused by the exposition of necrotic bone, the dehiscence of the mucous membranes and the difficulty in maintaining proper oral hygiene.

The choice of azithromicin was dictated by the fact that the patients had previously been treated with various types of antibiotics other than azithromicin. Moreover, clinical experience has shown treatment with azitromicinale to be the best, fastest and most durable remissions of local suppuration. To date, after several years of treatment of patients with ONJ, we believe that the drug is more 'in the appropriate population of patients' which came to our attention.

Table 1 shows the procedures for O_3 insufflation after 10 days of antibiotic treatment.

Before each treatment the condition of the mucosa and the stability of the necrotic bone must be checked to evaluate whether or not it needs to be removed.

Each patient was treated for with a minimum of 10 applications of O_3 gas once every three days on each lesion area. When patients showed necrotic bone sequestrum and spontaneous expulsion with O_3 gas applications were stopped after few insufflations . In patients with plurifocal locations, with extensive injury (and for which a insufflation bell Fig. 1 was packed and used for each side application) or in the case of abundant purulent secretions, a higher number of O_3 gas applications were administered because the concentrations of O_3 initially interacted with the bacteria and purulent or essudative secretions. Each O_3 gas insufflation lasted for 10 min.

When necrotic bone was not spontaneously expelled even if the sequestrum was present, the patients were eligible for surgical resection of the necrotic bone, rotation of the mucosa lap, and surgical joining of the two edges.

2.3.2. Assessments

Efficacy and safety evaluations were performed after each O_3 gas application and during the follow-up period scheduled at one, two, three, and four months after the completion of the treatment and then every six months. In particular at the end of all O_3 gas insufflation the damage of mucous tissues has been evaluated.

Moreover, pain intensity was assessed at each visit by means of a self-reported numerical rating scale (NRS). The treatment was stopped when patients showed clinical response (spontaneous expulsion of necrotic bone or surgery to remove the sequestrum) or undesirable adverse effects or when Performance Status worsened due to disease progression.

2.3.3. Study design and statistical considerations

This was a single-centre, open-label, Simon two-stage optimal design study [54]. The primary efficacy end point of the study was the clinical response, as previously defined. During stage one, an enrolment of nine patients was required. If no response was observed, then the study had to be terminated. If at least one response was observed, the trial could continue to stage two and an additional 15 patients enroled. After completion of the second stage of the study, the treatment would be considered worthy of further investigation if at least three responses were observed. The study design yielded a $>90\%$ probability of a positive result if the true response rate was $>25\%$, and $>90\%$ probability of a negative result if the true response rate was $<5\%$. The study design incorporated monitoring of treatment associated toxicity, with a Bayesian stopping rule in case of a 90% posterior probability of a toxicity rate greater than 10%. The response rate was computed as the percentage of responding patients over the total number of patients accrued. Calculation of the corresponding 95% confidence interval (95% CI) was based on the binomial distribution.

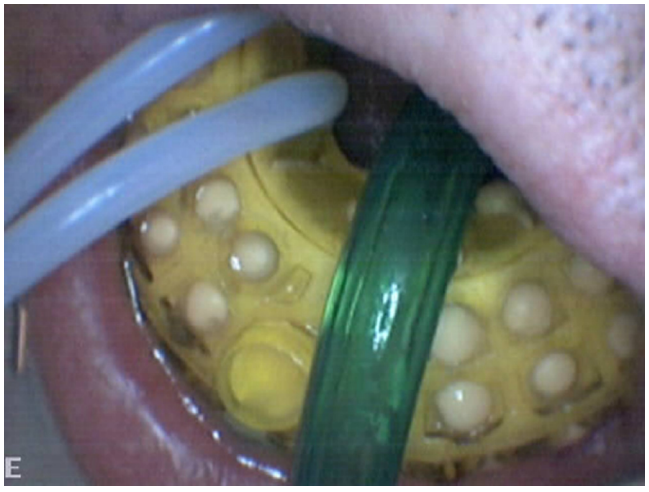

Fig. 1. Bell used during insufflation on the ONJ lesion to avoid O_3 gas diffusion.

Table 1
Procedures performed to administer O_3 gas.

- The exposed bone and osteomucosal edge were cleaned with a tartar supersonic scaler in order to reduce the infections at gum level and favour the penetration of O_3 gas through the mucosa around the ONJ lesion.
- An impression of the affected arch was taken using a perforated impression tray and addition silicone impression material.
- Once the impression has been removed from the mouth, the marginal seal of the impression material on the healthy gum around the lesion was checked; if necessary a demographic pencil was used to make ONJ lesion reference points on the impression material.
- Having identified the area corresponding to the lesion site, the "insufflations chamber" was prepared, creating a hollow in the impression by carefully removing the impression material to obtain a space in which the gas to be insufflate onto the treatment area can circulate (Fig. 1).
- We make calibrated holes in the perforated impression tray, at the proximal and distal margins of the lesion, for the tubes delivering the ozone gas to pass through.
- Once in position, we checked the fit of the "insufflations chamber", by checking the stability and seal on the marginal mucosa.
- The tubes were then connected to the ozone dispensing device, along with the return pipe that, using an aspiration pump, allows a perfect fit on the mucosa at the chamber's seal margin, thus making it possible to recover any ozone present after contact that is not transformed into oxygen inside the chamber.
- Insufflations, were monitored constantly by the practitioner who can, when necessary, use a flow-metre to adjust the amount of ozone applied, which must be kept constant at 20 ppm $+/-1$.
- Flow control was made possible by constant monitoring of the ozone produced thanks to the presence in the device of a mass spectrophotometer that allows real-time concentration control.
- At the end of the treatment, the insufflations chamber was removed from the mouth, then washed and disinfected by emersion in sterilising product for subsequent use.

3. Results

The patients' demographics, baseline disease characteristics and outcomes with medical O_3 gas therapy are shown in Table 2. The patients received a number of zoledronic acid infusions ranging from 10 to 18. At the time of ONJ diagnosis no patient presented risk factors such as diabetes or the use of corticosteroids.

Twenty-four patients (mean age 62.5, range 41–80, 12 female) with bone metastases due to breast (11), prostate (4)and lung (4)cancers, myeloma (2), or osteoporosis (3), and with ONJ lesions >2.5 cm previously treated with zoledronic acid, were enroled in the study.

No patient underwent to preventive dental screening before starting BPs infusions. All patients received various cycles of antibiotic therapies after diagnosis of ONJ until the initiation of O_3 treatment without any clinical or radiological evidence of ONJ healing. Two patients underwent hyperbaric oxygen therapy without ONJ healing.

At the time of this investigation, no patient reported spontaneous ONJ healing. Three patients (number 5, 10 and 14) presented with relapsing ONJ after surgical therapy performed to cure ONJ in other hospitals (Table 2).

ONJ was diagnosed by an experienced maxillofacial dentist on the basis of the following criteria: presence of exposed bone in the maxillofacial region with no evidence of healing after six weeks of appropriate dental care [10]and in some cases of CT evaluation.

According to Weitzman classification [43] 15 patients had a single ONJ lesion (4A) and 9 patients had multiple lesions (4B) or a "multifocal" lesion (Table 2).

Table 2
Patients' demographics, baseline disease characteristics and outcomes with medical O_3 gas therapy.

Patient	Age	Gender	Primary cancer or osteoporosis	ONJ lesion size grading[a]	Time (days) from diagnosis of ONJ to first O_3 gas insufflations	Number of O_3 gas insufflations[b]	Clinical outcomes
1	73	M	Prostate	4A	1080	Three applications than interruption	Dropped-outstop of O_3 therapy due to ODP[c].
2	53	M	Lung	4A	240	10	Surgery to remove NB after sequestrum. No ONJ relapse.
3	70	F	Breast	4A	540	7	NB sequestrum+spontaneous expulsion+ re-epithelisation. No ONJ relapse.
4	74	F	Breast	4A	30	4	NB sequestrum+spontaneous expulsion+ re-epithelisation. No ONJ relapse.
5	41	M	Osteoporosis	4A	840 ONJ relapse in area 44–48 after surgery	38 on single lesion	Surgery to remove NB after sequestrum. No ONJ relapse.
6	64	M	Prostate	4B	510	17 applications than interruption	Dropped-out, stop of O_3 therapy due to ODP.
7	65	M	Myeloma	4A	30	9	NB sequestrum+spontaneous expulsion+ re-epithelisation. No ONJ relapse.
8[d]	60	M	Prostate	4A	90	12 bone sequestrum ready to be removed surgically	No surgical removal of NB because of ODP.
9	64	M	Myeloma	4B	570	17	Surgery to remove NB after sequestrum. No ONJ relapse.
10	49	F	Breast	4A	390 ONJ relapse after partial resection of the left maxillary not responsive to antibiotics	16	Surgery to remove NB after sequestrum. No ONJ relapse after O_3 gas applications.
11	79	M	Lung	4B	270	15+12 multiple lesions on sx and dx sites	NB sequestrum and spontaneous expulsion+mucosal re-epithelisation at all sites. No ONJ relapse nor decubitus after denture placement in the inferior arch.
12	80	F	Breast	4B	720	Two applications than interruption	Dropped-out stop of O_3 therapy due to ODP.
13	67	F	Breast	4B	420	10+9 multiple lesions on sx and dx sites.	Surgery to remove NB after sequestrum. No ONJ relapse.
14	66	F	Osteoporosis	4A	120	One application than interruption.	Dropped-out. Stop of therapy for fear of adverse effects.
15	79	M	Breast	4A	420	9	NB sequestrum and spontaneous partial expulsion+ re-epithelisation. Stop of therapy for ODP.
16	51	F	Breast	4B	300	15 applications than interruption.	dropped-out stop of therapy for ODP.
17	47	F	Breast	4A	90	Six applications than interruption.	Dropped-out stop of O_3 therapy due to ODP.
18	63	M	Lung	4A	44	6	NB sequestrum+spontaneous expulsion+ re-epithelisation. No ONJ relapse.
19	77	M	Prostate	4A	82	10	Surgery to remove NB after sequestrum. No ONJ relapse.
20	60	F	Osteoporosis	4B	476	16	Surgery to remove NB after sequestrum. No ONJ relapse.
21	58	F	Breast	4B	360	15	Surgery to remove NB after sequestrum No ONJ relapse.
22	43	M	Lung	4A	286	8	Surgery to remove NB after sequestrum. No ONJ relapse.
23	62	F	Breast	4B	375	9	Surgery to remove NB after sequestrum. No ONJ relapse.
24	55	F	Breast	4A	266	10	Surgery to remove NB after sequestrum. No ONJ relapse.

NB=Necrotic bone.

[a] Classification according to Weitzman et al. Ref. [43]:lesion size measured as the largest diameter 4A=single lesion >2 cm; 4B multiple lesions, largest >2 cm.

[b] The patients who received more than 10 insufflations of O_3 medical gas had multiple lesions or single lesion but copious purulent discarge even if treated with antibiotic prophylaxis.

[c] ODP oncological disease progression.

[d] The patient received two more insufflations of O_3 medical gas while he was waiting for the surgery.

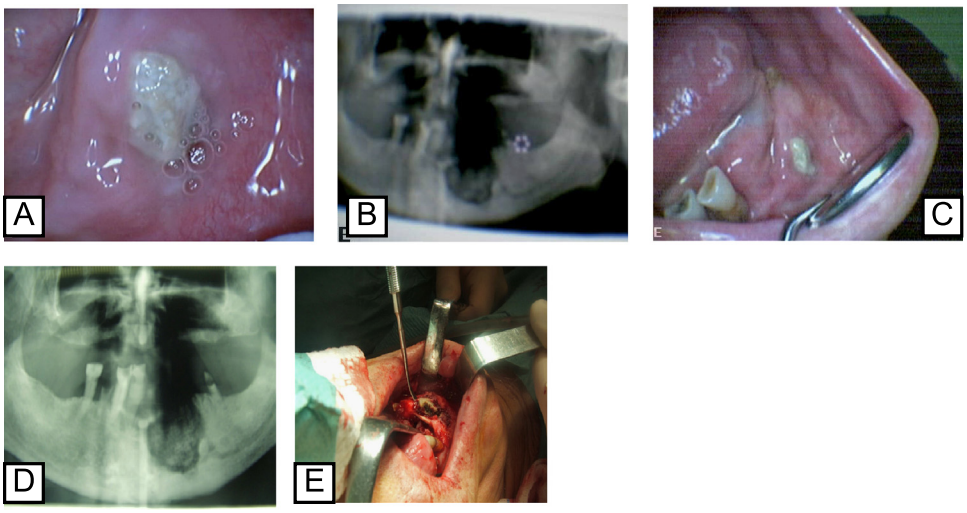

Fig. 2. ONJ before starting first O_3 gas insufflation, necrotic area produced after 10 insufflations and during surgery for the removal of the necrotic bone sequestrum area (patient no. 19): (A) ONJ lesion before the first O_3 gas insufflations, (B) X-ray before the first O_3 gas insufflations, (C) necrotic area post O_3 gas insufflations, (D) X-ray post insufflations and (E) Surgical removal of necrotic bone with periosteum dissector (no need to use devices to cut bone walls).

Six patients interrupted the treatment with O_3 gas application for disease progression (five patients) and for fear of an experimental therapy (one patient). The drop-outs were considered as failure in the Intention to treat (ITT) analysis, and excluded, in the per-protocol (PP) analysis.

No patients reported adverse events during O_3 insufflations and the days after; moreover no objective or subjective symptoms of intolerance to the O_3 gas applications were observed.

In 10 patients with the largest and deeper ONJ lesions, O_3 gas therapy produced the sequestrum of the necrotic bone. Nine patients required more than ten O_3 gas applications due to multiple lesions and/or abundant purulent secretion notwithstanding the concomitant antibiotic therapy. In all these patients surgery was necessary to remove the necrotic bone after sequestrum. Of interest, surgical removal was possible without the resection of healthy mandible edge because of the presence of bone sequestrum. One patient (number 8) received two further insufflations while waiting for the surgery because he had bone sequestrum ready to be removed surgically; however because of sudden disease progression and Performance Status worsening, he was not operated on.

Fig. 2 shows the ONJ lesion before starting O_3 gas insufflations (picture A), the X-ray before the first therapy with O_3 (it is possible to see the extent of necrotic lesion and the involvement of the loop of the mandibular nerve) (B), and the necrotic area post 10 gas insufflations, with the perfect soft tissue tropism, cannot be evaluated in the picture and the mobility of the necrotic area, easily detectable clinically (C).

The perfect condition of the soft tissues allows an extremely conservative treatment, with a perfect mucoperiosteal seal, once the necrotic bone has been removed. This is possible due to the paradoxical effect of ozone, which, being an oxidant, in the cell membranes of complex organisms, triggers a reparatory phase that leads to the healing of mucous tissues that had been overturned by the damage caused by the presence of necrotic tissue and the consequent bacterial and fungal superinfections produced in the lesion site.

Fig. 2 also shows the post-insufflation X-ray (D), where the complete demarcation of the area of necrotic bone and its particular nearness to the emergence of the mandibular nerve can be observed. In this case, the nerve is not at all involved in removal and the patient will not suffer any loss of sensitivity or paraesthesia. The photograph of removal of the necrotic fragment (E) in the operating theatre can also be seen. After the elevation of the mucoperiosteal, vestibular and lingual flaps, the necrotic fragment can be more easily removed using a periosteal elevator, without having to use tools for cutting bone walls. This allows special softness during the procedure, without causing any surgical traumas to the bone treated with bisphosphonates.

During the surgical phase, it is easy to lift the mucoperiosteal tissue, which is completely healthy and free of fibrosis and with excellent cleavage planes, contrary to what one would expect of an area that for months has harboured infections associated with purulent serous secretion (the paradoxical repair effect generated by ozone).

Six patients had complete sequestrum or partial (one patient) spontaneous expulsion of the necrotic bone with oral mucosa re-epithelisation, after 4 to 27 insufflations of O_3 gas.

Fig. 3 shows the patient number 3 before starting O_3 gas insufflations, the necrotic bone removed by a pinch and re-epithelizated area after the O_3 gas therapy. In the area 47 (A), it is possible to observe the infectious inflammatory damage to the soft tissues, despite the fact that the necrotic lesion is slightly infiltrating just below the mucosal margin. Picture (C) shows the mobile necrotic bone removed without anaesthesia during the seventh application, simply using an anatomical forceps in an outpatient setting. Insufflation treatment was performed as usual after removal. Picture (D) shows area 34 immediately after the removal of the necrotic bone in an outpatient setting. It is worth noting that the mucosal surface is completely different from the condition in regard with both colour and continuity. The area does not present any signs of bleeding as experienced in other cases, the mucous membrane has formed again between the healthy bone and the necrotic area, leading to the expulsion of the sequestrum.

No ONJ relapse was observed in any of the 18 patients treated with O_3 gas $\pm$ surgery, during a mean of follow-up of 18 months (range 1–3 years). After completion of the treatment, all patients were able to eat normally and prothesic dentures were adapted and re-positioned for those patients who had dentures. *The patients restarted the therapy only if their Performance Karnosfsky Status was >70; the dental team visited them every 6 months.*

All together, the response rate was 75% (95% CI, 53.3–90.2%) in the ITT analysis, and 100% (95% CI, 81.5–100%) in the per-protocol (PP) analysis.

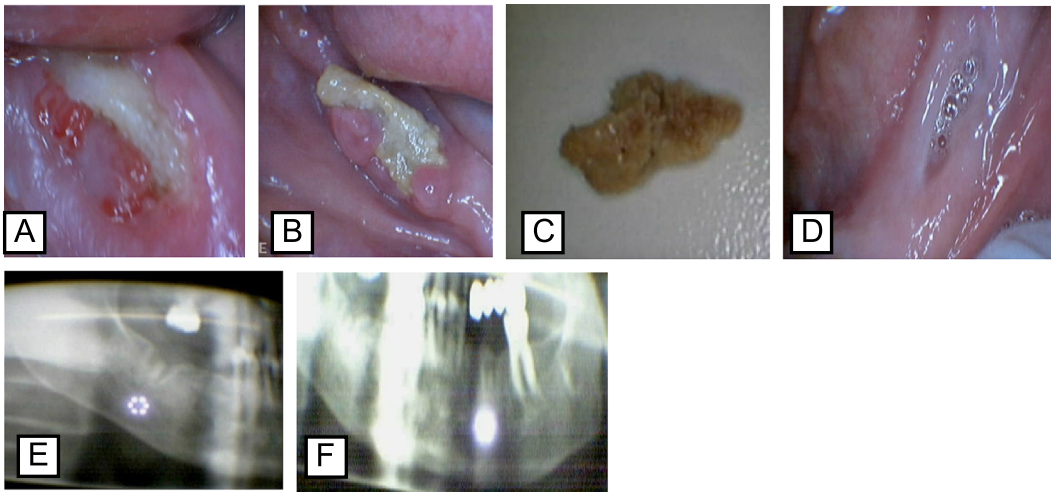

Fig. 3. ONJ before starting first O_3 gas insufflation, necrotic bone expulsed with the help of a pinch and re-epithelization (patient no. 3): (A) initial lesion (area 47), (B) post avulsion necrotic area (position 34), inflammatory processes infiltrating the mucosae, (C) ambulatory mobile necrotic bone removal without anestesia at the seventh application exclusively with an anatomic forcep; usual insufflation treatment has been followed after the drawing, (D) area 34 immediately after the ambulatory removal of the necrotic bone, (E) X-ray shows the poorly outlined necrotic area around the alveolus of 36 after the avulsion, and (F) X-ray shows a necrotic area in position 34.

4. Discussion

Unfortunately it was not possible to perfom a RCT comparing medical ozone gas therapy in respect to standard therapy because all our 24 patients had been previously treated with various cycles of antibiotic therapies before we could start the therapy with O_3 gas. Moreover the patients needed to wait for a long time (Table 2) the approval of Ministry of Health in using medical O_3 gas with the new indication for the treatment of patients with ONJ related to bisphosphonate treatments.

With the data on the role of preventive measure in reducing the risk of ONJ in patients treated with BPs [1–7,14,44–46]and the efficacy and tolerability of ozone gas [51–53]and medical O_3 oil in treating small ONJ lesions [47], we have another therapeutical option for treating ONJ lesions when they are >2.5 cm.

Our study shows that the topical insufflation of ozone medical gas on ONJ lesion >2.5 cm is able to produce a sequestrum of the necrotic bone with 1. its spontaneous or not traumatic explusion and the healing of the gum mucosa followed by re-epithelization 2. or surgical removal of the necrotic bone and healing of the gum mucosa. This is an effective and original method, in the absence of observable toxicity. Thus medical O_3 gas insufflations appears to be an effective and safe treatment for patients treated with BPs who developed ONJ lesions >2.5 cm.

Three are the most important results of our study and the result produced:

1. Six patients reached the sequestrum and had a spontaneous expulsion of the necrotic bone in respect to the 10/10 patients with ONJ ≤ 2.5 cm and treated with topical application of an oil suspension enriched with medical O_3 gas [47],
2. when surgical removal of the necrotic bone after sequestrum was necessary, the surgical resection of healthy mandible edge was not necessary because of the presence of the sequestrum,
3. no ONJ relapse appeared in our patients visited every six months and after a mean follow -up of 18 months (range 1–3 years).

Curiously, two patients with osteoporosis who completed the trial, requested the highest number of O_3 gas insufflations and this fact needs further investigation.

In conclusion, the preliminary results of this study demonstrate that medical O_3 delivered as topical gas insufflation can be considered a promising, effective, safe and simple therapeutic option for the treatment of ONJ lesions >2.5 cm.

- Emerging promising therapeutic options such as O_3 gas investigated in this study indicate that ONJ can be treated, allowing patients to recover and heal from this debilitating condition.
- These results add to the evidence that cancer patients and clinicians should weigh considerable benefits of BPs toward management of skeletal health as opposed to the possible risks posed by ONJ, an uncommon condition that may be prevented and managed.
- These results should be considered as preliminary and further investigations in a larger sample trial are required to validate findings.

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