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PII: S2468-7855(22)00158-6
DOI: https://doi.org/10.1016/j.jormas.2022.05.023
Reference: JORMAS 1208

To appear in: *Journal of Stomatology oral and Maxillofacial Surgery*

Received date: 12 October 2021
Accepted date: 27 May 2022

Please cite this article as: Arnal Etienne Andrea , van Maanen Aline , Van Eeckhout Pascal , Magremanne Michèle , Actinomycosis and osteonecrosis of the jaw: every why hides a why., *Journal of Stomatology oral and Maxillofacial Surgery* (2022), doi: https://doi.org/10.1016/j.jormas.2022.05.023

Original Article

Actinomycosis and osteonecrosis of the jaw: every why hides a why.

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Introduction

Osteonecrosis of the jaws (ONJ) is a chronic and challenging disease for most clinicians. Two types of ONJ are described with different etiologies and pathophysiological mechanisms: osteoradionecrosis (ORN) and Medication-Related Osteonecrosis of the Jaws (MRONJ).

The AAOMS described MRONJ as a combination of 3 features :

- Bone exposure or intra/extra oral probed fistula in the maxillofacial region for more than 8 weeks
- Together with current or previous anti-resorptive or anti-angiogenic drugs,
- Without any history of radiotherapy or metastases[1].

MRONJ is typically limited to the maxillofacial region, probably due to its membranous osseous origin and mandible is known to be twice more affected by MRONJ than the maxilla [2-3].

Even if the exact physiopathology of MRONJ remains unclear, two main theories comes into sight :

- The “Inside-out” theory suggests that anti-resorptive and anti-angiogenic agents would stop osteoclastic activity and neo-angiogenesis, causing inhibition of bone remodeling with secondary local infection. Mucosal involvement and bone exposure occur lately.
- The “outside-in” theory would argue that natural healing of oral mucosa is altered by toxic concentrations of BPs and anti-angiogenic agents, leading to subsequent primary microbial invasion and bone necrosis[4-5].

Osteoradionecrosis is a chronic disease defined by 3 characteristics:

- A persistent bone exposure for more than 3 months in an irradiated area,
- Without tumor recurrence, occurring mainly in the first three years after irradiation,
- Spontaneously or following a trauma of the bone, such as dental extraction[6].

ORN is proportional to radiation doses and mostly occur when doses exceed 60 Gy.

Radiation-induced fibrosis is the latest and most accepted etiopathogenic theory introduced by Delanian *et al* in 2004. Three distinct histopathological phases are described and interesting for this study:

1. **The first initial pre-fibrotic phase** with chronic nonspecific inflammatory response and changes in endothelial cells predominate. This inflammation is characterized by increased vascular permeability and oedema formation, and induce destruction of endothelial cells associated with vascular thrombosis and local ischemia.
2. **A constitutive organized phase** with abnormal fibroblastic activity in consequence of endothelial destruction and subconsequent exposure of connective tissue cells. This radiation induced fibrosis phase is composed of a patchwork of senescent fibroblasts (fibrocytes) and activated fibroblasts (myofibroblasts).
3. **A late fibroatrophic phase** with dense extracellular matrix, fibroatrophy and poor vascularization.

Activation and dysregulation of fibroblasts into myofibroblasts are the key event that leads to atrophic tissue within a previously irradiated area[7]. Fibrosis and chronic inflammation cause obliteration and hyalinization of arterial vessels which leads to a late microbacterial colonization[8].

Actinomyces is a Gram positive, filamentous, facultative and anaerobic bacteria that exists in the normal flora of oral cavity, gastrointestinal tract and female genital tract. It belongs to *Actinomycetaceae*, *Propionibacteriaceae* and *Bifidobacteriacea* species with *Actinomyces israelii*, *Actinomyces rencseriae* and *Actinomyces naeslundii / viscosus* representing more than 75% of them. However, the most common agent found into the oral cavity is *Actinomyces israelii*. *Actinomyces* infection causes on the head and neck region a suppurative and granulomatous infectious reaction with abscess formation, tissue fibrosis, and occasionally a soft tissue tumor-like mass. It usually follows a dental disease or a jaw osteomyelitis commonly called the "lumpy jaw syndrome"[9].

Histopathological examination shows sulfur granules in about 75% of *Actinomyces* infection that are generally seen macroscopically as yellowish little dots. These sulfur granules are constituted by conglomeration of bacteria trapped into a biofilm of 0.1 to 1mm in diameter. They bind together into a rosette form ("sun ray"), and are stabilized by a protein-polysaccharide complex to provide a resistance to host defenses by inhibiting phagocytosis[10]. Grocott-Gomori's Methenamine Silver (GMS) staining is also useful for demonstrating the filaments, which are not stained by the Hematoxylin and Eosin (HE), Periodic Acid Schiff (PAS) and Gram stain.

In the light of this knowledge, the aim of this study was to compare demographic and histological features between MRONJ and ORN patients.

Material and methods

A retrospective study was conducted from May 2015 to July 2020 in the Department Oral and Maxillofacial Surgery and the department of Pathology, Cliniques Universitaires Saint-Luc, Brussels, Belgium. All patients with samples of osteonecrosis in relation to radiotherapy and/or anti-resorptive drugs were included. The samples were examined by two head and neck pathologists and by a maxillofacial surgeon. Only microscopic analysis was used for the study.

Several data were collected such as systemic, local and demographic factors, previous radiation therapy, along with indication, time and type of anti-resorptive drugs. Local risk factors in past medical history were recorded (extractions, wearing prostheses, periodontal diseases), together with ongoing antibiotic treatments, patient's addictions (alcohol, tobacco), corticosteroid intake, and presence of type 2 diabetes.

Four types of bone biopsies were selected: curettages, bone spicules (< 5 mm), sequestras (> 5mm) and surgical bone resection. Samples were cut into 4 microns slices, embedded into formaldehyde, with a decalcification time ranging from 24 to 72 hours. Pathological examination was achieved with a Zeiss microscope using mostly 2X, 4X, 10X and 40X magnification. The analysis of *Actinomyces* and its biofilm were observed using a routine HE stain (Fig. 1). Other specific stains were used for the detection of *Actinomyces spp* when necessary. PAS colored into magenta *Actinomyces* colonies and filaments (Fig. 2). GMS is a chromic acid- sodium bisulfate stain that precipitates silver ions into polysaccharide walls of micro-organisms, producing a characteristic grey-black color on light microscopy (Fig. 3). This stain is useful to recognize fungal organisms such as *Candida spp*, or *Actinomyces* filaments. Eventually, Gram stain was used to help differentiate gram positive from gram negative germs, as gram positive bacteria were colored into a dark blue color on light microscopy (Fig. 4)[11].

Considering the stain used, *Actinomyces spp*, or other form of bacteria (biofilm) were sought in depth and on surface of samples (MRONJ and ORN), together with bone cells (osteoblasts, osteocytes, osteoclasts) and inflammatory cell reaction. One of the key histological differences between acute and chronic inflammation is seen in the sets of leukocytes that are present in the tissues. In acute inflammation polymorphonuclear neutrophils usually predominate, whereas macrophages and lymphocytes predominate in chronic inflammation. Other pathological features were examined such as bone architecture (mothed-like or lamellar), vessels aspect (normal or hyalinized) and pseudo-epithelial hyperplasia. Sulfur granules and the so called "sun-ray effect" are common pathognomonic features encounter in *Actinomyces* primary infections [12]

Study data was collected using Excel (*version 16.45)*. Continuous data was summarized using descriptive statistics (mean, standard deviation (SD), median). Categorical

data was summarized using frequencies and percentages. Group comparisons were obtained using Fisher Exact test for categorical data and Mann-Whitney U-test for continuous data. A Bonferroni-correction was applied to account for multiplicity. In all analyses, p-values were 2-tailed, and p-values < 0.05 were considered statistically significant. Analysis was performed using SAS software (version 9.4 of later; SAS Institute Inc, Cary, NC, USA).

Results

Population study : Table 1

Eighty-nine patients were included in the study with 107 bone specimens, some patients having biopsies at different stages of the disease. Were excluded all patients without bone in the sample. Two groups were distinguished: a first group with ORN lesions (13 patients, 20 bone specimens), and a second with MRONJ lesions (76 patients, 87 bone specimens).

The MRONJ group was separated into 2 subgroups: A first group was named “MRONJ K” (33 patients) for patients treated for cancer, the second was called “MRONJ OP” (43 patients) for patients treated for osteoporosis.

The samples observed were mainly characterized by sequesters at 57,9% (62/107), followed by bone spicules at 19,6% (21/107), 18,8% of curettage (14/107) and 3,7% of mandibular resections (4/107).

1. **Demographic factors :**

Overall, the median **age** was 70 years old (28-96). The male to female ratio was of 1:1,25 (40 males and 49 females) and the age was significantly higher in the group MRONJ OP versus ORN group (p=0,005).

There was a significant relationship between **gender** disparities, 76.9% of the ORN patients being men versus 21,2 % in the MRONJ OP group (p=0.015). In the MRONJ group, MRONJ OP has a higher percentage of female (78,8%) than MRONJ K group 46,5%), p=0,004.

2. **Systemic risk factors :**

Alcohol abuse was 5 times higher into the ORN group than into the MRONJ K group, but results were no significant (p=0.064). Tobacco consumption was twice superior into the ORN group compared to MRONJ OP and MRONJ K, without significant values.

No significant differences were found for the other factors (**diabetes** and **steroid intake**).

3. **Local risk factors :**

Periodontal disease was found in 58.4% (52/89) of the patients, but no clear differences were found between MRONJ and ORN groups.

Prosthetic cause was found to be a local risk factor by causing irritation, injuries and disrupture of oral mucosal when it was poorly adapted, especially for removable prosthesis. In total, 22.5% (20/89) of the patients developed an ONJ with a badly adapted denture. There was no statistical difference between ORN and MRONJ OP or MRONJ K groups.

Extractions : Tooth extraction was found to be one of the major precipitating factor for MRONJ and ORN development (67,4% in total). However, none of the comparisons showed significant results or even trends that could be interpretable in the study.

Histological study : Table 2

1. **Micro-organisms :**
   - ***Actinomyces*** was found in 95.3% (102/107) of the samples, without statistical differences between MRONJ and ORN samples (p = 0.074). In comparison to MRONJ biopsies, *Actinomyces* was further found into the surface of ORN samples (25.0%, 5/20) versus 8,0% (7/87) for MRONJ, but without statistical differences between the two groups. The presence of aggregates of *Actinomyces* surrounding the bone was found in 54.2% of all samples (MRONJ and ORN) without a significant difference between the groups. Unfortunately no statistical analysis could be done for the different type of samples (bone spicules, curettages, sequestras and mandibular resections) between MRONJ and ORN patients. This could be a potential bias, as the number of biopsies in each samples type was too low for a valid statistical analysis.

   - **Bacterial biofilm** was identified by Gram stain but histological study lacked to give a precise germ identification. Biofilm was found in 47.7% (51/107) with no significant difference between depth and surface of MRONJ and ORN bone samples. Although PCR technique could have been a better option to highlight biofilm behavior towards *Actinomyces* and inflammation, it was not a technique that could suits to histologic characterization of the samples.

2. **Inflammation :**
   Inflammation acts as an indirect infection type (primary or secondary infection).

- Chronic inflammation was significant into ORN group with 50.0%, (10/20) presenting chronic inflammation at the surface (p=0.018), compared to the MRONJ group.
- Acute inflammation was not a significant feature into MRONJ and ORN samples, but more than half of acute inflammation (51.7%; 45/87) was set at the surface of MRONJ samples. Another quarter (25.3%; 22/87), was present in both surface and depth of MRONJ biopsies whereas half (50,0%; 10/20) ORN samples had none acute inflammation reaction.

Although, the same findings were found : statistical analysis couldn't be done between the different samples types for ORN and MRONJ group, as the number of samples in each group was too low.

3. **Living cells and microscopic aspect :**
   - Very few **osteoclasts** were found into MRONJ and ORN samples (9.3% ,10/107), with no differences at all between the two groups. **Osteocytes** were observed in 13.1% (14/107), but were mainly found into MRONJ in comparison with ORN's (14.9%; 13/87 for MRONJ and 5.0%; 1/20 for ORN). However, no statistical differences were found between ORN and MRONJ.
   - Hyperemia was found in 44.9% (48/107) of all samples (ORN and MRONJ), with a small predominance in the MRONJ group with 43.8%(42/87) vs 30.0 % (6/20) for ORN samples. However, no significant differences were found between the groups.
   - Pseudoepithelial hyperplasia was found in 22.4%, (24/107) without statistical difference between ORN and MRONJ biopsies. Interestingly a small predominance was observed into the ORN group compared to MRONJ (30.0% for ORN vs 20.7% or MRONJ). These numbers come into conflict with the literature, but might be consequence of the small ORN sampling (20 samples) in comparison to MRONJ (87 samples).
   - Finally, microscopic lecture showed a lamellar-like appearance into the ORN group (55.0% ;11/20), while MRONJ samples revealed a moth-eaten aspect (69.0% ;60/87), however without any significant differences between MRONJ and ORN.

Overall, no statistical data could be done in between the different samples type for ORN and MRONJ group, as the number of biopsies in each subgroups were too low.

Discussion

The aim of this study was to compare histological and demographical findings between ORN and MRONJ specimens. Although distinct pathophysiology exists between MRONJ and ORN, we found little significant demographical or histological disparities. These results could be linked to the fact that we had less ORN specimens (20 samples) than MRONJ (87 samples) with numerous small samples compared to large specimens. Comparing the three patients groups (ORN, MRONJ OP and MRONJ K), only age and gender seemed to have significant values regarding demographical features, linked to the fact than ORN population was much lower (13 patients) than MRONJ OP (33 patients) and MRONJ K (43 patients).

Considering histological features, *Actinomyces* was widely found into MRONJ and ORN samples (95.3%), without any significant difference between the two groups. De Ceulaer et al described similar results ranging from 72 to 100%, but only for MRONJ specimens[12]. Biofilm examination was limited by microscopical research, skewed by the overpresence of *Actinomyces*, essentially colored with HE stain. More living cells and osteocytes were found into MRONJ samples, and *Actinomyces* was rather located both at the surface and at depth of MRONJ group, with acute inflammation embedding the surface of bone. Kaplan *et al* described the same features with acute inflammatory cells surrounding bone sequesters and Actinomyces colonies, with some bone cells such as osteocytes and osteoclasts living nearby[13]. These observations suggest there might be bacterial front reaching the bone core ahead an acute inflammatory reaction supporting the "outside-in" theory knowing that *Actinomyces* can adhere to bone and mucosal through small adhesion proteins called "Microbial Surface Components Recognizing Adhesive Molecule Molecules" (MSCRAMM), and link with other virulent strains[14, 15]. Direct bone adhesion was found between gram negative bacteria (such as Actinomyces) and the amino group of anti-resorptive drugs when a rupture of mucosal barrier happens [16, 17]. This initial stage of mucosal disruption could correspond histologically to the pseudo-epithelial hyperplasia described by Hansen and al [8]. However, in this study, no statistical differences were found in between MRONJ and ORN groups for this feature.

Chronic inflammation into the surface of ORN biopsies was the only histological feature found to be significant (p=0.018). Some trends showed a rather macroscopic "lamellar" aspect of the bone whereas MRONJ holds a rather "moten-like" bone feature. Although no statistical difference was found, it is interesting to note that similar observations were described by Russmueller *et al* regarding ORN, showing hyalinized vessels due to fibrosis as well as a regular or "lamellar" appearance of the bone, invaded by chronic inflammation and colonized on the surface by microorganisms such as *Actinomyces spp* [18]. Much over, Konstantinos *et al* detected an overexpression of collagen type I, with predominant chronic inflammation and fibrosis into ORN specimens [19]. Even if these findings could match the current view of the radiation-induced fibrosis theory for ORN, some authors found *Actinomyces spp* and other bacterias into the depth of bone, rejecting

the hypothesis of a superficial colonization[20]. In the other hand, bacterial infection in ORN might be one of the reasons why Meyer's theory became the foundation for the popular use of antibiotics before and after surgery in an irradiated area[21-22].

Unlike the rest of the body, low mucoperiostal coverage of oral mucosa, common local pathological conditions (periodontal disease, decay, periapical diseases, etc.), or surgical procedures (dental extraction), can trigger microorganisms as *Actinomyces* to start bone invasion[23]. Interestingly, some authors found with molecular sequencing that oral flora from MRONJ patients hold different patterns than oral flora from healthy patients[24] . The top three bacteria ranked among the MRONJ group were Streptococcus (29%), Eubacterium (9%), and Pseudoramibacter (8%), while in the control group were Parvimonas (17%), Streptococcus (15%), and Fusobacterium (15%)[25].

Finally, the study tried to look into the different types of samples (curettages, bone spicules, sequestras and mandibular resections), to analyze their distribution and their difference in between ORN and MRONJ group. But no statistics could be done as the number of samples was too low. This is one of the major bias we found in the study. More than half of the samples were sequestras (57,9%) in comparison with mandibular resections (3,7%). Distribution of *Actinomyces* and inflammation features (surface, depth and surface, depth) into MRONJ and ORN samples could not be compared, and one could think that our results could merely show a secondary microorganism colonization of the bone.

With the aim of eliminating bacterial biofilm, Aghaloo *et al*, recently implemented a rigorous protocol by performing daily mechanical debridement of exposed bone in patients with MRONJ using a Chlorhexidine soaked brushing (CHX) 0.2%. Mechanical debridement is a crucial step to mess up biofilm and remove necrotic debris in order to achieve antibiotic and antiseptic penetration into the bone. Complete epithelialization of the mucous membrane was observed at 71% in stages 1 and 2, and even in stages 3, when patients were not good candidates for surgery[26]. Hydrogen peroxide is also known to be a molecule naturally present in the body, playing a role into cellular signaling, inflammation, and innate immune response. It is particularly active against Gram-positive bacteria at 3% concentration and is able to act in synergy with other antiseptics, in order to potentiate their bactericidal effect [27].

Indeed, chlorhexidine appears to be the most widely used antiseptic in dentistry, in comparison with iodine povidone, due to its cationic nature that binds naturally onto dental surfaces, oral mucosa and hydroxyapatite[28, 29]. It acts as a bactericidal and fungicide agent at 0.12% to 0.2% concentration and acts faster than iodine povidone in the oral cavity, with an upper remanence ranging from 4 to 5 hours after the first mouthwash[30].

With these observations, surgical debridement combined with local treatments such as hydrogen peroxide and CHX, could disrupt bacterial biofilm and allow the action of antibiotics on planktonic bacteria, before they stick to the necrotic bone. This is one of the major explanation why antibioprophylaxis prevents the adhesion and co-aggregation of bacterias between them[31]. Most microorganisms isolated in MRONJ and ORN and particularly *Actinomyces* are sensitive to penicillin. These drugs should be administered over a long period of months to more than a year, and various studies highlight the improvement of symptoms with prolonged penicillin antibiotic therapy, when *Actinomyces* was found, with a direct correlation with??clinical evolution[13]. No consensus on antibiotic therapy has been currently established, but a recent review shows that the mean mandibular bone concentration exceeds the MIC90 for doxycycline, penicillin, and clindamycin, but not for metronidazole[32]. Long-term antibiotic therapy and antiseptics could be a therapeutic avenue for sustainably treating ORN and MRONJ as is already the case for chronic *Actinomyces* infection.

Conclusion :

Actinomyces spp was found in almost all of the samples of MRONJ and ORN (95,3%), and could be a trigger factor for ONJ. As so, controlling *Actinomyces* and its biofilm with regular surgical debridement combined with local antiseptics and long-term antibiotics, would be an effective treatment option for the management of osteonecrosis. Although ORN and MRONJ hold different pathophysiological mechanisms, poor statistical differences were found in the study. Age and gender hold significant values, with more women in the MRONJ OP group in comparison with ORN and MRONJ K patients. These findings are linked to the fact that more women suffer from osteoporosis than men. Regarding histological features, only chronic inflammation was found to be significative for ORN specimens in comparison with MRONJ. These findings support the radio-induced fibrosis ORN theory described by Delanian *and al*. The weakness of this study is the little number of ORN patients and specimens compared to MRONJ, as for pieces of mandibular resections that were much lower in comparison with curettages, bone spicules and sequestras.

Ethical approval The study was carried out with approval of the Ethics Committee (2018/21FEV/074).

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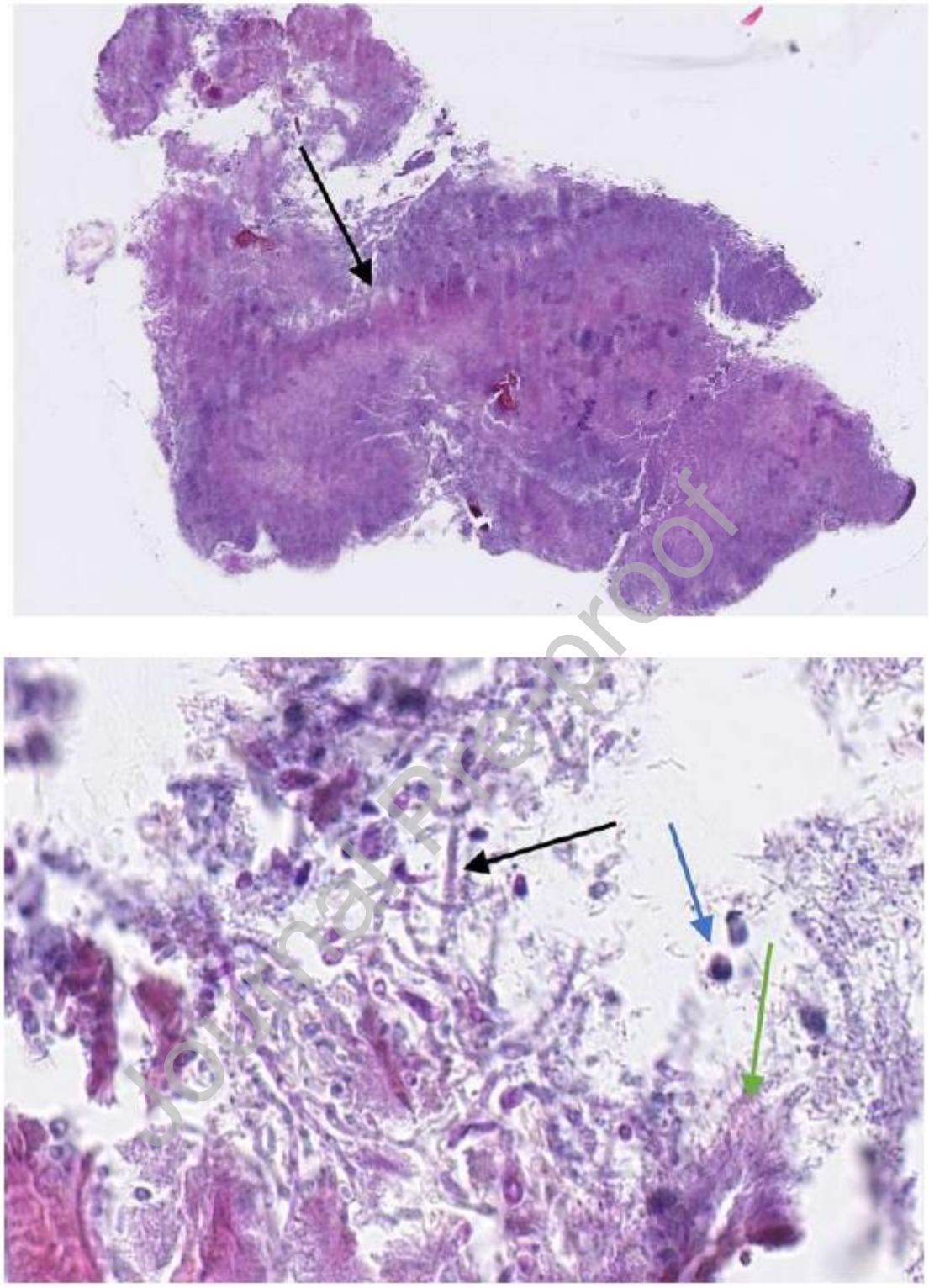


Fig. 1 : HE stain

(a) Magnification x 12. Actinomyces colony of a sulfur granule, showing the “sun ray effect” (black arrow).
(b) Magnification x 77. Candida (black arrow) embedded into Actinomyces filaments (green arrow) showing acute inflammation reaction with polymorphonuclear leucocytes (blue arrow). Notice the change of width in between Actinomyces filaments and Candida species.

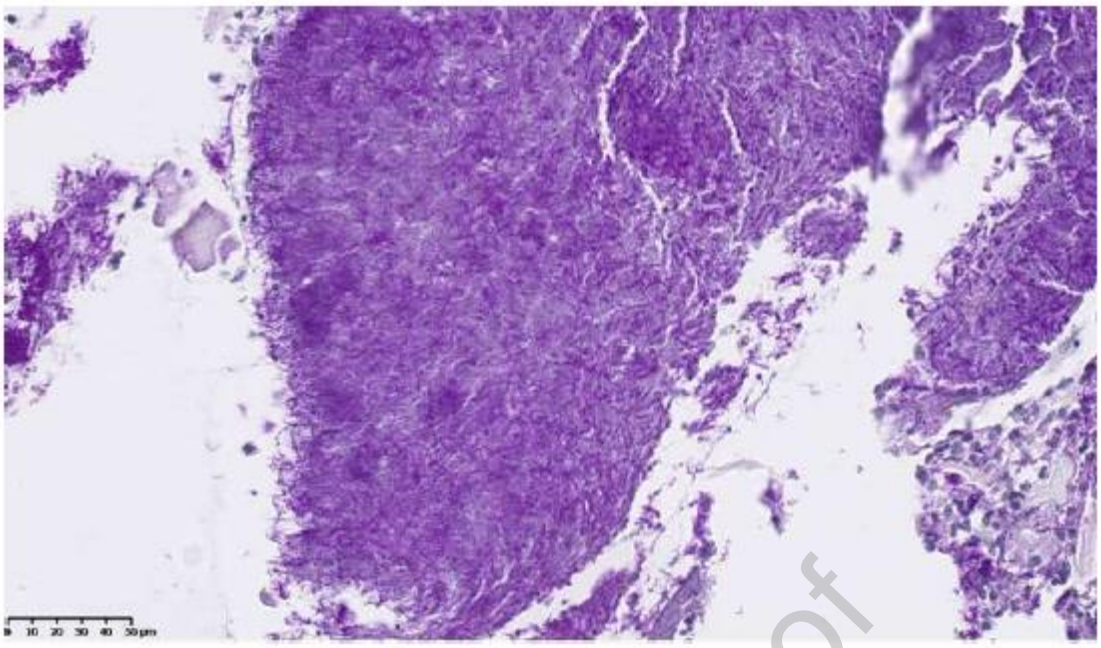


Fig. 2 : PAS stain.
Magnification x 55. Same view as fig. 1 showing aggregates of actinomyces filaments tint into a dense magenta color

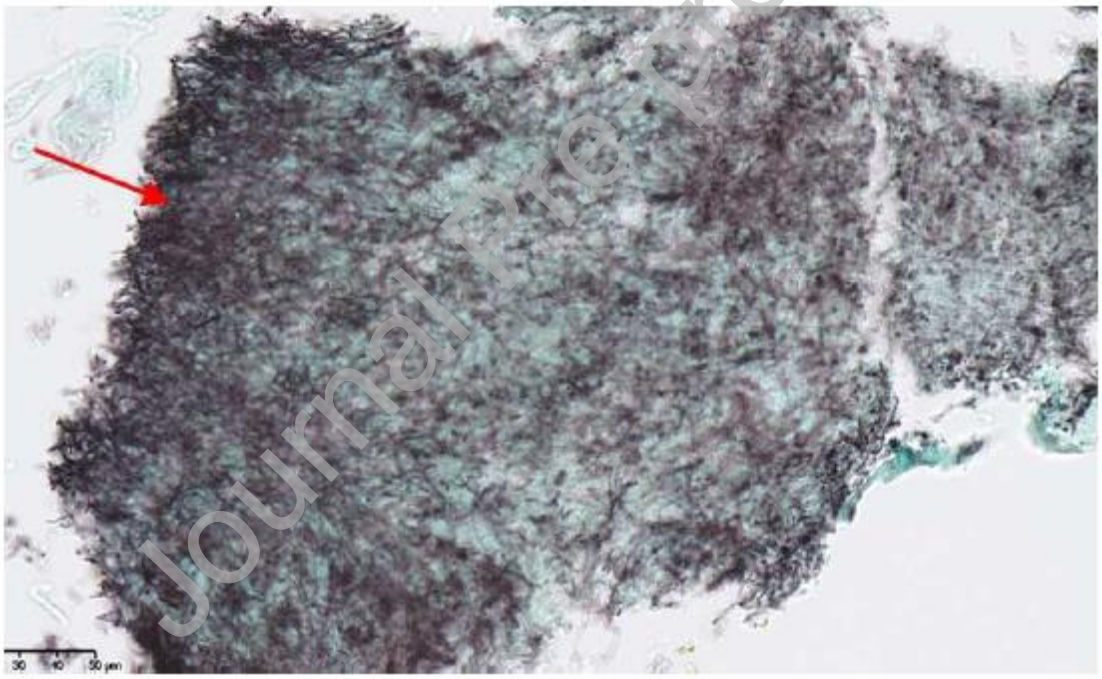


Fig. 3 : GMS stain
Magnification x 55. Same sample as in Fig. 1. Actinomyces filaments (red arrow) are colored into a gray-darkish color. Actinomyces species gather together into the periphery of the granule more obviously than in the PAS stain. No Candida species are seen on this specimen.

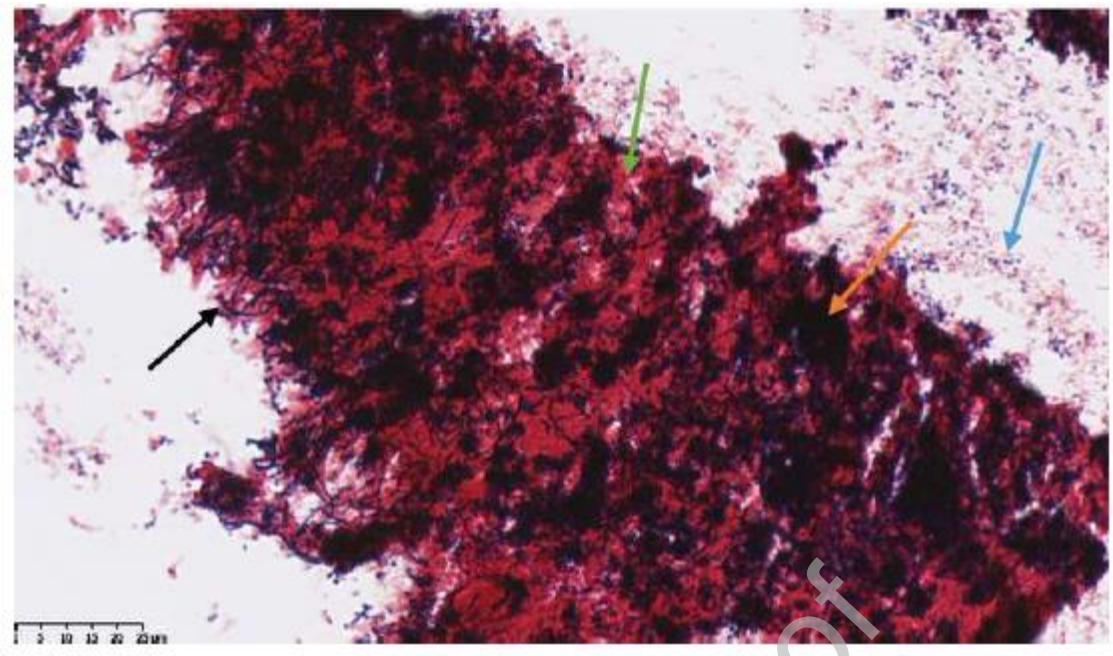
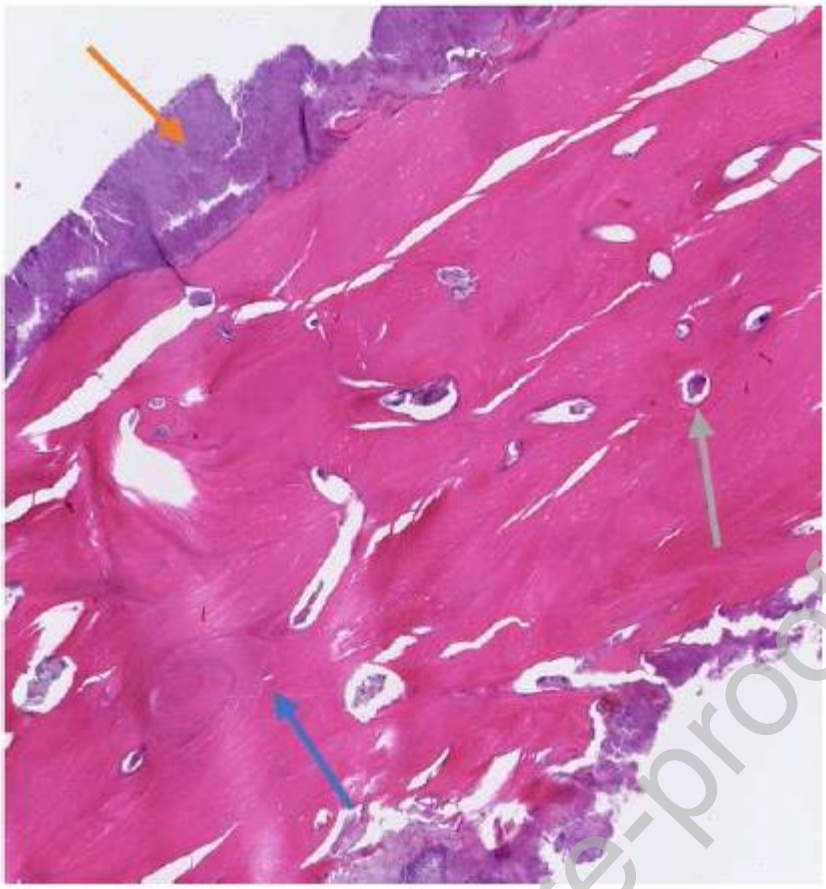
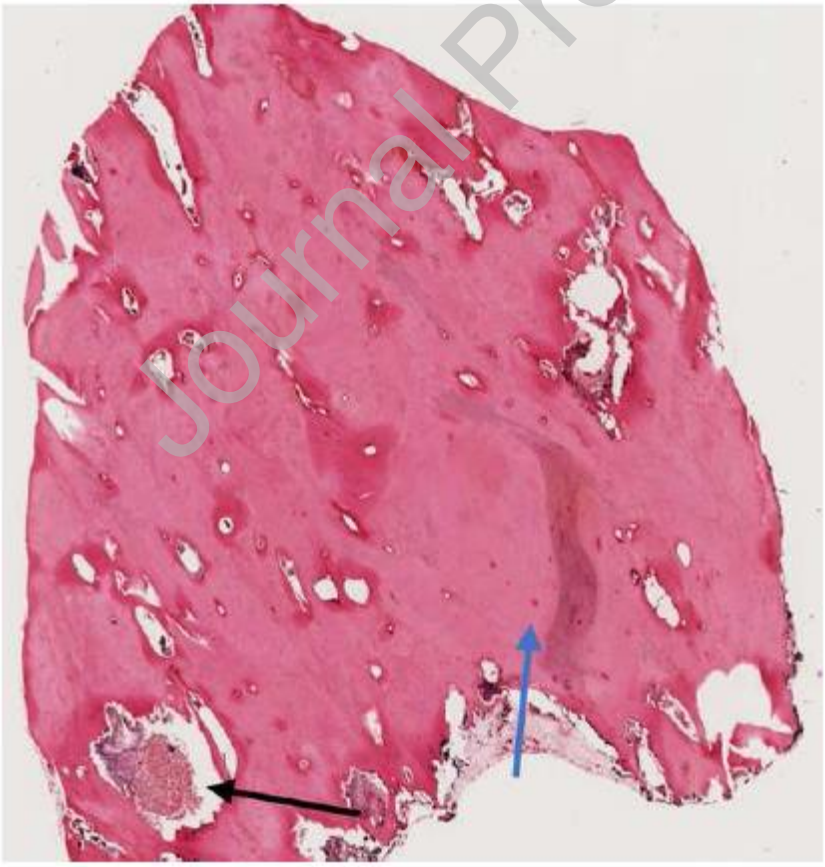


Fig. 4 : Gram stain

Gram-positive bacteria are colored in a dark blue whereas gram negative bacteria are colored in a reddish color. Gram positive bacteria (green arrow), Actinomyces filaments (black arrow), Actinomyces aggregates (orange arrow), Gram positive bacteria showed as a little blue dot (blue arrow). Actinomyces filaments gather themselves into small dot-colonies, embedded with a gram positive environement, showing interactions in between filamentous, gram positive and gram negative bacterias. These diverse assembly of germs cooperating together into a complex polysaccharide matrix called "biofilm".





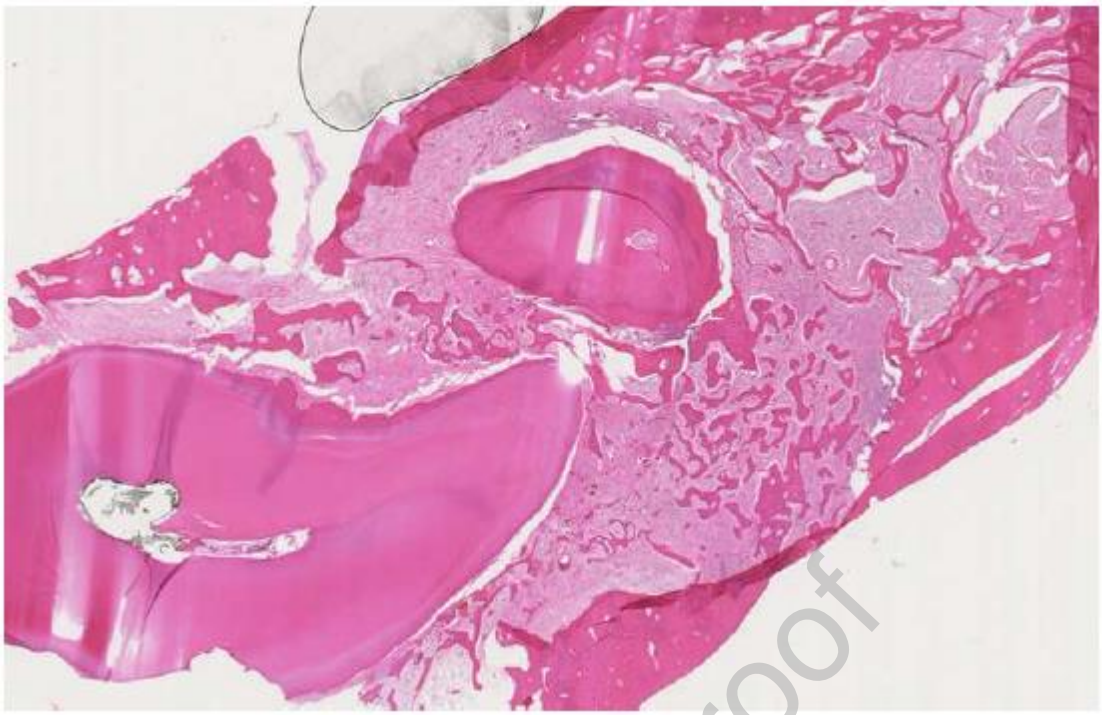
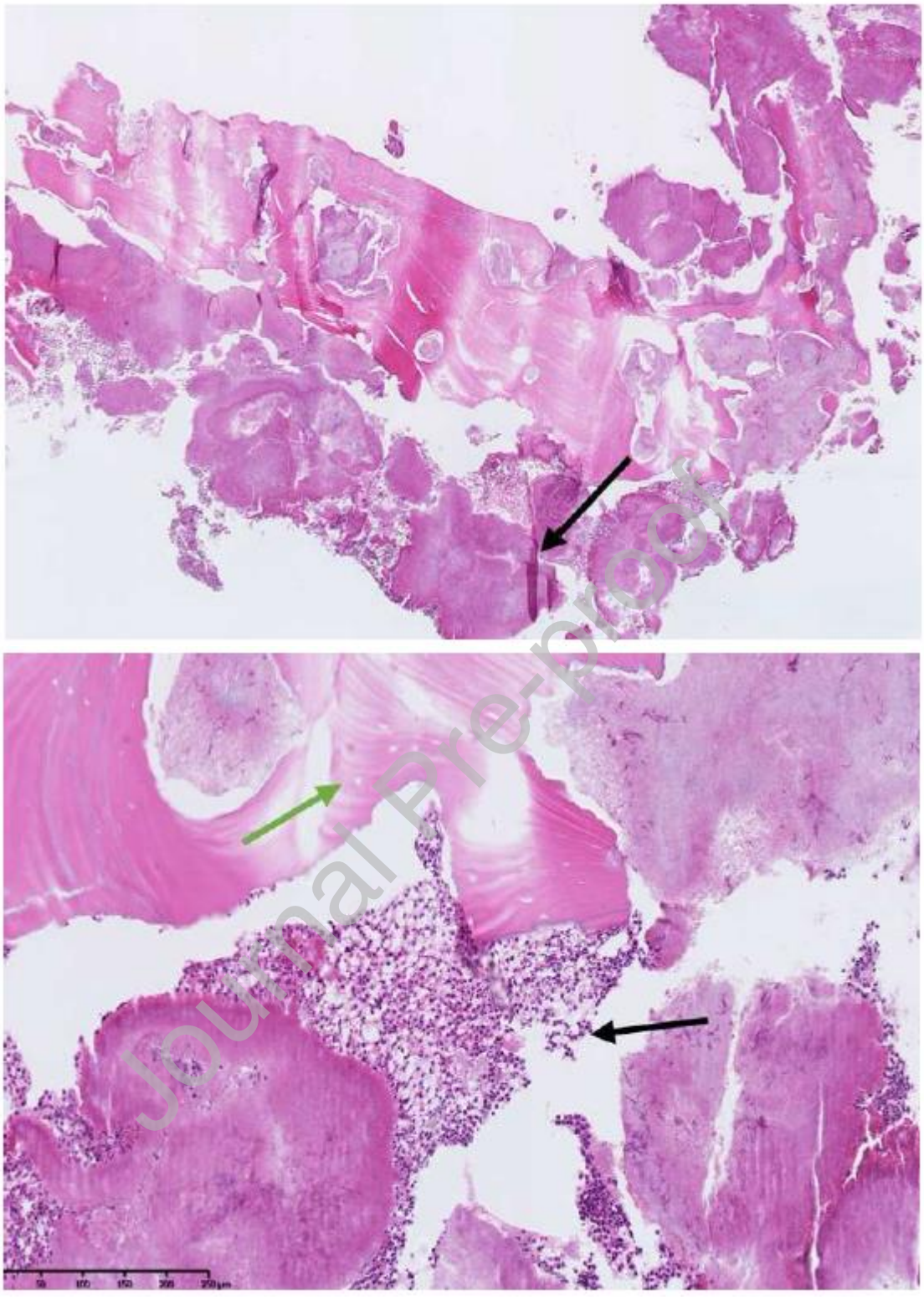


Fig 5. Microscopic aspect of a ORN bone sample :
Lamellar aspect of the dead bone into (a), (b) and (c) samples.
(a) Magnification x3. Osteoradionecrosis 3 years after radiotherapy.
Dead osteocytes are shown with a blue arrow, no osteoclasts are visible. A large biofilm of Actinomyces is shown all around the bone (orange arrow), with little change into the morphology of the bone. Some bacterias are visible into the lacunae (grey arrow). Very few inflammation reaction is visible. Patient had 15 days of antibiotics before the removal of the sequester.
(b) Magnification x3. Osteoradionecrosis sequester 2 years after radiotherapy. No actinomyces or living bone cells are visible on this sample (blue arrow). We can notice a discret chronic inflammation reaction with a hyalinized vessels (black arrow). Features that are commonly found into ORN samples. No antibiotics were given to the patient before the removal of the sequester.
(c) Magnification x 1. Osteoradionecrosis of a sample of a mandibulectomy 3 years after radiotherapy. Lamellar aspect of the bone with large areas of fibrosis. Absence of inflammation, actinomyces or any kind of bacteria. No living osteocytes or osteoclasts is seen in this sample. The patient had 30 days of Pentoxyphylline treatment combined with Amoxicilline before mandibulectomy.



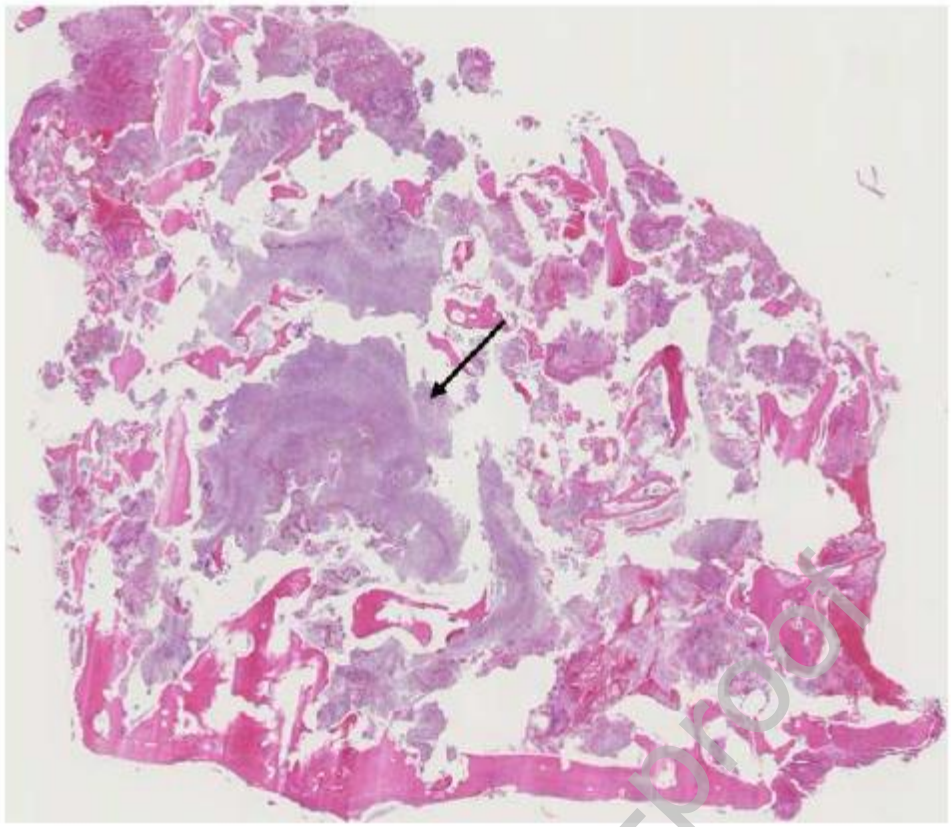


Fig 6. Microscopic aspect of a MRONJ bone sample :
(a) Magnification x3. The patient had 10 years of oral BP and the specimen was taken 1 year after the end of the treatment. Characteristically we can see the moten eaten aspect of the bone. Sulfur granules of actinomyces are shown all around the bone (black arrow)
(b) Magnification x17. Same sample as figure (a) showing dead osteocytes (green arrow) and acute inflammation (black arrow) next to a sulfur granule.
(c) Magnification x2. The patient had 17 years of oral BP followed by 2 years of Denosumab. The sequester was taken 1 year after the end of the treatment. We can see the moten eaten aspect of the dead bone, with large colonies of bacterias and actinomyces into the sequester (black arrow)

Table 1 : Population study - Risk factors MRONJ K versus MRONJ OP versus ORN

Risk factors (n=patients)	MRONJ K (n=43)	MRONJ OP (n=33)	ORN (n=13)	Total (n= 89)	p-value
Age (yo)	69 (28-96)	75 (58-92)	61(37-83)	70.0 (28-96)	
MRONJ K VS MRONJ OP					0,110
MRONJ K VS ORN					0,212
MRONJ OP VS ORN					**0,005**
Sex					
Female	46,5% (20/43)	78,8% (26/33)	23,1% (3/13)	55,1% (49/89)	
Men	53,5% (23/43)	21,2% (7/33)	76,9% (10/13)	44,9% (40/89)	
MRONJ K VS MRONJ OP					**0,015**
MRONJ K VS ORN					0,601
MRONJ OP VS ORN					**0,004**
Tobacco	23.3% (10/43)	27,3%(9/33)	53,8% (7/13)	29,2% (26/89)	
MRONJ K VS MRONJ OP					0,999
MRONJ K VS ORN					0,139
MRONJ OP VS ORN					0,502
Alcohol	4,7% (3/43)	9,1% (3/33)	30,8% (4/13)	10,1% (9/89)	
MRONJ K VS MRONJ OP					0,999
MRONJ K VS ORN					0,065
MRONJ OP VS ORN					0,260
Diabetes	16,3% (7/43)	9,1% (3/33)	7,7% (1/13)	12,4% (11/89)	
MRONJ K VS MRONJ OP					0.999
MRONJ K VS ORN					0,999
MRONJ OP VS ORN					0,999
Steroid intake	18,6% (8/43)	24,2% (8/33)	7,7% (1/13)	19,1% (17/89)	
MRONJ K VS MRONJ OP					0.999
MRONJ K VS ORN					0,999
MRONJ OP VS ORN					0,999
Periodontal disease	60,5% (26/43)	48,5% (16/33)	76,9% (10/13)	58,4% (52/89)	
MRONJ K VS MRONJ OP					0,999
MRONJ K VS ORN					0,999
MRONJ OP VS ORN					0,318
Prosthesis	27,9% (12/43)	21,2% (7/33)	7,7% (1/13)	22,5% (20/89)	
MRONJ K VS MRONJ OP					0.999
MRONJ K VS ORN					0,777
MRONJ OP VS ORN					0,999
Extractions	65,1% (28/43)	63,6% (21/33)	84,6% (11/13)	67,4% (60/89)	
MRONJ K VS MRONJ OP					0,999
MRONJ K VS ORN					0,911
MRONJ OP VS ORN					0,859

Table 2 : Histological features - MRONJ versus ORN

Histological	MRONJ (n=87)	ORN (n=20)	Total	p-val

features (n=biopsies)											(n=107)	ue
	Bone spicules (n=19)	**Curettages (n=13)**	**Sequestras (n=52)**	**Mandibular resections (n=3)**	**Total (n=87)**	**Bone spicules (n=2)**	**Curettages (n=7)**	**Sequestras (n=10)**	**Mandibular resections (n=1)**	**Total (n=20)**		
Actinomyces												0,074
Surface	2/19	0/13	5/52	0/3	8,0% (7/87)	0/2	1/7	4/10	0/1	25,0% (5/20)	11,2% (12/107)	NA*
Surface and depth	15/19	12/13	46/52	3/3	87,4 (76/87)	2/2	6/7	6/10	0/1	70,0% (14/20)	84,1% (90/107)	NA
None	2/19	1/13	1/52	0/3	4,6% (4/87)	0/2	0/7	0/10	1/1	5,0% (1/20)	4,7% (5/107)	NA
Actinomyces Aggregates/sulfur granules												0,327
Presence	7/19	5/13	32	1/3	51,7% (45/87)	1/2	5/7	7/10	0/1	65,0% (13/20)	54,2% (58/107)	NA
Absence	12/19	8/13	20	2/3	48,3% (42/87)	1/2	2/7	3/10	1/1	35,0% (7/20)	45,8% (49/107)	NA
Biofilm												0.203
Surface	5/19	2/13	8/52	0/3	17,2% (15/87)	2/2	0/7	5/10	0/1	35,0% (7/20)	20.6% (22/107)	NA
Surface and depth	5/19	4/13	14/52	0/3	26.4% (23/87)	0/2	3/7	3/10	0/1	30,0% (6/20)	27,1% (29107)	NA
None	9/19	7/13	30/52	3/3	56,3% (49	0/2	4/7	2/10	1/1	35,0% (7/20)	52.3% (56/	NA

					/87)						107)	
Chronic inflammation												**0.018**
Surface	11/19	0/13	13/52	0/3	27,5% (24/87)	0/0	4/7	6/10	0/1	50,0% (10/20)	30,8% (33/107)	NA
Surface and depth	4/19	3/13	13/52	2/3	25,3% (22/87)	1/2	3/7	2/10	1/1	35,0% (7/20)	27,1% (29/107)	NA
None	4/19	10/13	26/52	1/3	47,1% (41/87)	1/2	0/7	2/10	0/1	15,0% (3/20)	41,1% (44/107)	NA
Acute Inflammation												0.065
Surface	9/19	7/13	28/52	1/3	51,7% (45/87)	2/2	0/7	4/10	0/1	35,0% (7/20)	47,6% (51/107)	NA
Surface and depth	3/19	4/13	14/52	1/3	25,3% (22/87)	0/2	2/7	2/10	0/1	15,0% (3/20)	24,3% (26/107)	NA
None	7/19	2/13	10/52	1/3	23,0% (20/87)	0/2	5/7	4/10	1/1	50,0% (10/20)	28,0% (30/107)	NA
Hyperemia												0.212
Presence	4/19	10/13	25/52	3/3	48,3% (42/87)	0/2	4/7	2/10	0/1	30,0% (6/20)	44,9% (48/107)	NA
Absence	15/19	3/13	27/52	0/0	51,7% (45/87)	2/2	3/7	8/10	1/1	70,0% (14/20)	55,1% (59/107)	NA
Osteocytes												0.460
Presence	4/19	3/13	4/52	2/3	14,9% (13/87)	0/2	0/7	1/10	0/1	5,0% (1/20)	13,1% (14/107)	NA
	15/	10/1	48/5	1/3	85,	2/2	7/7	9/10	1/1	95,0%	86,9	NA

Absence	19	3	2		1% (74/87)					(19/20)	% (93/107)	
Osteoclasts												NA
Presence	0/19	1/13	7/13	0/3	9,2% (8/87)	0/2	0/7	2/10	0/1	10,0% (2/20)	9,3% (10/107)	0.999
Absence	19/19	12/13	45/13	3/3	90,8% (79/87)	2/2	7/7	8/10	1/1	90,0% (18/20)	90,7% (97/107)	
Pseudoepithelial hyperplasia												NA
Presence	0/19	6/13	10/52	2/3	20,7% (18/87)	0/2	2/7	4/10	0/1	30,0% (6/20)	22,4% (24/107)	0.382
Absence	19/19	7/13	42/52	1/3	79,3% (69/87)	2/2	5/7	6/10	1/1	70,0%(14/20)	77,6% (83/107)	NA
Microscopic aspect												NA
Lamellar	7/19	4/13	13/52	3/3	31,0% (27/87)	0/2	4/7	7/10	0/1	55,0% (11/20)	35,5% (38/107)	0.068
Moth-eaten	12/19	9/13	39/52	0/3	69,0% (60/87)	2/2	3/7	3/10	1/1	45,0% (9/20)	64,5% (69/107)	NA

NA* : Not Appliable. Statistical analysis could not be performed due to small samples numbers.