

# Oral Pathology and Medicine

## LNCrNA hotair expression, a promising prognostic factor in head and neck squamous cell carcinoma: a meta-analysis

**Caponio Vito Carlo Alberto\***, **Troiano Giuseppe\***,  
**Zhurakivska Khrystyna\***, **Santarelli Andrea\*\***, **Guida Laura\***, **Lo Muzio Lorenzo\***

\*Department of Clinical and Experimental Medicine, University of Foggia, Via Rovelli 50, Foggia 71122, Italy

\*\*Department of Clinical Specialistic and Dental Sciences, Polytechnic University of Marche, Ancona, Italy;

**Aim:** Head and neck cancers (HNCs) are the ten most frequent malignant neoplasia in humans. Despite improvements obtained in both surgical and adjuvant therapies, the 5-years survival rate for HNCs has shown very little improvements in the last decades. For such reasons, we are in need of new biomarkers for improving diagnosis and prognosis outcomes, to provide clinicians new tools for the treatment of these diseases. Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides in length previously defined "junk DNA". HOX transcript antisense intergenic RNA (HOTAIR) is one of the most studied lncRNAs. Different studies focused on HOTAIR capacity to cooperate with different chromatin modifying complexes, above all with the Polycomb Repressive Complex 2 (PRC2), through its 5'-terminal binding domain. The former lets latter to recognize the target gene, leading to Histone H3 lysine-27 trimethylation, with a silencing effect. The aim of this meta-analysis is to investigate the link between HOTAIR expression and patient prognosis in HNCs, to highlight its role as prognostic biomarker

**Methods:** Literature research was performed on the following databases: PUBMED, SCOPUS, EMBASE and Web of Science, focusing on the expression of HOTAIR on HNSCC including more than 50 patients

in total and showing analysis of correlation between different levels of HOTAIR expression and overall survival. The results of selected studies were collected as Hazard Ratio (HR) for 5-years overall survival and Odds Ratio (OR) for the link between HOTAIR expression and Lymph-node metastasis (LNM), Tumour Clinical Stage and Histological Grade. Heterogeneity was investigated using both the Higgins index and the Q test. Of 135 articles screened, only 8 were read in full text and 5 of these were included in the meta-analysis for a total of 431 patients analysed.

**Results:** Results of meta-analysis, on the basis of three studies, revealed interesting correlation between high expression of HOTAIR and higher level of histological grade and advanced tumor stages. We could not show a correlation between high HOTAIR expression and the rate of LNM. Analysis of overall survival (OS) showed that high expression of HOTAIR was associated with a poor OS in patients with head and neck cancer. These results suggest a potential important role for HOTAIR as biomarker of aggressivity in head and neck cancer.

**Conclusion:** In conclusion higher expression level of lncRNA HOTAIR are associated with poor prognosis, higher tumor stages and higher histological grade. This study encourages the execution of further studies on human samples in order to confirm such preliminary findings and to understand the HOTAIR role in the biology of tumor to figure out new therapeutic strategies and better prognosis evaluation.

## Oral microbiome, salivary nitric oxide and hypertension: is there a relationship?

**Coccia Erminia\***, **Addesa Angelo\***, **Rappelli Giorgio\***,  
**Barbadoro Pamela\*\***, **Ponzio Elisa\*\***, **Santarelli Andrea\***,

**Piemontese Matteo\*, Procaccini Maurizio\***

\*Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche, Ancona

\*\*Dipartimento di Scienze Biomediche e Sanità Pubblica, Ancona

**Aim:** The association between oral microbiome and nitric oxide (NO) has been yet demonstrated. In the oral cavity there are more than 300 different bacterial species of which a variety of facultative anaerobic bacteria responsible for the nitrate-nitrite-NO transformation. Recent research has revealed that the NO participates in a vast number of physiological functions including vasodilation, endothelial function, antiaggregation of platelets, host defence, metabolism and mitochondrial function. However, the results on the correlation between NO salivary concentration and blood pressure are conflicting. Therefore, the aim of this study is to evaluate the correlation between oral microbiome characteristics, salivary NO concentration and their association with the presence of hypertension.

**Methods:** 48 subjects between 50 and 70 years old were visited the dental clinic of the Polytechnic University of Marche. The protocol of the present study was approved by the Ethics Committee of Marche. Salivette® kits (Sarstedt Aktiengesellschaft & Co., Nümbrecht, Germany) were used to saliva samples collections. A commercial enzyme immunoassay kit to determine Total NO in saliva (R&D Systems, Inc., Minneapolis, USA) was used according to manufacturer's instruction. Supragingival and subgingival plaque sampling were collected using sterile paper points point (ISO 30, Dentsply-De-Trey, GmbH, Germany). Bacteria specific TaqMan probe and primers sets were designed from the specie-specific region on the 16S rRNA. In addition, a universal primer pair based on the conserved region of the 16S rRNA gene was used to quantify the total amount of eubacterial species in the specimens. Real-time PCR was carried out using a Rotor-Gene 3000 (Corbett-Research, Sydney, Australia). The bacterial DNA level were quantified by qRT-PCR and converted to theoretical cell numbers.

**Results:** 48 subjects have been included in this study: the case group consisted in 23 people diagnosed with hypertension, 14 males (60.9%) and 9 females (39.1%); the control group consisted in 25 people with normal blood pressure levels, 16 females (64.9%) and 9 males (36%). A statistically significant difference in nitric oxide levels has been found in the groups, in fact, NO level in saliva were higher in normotensive subjects than in hypertensive ones (respectively  $165.77 \pm 61.7$ ;  $57.49 \pm 19.61$ ;  $p=0.023$ ). Bacterial concentration of the supragingival plaque

was significantly higher in hypertensive people ( $4.73 \cdot 10^7 \pm 4.33 \cdot 10^7$ TCN) than in normotensive ones ( $4.02 \cdot 10^7 \pm 4.00 \cdot 10^7$ ;  $p=0.024$ ), while the bacterial concentration in subgingival plaque was not significantly different.

Some bacterial species had higher concentration in different kind of oral plaque in hypertensive patients. Nevertheless, other bacterial species, such as *Neisseria subflava*, resulted significantly more present in normotensive people than in hypertensive ones (respectively  $9.09 \cdot 10^3 \pm 5.48 \cdot 10^3$ ;  $4.79 \cdot 10^3 \pm 4.35 \cdot 10^3$ ;  $p<0.001$ ).

Multiple logistic regression model has highlighted the association between hypertension and a lower concentration of *Neisseria subflava* in subgingival plaque as a risk factor (OR 5.75, 95% CI 1.11-29.78); furthermore, a high NO salivary concentration resulted to be a protective factor against hypertension (OR 0.33, 95% - CI 0.13-0.86).

**Conclusion:** The results have illustrated the influence of NO in hypertensive disease: significantly higher level of NO have been found in normotensive patients; disposable data show that NO concentrations similar to the ones found in our sample can influence blood pressure levels. Moreover, the results of our clinical study highlight specific microbiological differences between the oral flora of hypertensive and normotensive people. According to these results, it is possible to reduce blood pressure by increasing the levels of salivary NO and the concentration of some specific microbial species in the mouth.

### Genistein as drug for oral squamous cell carcinoma of tongue: anticancer properties and metabolic profiling

**Ardito F.\*, Perrone D.\*, Troiano G.\*, Di Fedè O.\*\*\*, Mascitti M.\*\*\*, Lo Muzio L.\***

\*Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

\*\*Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy

\*\*\*Department of Clinical Specialistic and Dental Sciences, Marche Polytechnic University, Ancona, Italy

**Aim:** The tongue cancer is extremely aggressive and characterized by poor prognosis. It is a complex disease to be treated and therapies in use have led to mediocre results and many side effects. Genistein is a soy-derived isoflavone and phytoestrogen with antineoplastic activity. There are not many studies in literature on the effects of genistein on the tongue cancer and, for this reason, we evaluated in vitro the antitumor action of genistein and the possible variations of cellular metabolism.

**Methods:** We used 3 cell lines of TSCC, PE/CA-PJ15, PE/CA-PJ49 and HSC-3 cell lines. Three concentrations of genistein, 20, 50 and 100  $\mu\text{M}$ , have been used to assess adhesion, proliferation, migration, cell viability at three time points, and we calculated the IC50 values. In addition, we studied the expression of OCT4 and survivin, two important proteins in promoting tumorigenesis, after treatment with 20, 50 and 100  $\mu\text{M}$  of genistein at 24, 48 and 72 hours to treatment. Respirometric assay, ATP-measurement methods, LDH Cytotoxicity Assay, Calcium Colorimetric Assay Kit, quantitative determination of intracellular ROS level, Analysis of  $\Delta\psi$  by fluorescence assay were used to determinate alteration of metabolic profiling.

**Results:** Cell adhesion was inhibited especially between 20 and 50  $\mu\text{M}$  of genistein treatment. Proliferation was reduced by 50% for treatments with 20  $\mu\text{M}$  at 24 hours, with 20 or 50  $\mu\text{M}$  at 48 and 50  $\mu\text{M}$  at 72 hours ( $p < 0.0001$ ). Viability tests confirmed a proportional reduction at concentration of genistein and at duration of treatments. Even the migration was reduced with high significance ( $p < 0.001$ ). Genistein down-regulated vitronectin, Oct4 and survivin. It was showed a reduction of ATP, an increased amount of intracellular calcium and LDH release. Intracellular ROS and  $\Delta\psi$  were reduces after treatment of genistein.

**Conclusion:** This in vitro study points out the antitumor effect of the genistein on tongue carcinoma and its role in metabolic alterations of TSCC. In vivo studies are necessary to confirm these data and to be able to manufacture a suitable delivery system that is acting directly in the tumor site.

### Brain abscesses caused by oral infection: case series

**Bacci Christian, Tomasin Lisa, Cerrato Alessia, Lucchiari Nicola, Berto Alessandro**

Dipartimento di Neuroscienze (Direttore Prof. A. Martini)  
Sezione di Clinica Odontoiatrica (Direttore Prof. E. Stellini)  
Università degli Studi di Padova

**Aim:** To describe 7 rare cases of brain abscess origin from dental infection

**Methods:** From January 2014 to December 2016, 7 cases of brain abscess from dental infection has been documented.

**Results:** This report described seven cases of brain abscess from dental foci. 4 patients were allowed to access to the emergency room for persistent headache and epileptic syndromes, 2 patients were for long time hospitalized and developed a brain abscess as aggravation of pre-existing conditions,

a case has been transferred from another hospital, comatose. 5 cases underwent craniotomy and brain abscess drainage and drained material was collected and sent for microbiological examination, in two cases it was not possible because of incompatibility with the clinical condition. Teeth with lesions were extracted with careful curettage of the same, and even these samples were sent for microbiological examination. In 5 cases there were apical lesions, in two cases, apical and periodontal lesions combined and in one case in particular was present serious oral health impairment. Microbiological examination confirmed in 5 cases the dental origin of brain abscess, in the other cases indirect proving were obtained. In 4 cases the radiographic examination was performed the panoramic radiograph and CT scan with axial, sagittal processing, coronal and Dentscan, in the other 3 only CT was performed.

In one case the subject presented immunodepression related to HIV infection, while in one case was present because in treatment with anti-rejection drugs for liver transplantation. The case with severely impaired state of oral health was a young woman (29 years old), hospitalized in intensive care at another hospital with odontogenic abscesses of the submandibular fossa, temporal, then developed brain abscess and transferred to our hospital in coma, due to lack of dental treatment for phobia. There were two deaths, one case described above and the other the case of pz transplanted liver. In a case in particular, it was present in a very small dental apical lesions of 11, visible only in CT, which was removed together with the element tooth involved. The microbiological examination of comparison between the endodontic lesion and brain lesion confirmed the same pathogen, as in other cases.

**Conclusion:** Over the years, there have been a number of case reports of brain abscess, in which the microorganisms were thought to have arisen from a dental source. The oral cavity is well recognized as being home to a rich and abundant microflora. Bacteria gaining access to the blood stream may then spread to distant sites. Brain abscess is a rare, but life-threatening infection in which a localized area of suppuration develops within the brain parenchyma. The most common sites are the temporal lobes, followed by the cerebellum. The aspect that we want to emphasize is that the very low number of cases reported in the literature of brain abscesses of odontogenic origin can also be sought in the lack of diagnostic capacity. What we want to emphasize is find everything to work together different medical specialties in order to allow the patient to receive the best diagnosis and treatment possible.

## Metalloproteinases and their expression in oral disease: a review

**Baldrini A.\***, **Caruso D\*\*\***, **Riccelli U.\*\*\*\***, **Tortosa C.\*\*\*\***,  
**Cordaro R.\*\*\*\***, **Lo Faro C.\*\*\*\***, **Cristofaro M.G.\*\***

\*Biotechnologist, Unit of General Pathology

\*\*Adjunct Professor, Unit of Oral and Maxillofacial Surgery

\*\*\*Researcher Fellow, Unit of Oral and Maxillofacial Surgery

\*\*\*\*Resident, Unit of Oral and Maxillofacial Surgery

University "Magna Graecia" of Catanzaro, Italy - Department of Experimental and Clinical Medicine

**Aim:** The matrix metalloproteinases (MMPs) belong to a family of structurally related with proteolytic enzymes zinc related, which are known to play a key role in the catabolic turnover of extracellular matrix components (ECM). Until now, research studies have shown that MMPs regulate the activity of different bioactive substrates non-ECM related, including growth factors, cytokines, chemokines and cell receptors, which determine the tissue microenvironment. This activity has been implicated in a number of essential pathophysiological processes like embryonic development, wound healing and differentiation related to tissue remodeling. However, overexpression of different MMPs in tumors can be associated to invasion, metastasis, angiogenesis unregulated development, inflammation and even cell destruction. The properties of MMPs is highlighted in research of topical dermatology and periodontology. The aim of this study was to evaluate the MMP as a predictive biomarker in oral disease in body fluids, using literature review.

**Methods:** The Authors performed a literature search using literature review made by the Bank PUBMED data, in the period 2010 - 2016, on the role of MMPs in oral diseases. Of the 58 articles viewed, we analyzed only 4 scientific review on the expression of MMPs in biological fluids such as saliva and blood. The relative remaining research were excluded because for not presented inclusion criteria. Whole-blood specimens collected from the controls and patients with OSCC were placed in tubes containing ethylenediaminetetra-acetic acid (EDTA), immediately centrifuged, and stored at -80 °C. Measurements of plasma LCN2, MMP-9, and LCN2/MMP-9 levels by an enzyme-linked immunosorbent assay (ELISA). Lipocalin2, MMP-9, and LCN2/MMP-9 levels in plasma samples were, respectively, analyzed using human LCN2, MMP-9, and LCN2/MMP-9 ELISAKits. From each plasma sample, 100µl was directly transferred to the microtest strip wells of the ELISA plate and then assayed according to the manufacturer's instructions.

**Results:** The biomarkers used for prediction of oral disease, are substantially present in biological fluids such as saliva and serum. The saliva can be used as a diagnostic medium for paradontitis. The inflammatory biomarkers are MMP-8 and MMP-9. MMP7 levels are significantly increased in the saliva and in Gingival crevicular fluid (GFC) in patients with paradontitis. A multivariate analysis shows that MUC4 together MMP7 can accurately discriminate the paradontitis from healthy individuals. In the blood they were measured the expression levels of MMP-9, and the complex LCN2 (LCN2 / MMP-9) in patients with oral squamous cell carcinoma (OSCC). In total they were recruited 195 patients with OSCC and 81 controls; to which patients with OSCC have high levels of LCN2, MMP-9 and LCN2 / MPP-9 compliance controls. Plasma high levels of these proteins are associated with lymph node or distant metastases metastases.

**Conclusions:** Despite the large number of markers used in various pathologies, from this review it is emerged as MMPs also have an important role in prognosticate the onset of various diseases. The most important ones treated in this article, are the MMPs that affect oral pathology, taken from the serum and saliva of patients. Despite this MMPs to date are not used as main prognostic markers, although they have the advantage of being able to be detected by non-invasive methods to monitor the evolution of oral pathology.

## Advanced implant rehabilitation of a haemophilic patient: case report

**Bardhi Elisa\***, **Zanon Ezio\*\***, **Cerrato Alessia\***, **Tomasin Lisa\***, **Zanette Gastone\***, **Bacci Christian\***

\*Section of Dentistry (head Prof. E. Stellini), Department of Neurosciences (head Prof. A. Martini), University of Padua, Padua, Italy

\*\*Department of Cardiac Thoracic and Vascular Sciences (head Prof. S. Iliceto), Haemophilia Centre Unit of Coagulopathies, University Hospital of Padua, Italy

**Aim:** The aim of this study is to report a case of implant treatment of a patient suffering from haemophilia A.

**Methods:** A 41-year-old male patient, affected by Haemophilia A, attended the Dental Clinic of University of Padua reporting pain of the lower right second molar. The positive percussion test indicates the presence of inflammation in the periodontal ligament. X-ray examination (Panoramic Radiography) showed an area of radiolucency in correspondence of the apex, indicating bone resorption. Previously, the patient



received endodontic retreatment of the tooth. Even doing so, the pain persisted, so as the radiolucency. Due to oral and radiographic examination, the tooth was deemed hopeless, so the treatment proposed and accepted was the extraction of the lower right second molar with socket preservation, for future implant placement. The patient received recombinant Factor VIII injected intravenously 3000 IU prior to dental extraction and 1000 mg of Tranexamic Acid. Anaesthesia by paraperiosteal and troncular infiltration was performed. An envelope flap was created by incision in the gingival sulcus, extended from third to first molar. The surgical extraction of the tooth was followed by the removal of the periapical tissue, that was sent for histopathological examination. Bone graft (Bio-Oss) was added and covered with a resorbable membrane (OsseoGuard, BIOMET3i) sutured to the buccal flap, with exposed membrane left at the occlusal aspect of the extraction socket. The patient was seen with the results of the histopathological examination, that confirmed an apical cyst. After three months, the postoperative site showed good bone healing. One hour prior the surgery the patient received a single infusion of recombinant Factor VIII 2000 IU, and 1000mg of Tranexamic Acid. Flapless implant placement was performed under local paraperiosteal anaesthesia. A tissue punch was used to perforate the gingival tissue to gain access to the bone. After osteotomy preparation, there was placed a 4mm x 11.5mm titanium implant (Osseotite, BIOMET3i) and a healing abutment. A Panoramic Radiography was done at the end of the surgery to control the position of the implant. At the patient was prescribed chlorhexidine digluconate gel, pain killers and antibiotic therapy.

**Results:** There were no haemorrhagic complications neither during first and second surgery, nor in postoperative time.

**Conclusion:** Haemophilic patients suffer of an inherited bleeding disorder caused by missing or defective blood coagulation factors: factor VIII (Haemophilia A) or factor IX (Haemophilia B). Bleeds can occur internally, into joints and muscles, or externally, from minor cuts, dental procedures or trauma. Because of the risk of haemorrhagic complications, haemophilic patients require special care during invasive dental procedures, such as extractions and implant placement. The correct haemostasis is necessary in order to not compromise the osseointegration of the implants. To the best of our knowledge, this is the first case described in literature of implant regenerative surgery in haemophilic patient.

### Cutaneous vasculitis mimicking odontogenic

### fistula treated with platelet rich fibrin. A case study

**Barone S., Giudice A., Bennardo F., Antonelli A., Fortunato L.**

School of Dental Medicine, Department of Health Sciences, "Magna Graecia" University of Catanzaro

**Aim:** Cutaneous vasculitis is defined as a pathological process characterized by inflammation of the skin blood vessel wall. The disease process causes an alteration of the blood flow, ischemia and damage to the neighbouring tissue. Clinical variants of cutaneous vasculitis are related to the severity, etiology and evolution of the disease.

A 54-years-old woman came to our attention with severe pain and asymmetry of the lower third of the face. At clinical examination, the left mental region showed a fistulised swelling with leakage of exudate. Initially, the localization and clinical signs of the lesion could mimic an odontogenic etiology. On clinical, radiological and histological findings, a cutaneous small vessel vasculitis (CSSV) was diagnosed. The aim of this study is to show the effectiveness of the autologous platelet rich fibrin (PRF) in the treatment of chronic ulcers.

**Methods:** When the patient came to us a biopsy was immediately performed. After considering lesion's clinical evolution and analysing histological report, treatment of this fistula was performed by the use of autologous platelet-concentrate either in the solid form (L-PRF) or in the liquid one (i-PRF). To prepare the L-PRF 40 ml of autologous venous blood was taken and collected into four tubes of 10 ml (with clot activator additive) that were immediately centrifuged at 2700 rpm for 12 minutes. To prepare the i-PRF 36 ml of autologous venous blood was taken and collected into four tubes of 9 ml (without additives) that were immediately centrifuged at 700 rpm for 3 minutes. Treatment protocol was based on two phases. During the first one an intralesional application of L-PRF membranes was performed and i-PRF was injected around area of injury using an insulin syringe. Then perilesional injections of liquid platelet-concentrate were repeated each week for 6 weeks.

**Results:** After using autologous platelet rich fibrin in the treatment of this cutaneous ulcer there was a clear improvement of clinical conditions. A reduction in lesion size and a diminution of pain were gradually appreciated. Healing process was initially characterized by the formation of an eschar, then it was defined by the partial healing of the lesion with minor leakage of exudate and finally a complete wound healing was obtained. Successful ulcer closure and epithelization was completed

after 45 days from the first injection.

**Conclusions:** Non-healing cutaneous lesions represent a challenging problem and are commonly related to peripheral vascular disease, trauma, infection, neurologic and immunologic condition, as well as neoplastic and metabolic disorders. Therapeutic potential of autologous PRF is based on platelet ability to release growth factors and cytokines that accelerate the healing process. In particular, growth factor bioactivity involves in tissue repair mechanisms such as chemotaxis, cell proliferation and differentiation, angiogenesis, intracellular-matrix deposition, antimicrobial effect and remodelling. Platelet growth factors work as messengers to regulate a complex series of events that promote healing of chronic ulcers through the formation of granulation tissue in the early healing phase. As it's showed in our clinical case, autologous platelet concentrate can be a valid alternative in the treatment of cutaneous ulcers.

### Socket preservation using calcium sulphate with and without addition of PRP

**Basili M., Venditti A., Sottile G., Bonifazi E., Di Trani G., Marsella G., Viscogliosi O., Ferro R., Barlattani A., Bollero P.**

Department of diagnosis, hygiene and oral prevention for health and social vulnerable subjects with dental day hospital, Polyclinic of Rome Tor Vergata. School of dentistry, University of Rome Tor Vergata

**Aim:** After teeth extraction, the healing process and the post-extraction alveolus rearrangement lead to an inevitable loss of bone tissue in terms of vertical and horizontal volume. The use of filling materials limits the proliferation of epithelial tissue inside the alveolus to the advantage of the formation of new regenerated bone. Some materials also have the ability to induce and catalyze the proliferation of tissues. Calcium sulphate is able to stimulate the growth of bone tissue during the healing process, while the prp has the ability to accelerate the healing of both hard and soft tissues.

The purpose of the study is the comparison between the single calcium sulfate and mixed with PRP when used as regeneration material in extraction sockets.

**Methods:** It proceeded with the review of the literature on the search engine PUBMED / MEDLINE, setting the keywords "socket preservation" and "calcium sulfate". The selection criteria were: articles conducted in clinical trial mode, on the human and published between 2012 and 2017.

**Results:** From the research were obtained 3 scientific articles in which was analyzed the amount of regenerated bone after tooth extraction and use of calcium sulphate. Only two of the three items were

taken into account as they were considered valid for the comparison of materials. All cases treated in the studies involved the extraction of a dental element, by regeneration of the graft material and X-ray control after three months. In the analyzed cases no membrane was applied as it is proceeded to an alveolar periosteal coverage using drop-down flaps. In all cases treated was calculated the vertical bone height taking as reference coronally the cortical bone and apically the tangent point to the cortical bone and place at the level of the apex of the mesial tooth provided for reference. It was then calculated the difference between the initial height of the ridge with the tooth in the dental arch and the residual height at three months from extraction. In cases where calcium sulfate was used as a single material, the average residual bone height at three months was equal to -0,23mm. Where calcium sulphate was added to PRP, the average remaining height at three months was -0.4 + -0,4mm in the mesial side and -0.5 + -0,7mm in the distal side.

**Conclusions:** An analysis of the literature shows that bone regeneration in post-extraction sockets is better when used calcium sulphate as the only material without mixing with prp. The use of calcium sulphate as a single material allows to obtain a residual gain bone height equal to 0.2 mm in comparison to cases where it is mixed with PRF.

### Dual antiplatelet therapy in oral surgery: actuality and critical issues

**Basilicata M., Franco R, Miranda M., Rosa A., Barlattani A. jr, Agrestini C., Tiberti U., Bollero P.**

UOSD Diagnosis, Prevention and Oral Hygiene with Day Hospital Medical-Surgical General and Special for vulnerable patients, Polyclinic of Rome Tor Vergata, Rome, Italy

**Aim:** Dual antiplatelet therapy consists of the combination of two antiaggregant drugs (usually clopidogrel and aspirin) and has two indications: the prevention of thrombotic processes (cerebrovascular accidents, CV, and the prevention of acute myocardial infarction (MI) in patients with acute coronary syndromes or patients subjected to percutaneous coronary intervention (stent). Platelets play a central role in the pathogeny of thrombotic processes; platelet inhibiting drugs are used to prevent these processes. Clopidogrel irreversibly inhibits adenosine diphosphate, which is necessary for platelet aggregation while aspirin works through inactivation of the enzyme cyclooxygenase. Due to the increase of cardiac and cerebral diseases more and more patients taking this class of drugs and also require oral surgery

treatments. Therefore to be able to treat these patients need close cooperation between dentist and hematologist / cardiologist physician. Several articles in the literature, conducted on patients taking clopidogrel and aspirin, contemplate in general surgery the suspension of clopidogrel at least 5 days prior to surgery and the continuation of aspirin. Moreover it is clear from these studies that the risk of thromboembolic events increases exponentially at least 5% after discontinuation of drugs in question. Therefore it assumes great importance the ability to perform oral surgery without suspending these drugs. The purpose of this study is to evaluate the management of these drugs during oral surgery procedures.

**Methods:** It has been performed a search of PubMed and Medline using as keywords: "aspirin, plavix, oral surgery". They found 160 articles of which were selected only 4 covering the subject examination. They were excluded from the search bibliografiche reviews and articles that consider clinical trials in a single antiplatelet agent.

**Results:** Sanchez in his clinical study describes the 32 patients bleeding rates underwent oral surgery without the discontinuation of dual antiplatelet. The results showed the absence of uncontrolled bleeding. Grobe described 214 interventions mandibular osteotomy without suspension of the double antiplatelet comparing them with a control group of 281 patients. The results showed an incidence of 3.3% bleeding compared with 0.7% of the control group. However, the bleeding was well managed with local hemostatic maneuvers. Dudek has performed on a sample of 55 patients interventions of minor oral surgery. There were no excessive bleeding and uncontrolled in the study. Lillis has analyzed a sample of 111 patients taking clopidogrel, aspirin or aspirin and clopidogrel and compared them with a control group of 532 patients. The study demonstrated that the group that assumes clopidogrel and aspirin has a greater bleeding time compared to the control group and to the group of patients that only assume one of antiplatelet agents in question. However, the bleeding was well run with local hemostatic maneuvers.

**Conclusions:** All literature studies agree on the possibility to continue the therapy with the double antiplatelet and thereby can avoid thromboembolic risks for the patient. Bleeding in all the studies considered is controllable through local hemostatic maneuvers.

### Medication-related osteonecrosis of the jaw in patients with metastasis of renal cell carcinoma or GIST receiving sunitinib: report of 2 cases

### with clinical implications

**Erovigni F., Dell'Acqua A., Brega M., Gambino A., Carossa S.**

Surgery Department – Turin University CIR Dental School

**Aim:** Sunitinib is an orally administered multitargeted receptor tyrosine kinase (RTK) inhibitor, including vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptor (PDGFR), which are over-expressed in tumors. Recently, it has been showed that patients treated with this antiangiogenetic therapy could even develop osteonecrosis of the jaw.

**Methods:** We describe 2 cases of MRONJ in patients treated with Sunitinib. In February 2016, a 57-year-old man affected by clear cell renal carcinoma (CCRCC) with rhabdoid areas (IV grade sec Fuhrman pT3a N0 and papillar renal cell carcinoma type 1), presented to the Oral Surgery Department at Turin University Faculty of Dentistry (Dental School), complaining pain and swelling on the right lower jaw, in correspondence of the only two residual teeth (the inferior mandibular premolars). The patient had been visited a year before (9/5/2014) for a dental evaluation in order to begin endovenous bisphosphonate, but never started that. At that time the patient was completely edentulous in the upper jaw, while in the mandible persisted the 2 inferior right premolars. In February it was decided to extract the two residues premolars, with a drug holiday of 28 days. He was followed for other 15 days, once a week. A month later (April 27th), the patient came back to our Department with swelling, pain and pus excretion from the residual socket, with bone exposure at probing through the socket. The patient stated that he experienced episodes of swelling and abscess, apparently correlated with the sunitinib treatment courses. OPT and TC stated a complete disintegration of the alveolar crest in the 43-46 area with a relevant loss of the vestibular cortical bone. Diagnosis of Medication related osteonecrosis of the jaw (MRONJ) was made. In August 2016 a 77 years old patient affected from gastric GIST presented to the Oral Surgery Department at Turin University Faculty of Dentistry (Dental School), showing an intraoral bone exposure with a diameter of 8 millimeters, located on the milohyoid line among the first and the second mandibular left molars, asymptomatic. In 2000 the patient had been surgically treated for a breast cancer with RT. In 2009 she underwent to a carotid artery unblocking, and a Gastrointestinal Stromal Tumour was diagnosed. In 2012 she was gastro-resected and started Imatinib (a tyrosine-kinase inhibitor) as an adjuvant therapy. The patient suspended Imatinib in October 2012 for a

cutaneous reaction. In January 2013 she restarted Imantinib in association with an antihistaminic. In April 2016 she definitively suspended Imantinib due to heartburn and started Sunitinib. After 4 months bone exposure emerged.

**Results:** Both the patients underwent to surgery after the suspension of the antiangiogenetic drug 14 days before and after the surgery. They underwent to surgical debridement of the necrotic bone. An antibiotic therapy (Amoxicilline + Clavulanic Acid 1gr/3 times a day starting 2 days before the surgery; Metronidazole 500 mg/ 3 times a day starting 2 days before surgery) and antibacterial rinses (Chlorexidine 0,12% 3 times a day starting 1 week before surgery) were prescribed. The surgery was conducted opening a full thickness flap without release incisions. Then, with a piezoelectric shovel, the necrotic bone was removed until bleeding of the bone could be clinically appreciated.

**Conclusions:** There is emerging evidence that Sunitinib is independently related to osteonecrosis of the jaw and that discontinuation may lead to clinical improvement, often in association with surgery.

### Osteonecrosis of the jaw associated with denosumab: case series

**Brizzi Luciano\***, **Riccelli Umberto\*\***, **Novembre Daniela\*\***, **Tortosa Claudio\*\***, **Baldrini Antonio\*\*\***, **Cristofaro Maria Giulia\*\*\*\***

\*Student

\*\*Resident, Department of Experimental and Clinical Medicine. Unit of Oral and Maxillofacial Surgery

\*\*\*Biotechnologist, Department of Experimental and Clinical Medicine. Unit of General Pathology

\*\*\*\*Adjunct Professor, Department of Health Sciences. Unit of Dentistry University "Magna Graecia" of Catanzaro, Italy

**Aim:** Medication-related osteonecrosis of the jaw (MRONJ) is a severe adverse drug reaction, consisting of progressive bone destruction in the maxillofacial region of patients; caused by two pharmacological agents: Antiresorptive (including bisphosphonates (BPs) and receptor activator of nuclear factor kappa-B ligand inhibitors) and antiangiogenic drug therapy. There are also few cases reported of ONJ related to the use of other pharmaceutical agents, such as denosumab – a monoclonal antibody that lowers osteoclast activity, by inhibiting RANK-L during osteoclastogenesis reducing bone resorption, used as therapy for the treatment of osteoporosis and anti-cancer agent. There are several suggested hypothesis that could explain its unique localization to the jaws. Inflammation or infection, microtrauma,

altered bone remodeling or over suppression of bone resorption, angiogenesis inhibition, soft tissue BPs toxicity, peculiar biofilm of the oral cavity, terminal vascularization of the mandible, suppression of immunity, or Vitamin D deficiency. The purpose of this work is to highlight the potential effects of this monoclonal antibody on bone turnover and the subsequent results of osteonecrosis of the jaw no bisphosphonates related.

**Methods:** we report case series of 4 patients who underwent to subcutaneous injection of denosumab at dosage of 180 mg every four weeks for >6 months (prostate cancer, breast cancer and rheumatoid arthritis) with no pharmacological association of bisphosphonates with incoming of ONJ. The patients 1 man and 3 women with an average age of 65,3 (range 58-76); ONJ stage 2 to 3. They are in treatment from the year 2014 to 2016; good oral hygiene, limiting alcohol intake and stopping smoking were suggested for all patients requiring such treatments. All patients were strongly recommended to take daily calcium and vitamin D supplements.

**Results:** We observed in all patients ONJ Denosumab related. This drug reduces bone turnover and so had good results for treatment of osteoporosis and as anti-cancer agent. Denosumab elimination is likely through nonspecific catabolism in cells of the reticuloendothelial system similar to that of other therapeutic monoclonal antibodies and is not reliant on renal function; thus, denosumab represents a therapeutic option for patients with bone metastases who have chronic renal failure and renal insufficiency. 3 of these patients presented hypocalcemia; in an other patient there was a spontaneous loss of dental implants related to osteonecrosis.

**Conclusions:** denosumab represent a viable alternative to bisphosphonates for the treatment of osteoporosis or bone metastases. However it can also develop, like other monoclonal antibodies, ONJ with no association of bisphosphonates. Several cases of ONJ in patients with cancer who underwent denosumab therapy have been reported and it seems that the overall incidence of denosumab-related ONJ is similar to that for bisphosphonates-related in this population, ranging between 1-2%. In patients with advanced cancer and malignancy-associated hypercalcemia undergoing denosumab therapy, enquiry into current dental health and dental examination is mandatory.

### Epidemiological study of elongated styloid process on panoramic radiographs

**Giovanni Bruno\***, **Alberto De Stefani\***, **Paolo Balasso\*\***,



**Antonio Gracco\*\*\*, Edoardo Stellini\*\*\***

\*Department of Neuroscience, Dental School

\*\*Department of Management and Engineering

\*\*\*Department of Neuroscience, Dental School  
University of Padua

**Aim:** Eagle's syndrome is a disorder caused by an elongated or deformed styloid process or an ossified or calcified stylohyoid ligament. This condition can be confused with some other craniofacial disorders and oral diseases. Styloid process is a bony projection of cylindrical shape of approximately twenty to thirty mm in length. A styloid process is elongated when it is longer than the normal (longer than thirty mm). Differential diagnosis is possible because of imaging evaluation can easily allow to identify pathological styloid process or calcified stylohyoid ligament. In medical literature there are only a few studies that evaluate the prevalence of the elongated styloid process in different populations. The aim of this epidemiological study is to investigate the prevalence and the dimension of the elongated styloid process on digital panoramic radiograph in a North Italian population and how it is related to gender, age and side.

**Methods:** This study is a retrospective analysis on digital panoramic radiographs of 800 patients (421 females and 379 males, between 6 and 83 years old). Patients were taken from Padua University Dental Clinic database. The radiographs were performed using a Sirona Ortophos XG (Dentsply Sirona) The styloid process length were measured using the measuring tool of Sidexis Software. The process were measured always by the same operator from the point where it leaves the tympanic plate to its tip and it is considered elongated with a length greater than 30 mm.

**Results:** 33,1 % of the panoramic radiographs that were analysed presented an elongated styloid process. 55,6% of the patients presented the abnormal structure on both right and left side, while the others 44,4% of the patients showed it only on one side. The disease doesn't seem to have a correlation with the gender because the percentages of females and males are similar.

**Conclusions:** The presence of elongated styloid process in the evaluated population is high and a progressive increase of the elongation was found in the older group. The results found in this study are in agreement with the results showed in other similar studies made for other populations.

**Usefulness of auto-fluorescence of the bone to surgical resection margins in medication-****related osteonecrosis of the jaw (MRONJ)****Buffone C., Giudice A., Brancaccio Y., Turco A., Liborio F., Pacifico D., Fortunato L.**School of Dental Medicine, Department of Health Sciences,  
"Magna Graecia" University of Catanzaro

**Aim:** Medication-related osteonecrosis of the jaw is a disease characterized by necrotic lesions with bacterial superinfection which causes persistent bone exposure within oral cavity for a minimum period of 8 weeks. The condition is related to the assumption of anti-resorptive and anti-angiogenic drugs, such as bisphosphonates and monoclonal antibodies, for long periods in patients treated for malignant disease or bone diseases and never subjected to radiotherapy in the head-neck area. Surgery is one of the therapies performed to treat severe stages of the disease removing the necrotic bone. Currently there is no an instrumental method that leads surgery in distinguishing necrotic bone margins and the use of autofluorescence (AF) of the bone as a possible guide to visualize necrotic bone was proposed. The aim of this study is to evaluate the efficacy of AF using the VELscope as an intraoperative guide to delimit surgical resection margins for necrotic bone in patients with MRONJ.

**Methods:** In our report we considered 8 patients with clear signs of MRONJ and eligible candidates for resective surgical therapy. The patients had not received any radiotherapy in the head and neck area, but had got bisphosphonates orally or through intravenous injection for the treatment of diseases such as osteoporosis and tumors metastasizing to bone. Diagnosis was made by clinical examination, confirmed by instrumental exams such as orthopantomography and/or Cone Beam Computed tomography (CBCT). Classification was made according to SIPMO-SICMF MRONJ classification.

All patients received an antibiotic therapy of amoxicillin (1g each 12h) and metronidazole (250mg each 8h) from three days before surgery, together with professional dental hygiene and mouth rinse with nystatin and chlorhexidine 0.2%. Surgery was performed under local anaesthesia, bone samples were removed by piezoelectric surgery and subsequently subjected to histopathological analysis. Velscope was used to detect healthy bone and check surgical resection margins basing on the different coloration of tissues reflecting polarized UV light: the pathological tissue appeared dark-colored rather than the healthy one which was green.

**Results:** In this study 13 bone samples were collected, taken from 8 patients participants and removed surgically basing on the reduction or absence

of auto-fluorescence of the bone detected by VELscope. The results of histopathologic examination confirmed that almost all of the samples taken were consistent with the diagnosis of necrotic bone and all compatible with lesions at 2nd and 3rd stage.

**Conclusions:** The results of this study show that VELscope may be useful for the determination of the surgical resection margins of necrotic bone areas in patients with MRONJ, standardizing the procedure that is today strongly dependent on surgeon's sensations. However it is important to specify that VELscope can only be used as a help, especially in cases where surgery is carried out under local anaesthesia, which allows a limited surgical access: for these reasons future case-control randomized studies could give scientific evidence of its real efficacy.

### Initial evaluation for the role of HPV in the prognosis of oral cancer

**Cabras M., Campolongo M., Valpreda L., Broccoletti R., Molinaro L., Arduino P.G.**

Oral Medicine Section, Department of Surgical Sciences, University of Turin, Turin, Italy

**Aim:** To determine the prevalence and the potential prognostic role of HPV in a group of 26 patients with OSCC.

**Methods:** From the files of the Pathology Unit of "Azienda Ospedaliera Universitaria Città della Salute e della Scienza" of Turin, we selected a series of invasive squamous carcinomas of the oral cavity, as defined by morphological features. Samples were anonymized prior to analysis and representative haematoxylin and eosin (H&E)-stained sections of each case were reviewed by a dedicated pathologist (LM). In situ hybridization (ISH) for Human papilloma virus (HPV) was performed using the Bond TM Ready-to-Use ISH HPV Probe that targets the following subtypes: 16, 18, 31, 33, 51. ISH was carried out following manufacturer's instruction on the automated Leica BOND system (BOND-MAX, Leica).

**Results:** Eight (31%) out of 26 patients were positive for HPV. No statistically significant differences were found between the HPV-positive patients and the HPV-ones, with regards to gender ( $p=0.797$ ), tumour localization ( $p=0.289$ ), tumour size (T), lymph node metastasis (N), to distant metastasis (M), ( $p=0.06$ ;  $p=0.36$ ;  $p=0.50$ , respectively), histological grading (G), ( $p=0.90$ ), and therapeutical options ( $p=0.79$ ;  $p=0.28$ ;  $p=0.40$ , for surgery, chemotherapy and radiotherapy respectively). With regard to smoking, 7 out of the 26 patients (26.9%) were smokers;

interestingly, all of them belonged to the group of HPV-negative patients; concerning this behavioural risk factor, a statistically significant difference was detected ( $p=0.04$ ). Furthermore, 3 (11.5%) of the 8 HPV-positive patients died; on the other hand, only one (3.8%) of the 18 HPV-negative patients died: therefore, exitus rate was also significantly different between the two groups ( $p=0.04$ ).

**Conclusions:** HPV is an important risk factor for head and neck cancer, specifically oropharyngeal cancer; however, the pathological role of HPV infection in the carcinogenesis of the oral cavity remains unclear. According to literature, HPV seems to play a minor role in the carcinogenesis of the oral cavity and there is not yet a standard assay for HPV detection: current methods include consensus and type-specific polymerase chain reaction (PCR) techniques, real-time PCR assays to quantify viral load, DNA ISH, and immunohistochemical detection of surrogate biomarkers. Regarding the results acquired from the collected data, even though smoke habit and death rate were found to be statistically different between the two groups, the small number of patients involved is the main limit of our investigation. Further studies with larger samples of patients are needed.

### Assessment of disease activity in primary Sjögren's syndrome using the ESSDAI index (Eular Sjögren's Syndrome Disease Activity Index)

**Cafaro A.\*, Romagnoli C.\*, Romagnoli E.\*, Arduino P.G.\*, Cabras M.\*, Broccoletti R.\***

\*Oral Medicine Section, Department of Surgical Sciences, University of Turin, Turin, Italy

\*\*Department of Surgical Sciences and Integrated Diagnostics, Genoa University, Genoa, Italy

**Introduction:** Primary Sjogren's syndrome is a chronic autoimmune disease, characterized by lymphocytic infiltration into the salivary and lacrimal glands with hyperactivity of lymphocytes B. Besides decreased saliva and tear secretion, the disease may present a wide range of extraglandular systemic manifestations involving the entire body, with also an increased risk of developing non-Hodgkin lymphoma. Like all chronic diseases, Sjogren's syndrome is characterized by phases of activity and inactivity, and, given the complex aspect of the disease, it is indispensable to monitor patients over time and to revise the possible different treatment modalities. One of the assessment tools of the disease is the ESSDAI index.

**Aim:** The aim of the study is to evaluate the ESSDAI

index in a population of patients with primary Sjogren's syndrome.

**Methods:** Thirty-three patients (32 females and 1 male, mean age 70.27), diagnosed with primary Sjogren's syndrome according to the AEGC and ACR / EULAR classification criteria, were selected from those attending the follow-up visits at the Oral Medicine Section of the CIR-Dental School, Turin, Italy. The ESSDAI index (EULAR Sjögren's Syndrome Disease Activity Index), which provides for the assignment of a final score based on 12 questions regarding all signs and body districts, was rated for every case. The medical records of these patients, plus instrumental and recent blood investigation, and together with the responses of immunologists and / or rheumatologists, were considered in order to acquire the necessary information to determine the final score indicating the disease activity index.

**Results:** Of the 33 patients studied, 7 were awarded an index > 20 (of which one 35 and one 30), for 14 patients the index was > 10, while 12 patients had an index < or equal to 10. The body part most affected in patients examined was the osteoarticular district, and the most common sign was the presence of lymphadenopathy particularly in the facial region.

**Conclusions:** The disease activity index, whose theoretical maximum value is 123, is a method of assessing the effects of the disease on patients and how the disease may have affected other organs and systems beyond the salivary and lacrimal system. As reported in the literature, the ESSDAI is useful in monitoring the progress of the disease and to evaluate the effectiveness of treatments, also for cases with only oral involvement.

### A rare case of lichenoid reaction in patient treated with Pembrolizumab

**Campolongo M.G.\*, Carbone M.\*, Conrotto D.\*, Arduino P.G.\*, Gambino A.\*\*\*, Broccoletti R.\***

\*Department of Surgical Sciences, Oral Medicine Section, CIR Dental School- University of Turin, Turin, Italy

\*\*Department of mechanical and aerospace engineering, Politecnico di Torino, Turin, Italy

**Aim:** Pembrolizumab is a anti-programmed cell death receptor-1 (PD-1) humanized monoclonal antibody. Nowadays pembrolizumab represents an effective treatment option for metastatic melanoma as well as for other cancer entities. It acts via blockade of the PD-1 receptor, an inhibitor of the t-cell effector mechanisms that limits responses against tumors. As reported for other anti-PD-1 antibodies, Pembrolizumab can induce immune-related adverse events: Pneumonitis is the most common among

these. Skin rash, diarrhea, endocrinopathies and epatitis are other adverse effects, most of them appear between two and six months after the beginning of the treatment. Oral lichen planus is considered a rare adverse effects. Literature reports only two cases of lichenoid lesions developed after the beginning of anti PD-1 therapy.

**Methods:** In this case report was described a recent case observed in our university clinic, concerning a oral lichenoid reaction probably due to assumption of Pembrolizumab. A review of the literature was performed, with the purpose to find any similar cases.

**Results/case report:** a 61 years-old male patient with metastatic melanoma stage IV with BRAF mutation arrived at the oral medicine unit of CIR-Dental School of Turin in October 2016, referring the presence of multiple white lesions on the oral mucosa, notice for the first time by the generic dentist. He smokes 3-4 cigarettes/daily and drinks occasionally wine. He hasn't amalgam restorations o removable prosthesis. Melanoma, which originated on left leg, was diagnosed in February 2015, following a visit for a inguinal-iliac lymphadenopathy. Surgical resection was performed in March 2015. Next, PET showed abdominal and pelvic adenopathy, for this reason he started a medical therapy, first with Ipilimumab, then in December 2015 with Pembrolizumab. Pembrolizumab was administered for 120 mg e.v. every three weeks. In September 2015 during the periodic inspection by his dentist, he showed no oral lesions. In September 2016 he showed first oral signs, in addition to skin psoriasis. At the moment of first visit, he showed spread papular lesions located on bilateral buccal mucosa, tongue and gingiva, totally asymptomatic. Clinical suspicion was Oral Lichen Planus or Lichenoid lesions, therefore was performed in November 2016 a biopsy and histopathological test of a papular lesion, which gave outcome of lichenoid reaction.

**Conclusions:** To the best of our current knowledge, this could be the thirst described case of adverse reaction to Pembrolizumab who presents lichenoid-like lesions of the oral mucosa.

### 18-β-glycyrrhetic acid effect on oral squamous cell carcinoma

**Cantiani M.\*, Gallenzi P.\*, Patini R.\*, Giordano A.\*, Nocca G.\*\*\*, Cordaro M.\***

\*Institute of Dentistry, Università Cattolica del Sacro Cuore, Policlinico A. Gemelli, Rome; dean Prof. M. Cordaro;

\*\*Institute of Biochemistry and Clinical Biochemistry, Università Cattolica del Sacro Cuore, Rome;

**Aim:** 18-β-glycyrrhetic acid (GA) is a triterpene



found in the roots of licorice plants (*Glycyrrhiza glabra*). GA is the most important active ingredient in the licorice root, and possesses a wide range of pharmacological and biological activities (anti-inflammatory, antiviral, antiallergic and anti-cancer properties). GA exhibits remarkable cytotoxic and anti-tumor properties; in particular this molecule inhibits cell proliferation in a series of cancer cells, including human cervix cancer cells, melanoma cells, hepatocarcinoma cells, human epithelial ovarian cancer cells, gastric cancer cells, human breast cancer, lung cancer cells etc. GA exhibits dual activity in cancer: anti-cancer and cancer chemopreventive activities. A large number of studies have investigated the molecular mechanisms underlying both activities. GA enhances antioxidant enzymes, inhibits oxidative enzymes and GA is an 11 $\beta$ -HSD2 inhibitor. Moreover, GA exerts its cytotoxic activities via different mechanisms, including either the inhibition of NF- $\kappa$ B, PKC, Ras and other anti-apoptotic proteins or the activation of Bid, kinase inhibitors, caspases and other pro-apoptotic proteins. View of the important GA action on many tumor lines, the aims of this study were:

- 1) Verify the cytotoxic effects of this molecule on PE/CA PJ15 (a squamous cell carcinoma of oral cavity)
- 2) Verify the cytotoxic effects of this molecule on normal gingival fibroblasts (HGFs).

**Methods:** Isolation and culture of HGFs: Cells were obtained (with informed consent) from patients subjected to gingivectomy of the molar region. The specimens were plated in tissue culture flasks with complete DMEM, at 37°C, 5% CO<sub>2</sub> atmosphere. The HGFs were used before the fifth passage. Culture of PE/CA PJ15 cell line: Cells were plated in tissue culture flasks with complete ISCOVE, at 37°C, 5% CO<sub>2</sub> atmosphere. Cytotoxic Assay: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to determine the GA concentration value able to provoke cytotoxicity. PE/CA PJ15 and HGFs were seeded into 96-wells culture plates and exposed to different concentrations of GA (range from 10  $\mu$ mol/L to 500  $\mu$ mol/L). Cells were incubated with 0.5 mg/mL MTT for 4 h at 37 °C. Purple formazan crystals were solubilized by adding 100  $\mu$ L of DMSO and the absorbance was measured using a microplate reader at a wavelength of 540 nm. Statistical Analysis: Data were expressed as mean  $\pm$  Standard Deviation. Analysis was performed by ANOVA,  $p < 0.05$  was considered significant.

**Results:** Obtained results showed that the cytotoxic effects of GA were present at concentration values higher than 100  $\mu$ mol/L in PE/CA PJ15, while no cytotoxic effects were observed on HGFs; in particular, on fibroblasts, an intriguing proliferative effect (at the same concentration values) was

noted, even if not statistically significant.

**Conclusion:** In conclusion, this compound seems to be promising in oral cancer cells proliferation inhibition (although, its mechanism of action is not completely understood), the effect on healthy gingival fibroblasts will be further evaluated in future studies.

### **In vivo diagnosis of oral squamous cell carcinoma using optical coherence tomography: three cases reports**

**Capocasale Giorgia\***, **Mauceri Rodolfo\***, **Giancola Francesco\***, **Dioguardi Mario\*\***, **Giannatempo Giovanni\*\***, **Panzarella Vera\***, **Campisi Giuseppina\***

\*Dept. Surgical, Oncological and Oral Sciences, University of Palermo, Palermo

\*\*Dept. Clinical and Experimental Medicine, University of Foggia, Foggia

**Aim:** Optical coherence tomography (OCT) is a new non-invasive biomedical optical technology that provides high resolution of the cross-sectional tissue images applied clinically in ophthalmology for diagnosis of retinal macular diseases.

Several studies have investigated the potential validity of OCT use in the assessment of oral lesions but, to date, there doesn't exist a bank of OCT in vivo data of oral tissues.

We report three cases of oral squamous cell carcinoma (OSCC), analyzing in vivo OCT use and comparing data with microscopic evaluation.

**Methods:** For these evaluations, we used in vivo VivoSight® OCT (Michelson Diagnosis). We decided to use the dermatological probe because clinical researches independently conducted have demonstrate the effectiveness of this new medical technology. Dermatologic diseases are very similar to the oral cavity diseases and also other ex vivo studies have used the technology of Michelson Diagnosis for the evaluation of oral cavity lesions.

A standardized protocol consisting of I) clinical examination and a classification of the lesions (exophytic, verrucous endophytic), II) an OCT analysis of the lesions; no preparation are necessary to performed OCT imaging. The operator obtained an OCT scan over a 6mm diameter central area where the surgeon then performed incisional biopsy. III) Then, incisional biopsy was performed. The biopsy specimens were processed routinely in 10% formalin and embedded in paraffin. Representative section of lesions were selected by pathologist and photographed under light microscopy.

**Results:** OCT assessment showed inhomogeneity of epithelial layers with epithelial cleavage, probably



associated with infection, and neoplastic epithelial tissue islands that invades the connective; also in endophytic lesion we can find hypo-reflective keratin layer or no layer due to structural damage from ulceration. All malignant lesions showed clearly the breakdown of the basement membrane.

**Conclusion:** Oral cancer is the eighth most common cancer worldwide. If detected at an early stage, survival is better than 90% at 5 years. To date, most oral cancers (60%) are diagnosed at advanced stages (III and IV). Also it is noteworthy that many OSCC develop from potentially malignant disorders (PMDs). Then, correct diagnosis and timely treatment of PMDs may help prevent malignant transformation in oral lesions. The current approach to detecting the transformation of leukoplakia/erythroplakia to OSCC is regular surveillance combined with biopsy or surgical excision. However, biopsy techniques – the current gold standard – are invasive and unsuitable for regular screening of high-risk sectors of the population. With the recent developments in optical engineering and biomedical imaging, several studies have investigated the potential validity of OCT non-invasive use in other medical specialization. It could be a new approach that will help improve the diagnosis and the follow up of oral lesions. The validity of OCT in ex vivo oral lesions is confirmed in literature, while in vivo OCT validity should be supported by comparison of data of several PMDs: further researches are needed.

### Medical-related osteonecrosis of the jaw or actinomyces-related osteonecrosis of the jaw? A retrospective study and review of the literature

**Cerrato A.\*, Valente M.\*\*, Bacci C.\***

\*Clinical Dentistry (Head Prof. E. Stellini), Department of Neurosciences (Head Prof. A. Martini), University of Padua

\*\*Pathological Anatomy, Department of Cardiac, Thoracic and Vascular Sciences (Head Prof. S. Illiceto), University of Padua

**Aim:** The American Association of Oral and Maxillofacial Surgeons (AAOMS) Special Committee recommended in 2014 a changing in the nomenclature from BRONJ to the term Medication-Related Osteonecrosis of the Jaw (MRONJ), because of the growing number of osteonecrosis cases associated with other antiresorptive (denosumab) and antiangiogenic therapies. The histopathological findings include, in most cases, a combination of necrotic bone areas with devitalized trabecular bone and empty lacunae, with evidence of osteoclasts apoptosis without inflammatory infiltrate, and surface colonies of Actinomyces. The aim of the

study is to report on Actinomyces prevalence among the cases of MRONJ, taking into consideration also antiresorptive and antiangiogenic therapies, according to the new classification of the AAOMS 2014. Another aim is to report on the presence of those bacteria in our own MRONJ cases, higher than the prevalence found by other authors, in order to support the hypothesis of an infectious aetiology of these lesions.

**Methods:** The systematic review was performed using the database Medline, using the keyword "Actinomyces", in combination with "osteonecrosis" and one of the following terms "bisphosphonates", "denosumab", "sunitinib", "antiresorptive therapy", "antiangiogenic therapy". The results included clinical studies, case series, case reports, from 2004 and 2014. No publications have been excluded, except in-vitro studies, animal studies and studies about the linkage between Actinomyces infection and osteoradionecrosis. No studies have been excluded because of the language, on the contrary an appropriate translation has been performed. In many studies about ONJ, the presence of Actinomyces colonies was detected, but the exact percentage was not calculated: those studies have been excluded. The retrospective study was conducted on 36 patients, referred to the Dental Clinic, University Hospital of Padua, between May 2005 and January 2016. Those patient presented clinical and radiological manifestations of MRONJ and referred a history of bisphosphonates, antiresorptive or antiangiogenic therapies. Patient with a history of head-and-neck radiation therapy were excluded. Patient with a non-confirmed diagnosis of ONJ were excluded too. All the patients underwent surgical excision of the lesion and a biopsy was sent for histological examination. Criteria used for the diagnosis of Actinomyces colonies include the presence of filamentous bacteria, aggregated to constitute a mass, with color shades between the center and periphery on the colony, visible with H&E stains (the so called "sun-ray" effect) and with the Gram stain.

**Results:** A total of 42 articles were found. A total of 33 publications have been taken into consideration for the systematic review. 9 studies have been excluded due to the previously listed exclusion criteria. A total of 600 patients affected by ONJ have been taken into consideration, 438 showed the presence Actinomyces (73%). As far as our patients are concerned, 34 of 36 patients showed the presence of Actinomyces colonies at the histological examination (94.4%).

**Conclusions:** The reason why many drugs, characterized by different molecular reactions and administered for such different diseases, induce the same condition affecting the bones is still unclear.

On the contrary, the association between MRONJ and Actinomyces presence is undoubtedly strong, although it is unclear whether MRONJ is caused by Actinomyces or the presence of Actinomyces species is a secondary infection. Our hypothesis is that bisphosphonates, antiresorptive drugs and antiangiogenic therapy (especially if steroids are simultaneously administered) render bones more susceptible for osteomyelitis, even if the pharmacology is different.

### Ozone therapy for the treatment of oral lichen planus: a case-control study

**Bardellini Elena\***, **Amadori Francesca\***, **De Giuli Maria Cristina\***, **Conti Giulio\*\***, **Chiappini Davide\***, **Majorana Alessandra\***

\*Department of Medical and Surgical Specialities, Radiological Sciences and Public Health, Dental School, University of Brescia, Brescia, Italy.

\*\*University Vita-Salute S. Raffaele, Milan, Italy

**Aim:** Ozone therapy can be defined as a versatile bio-oxidative therapy with immunostimulant, analgesic and antimicrobial properties. The aim of this study was to assess ozone effectiveness in the treatment of erosive oral lichen planus.

**Methods:** A case-control study was carried out. Patients with erosive oral lichen planus visited at the Dental Clinic of Brescia from September 2016 to January 2017 were enrolled. Patients were randomly divided into two groups: group A received ozone therapy twice a week for two weeks; group B received cortisone topical therapy (betamethasone 4mg/ 2ml, two rinses a day for two weeks). For every patient, the area (cm<sup>2</sup>) of the largest erosive lesion was taken at T0 (first visit) and T1 (after two weeks). Pain assessment was evaluated using a Verbal Rating Scale (VRS) where 1=no pain, 2=mild pain, 3=moderate pain, 4=severe pain.

**Results:** A total of 20 patients were included in the study, divided into group A (n=10) and group B (n=10). The difference in the decline of the areas of the erosive lesions between the two groups resulted not statistically significant. A statistically significant difference in pain reduction was observed.

**Conclusions:** Ozone therapy is effective in reducing pain due to erosive Oral Lichen Planus.

### Conservative treatment of a Riga-Fede disease: a case report

**Ciaravino C.\***, **Mauceri R.\***, **Seidita F.\***, **Laurenziello M.\*\***

**Mastrangelo F.\*\***, **Giancola F.\***, **Campisi G.\***

\*Dept. Surgical, Oncological and Oral Sciences, University of Palermo, Palermo

\*\*Dept. Clinical and Experimental Medicine, University of Foggia, Foggia

**Aim:** Riga-Fede disease (RFD) is clinically characterized by the presence of a traumatic ulceration on the ventral surface of the tongue in newborns and infants. It is frequently associated with prematurely erupting primary teeth, but may also occur after the eruption of primary lower incisors in older infants with repetitive tongue thrusting habits and in children with familiar dysautonomia (insensitivity to pain). In addition to trauma of the tongue, complications related to RFD are the increasing risk of infection of the wound, the discomfort during suckling and the inadequate intake of nutrients that can cause poor development of the patient. Usually, the treatment of RFD is the surgical extraction of teeth that could be associated with the excision of the lesion. Rarely, conservative therapy is adopted, by smoothing out sharp teeth surfaces or applying a thin layer of composite to the incisal edges of the teeth. The aim of this study is to describe a case of RFD in an infant, treated by our Sector.

**Methods:** An 8-month-old male baby was referred to our Sector for an ulceration of the tongue. The lesion was approximately 10mm diameter and was located at the midline of the ventral surface of the tongue. The teeth showed a physiological eruption, normal structure of the crown and absence of mobility. The clinical examination of the lesion showed a relationship with the mandibular anterior incisors, due to repetitive traumatic injuries of the tongue against the teeth. The palpation of ulcerated area elicited an acute pain response from the child. All these conditions also had interfered with proper suckling; furthermore, the parents reported the finger-sucking habit of their son. According to our collaborator Pediatric Dentist, this case was treated with a conservative approach, removing the traumatic agent and modifying sharp teeth surfaces. The parents, moreover, was informed that the finger sucking habit could delay healing, and should be limited.

**Results:** The patient returned after 1 month showing a good healing of the lesion. After 2 months, it was completely healed and infant was feeding normally.

**Conclusions:** In literature, there are several studies regarding RFD and the treatments proposed are different. The first option for RFD treatment is surgical extraction of the teeth; however, it must be considered that premature loss of primary teeth may result in aesthetic, orthodontic, and phonetic problems. Moreover, it can promote malocclusion's

complication and the child could develop a wrong tongue posture, which also jeopardize speech. It can also induce a gingival fibrosis in the area, which could interfere with the eruption of the permanent tooth. The conservative approach, instead, avoids these consequences, is less invasive and more tolerable by the little patient. By working together, the Oral Medicine specialist, the Pediatric Dentist and the parents can achieve positive results in a short period with minimal trauma to the infant. This disease makes it difficult for the infant to suck and feed, putting the baby at risk of nutritional deficiencies. It is important that professionals are able to recognize the RFD's injury and the causal agent, so that a proper diagnosis and treatment can be performed.

### Oral mucosal complications in orthodontic treatment

**Lucchese A., Manuelli M., Ciuffreda C., Lissone A., Abati S.**

Dept. of Dentistry (Chairman Prof. EF Gherlone) University Vita-Salute San Raffaele, Dental School, Italy  
Dept of Orthodontic, University Vita-Salute San Raffaele, Dental School, Italy

**Aim:** Fixed orthodontic therapy is a valid method to solve functional and aesthetical oral problems, but the use of fixed oral devices can also cause negative effects in the oral cavity, if the treatment is not steadily under control. The aim of this study is to evaluate tooth, bone and soft tissues lesions due to fixed orthodontic appliances.

**Methods:** 100 patients with fixed orthodontic appliances were included in the study. In particular, 20 patients wear REP, 20 patients wear Forsus appliance, 20 patients had just the upper vestibular multibracket treatment, 20 patients had just the lower vestibular multibracket treatment while 20 patients had both upper and lower vestibular multibracket treatment. An accurate oral examination of the oral cavity, comprehending teeth, bone and soft tissues, was processed, in order to find possible lesions caused by the fixed orthodontic treatment.

**Results:** As regard REP, 35% patients had reversible palatal lesions, while 45% patients had the impression of the appliance on the tongue. Periodontal damages were observed in 5% patients, while tooth lesions such as dental caries were found in 5% patients. 20% of the patients with Forsus appliance experienced the lesion on the cheek mucosa, while 10% individuals reported periodontal problems, and 15% of the subject suffered for WSL (white spot lesion) and dental caries. Upper vestibular multibracket appliance

complained for superior labial lesions (15%), cheek mucosal lesions (20%), gingivitis (55%), WSL of superior teeth (15%), while dental recessions and periodontitis due to the appliance were rarely observed (5%). Lower vestibular multibracket appliance was frequently the cause of inferior labial lesions (15%), cheek mucosal lesions (15%), gingivitis (50%), WSL of inferior teeth (20%), and also in lower arch dental recessions and periodontitis due to the appliance were rarely observed (5%). Patients with both superior and inferior multibracket appliance experienced upper and /or lower lip lesions (25%), lesions of cheek mucosa (25%), gingivitis (65%) and WSL (30%), and just in few cases periodontitis (10%). Data shows a more critical oral situation in patients with both superior and inferior appliances than people with one-arch therapy, especially concerning inflammations such as gingivitis and other problems related to oral hygiene. Intra-oral photographs were taken to record the characteristic of the oral lesions caused by these oral devices.

**Conclusion:** An accurate assessment of the patients before the application of fixed orthodontic treatment is necessary. Oral hygiene instructions and motivation are very important, as well as periodic controls of the fixed oral device. The orthodontists are medical professional, and not only a mechanic, so their examination doesn't interest exclusively tooth movements, but comprehends the evaluation of the whole oral cavity, thus teeth, periodontal structures, bone and soft tissues have to be always observed.

### Bio-impedance analysis of mucosal tissues of the oral cavity: a comparison between healthy patients and patients affected by oral lichen planus

**Cocco M., Cerrato A., Pampaloni L., Bacci C.**

Clinical Dentistry (Head Prof. E. Stellini), Department of Neurosciences (Head Prof. A. Martini), University of Padua

**Aim:** Lichen Planus is a chronic inflammatory autoimmune disease that may affect the skin, the mucous membranes of the oral cavity and also other mucosae. According to the Literature, Oral Lichen Planus (OLP) has a higher incidence rate in the fourth decade of life and the female-to-male ratio is 1.4:1. The prevalence is estimated to be around 1–2% among population worldwide and oral manifestations may appear under various aspects, namely reticular, plaque-like, erosive, atrophic or bullous. The buccal mucosa, the tongue and the gingivae are the most commonly involved, but other

areas may be affected too. Erosive OLP shows the highest risk of malignant transformation among the various subtypes. Bioelectrical Impedance analysis is a method used for an accurate evaluation of tissues composition. The bioelectrical impedance data are a combination of capacitive and resistive type elements which, as a result, provide reactance and resistance. Reactance ( $X_c$ ) should be representative of the opposition of body tissues to the flow of electrical current or voltage and resistance ( $R$ ) should be representative of the conduction of fluid of the direct or alternating current. According to resistance and reactance, a phase angle (PA,  $\phi$ ) is calculated. PA values may range from  $90^\circ$  to  $0^\circ$  and a correlation between PA values and body cell mass is demonstrated. For example, if a tissue would have been composed of cells membranes only, it would have higher resistance, lower capacitance and a PA value of  $90^\circ$ , whereas a tissue composed mainly by fluids would show lower resistance, higher capacitance a PA value of  $0^\circ$ . According to these evidences, the PA values of neoplastic tissue are expected be lower than those of normal tissue. The aim of the present study is to compare the values of bioimpedance analysis of healthy patients to the values of patients affected by OLP and, if possible, to establish some cut-off values, which could be related to an higher or lower risk of malignant transformation.

**Methods:** Bioelectrical impedance analysis and PA measurements have been obtained using a special device, (Akern, Bia 101 Anniversary). The measurements were carried out by the same clinician. We evaluated 8 consecutive cases affected by reticular LP, already confirmed at the histological examination (case-group) and 9 healthy patients (control-group). The measurements have been performed in the buccal mucosae, the tongue, hard palate and upper anterior gingiva.

**Results:** The phase angle, reactance and resistance were generally higher in the case-group than in the control-group. The median PA of hard palate was found to be 23.9 in case-group and 14.8 in the control-group, the median PA of gingiva was 14.1 among the cases and 12.0 among healthy patients, the median PA value of the tongue was 14.2 for the first group and 14.4 for the second one. As far as the buccal mucosae are concerned, the median PA values among patients affected by OLP and among healthy patients were 13.1 and 12.4 on the right side and 14.1 and 12.1 on the left side, respectively.

**Conclusions:** The results of the study are not statistically significant, the sample size should be increased.

### The diagnosis of secondary syphilis of the oral

### cavity: a complex challenge for the dentist

**Conrotto Davide, Carbone Mario, Broccoletti Roberto, Gambino Alessio, Cabras Marco, Arduino Paolo Giacomo**

Department of Surgical Sciences, CIR-Dental School, University of Turin, Via Nizza 230, 10126 Torino, Italy

**Aim:** Syphilis can induce oral lesions in all its three stages; secondary syphilis, that occurs between 2 to 12 weeks after exposure, is the most frequent, but the large variety of clinical manifestation can often induce clinicians in mistake; lesions include aphthous like lesions, papules, plaques, irregularly shaped, with whitish edges, which can be single or multiple and involve the tongue, the lips and the oropharynx. The diagnosis of syphilis with a biopsy can be difficult, because the histological exam is often not specific. Therefore, a good anamnesis added to serological and microbiological essays is fundamental.

**Methods:** *Treponema pallidum* has tropism for many organs and tissues. For this reason, a unique initial presentation of secondary syphilis of the mouth is rare.

**Results:** We report the case of a patient, 50 years old, caucasian, with a good general health who attended to the Oral Medicine Section, Cir Dental School Lingotto, University of Turin with a large erythematous plaque of the palate, partially erosive, with white papules and difficulty in swallowing. The anamnesis did not added any important data. No other skin lesions were referred. The response of the histological exam was of lichenoid mucositis with plasmacells, the direct immunofluorescence for IgA, IgG, IgM and C3 was negative. The appearance of a new lesion on the penis and a more accurate anamnesis that revealed sexual risk habits suggested a provisional diagnosis of secondary syphilis. The positiveness for VDRL (1:16) and TPHA (>1:5120) confirmed the diagnosis. After a treatment with penicilline-G benzathine for 15 days, the oral lesions healed.

**Conclusions:** Syphilis has been called "the great imitator", for the large variety of lesions that can induce. Despite 12.000.000 of estimated new cases that occur every year in the world, syphilis is often undetected and without a proper treatment. The situation is particularly difficult when the oral lesion is the first or unique manifestation of the disease; therefore, dentists should have a particular training to play a role in detecting suspected lesions. Infection is mainly due to unprotected sexual intercourses. A correct anamnesis is the first step for the diagnosis of the disease and should be central in the visit of the patient.



## Traumatic ulcerative granuloma with stromal eosinophilia of the tongue (TUGSE): differential diagnosis

**Cordaro R.\*, Colangeli W.\*\*, Novembre D.\*, Tortosa C.\*, Lo Faro C.\*, Barca I.\*\***

\*Resident

\*\*Medical Doctor

University "Magna Graecia" of Catanzaro, Italy - Department of Experimental and Clinical Medicine. Unit of Oral and Maxillofacial Surgery.

**Aim:** Traumatic Ulcerative Granuloma with Stromal Eosinophilia (TUGSE) is an uncommon condition characterized by the presence of a solitary ulcer in the oral mucosa with raised and indurated margins, usually affecting the tongue, cheeks, or, less frequently, lips. Histologically, it is characterized by a diffuse polymorphic inflammatory infiltrate composed predominantly of eosinophils, B and T lymphocytes and macrophages, involving the superficial mucosa and extending deep into the submucosa with infiltration of the underlying muscle layer. The present case highlights the clinical aspects, aetiopathogenesis and histopathology of TUGSE.

**Methods:** An 11-year-old female presented herself at the Department of Oral and Maxillo Facial Surgery University Magna Graecia of Catanzaro with isolated painful ulcer of the tongue since 7 days. Intraoral examination revealed an ulcer on the dorsal surface and left side of the tongue measuring 2 cm x 1,5 cm in size, surrounded by a small area of erythema, painful on touch. The ulcer was smooth, tender and firm in consistency, with well defined margins and induration. No dentoscheletrical and intraoral alterations were found. The patient gave history of moderate and continuous pain, radiating to the cheek and neck region. Ultrasonographic exam of lymph nodes in head and neck showed reactive lymph nodes in left submandibular region. Blood and chemistry tests were performed to exclude haematological or metabolic disorders. Furthermore microbiological and serological tests were also carried out to investigate leishmaniasis, toxoplasmosis, EBV, CMV, HBV, HCV, HIV, zygomycosis, aspergillosis, histoplasmosis, blastomycosis, siphilis, Lupus erythematosus, Reiter's syndrome (HLA-B27) and Wegener's disease (ANCA). Tuberculosis skin test was added too. An incisional biopsy was performed under local anesthesia for histopathological examination.

**Results:** The differential diagnosis of lesions morphologically presenting as ulcers of the tongue can be very complex. It should include: infectious diseases, metabolic or autoimmune disorders,

malignancies, aphthous-like and traumatic lesions. In our case negative results of laboratory exams excluded a systemic condition, and then biopsy was recommended to define diagnosis. Therefore histopathological analysis revealed infiltrated with mixed inflammatory cells chiefly composed of CD 20+ and CD3+ B and T lymphocytes, eosinophilic granulocytes, CD 68+ histiocytes, CD 31+ cells and no atypical cells. A marked improvement of the lesion was observed after biopsy with a complete resolution at the outpatient follow-up visits at 1 and 6 months, indicating a full recovery. The age of patient, history of an accidental bite and short duration of lesion related to typical clinical picture and histopathological exam led to diagnosis of TUGSE.

**Conclusions:** TUGSE is a benign lesion of the oral mucosa of an unclear pathogenesis. This condition likely represents a group of related disorders with overlapping clinical and histopathological features and may be easily mistaken for a cancer, infection or autoimmune disease. According with literature, some cases show correlation between TUGSE and recurrent traumatic injury to the tongue. So the clinicopathological aspect related to spontaneous self-healing suggests diagnosis of TUGSE as benign entity excluding other similar ulcerative lesions. Awareness of this entity is important to emphasize the correct diagnosis of indurated ulcerated lesions and deliver appropriate and effective treatment.

## Immunohistochemical expression of MMP-9 in squamous cell carcinomas of oral tongue (SCCOT)

**Di Buò Sonia\*, Mascitti Marco\*, Bambini Fabrizio\*, Rubini Corrado\*\*, Lo Muzio Lorenzo\*\*\*, Troiano Giuseppe\*\*\*, Santarelli\* Andrea, Procaccini Maurizio\***

\*Department of Clinical Specialistic and Dental Sciences, Marche Polytechnic University, Ancona, Italy

\*\*Department of Biomedical Sciences and Public Health, Marche Polytechnic University, Ancona, Italy

\*\*\*Department of Clinical and Experimental Medicine, Foggia University, Foggia, Italy

**Aim:** Squamous cell carcinoma represents more than 90% of the carcinomas of the oral cavity, and tongue is the most commonly affected site within the mouth. The metalloproteinases (MMP) are a family of zinc-dependent proteins involved in the degradation of the extracellular matrix and in the processes of tumoral growth, invasion and metastasis. MMPs are frequently overexpressed in malignant tumors and have been associated with a worse prognosis. The aim of this study was to perform an immunohistochemical evaluation

of metalloproteinase 9 (MMP-9) in squamous cell carcinomas of oral tongue (SCCOT) and its correlation with clinicopathological factors, in order to use it as prognostic marker.

**Methods:** This study includes 60 surgical specimens of SCCOT obtained from patients undergoing glossectomy or hemiglossectomy. Data were retrieved and cataloged from clinical records and from the archive of the Institute of Pathology of the Polytechnic University of Marche by a single operator, in order to ensure the uniformity of the collected data.

Serial sections (4 µm) from formalin-fixed, paraffin embedded blocks were cut for each case and mounted on poly-L-lysine-coated glass slides, and 1 section stained with hematoxylin-eosin was used to confirm the histopathologic diagnosis. The sections were incubated for one hour at room temperature, with the anti-CD56 antibody diluted 1:200 (clone 56-2A4). The expression of MMP-9 was evaluated in all the samples at the invasive front. MMP-9 immunostaining was classified as negative (<5% of cells expressing MMP-9), 1+ (5-50%), and 2+ (>50%).

**Results:** There was a significant correlation between MMP-9 expression at the invasive zone with lymph node status and clinical stage of the disease, while it was not observed significant correlation with the other clinicopathological parameters. We observed a correlation between overexpression of MMP-9 and lymph node metastases. In fact, no cases with negative score showed lymph node involvement, while cases with score 2+ always presented lymph nodes tumor extension. Furthermore, a correlation between overexpression of MMP-9 and advanced clinical stage was found. We observed that the score of MMP-9 expression in the cases with stage I and II was largely negative (75.9%), while most of the cases with stage III and IV showed a score 2+ (67.7%). Lastly, we did not observe any significant relationship between the score at the invasive front and the parameter T.

**Conclusion:** According to these data, the MMP-9 was overexpressed in more aggressive SCCOT. In fact, MMP-9 expression correlated with cell proliferation, tumor growth, invasiveness and metastases.

### Contemporary diagnosis of mucoepidermoid carcinoma of the hard palate and intracranial cavernous angioma: a case report of a young adult

Di Fede O.\*, Capocasale G.\*, Bruno E.\*, Laino L.\*\*\*, Giuliani M.\*\*\*, Di Lorenzo S.\*, Panzarella V.\*

\*Dept. Surgical, Oncological and Oral Sciences, University of Palermo, Palermo

\*\*Dept. Clinical and Experimental Medicine, University of Foggia, Foggia

**Aim:** The specialisation of Oral Medicine provides clinical care to patients with a wide variety of oro-facial conditions, including: diseases of the oral mucosa; syndromes involving oro-facial pain; disorders of the salivary glands; and other oral manifestations of systemic diseases. The management of multiple, oro-facial diseases is the remit of the Oral Medicine specialist (OMs). Ranging from taking an accurate medical history to a detailed intra- and/or extra-oral examination, the OMs should be able to diagnose the most complex and systemic of diseases. We report a case of a young adult patient with two brace of diagnoses: mucoepidermoid carcinoma (MEC) of the palate and intracranial cavernous angioma.

**Case report:** A 35 -year-old Caucasian man referred by his dentist to Oral Medicine Unit of Dept. of Surgical, Oncological and Oral Sciences (University of Palermo) for a swelling of hard palate, with a slow growing during the last month. Intra-oral examination showed a fixed, rubbery, and painless mass on the hard palate; extra-oral inspection revealed unilateral proptosis of the left eye, appeared from seven days. Then, we programmed the following investigations: I) incisional biopsy of intra-oral lesion and histological evaluation; II) maxillofacial contrast-enhanced computed tomography (CT) to assess the extension of the palatal mass and the unilateral proptosis. Report and images of CT scan indicated the absence of palatal bone involvement of the intra-oral lesion and the presence of suspected intracranial lympho-proliferative mass that was compressing and dislocating the optic nerve. No relationship between two lesions have been appreciated. After, histopathological confirmation of mucoepidermoid carcinoma (MEC) of the oral cavity, the patient was firstly referred to the Plastic and Reconstructive Surgery (University of Palermo) for a total resection of intra-oral lesion. Subsequently, he was transferred to the Neurosurgery Unit for the management of intracranial lesion. Histological report of neurosurgical resection was cavernous angioma.

**Conclusion:** The Oral Medicine specialist may be able to intercept systemic diseases or diseases that affect multi-anatomical areas. Contemporary presence of two histo-morphological different disease in the same oro-facial district is rare and the correct intra-oral and extra-oral examination and management was fundamental for timely diagnosis and successful treatments.

### Treatment of localized langerhans' cell histiocytosis of the palate with a combined

## steroid therapy: report of a case

**Limongelli L., Tempesta A., Crincoli V., Favia G.**

Complex Operating Unit of Odontostomatology, "Aldo Moro"  
University of Bari, Italy

**Aim:** Langerhans' cell histiocytosis (LCH) can involve virtually any site and organ of the body, either as an isolated lesion or as a widespread systemic disease. Bone can be involved as a part of generalised disease or as a separate entity, formerly known as localized Langerhans cell histiocytosis (LLCH) or eosinophilic granuloma. Solitary bone lesions mainly affect the skull, mandible, and vertebrae with symptom that ranging from none to pain, swelling and pathological fracture. The aim of this work is to describe the treatment of a case of LLCH of the palate.

**Case report:** A 46-year-old woman came to our attention at Complex Operating Unit of Odontostomatology (University of Bari) referred from her general practitioner for an aggressive periodontal disease. The intraoral examination revealed a reddish bleeding pseudo-granulomatous mucosa lining the posterior third of hard palate bilaterally, an important gum line recession on 1.7 and 2.7, an attachment level loss of 9 mm and 8 mm respectively measured using a WHO probe on 1.7 and 2.7, mobility of grade I of this two teeth with spontaneous bleeding and pus discharging of the closed palatal marginal gingiva. The orthopantomography (OPT) revealed a horizontal and vertical resorption of maxillary alveolar bone around 1.7 and 2.7. The patient referred occasional pain in the region analysed. The total body enhanced spiral computerised tomography highlighted only the presence of osteolytic lesions in the posterior third of hard palate. Histological evaluation of the intraoral biopsy sample established a LLCH. Having excluded a multifocal form of LCH, a topical medical treatment was chosen. The patients underwent ten injection (once a month) of dexamethasone fial 4 mg/ml mixed with 0.8 ml of mepivacaine hydrochloride 20mg/ml with adrenaline 1:100000 in order to reduce the pain during and immediately after the steroid injection and to increase the duration of the steroid. Moreover, for ten months the patients had a domiciliary treatment consisting on the application of a gel of clobetasolo propionate twice a day on the palate using an individualised dental tray. Symptoms of pain were quickly relieved. Twelve months after the diagnosis, intraoral examination revealed the absence of the pseudo-granulomatous appearance of the mucosa lining the posterior third of the hard palate, the stability of 1.7 and 2.7. OPT and TC revealed a good osseous consolidation.

**Conclusion:** The monthly local injection of steroid and their daily domiciliary application completely resolved the case herein described so this medical treatment protocol could be considered successful and a valid non-invasive treatment option especially to preserve involved teeth.

## Oral squamous cell carcinoma: an epidemiological retrospective study of a thirty-year experience

**Limongelli L., Tempesta A., Crincoli V., Favia G.**

Complex Operating Unit of Odontostomatology, "Aldo Moro"  
University of Bari, Italy

**Aim:** Oral Squamous Cell Carcinoma (OSCC) is the most common malignant neoplasia of the oral cavity, arising from the epithelial lining of this one. The OSCC is one of the ten most frequent cancers in the world and the ranking varies a great deal among countries. It occurs mainly in middle-aged and older people. The aim of this study is to report on an epidemiological retrospective study of our thirty-year experience.

**Methods:** We report on 843 cases of OSCC diagnosed at the Complex Operating Unit of Odontostomatology (Policlinic of Bari) from 1986 to 2016. Data were collected using medical records. Statistical analysis of epidemiological data was carried out using Microsoft Excel. The staging was identified using WHO TNM cancer staging system.

**Results:** The analysis of the epidemiological data revealed that 545 OSCC occurred in males and 298 in females, with a male female ratio of 1,8:1. The average was 64,7 years (range 24-92); 67% occurred after the sixth decade of life, 19,1% in the sixth one, 9,4% in the fifth one, 2,9% in the fourth one and 1,6% before the thirty years. The 38% of the OSCC were on the tongue, 19% on the gingiva, 12% on the cheek, 7% on the palate, 5% on the mucous side of the lip, 3% on the floor of the mouth and 2% on the trigon; 14% of the OSCC involved more sites (gingiva-floor of the mouth-tongue, cheek-gingiva-palate). About the main type of presentation, the 44% were ulcers, 22% were nodules, 10% were leukoplakia and 4% were erythroplakia; the remaining 20% showed as a mix of the previously described presentation. Regarding the stage, 1% of the OSCC diagnosed were stage 0, 25% were stage I, 27% were stage II, 24% were stage III and 23% were stage IV. The average 5-year relative survival rate was 95% for cancers that have not spread (stage 0-I-II) and were treated whit our tridimensional surgical protocol, 62% for cancers that have spread to nearby lymph nodes

(stage III) and 38% for cancers that have spread to distant parts of the body. The percentage of the 5-year survival rate was respectively 38% and 20% for stage III and IV when the cancers occurred on floor of the mouth. The 70% of cancers that have not spread (stage 0-I-II) were early detected by general dentists; general practitioner, instead, often identified cancers in advanced status of disease (60% of stage III and IV).

**Conclusion:** The results of the statistical analysis carried out from the data collected during the retrospective study are perfectly agreed with the epidemiological data reported in the current scientific literature. Considering that approximately the 50% were diagnosed in advanced status of disease (III and IV stages) with low 5-year survival rates, the identification of rescue categories (middle-age and older people), most frequent site of occurrence (tongue) and type of presentation (ulcer), could allow as to set up programs of prevention and early detection more effective to propagate among the general practitioners that represent the first reference point for patients.

### High-level laser therapy in the treatment of the burning mouth syndrome

**Bardellini Elena\***, **Amadori Francesca\***, **Ferri Anna\***, **Conti Giulio\*\***, **Alessandra Majorana\***

\*Department of Medical and Surgical Specialities, Radiological Sciences and Public Health, Dental School, University of Brescia, Brescia, Italy.

\*\*University Vita-Salute S. Raffaele, Milan, Italy

**Aim:** This study aims to evaluate the efficacy of high-level laser therapy (HLLT)- in terms of pain and of quality of life- in patients affected by burning mouth syndrome (BMS).

**Methods:** This study was designed as a randomized double-blinded prospective study. Patients with diagnosis of BMS in the period from June 2012 to June 2015 were enrolled. The patients were randomized into two groups: group A received laser therapy (K Laser Cube 3®) once a week for ten weeks and group B sham therapy (placebo) with the same timing. Pain was evaluated through the Visual Analogue Scale (VAS); quality of life was assessed using the short form of the Oral Health Impact Profile (OHIP-14). The assessment was done at baseline and at the end of each treatment session. The researchers were blinded for the randomization allocation.

**Results:** A total of 85 patients were analysed. Group A (laser treatment) was composed of 43 people while group B (sham therapy) of 42 people. After

the therapy ended, the patients treated with HLLT showed a significant decrease in symptoms ( $p=0.0008$ ) and an improvement in the quality of life related to oral health ( $p=0.0002$ ).

**Conclusions:** HLLT demonstrated beneficial effects for the relief of the symptoms and for the quality of life in patients with BMS.

### The treatment of thrombocytopenic patients: a clinical trial

**Franco R.**, **Miranda M.**, **Basilicata M.**, **Rosa A.**, **Barlattani A. jr.**, **Agrestini C.**, **Tiberti U.**, **Bollero P.**

UOSD Diagnosis, Prevention and Oral Hygiene with Day Hospital Medical-Surgical General and Special for vulnerable patients, Polyclinic of Rome Tor Vergata, Rome, Italy

**Aim:** Chronic liver disease (CLD) is a progressive disease of destruction and regeneration of the liver leading to liver cirrhosis (LC). LC is the end stage of CLD. In addition to thrombocytopenia, patients with CLD suffer from several serious symptoms leading to liver failure. When medications fail to control CLD, the only effective therapy is liver transplantation. The patient with cirrhosis presents with a frequency ranging from 15-7% thrombocytopenia and clotting difficulties due to the lack of production of clotting factors. The etiology of thrombocytopenia is multifactorial. It depends on the portal hypertension which creates splenomegaly cause an increase of platelets within the organ and their degradation, other causes of thrombocytopenia can be the virus' HCV and alcohol abuse. A final cause in the process of study, in the case of autoimmune diseases, opsonization of platelets, increasing the splenic sequestration. Moreover, the same treatment with IFN-alpha for the treatment of viral liver disease produces a reduction of platelets. Thrombocytopenia is classified according to the platelet count in mild (75000-150000/mm<sup>3</sup>), moderate (50000-75000/mm<sup>3</sup>), severe (50,000/mm<sup>3</sup>). However, liver transplantation is associated with serious problems, such as donor shortage, surgical complications, organ rejection, and high cost. Before the transplant necessary to eliminate any possible infection of the mouth. The purpose of this study is to assess the ability to perform oral surgery on patients of patients with platelet counts less than 60,000/mm<sup>3</sup> in security.

**Methods:** Two thrombocytopenic patients due to liver cirrhosis and in need of liver transplant (Patient A 56,000/mm<sup>3</sup>; Patient B 55,000/mm<sup>3</sup>), were treated at the UOSD diagnosis, oral hygiene and prevention day hospital. Patients have turned to our center to eliminate any possible infection of the mouth



that could affect the transplant. The extraction were performed for oral surgery using atraumatic techniques. Before starting the surgical procedures it was performed infiltration of local anesthetic with vasoconstrictor (articaine 1/ 100.00). The vasoconstrictor is used as an adjuvant to decrease bleeding. For both patients have been performed multiple extractions (more than 3 dental elements). After the extractions was performed a thorough alveolar curettage, a wash of extraction sockets with hydrogen peroxide and was engaged sponge fibrin oxidized. At the end of the intervention the socket was sutured with 3/0 silk. The patients were observed for at least 3 hours afterwards. Patients have been revised to 7 days apart for the control of the wounds and the removal of the sutures.

**Results:** During the post-operative period and until the sutures were removed, there were no uncontrolled bleeding or issues worthy of clinical notes. Patients also showed good healing of the sockets.

**Conclusions:** We performed two operations on thrombocytopenic patients because of liver cirrhosis and we did not detect the excessive bleeding issues or other events worthy of clinical notes. In the literature there are many studies which claim the ability to perform safely dental operations on patients who have a platelet count above or equal to 50000/mm<sup>3</sup> and an INR greater than 2.5. This data is very important because you can avoid the risk of autoimmune response or infectious risk for the patient caused by the transfusion of blood components. In conclusion, patients with thrombocytopenia can be treated surgically by applying appropriate local hemostatic measures, such as the use of tranexamic acid and fibrin sponge oxidized, and performing surgery in protected regime.

### Glanzmann's thrombasthenia a rare disease: oral implication

**Franco R, Basilicata M., Miranda M., D'Ovidio N., Vazzana G., Rosa A., Barlattani A. jr, Agrestini C., Tiberti U., Bollero P.**

UOSD Diagnosis, Prevention and Oral Hygiene with Day Hospital Medical-Surgical General and Special for vulnerable patients, Polyclinic of Rome Tor Vergata, Rome, Italy

**Aim:** Glanzmann's thrombasthenia is one of the most frequent inherited disorders of platelet function. It is characterized by a marked reduction in or absence of the platelet fibrinogen receptor (gp IIb/IIIa), which leads to a clinical picture that includes mucocutaneous bleeding, normal or slightly decreased platelet count and normal platelet

morphology. These patients are often submitted to platelet and/or blood transfusions due to frequent bleeding and they may develop antibodies against the fibrinogen receptor gpIIb/IIIa glycoprotein. These sera do not usually react with thrombasthenic platelets. Thus it is often difficult to find a suitable donor for bleeding therapy and/or prevention in thrombasthenic patients. The importance of platelet aggregation disease forces patients to turn to hospitals to perform the scheduled dental procedure safely.

**Methods:** At the UOSD diagnosis, oral hygiene and prevention DH was performed dental extraction of elements 4.6-4.7 in a patient with glanzmann's thrombasthenia. The patient has followed a path of pre-hospitalization performing a visit with anesthesiologists, complete blood tests and cardiological visit. The patient was instructed on the pre-operative procedure to be performed. In fact, the patient 3 days before the intervention has performed a programmed therapy for the mouth with tranexamic acid (6 vials per day). The patient presented on the morning under antibiotic coverage, and after performing the above-mentioned prophylactic tranexamic acid. A peripheral block of the inferior alveolar nerve 'was performed together with a' plexus anesthesia using anesthetic with vasoconstrictor. The surgery was performed with atraumatic technique avoiding performing osteotomies and mucoperiosteal flaps. The dental extractions were performed and was applied in the socket sponge fibrin oxidized. At the end of the intervention it was applied with a 3/0 silk suture and was performed a cleaning with hydrogen peroxide. The patient was applied compressive gauze soaked in tranexamic acid. The sutures were removed at 7 days apart.

**Results:** The patient did not present excessive and uncontrolled bleeding during the postoperative period and during the days following the operation.

**Conclusions:** This study showed, although with the narrowness of the sample, which is possible to carry out simple oral surgery on patients with this rare disorder by applying local hemostatic maneuvers. The literature shows a lack of data on this subject, given the low frequency of the disease. There are 2 studies with 1 anticipates the need to transfuse platelets to the patient prior to the oral surgery and a second study provides an opportunity to carry out, with due attention paid local hemostatic, surgery scheduled oral surgery. New studies are necessary and the presence of a sample of the most recent patients to be able to deduce the clinically valid conclusions.

### Intratumour heterogeneity of OSCC revealed

## by multiple regions sequencing and MTDNA analysis: new insight on tumour biology and usefulness of circos plot in graphic visualization

**Gabusi A.\***, **Tarsitano A.\*\*\***, **Gissi D.B.\***, **Sozzi L.\***, **Luccarini L.\***, **Amadasi C.\***, **Morandi L.\*\***

\*Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences, University of Bologna, Italy

\*\*Department of Biomedical and Neuromotor Sciences, "M.Malpighi" Surgical Pathology Section at Bellaria Hospital, University of Bologna, Italy

\*\*\*Department of Biomedical and Neuromotor Sciences, Section of Oral and Maxillofacial Surgery at S.Orsola Hospital, University of Bologna, Italy

**Aim:** Intratumour heterogeneity (ITH) describes the hypothesis that genetic differences may exist among neoplastic cells inside the same tumour as effect of chromosomal instability and clonal evolution. So far, this theoretical model of tumour biology have only been suggested by the observation that histologic analysis of single tumours may in some cases undercover regions with different morphological alterations (multicentricity). Only recently, however, thanks to ultradeep sequencing technology, intra tumour heterogeneity can be properly demonstrated. Intratumour heterogeneity is believed to have significant implications in prognosis as it may explain cases of unresponsiveness to treatment especially in head and neck squamous cell carcinoma where relapses or nodal metastasis usually jeopardize surgical treatment. Aim of this study was to investigate intratumour heterogeneity in one case of Oral Squamous Cell Carcinoma (OSCC).

**Methods:** Multiple spatially separated samples were obtained from a single tumour and related metastasis of a 63 years old male patient with a diagnosis of T3N2cM0 oral squamous cell carcinoma(OSCC) of the tongue. 4 samples were obtained from the bulk tumour, 2 with histological features of invasive carcinoma and the other 2 with in situ carcinoma, 4 samples were obtained from nodal metastasis and 2 samples were obtained from regions histologically free from neoplasia for a total of 10 samples. Samples were genetically investigated for mutations in a panel of genes frequently mutated in OSCC (BRAF, P53,NOTCH1,PIK3CA, CTNNB1). MtDNA analysis was also performed. Values of mutations were graphically converted in circos plots using www.circos.ca web based platform, while MtDNA results were used to build phylogenetic trees.

**Results:** All genes investigated showed different degrees of mutational rates in the different regions of the tumour. In particular BRAF resulted mutated only in one out of 6 regions of the bulk

tumour and was not mutated in the remaining regions from nodal metastasis, suggesting that mutational profiles do vary from region to region of a same tumour. Also mtDNA analysis confirmed heterogeneity among samples. In some cases samples from histological or topographical similar regions resulted in belonging to distant branches of the phylogenetic tree. In particular one sample from histological normal mucosa showed phylogenetic similarity at MtDNA level with one sample from nodal metastasis suggesting metastatic potential in histological normal looking mucosa as well as " field cancerization".

**Conclusion:** Multiple region sequencing and mtDNA analysis of oral squamous cell carcinoma is able to demonstrate complex mutational landscapes within the same tumour, that may play a role in aggressive clinical behaviour such as metastatic development or relapses resulting from field effect. Circos plot and phylogenetic tree are useful graphic tools to visualize complex mutational patterns in oral squamous cell carcinoma.

## Osteonecrosis of the jaw in patients treated with Denosumab : a case series

**Gambino A.\***, **Erovigni F.\*\***, **Fusco V.\*\*\***, **Migliario M.\*\*\*\***, **Fasciolo A.\*\*\*\*\***, **Carossa S.\*\***

\*Department of Mechanical and Aerospace Engineering, Politecnico di Torino,Turin.

\*\*Department of Surgical Sciences, CIR Dental School,University of Turin, Turin.

\*\*\*Medical Oncology Unit, Ospedale SS Antonio e Biagio e C Arrigo, Alessandria.

\*\*\*\*Health Sciences Department, University of Eastern Piedmont "A. Avogadro," Novara.

\*\*\*\*\*Maxillofacial Surgery Unit, Ospedale SS Antonio e Biagio e C Arrigo, Alessandria.

**Aim:** This case series describes the course of osteonecrosis of the jaw (ONJ) in ten patients with metastatic bone tumors or osteoporosis treated solely with denosumab. Patients on denosumab may be more prone to developing ONJ even without a risk/precipitating factor and they may develop ONJ early in their denosumab therapy.

**Methods:** Ten patients were referred to Dental Services of the Oncological Network of Piedmont and Valle d'Aosta for evaluation of oral complaints such as exposed bone, jaw pain, non-healing extraction sites, and tooth mobility. All patients were treated with denosumab for management of metastatic bone tumors. No history of other antiresorptive agents was reported. The following clinical information was reviewed: demographics; primary cancer diagnosis;

sites of bone metastasis; history of chemotherapy; follow-up period (defined as time from onset of ONJ to last follow-up visit; history of active tobacco use; co-morbidities; site of ONJ lesion; precipitating factors for the development of ONJ; drug discontinuation; duration of drug holiday; and the clinical outcome and size of ONJ at last follow-up visit following drug holiday. ONJ outcome was divided into four categories: resolution (complete mucosal coverage of prior exposed bone); partial resolution (reduction in size of exposed bone); no change and progression (increase in size of exposed bone).

**Results:** The outcomes of ONJ in ten patients following a period of denosumab discontinuation after the onset of ONJ were: Ten patients (female n = 6, male n = 4; ages 52–78 years) presented with exposed bone in the jaw. The primary cancer diagnoses are as follows: 4 patient with breast cancer, 2 patients with prostate cancer, 1 patient with renal cell cancer, 1 patients with lung cancer. The patients with osteoporosis are 2. Nine patients had no history of smoking and no comorbidities (steroid use, diabetes mellitus and rheumatoid arthritis). At ONJ onset, the number of doses of denosumab treatment ranged from 4 to 20 doses, eight cases of ONJ involved the mandible. All patients presented non specific radiographic examination (TC) for ONJ; 4 had complete resolution of symptoms, 2 patients' ONJ progressed, 2 patients' ONJ was unchanged and in 2 patient there was partial ONJ resolution.

**Conclusions:** ONJ may develop earlier in patients receiving denosumab and the role of drug discontinuation prior to an invasive dental procedure or after the onset of ONJ still remains debatable. The role of drug discontinuation prior to an invasive dental procedure or after the onset of ONJ still remains debatable. The number of patients in this series is inadequate to draw any definitive conclusion or comparison, and further retrospective analyses as well as prospective studies of the effectiveness of denosumab holidays for ONJ prevention are indicated.

### Surgical treatment of medication-related osteonecrosis of the jaws

**Garrone Marco, Marino Roberto, Berrone Mattia, Val Matte, Gandolfo Sergio, Pentenero Monica**

Department of Oncology, Oral Medicine and Oral Oncology Unit, University of Turin, Italy

**Aim:** Bisphosphonates, antiresorptive and antiangiogenic therapies are widely used in the

management of metastatic disease involving the bone and in the treatment of osteoporosis. All these drugs are now recognized as potentially inducing Medication Related Osteo-Necrosis of the Jaws (MRONJ). Patients are referred to our clinic aiming to: 1) assess any need of oral treatment before starting the assumption of the drug in order to reduce the risk of MRONJ onset; 2) follow-up patients assuming or having assumed the drug in order to early detect the onset of MRONJ; 3) manage incident MRONJ patients.

**Methods:** Data on patients observed in a 6 years (January 2011–February 2017) period have been collected with particular attention for indications to treatment, medication type, incidence, surgical treatment and prognosis of MRONJ.

**Results:** 305 patients (162 males, 143 females) taking bisphosphonates or antiresorptive or antiangiogenic drugs were referred to our Department. MRONJ occurred in 54/305 patients (18%), after a mean latency time of 36 months after the first assumption of medication. Most of patients developing MRONJ suffered from bone metastases from oncologic diseases (76%) : breast cancer (41%), prostate cancer (34%), multiple myeloma (10%), other malignant tumors (15%). Nevertheless 24% of patients had osteometabolic disorders treated with oral bisphosphonates (alendronate, risedronate or ibandronate). The most relevant complication was pain, observed in 74% of cases. Mandible was the main site of MRONJ (70%). Half of MRONJ were related to dental extraction, while periodontal diseases or peri-implantitis were recognized in only 7% of cases. Of note 24% of cases had a spontaneous onset and wearing removable denture represented a trigger event in a high rate of cases (18%). Surgical treatment was performed in case of exposed and necrotic bone or fistulas that probes to bone with or without infection (AAOMS-SS stage 1–2). Therefore, 43 patients were treated, with 29 patients presenting relapse in less than 6 months, corresponding to an overall success rate of 67%. 27 Patients were treated under local anaesthesia, only performing debridement of necrotic bone. Fourteen of them were stage 1 and had a success rate of 79%. Conversely such kind of treatment performed in thirteen stage 2 patients resulted in a success rate of 46%. Two of the stage 2 patients undergoing relapse were later successful treated by marginal ostectomy performed under general anaesthesia. 71% of stage 2–3 patients undergoing at first marginal ostectomy under general anaesthesia had a successful treatment. Segmental ostectomy was performed in just two stage 3 cases without any relapse.

**Conclusions:** MRONJ management aims not only to cure patients but also to reduce morbidity and to

improve the quality of life in patients who have often a reduced life expectancy. With such premise, indications to conservative treatments should be guided by both life expectancy, performance status and MRONJ stage.

### Incidental findings detected with panoramic radiography: prevalence calculated on a sample of 2000 cases

**Ghassemzadeh S., Bacci C., Sbricoli L., Stellini E.**

Clinical Dentistry (Head Prof. Stellini E), Department of Neurosciences (Head Prof. Martini A), University of Padova

**Aim:** The aim of this study was to assess the prevalence of incidental findings, not strictly related to dentistry, viewed with panoramic radiography.

**Methods:** Between December 2013 and June 2016, panoramic radiographs (OPG) performed in University Hospital of Padova were retrospectively collected. These images were analyzed, searching for incidental findings, such as carotid artery calcification (CAC), elongated stylohyoid process (ESP), maxillary sinus pathology and any other pathologic condition, not strictly related to dentistry. All the information collected, was statistically analyzed, in order to find the prevalence, relating to sex, age and localization (right, left or both sides).

**Results:** A total of 2307 panoramic radiographs were analyzed and 2017 of them were included in the study. The mean age of the patients was 47 years and 53% of them were women, while 47% were men. The prevalence of CAC was 8.28% in total population, and it was higher in women (9.82%) than in men (6.54%). The mean age of people presenting CAC was 65.3 years. 48.5% of CAC were bilateral. When unilateral, the right side showed a higher prevalence, because 32.34% and 19.6% of CAC were assessed on the right and on the left side, respectively. The prevalence of ESP was 12.64% in total population (men: 13.82%; women: 11.60%). 84.71% of ESP were bilateral and, when present unilaterally, no difference was seen between the right and the left side. Only 13.33% of the ESP appeared segmented. In the panoramic radiographs where ESP was > 25mm, the highest prevalence of ESP was found in the 30-40mm-long range. The prevalence of maxillary sinus pathologies was 1.78% (men: 2.32%; women: 1.31%). Only 8.33% of these pathologies were bilateral, and, when unilateral, they were mostly present on the right side. Antral pseudocyst was the most commonly seen maxillary sinus incidental finding. Between the 71 other incidental findings (prevalence: 3.52%), sialoliths and tonsilloliths were assessed most

frequently (with a prevalence of 0.89% and 0.74%, respectively).

**Conclusion:** Due to the high prevalence of incidental findings detected with panoramic radiography, dental practitioners should be aware about the various pathologic conditions seen on the panoramic radiographs. Thus, an in-depth analysis of OPG may help to diagnose also pathologic conditions not strictly related to dentistry and a positive diagnostic finding on a routine panoramic radiograph may suggest further medical attention.

### Smiling to Wiskott-Aldrich syndrome: oral and orthodontic manifestations

**Albertini P.\*, Bassani L.\*, Ghilardi G.\*, Aiuti A.\*\*\*, Cicalese M.P.\*\*\*, Ciuffreda C.\*, Manuelli M.\*\*\*, Lucchese A.\*\*\*\***

\*Resident, Department of Orthodontics, Vita Salute San Raffaele University, Milan, Italy; Unit of Dentistry, Division of Orthodontics, Research area in Dentofacial Orthopedics and Orthodontics

\*\*Pediatric Immunohematology and Bone Marrow Transplantation Unit

\*\*\*Assistant Professor, Department of Orthodontics, Vita Salute San Raffaele University, Milan, Italy; Unit of Dentistry, Division of Orthodontics, Research area in Dentofacial Orthopedics and Orthodontics

\*\*\*\*Aggregate professor, Department of Orthodontics, Vita Salute San Raffaele University, Milan, Italy; Unit of Dentistry, Division of Orthodontics, Research area in Dentofacial Orthopedics and Orthodontics  
IRCCS San Raffaele Scientific Institute, Milan, Italy.

**Aim:** The oral cavity is an anatomical structure characterized by the juxtaposition of soft and hard tissues which is continuously subject to challenge by the external environment and foreign material. Disorders and discomforts caused by oral microorganisms are very common, in particular dental caries, periodontitis and halitosis. Oral diseases can be secondary to systemic pathologies such as Wiskott-Aldrich syndrome, a rare X-linked primary immunodeficiency. Despite progress in its diagnosis and treatment, prognosis for this condition remains poor. The aim of the present study is to find any common oral and more specifically orthodontic manifestations among individuals with Wiskott-Aldrich syndrome, in order to establish precautions that may help to improve patients' quality of life with particular regard to dental aspects.

**Methods:** Eighteen patients (18 males, mean age 11 ± 1 years) suffering from Wiskott-Aldrich syndrome were recruited between 2010 and 2015 from referrals to the Immunoematology Department at San Raffaele Hospital, Scientific Institute, Milan, Italy. Routine diagnostic records were obtained. The



following exams were performed for all patients: laboratory tests, microbiological evaluation, hematological and immunological evaluation, brain MRI, brain TC, orthopantomography and hand-wrist radiography, in order to evaluate the effects of Wiskott-Aldrich syndrome on growth. Finally, a thorough oral examination was performed.

**Results:** Medical signs and symptoms such as recurrent infections, eczema, bleeding, thrombocytopenia, anemia, petechiae, ecchymosis, hemorrhagic diathesis, major bleeding were usually observed. The oral examination revealed gingivitis, periodontitis, aphthous lesions, gingival bleeding, oral petechiae and severe oral infections (caries, pulpitis, abscesses). In regard to orthodontic aspects, a higher incidence of alterations in the physiological eruptive sequence with more cases of inclusions and transpositions was observed, probably due to untreated inflammatory and infectious processes. Infective processes affecting the permanent tooth may also result in malocclusion, which could possibly pave the way for future skeletal problems. Wiskott-Aldrich syndrome could also compromise and interfere with the orthodontic treatment which has the purpose of aligning teeth and solving skeletal issues.

**Conclusion:** Since Wiskott-Aldrich syndrome presents with a wide spectrum of symptoms and complications, it is mandatory to increase awareness of this entity and to apply a multidisciplinary approach that should include the dentist, in order to intercept any pathologies of the oral cavity and to improve patients' quality of life.

### Enrichment and characterization of cancer stem-like cells from an OSCC cell line

**Arena C.\*, Dioguardi M.\*, Giannatempo G.\*, Capocasale G.\*\*\*, Mastrangelo F.\*, Lo Muzio L.\***

\*Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

\*\*Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy.

**Aim:** Several recent studies showed that solid tumors contain a subpopulation of cancer stem cells (CSCs). CSCs play an important role in tumor initiation and progression and they are most likely the reason of cancer recurrence and metastasis. For further elucidation of the mechanism underlying the characteristics of CSCs, it is necessary to develop efficient culture systems to culture and expand CSCs. In this study, a nonadhesive culture system was used to generate spheres from the OSCC cell line, HSC-2. Subsequent investigations of their CSC properties, including colony formation capacity,

self-renewal potential, and cell invasion, were also performed.

**Methods:** A nonadhesive culture system was used to generate spheres from the HSC-2 cell line. The cell line was cultured in culture plastic wares with nonadhesive surface. 10 cm dish are made of nonadhesive for cells by coating with agarose thin films. Cells were plated at a density of 50000live cells/10 cm dish, and the culture medium was changed every other day until the sphere formation. Cell viability and migration were determined by MTT and wound healing assay.

**Results:** Spheres were formed cost-effectively and time-efficiently within 7-10 days. Moreover we proved that these spheres showed tumor initiating and self-renewal capabilities.

**Conclusion:** Typical tumor spheres appeared within 7-10 days. Compared to HSC-2 adherent cells, sphere cells showed a higher self-renewal and migration ability. Further studies will be performed to evaluate their tumorigenic ability in vivo and expression of stem cell specific markers (like OCT4, Sox2..) and drug resistance. Using this cost-effective and time-efficient culture system, a reliable model of enriching CSCs from OSCC cell line was established that can be used in cancer research.

### The malignant potential of oral lichen planus and oral lichenoid lesions: a systematic review

**Lajolo Carlo\*, Gioco Gioele\*, Lo Muzio Lorenzo\*\*, D'Addona Antonio\*, Troiano Giuseppe\*\*, Michele Giuliani\*\***

\*Department of Oral Medicine, Oral Pathology and Oral Surgery, Catholic University of the Sacred Heart, Rome, Italy

\*\*Department of Clinical and Experimental Medicine, University of Foggia, Foggia, Italy

**Aim:** Lichen Planus is a mucocutaneous inflammatory disorder which can affect either skin and/or mucous membrane; even if the etiology is still unknown, it accounts for an immune pathogenesis and affects from 0,5 to 2% of the studied population. Oral Lichen Planus (OLP) has been classified as a Potentially Malignant Disorder (PMD), nevertheless clinical and pathological diagnosis and its malignant transformation are still major points of debate. Aim of the present study is to perform a systematic review of the literature on the rate of transformation of OLP and OLL according to WHO or modified diagnostic criteria; furthermore clinical parameters predictive of transformation will be investigated. A secondary objective is to look for any evidence of a difference between OLP and OLL with regard to malignant transformation.

**Methods:** The systematic search and review processes

were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement criteria. PubMed, Scopus and Web of Science were used as search engines. Literature review considered paper published until June 2016. Inclusion criteria: full papers, English literature, peer-reviewed journals, observational studies both prospective and retrospective (i.e. cross-sectional, cohort, case-control or retrospective studies), more than 50 subjects. Exclusion criteria: case reports, reviews and papers where no distinctions were made between cutaneous and oral lichen planus. PRISMA protocol was used to evaluate and present results. PROSPERO registration code is CRD42016048529.

**Results:** The preliminary search retrieved 447 references through PubMed, 358 results through Scopus and 284 results through ISI web of Science. After the exclusion of studies that did not meet the inclusion criteria and removal of duplicates. All full texts of the articles considered valuable for the aim of our review were obtained and a critical evaluation was performed. Hand search of the reference list accompanying published articles gave no additional eligible articles. Our search identified a total of 40 publications. A total of 15,429 patients (mean age of 54 years) were followed for a mean period of 80 months and developed 174 Oral Squamous Cell Cancer (OSCC), with an overall transformation rate (TR) of 1.12%, an annual TR of 0.17 and a monthly TR of 0.014. Considering the WHO modified criteria and OLL, a total of 6385 patients were studied (mean age of 55 years) were followed for a mean period of 93 months and developed 86 OSCC, with an overall TR of 1.34%, an annual TR of 0.17 and a monthly TR of 0.014. Considering only the WHO modified criteria, a total of 3172 patients were studied (mean age of 55 years) were followed for a mean period of 133 months and developed 38 OSCC, with an overall TR of 1.19%, an annual TR of 0.11 and a monthly TR of 0.009. No significant differences were detected among different study groups. Being a female and having an erosive clinical presentation seem to slightly increase the transformation risk.

**Conclusions:** This systematic review confirms that OLP is a PMD and suggests that erosive type and being a female should be considered as risk factors. A strict clinical and pathological follow up should be done for OLP patients and major efforts should be done to define clinical and histological criteria in the diagnosis of OLP and to perform more sound methodological observational studies.

### Melkersson-Rosenthal syndrome diagnosed by lip biopsy: a case report of a rare disease

Giovannini Stefano\*, Cancian Mauro\*\*, Annalisa

Angelini\*\*\*, Marny Fedrigo\*\*\*, Sivoletta Stefano\*

\*Department of Neurosciences, Section of Dentistry

\*\*Department of Medicine, General Hospital and,

\*\*\*Department of Cardiac, Thoracic and Vascular Sciences  
University of Padova

**Aim:** Melkersson-Rosenthal syndrome is a rare neuromucocutaneous disease of unknown etiology. Diagnosis is based mainly on clinical detection of a triad of characteristic symptoms, such as oro-facial swelling, relapsing facial palsy and fissured dorsal tongue. Oligosymptomatic and monosymptomatic forms, however, are more common than the complete triad. Thus, in some cases histopathological examination of a lip biopsy is essential in diagnosing the disease. The aim of this work is to present an uncommon case of recurrent, apparently idiopathic angioedema in which we were able to establish a final diagnosis of Melkersson Rosenthal only after biopsy of the lower lip.

**Methods:** A 71 years old female looked for urgent care at the emergency room for sudden onset of right hemifacial paraesthesia, oedema of the lower lip and accentuation of an already known tinnitus. After three days of steroid and antihistamine therapy a complete resolution of symptoms was observed. One week later, however, the patient was readmitted to E.R. due to symptoms' recurrence and a worsening of lip oedema. The initial diagnosis was of Varicella Zoster Virus infection, and specific therapy was initiated. One month later, on further admission at the E.R. with the same clinical picture, a Quincke's angioneurotic oedema was suspected and a new course of antihistamines was prescribed. Unfortunately, treatment was not effective and after a few days systemic steroids were added. Finally, lack of response to any of the previous therapies suggested the possibility of Melkersson-Rosenthal syndrome and forced to perform a biopsy of the affected lip.

**Results:** Histological findings were consistent with a diagnosis of Melkersson-Rosenthal syndrome, showing non caseating granulomas (Mischer cheilitis) that ruled out alternative hypotheses as Chron's disease or sarcoidosis.

**Conclusion:** This case report confirms that Melkersson-Rosenthal syndrome can be characterized by rather aspecific symptoms, such as oro-facial swelling and hemifacial paraesthesia, and may present also in patients older than usually expected. In this setting, a careful lip biopsy is crucial to reach a correct diagnosis.

### DNA methylation analysis from oral brushing as a non invasive method to early detect oral

## squamous cell carcinoma

**Gissi D.B.\***, **Tarsitano A.\*\***, **Gabusi A.\***, **Sozzi L.\***, **Luccarini L.\***, **Amadasi C.\***, **Kavaja S.\***, **Morandi L.\*\*\***

\*Department of Biomedical and Neuromotor Sciences, Section of Oral Sciences

\*\*Department of Biomedical and Neuromotor Sciences, Section of Maxillo-facial Surgery at Policlinico S. Orsola-Malpighi, Bologna, Italy

\*\*\*Department of Biomedical and Neuro-motor Sciences, Section of Anatomic Pathology "M. Malpighi" at Bellaria Hospital University of Bologna

**Aim:** The unfavorable prognosis of Oral Squamous Cell Carcinoma (OSCC) is frequently related to late diagnosis in advanced stages as well as to secondary tumours development. A non invasive screening tool for the early diagnosis of OSCC may have a major impact on survival and quality of life and also may be helpful for an accurate monitoring of OSCC surgically treated patients. In OSCC various gene promoters were recently identified to show an aberrant methylation pattern related to early diagnosis, and prognosis. Furthermore an altered methylation pattern seems to be reliably detectable starting from body fluids as saliva and exfoliated cells. Aim of the present study was to propose and assess the feasibility of a non invasive method for early OSCC detection based on DNA methylation analysis of various gene promoters by bisulfite-Next Generation Sequencing (NGS) starting from non invasive collection specimens obtained with oral brushing.

**Methods:** oral brushing specimens were collected from 25 patients with an histological diagnosis of OSCC. Two different brushing specimens were collected in OSCC group: first one in lesion area and second one in clinically normal distant mucosa (cheek opposite). Oral brushing specimens were also collected from clinically normal oral mucosa of 40 healthy donors. A set of 13 previously described methylated genes in OSCC (ZAP70, KIF1A, LRRTM1, PARP15, FLI1, NTM, LINC0059, EPHX3, ITGA4, MIR193, GP1BB, MIR296, TERT) were investigated by bisulfite-Target Next Generation Sequencing (NGS) using MiSEQ platform (Illumina, San Diego, CA). For each gene the most informative CpG island was identified and an algorithm was utilized in each specimen to combine the promoter methylation values from all 13 genes. ROC curve analysis was performed to obtain an appropriate cut off level and Kruskal Wallis test and multiple range test were used to evaluate the presence of any between-group significant difference;

**Results:** The mean value of data obtained by gene combination in OSCC samples was significantly

different from that in normal mucosa of healthy donors. The mean value obtained in the group of normal distant mucosa of OSCC patients significantly ( $P < .01$ ) differed from both OSCCs and normal mucosa of healthy donors. Only the combination of all thirteen genes resulted highly sensitive and specific (Area Under Curve = 1). 5/25 (20%) samples from normal distant mucosa in OSCC patients showed higher values with respect to the cut off value.

**Conclusions:** Preliminary results confirmed the presence of an aberrant methylation status in OSCC and also revealed the presence of an altered methylation pattern in normal mucosa distant from OSCC area. Early diagnosis of OSCC may be of importance for clinical management, particularly in high-risk populations and a non invasive procedure based on DNA methylation analysis could be a highly sensitive and specific method to early detect OSCC starting from a non invasive, easy to perform, type of sampling.

## A comparison between clinical and pathological diagnosis with and without a tissue fluorescence visualization device (Velscope®)

**Golin F.**, **Cerrato A.**, **Pampaloni L.**, **Bacci C.**

Clinical Dentistry (Head Prof. E. Stellini), Department of Neurosciences (Head Prof. A. Martini), University of Padua

**Aim:** Early diagnosis of oral lesions should be one of the main targets for dentists, in order to prevent the development of oral squamous cell carcinoma and improve the overall survival rate. Unfortunately, the misdiagnosis rate after naked-eye examination is still high, estimated around 31.5% of cases, and it arises in case of malignant lesions (78.9%).

Given that tissue biopsy and histopathological examination remains the gold standard, various techniques have been described at this purpose, like stainings, auto-fluorescence visualization devices, chemiluminescence, cytological techniques, molecular and genetic analyses.

Concerning of tissue fluorescence visualization devices, they rely on the different wavelength at which abnormal cells emit their own light while excited by light of an appropriate wavelength.

The aim of the present study is a comparison between the misdiagnosis rate of oral lesions with and without an auto-fluorescence visualization device (Velscope®).

**Methods:** A case-group of 1406 patients and a control-group of 47 patients have been enrolled for this study. They all underwent a dental visit between January 2006 and December 2016 at the

Padua University Hospital, referring the presence of oral lesions and heterogeneous symptoms, such as pain, burning sensation, masticatory traumas and dysgeusia. Some lesions were asymptomatic. The patients of the case-group underwent a simple naked-eye examination, whereas the fluorescence visualization device (Velscope®) was used for the control-group, in addition to the clinical examination. All lesions involving connective tissues, mucous membranes and glandular tissues have been included for this study, whereas the lesions located outside the oral cavity have been excluded, as well as insufficient or damaged biopsies. All lesions have been subjected to biopsy and the withdrawals have been sent for an histopathological examination. A total of 1566 samples have been taken among the case-group, and a total of 51 withdrawals have been taken from patients of the control-group. All the data concerning visits and dental treatments were outlined using a specific server (Galileo) and consulting clinical reports.

**Results:** For the case-group, the diagnostic errors pertained to 23.8% of the benign lesions, 78.9% of the malignant lesions and 17% of precancerous lesions. The overall misdiagnosis rate for the control-group was 25.5% (38 diagnostic hypotheses of 51 have been confirmed at the histological examination). The diagnostic error pertained 50% of the malignant lesions, 35% of the benign lesions and 16.6% of precancerous lesions.

**Conclusion:** The oral cavity lends easily to a clinical examination during the routine dental visits. Due to the features of the oral tissues, however, the detection and diagnosis of oral lesion is neither simple nor immediate. The significant number of erroneous clinical diagnoses confirms how crucial the histological examination is. Undoubtedly, the misdiagnosis rate varies according to the clinicians' skills and experience, but a fluorescence visualization device may lead to a more accurate diagnosis, especially for general practitioners, and in particular for the detection of malignant and precancerous lesions. Lastly, a continuous and collaborative relationship between the clinician and the pathologist is really essential.

### Infective endocarditis: pathogens involved and antibiogram analysis in a retrospective study

**A. Grigoletto, A. Cerrato, V. Dotto, M. Nocent, C. Bacci**

Clinical Dentistry (Head Prof. Stellini E), Department of Neurosciences (Head Prof. Martini A), University of Padua

**Aim:** Several guidelines have been formulated to

prevent IE and its complications, these guidelines involve several medical specialists in addition to Cardiologists and Infective Disease specialists. The pathogens causing IE and their susceptibility to available antibiotics were analyzed in order to plan the most effective prevention and therapy and to avoid resistance phenomena.

**Methods:** 70 patients, 21 females and 49 males, were selected for this study. Every patient suffered from IE according to the Duke criteria modified by Li et al. and was treated at the Infective Diseases ward of the Azienda Ospedaliera of Padova from March to July 2012. The pathogens involved in every case of disease were researched, and, where found, their antibiogram response was analyzed. The mortality during the hospitalization was noted.

**Results:** Pathogens were found in the 75,5% of patients, mainly from blood culture, one patient was affected by multiple microorganisms. Staphylococcus resulted the principal bacteria involved in IE (33,2%). Staphylococcus Aureus was the most common pathogen (25 %), the second main group was represented by Faecalis Streptococci and Enterococci (24, 9%) followed by Streptococci (12,8%). Those agents are part of the oral bacterial flora. Other pathogens were observed in less than 3% of cases. 13 pathogens resulted resistant to one antibiotic group, 4 of them were resistant to multiple antibiotic families: 1 Escherichia Coli, 1 Coryne bacterium striatum, 1 Enterococcus Faecium, 1 Haemolyticus Coagulase negative Staphylococcus. 9 cases resulted resistant to Aminoglycoside, especially to gentamicin, 3 cases to penicillin and 2 cases to levofloxacin. Ciprofloxacin, Clindamycin and ceftriaxone result involved in one case of antibiotic resistance each. 4 patients reached out to exitus: in one case culture was negative, 2 cases were imputed to Enterococci and one to Gemella Morbillorum. Causative Faecalis and Faecium Enterococci were respectively resistant to Clindamycin and to aminoglycoside.

**Conclusion:** The most common microorganism responsible of IE was represented by Staphylococcus, in accordance with the literature data for industrialized countries. On the contrary the percentage of Faecalis Streptococci and Enterococci resulted higher than the data, and was responsible of 2 deaths. The 24% of microorganisms were drug-resistant, the 30,8% of these resulted multi-resistant. No death cases occurred in patients affected by streptococcal infective endocarditis. Enterococci with antibiotic resistance caused half of the deaths cases. Our results exonerate the oral flora for drug resistance and the major infective endocarditis complications; Aminoglycoside (especially gentamicin) often prescribed in dermatology, resulted one of the less effective



antibiotic. One of the focus point of this study is to suggest a prudent use of antibiotics in order to avoid the dangerous increase of antibiotic resistance.

### Therapeutic use of chlorhexidine and hyaluronic acid gel in cases of gingival oral lichen planus

**Lotito F.P.\*, Gambino A.\*\*\*, Broccoletti R.\*, Carcieri P.\*, Cabras M.\*, Arduino P.G.\***

\*Department of Surgical Sciences, Oral Medicine Section, CIR Dental School—University of Turin, Turin.

\*\*Department of mechanical and aerospace engineering, Politecnico di Torino, Turin, Italy.

**Aim:** The purpose of the study was to compare the therapeutic effects of the non-surgical periodontal therapy followed by administration of PAROEX Gum 0.2% for group 1 and PAROEX Gum 0.2% and Flogogel (a hyaluronic acid gel) for group 2 in patients with desquamative gingivitis by OLP.

**Methods:** 19 patients were recruited: 15 women and 4 men aged between 52 and 81 years, then divided into two groups. For each patient, the following parameters were evaluated: VAS (Visual Analogue Scale) Activity Score (rating severity of clinical signs) FMBS (Full Mouth Bleeding Score), PI (Plaque Index) and OHIP-14 (Oral Health Impact Profile-14). The same day of professional hygiene, each patient in Group 1 began the home oral hygiene procedures as indicated and administration of 5 ml of the mouthwash Chlorhexidine (Paroex Gum 0.2%) for 4 weeks in a row 2 times a day; each patient in group 2 had to add the chlorhexidine mouthwash to even the application of Flogogel on the injuries caused by OLP, at least 3 times a day for a period of 4 weeks. After the home therapeutic period were reassessed the parameters examined.

**Results:** We considered the mean values. The VAS in group 1, decreased from 4.50 to 2.50, in group 2 the value is reduced from 4,44 to 2,33. The Activity score in group 1 was 10,20 and is reduced to 6,80; in group 2 decreased from 12,11 to 7,00. FMBS index in group 1 decreased from 52.865% to 27.885%; in group 2 is reduced from 56.806 to 26.725; Plaque Index varies from 0.847 to 0.269 in group 1; in group 2 the initial value was 1.004 and the final 0.237. The OHIP-14 in group 1 decreased from 11.60 to 2.674, and in group 2 decreased from 10,545 to 3.30. The Wilcoxon test results reveal that there is a statistically significant difference in both groups between T0 and T2 of all the indexes, in fact, all p-values < 0.05. Therefore statistical analysis has shown that there are no statistically significant differences between the improvements obtained with the two protocols.

**Conclusion:** We have to consider some risks of bias in this study: the low sample size, patient compliance, the home application of the hyaluronic acid gel used. Therefore, the key point is to achieve adequate removal of plaque and calculus together with a meticulous oral hygiene in patient with OLP, in agreement with the literature. In agreement with scientific literature it can be stated that a hyaluronic acid gel at 0.2% can be a valuable additional aid relatively more secure and may be considered as a treatment option based on a long term. The study shows that the results obtained for the two groups are similar, showing as the only non-surgical periodontal therapy with help of chlorhexidine to improve the patient's plaque control are sufficient to achieve excellent improvements of all the considered indices for the two groups of patients with OLP taken into consideration. The hyaluronic acid can accelerate healing of soft tissue promoting re-epithelialization.

### Tongue "heart" ulcer from bisphosphonate

**Mascitti Marco\*, Luconi Elisa\*, Bambini Fabrizio\*, Mangione Corrado\*\*, Giuliani Michele\*\*, De Lillo Alfredo\*\*, Procaccini Maurizio\*, Santarelli Andrea\***

\*Department of Clinical Specialistic and Dental Sciences, Marche Polytechnic University, Ancona, Italy.

\*\*Department of Clinical and Experimental Medicine, Foggia University, Foggia, Italy

**Aim:** Oral bisphosphonates are an important class of drugs for the treatment of metabolic and oncological diseases related to the skeletal system. Well-known side effects include ulcerations of the upper gastrointestinal tract and development of osteonecrosis of the jaws (BRONJ). However, there is little awareness regarding the risk to induce oral ulcerations, whose mechanism is not well understood. The aim of this study is to present an unusual case of oral ulcerations due to incongruous administration of oral bisphosphonates.

**Methods:** A 71-year-old woman was referred because of a 9-month history of erosive mucositis of the tongue, accompanied by intense pain and dysphagia. Examination showed a large heart-shaped ulceration of the tongue, 2 by 3 cm in diameter, with regular invaginated margins and a necrotic center. Patient's drugs for systemic conditions included a proton pump inhibitor, dual antiplatelet therapy, a benzodiazepine plus a selective serotonin reuptake inhibitor, an ACE inhibitor plus diuretic plus calcium antagonist and an oral bisphosphonate. Routine blood examination was normal, direct and indirect immunofluorescence were negative, histologic and microbiologic evaluations, including those for

cancer, pathological bacteria, mycosis, infectious and non-infectious granulomatous disease were unrevealing. At subsequent direct questioning, the patient did report taking oral alendronate (70 mg per week) for 8 months for severe osteoporosis, allowing the tablet to dissolve in her mouth since her general practitioner indication, as she stated.

**Results:** Chronic inflammatory ulceration due to alendronate was diagnosed. The patient was instructed to swallow the alendronate whole and with plenty of water without holding the tablet in her mouth. After 2 months, the ulcer had substantially improved.

**Conclusion:** There are few case-reports in literature related to bisphosphonate-induced oral ulcerations; nevertheless, they seem to be more common as the small number of published cases indicates. However, considering the widespread use of bisphosphonates among people, the incidence is still low. This side effect is mostly associated with alendronate, but also etidronate and risedronate have been reported in literature as possible cause of oral ulcerations. The majority of patients affected by bisphosphonate-induced oral ulcerations are women, with a mean age of 70 years. Most of patients assumes a daily dose of alendronate (10 mg per day), while the remaining cases assume a once-weekly dose of 70 mg. Data in the literature suggest that a once-weekly dosing regimen can improve upper gastrointestinal tolerability and it might help to decrease the adverse effect of oral ulcers. The most affected areas are lips, tongue and hard palate; ulcerations are accompanied by burning, pain and, consequently, impaired eating and swallowing habits. The mean time of drug use until the onset of oral ulcers is less than 3 weeks, ranging from 2 days to 13 months. Effective treatments for the remission of these ulcerations are the discontinuation of oral bisphosphonates, the change of the drug's route of administration, or the reeducation about the correct intake of oral bisphosphonates. Complete healing requires from some weeks to several months.

### The interaction of PPI (proton pump inhibitors) on bone turn over

**Maggio S., Ragazzoni F.M., Carloni G., Roselli L., Papa E., Plini M., Di Trani G., Maltese F.**

Diagnosis, hygiene and oral prevention for heart and social vulnerable subjects with dental day hospital. Polyclinic of Rome, Tor Vergata. University of Rome, Tor Vergata

**Aims:** the topic of our review is about the interaction between gastroprotective drugs and bone turn over on patients that dealt with a protective gastric

therapy. After having analysed the studies of the latest 5 years, it has been treated the possibility to have an increasing risk in bone problems (fractures, osteoporosis and bone turn over alteration) considering that this drug should have also an effect on osteoclastic H<sup>+</sup>-ATPase so that they would be unable to perform their task.

**Methods:** every study has been based on different diagnostic investigation that focused on osteoblastic and osteoclastic cells. The patients that we collected need a gastroprotective therapy: they have used non steroidal drugs for a long period and they also present disorders linked to gastric mucosa. The most part of the patients analysed are between 50 and 60 years and they don't present any type of systemic pathology that could change studies results. The most widely used drugs are omeprazole, pantoprazole and rabeprazole. Cytological tests:

- Von Kossa staining and toluidine blue staining: evaluation of osteoclastic phenotype
- TRAP activities: evaluation of osteoclastic resorption in presence of RANKL
- MTT assay: quantification of cell viability
- gene expression: evaluation of influence of PPI on gene expression
- Western Blot: evaluation of omeprazole on the induction of expression NFAT C1

Haematological/biochemical tests: bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, azotemia, creatinine, serum calcium, osteocalcin, parathyroid hormone, Excretion of urinary calcium, Creatinine, DPD urine (urinary deoxyypyridinoline), gastrin, pepsinogen. Strumental tests:

- Endoscopy: this diagnostic procedure has been used in patients with dyspepsia or gastrointestinal bleeding during the study. Only patients with gastric ulcer that included bleeding were considered. Gastric ulcer was then defined by endoscopic and histological examination using the Sydney System.
- BMD: the measurement of 'bone mass density' has been implemented using dual energy X-ray absorptiometry using a Lunar-IDEX on the lumbar spine and femoral neck. The BMD was calculated individually based on the area expressed in square cm. The diagnosis of osteoporosis is been made considering a BMD lower or equal to 2.5. Patients who have shown a lower rate of BMD or equal to 2.5 before the beginning of the study were automatically excluded.

**Results and conclusions:** The development of alterations in cellular activity through a direct cellular damage caused by Pantoprazole appears unlikely. Different concentrations of omeprazole does not alter the cell viability nor of osteoclasts nor of osteoblasts but under the stimulation of the PPI, the ability of osteoclasts to resorb the mineralized bone

matrix seems to be inhibited, while the ability of osteoblasts to synthesize new matrix is increased. The PPI block the H + ATPase osteoclast inhibiting the physiological remodeling function. This resulted in a not significant change of bone turnover. What seems evident in agreement with multiple studies is that there still is a reduction of the basic BMD following a chronic administration.

### Geographic tongue: an easy algorithm for the young clinician

**Mainardi Beatrice, Lissoni Alessandra, Moreschi Chiara, Toma Fabiola, Gastaldi Giorgio, Abati Silvio**

University Vita-Salute San Raffaele, Dental School, Dept. of Dentistry, Chairman Prof. E.F. Gherlone, Milano, Italy

**Aim:** The aim of this study is to describe six iconic cases evidenced in the students' clinical training department of our dental clinic and to define a series of clinical features of this condition that might be useful for the diagnosis by younger clinicians. Geographic tongue (GT), also known as benign migratory glossitis or erythema migrans, is a chronic inflammatory immune-mediated oral disorder that affects the tongue and rarely other areas of the oral mucosa. GT is a common oral condition affecting about 3% of population and it occurs more frequently below fifty years. Its etiology is still unknown. No risks or severe consequences have been related with GT, although it has been demonstrated an association between GT and psoriasis; atopic allergies represent another frequent link with the condition, probably with a genetic origin. GT is often associated with fissured tongue (>10% of the cases). The typical lesions of GT are unstable and migrating. GT shows different appearances and sizes of the lesions with an alternation of red areas of atrophic epithelium frequently outlined with circinate whitish borders of regenerating filiform papillae. The lesions have characteristic patterns, oblate/circular, wavy or ring-shaped. The circular lesions tend to remain homogenous and they do affect the dorsum of the tongue. The ring-shaped ones appear as spirals and tend to last longer, while the wavy pattern affects more commonly the sides of the tongue. Patients usually refer to it as asymptomatic, and just in few cases it has been reported a mild irritation due to increased sensitivity in the affected areas particularly with salty, spicy and acidic foods. This condition alternates periods of remission and aggravation in severity of the symptoms described.

**Methods:** We administered a specific questionnaire to evaluate demography, history and clinical

features of the disease to six patients visited at the Dental School UniSR; the questions inquired clinical complaints and features based on the most common characteristics of GT as described in the evidence-based literature. On this basis, subsequently we developed a simple clinical-based diagnostic algorithm to help the dental students in the assessment of this condition during the oral examination.

**Results:** Six patients have been visited in the students training department at the Dental School UniSR, Milano, complaining alterations and/or symptoms affecting the tongue. The patients were (i) DG, female, 30 yrs (ii) AB, male, 42 yrs (iii) AS, female, 22 yrs (iv) EMJ, female, 29 yrs (v) LR, male, 43 yrs (vi) DPA, female, 36 yrs; all of them responded positively to the following questions: (1) do you have an unusual appearance of the tongue borders an/or dorsum? (2) The tongue alterations are red with or without whitish edges? (3) The tongue alterations change in shape, size and/or location over time? (4) Do you feel discomfort and/or burning in these areas, spontaneously or provoked by acidic/spicy foods or beverages? During the clinical examination all the patients showed alterations on the tongue borders or surfaces supporting the diagnosis of geographic tongue.

**Conclusions:** an oral clinician, although inexperienced, could recognize a GT with an adequate level of confidence, assessing the medical history of the patient and performing a thorough intraoral examination.

### Possible therapeutic role of resveratrol and polydatin in oral carcinoma. *In vitro* studies

**Mangione C.\*, Perrone D.\*, Ardito F.\*, Campisi G.\*\*\*, Mascitti M.\*\*\*, Lo Muzio L.\***

\*Department of Clinical and Experimental Study, University of Foggia, Foggia, Italy

\*\*Department of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo, Italy

\*\*\*Department of Clinical Specialistic and Dental Sciences, Marche Polytechnic University, Ancona, Italy

**Aim:** Nutraceuticals are natural substances with several effects (antiinflammatory, antineoplastic, immunomodulating and others). Moreover, nutraceuticals have safety pharmacological profile because of their natural origin. Polydatin and Resveratrol are nutraceutical object of this study. Both of them are used since a long time in Chinese traditional medicine. The aim of this study was to evaluate the *in vitro* effects of these nutraceuticals on the adhesion, migration, and invasion of three

different oral squamous cell carcinoma (OSCC) cell lines.

**Methods:** We used 3 cell lines of OSCC: PE/CA-PJ15, PE/CA-PJ15D and PE/CA-PJ-49 cell lines. At first, were evaluated the effects of Polydatin and Resveratrol on cell adhesion and proliferation by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay (MTT Assay) and xCELLigence RTCA-DP assays both on Resveratrol and on Polydatin at different timepoints (24-48-72h) and concentrations (10-20-40 µg/ml). By these early studies, Resveratrol showed more significant results than Polydatin, therefore were performed more studies, Scratch Wound-Healing assay and fluorescence analysis only on Resveratrol. In the Scratch Wound-Healing assay, the migration was monitored over time (30 minutes, 1.5-3-4.5-6-7.5 and 24 hours) through images acquisition at the optical microscope (Nikon Eclipse Ti-U) which allowed to visualize the wound repair process, and cell motility. The quantitative analysis of migration capabilities was conducted using the TScratch software. Fluorescence analysis was obtained through ReadyProbes® cell viability Imaging Kit (Invitrogen, CA, USA), using µ-Slide VI (Ibidi, USA) treated with different concentrations of Resveratrol (20-40µg/ml for 24-48-72h), following all the above manufacturer's notes. The images have been obtained using the fluorescence microscope EVOS FL™ Cell Imaging System (Thermo Fisher Scientific, USA). All experiments were conducted for three times, each of which with triplicated samples. All results are presented as mean±SEM. To establish statistical significance, it has been used t-Student test and ANOVA variance analysis to get cross comparison between the groups. The values of p-values were considered significant for p<0.05.

**Results:** Resveratrol and Polydatin treatments inhibited cell adhesion. Resveratrol treatment exhibited a significant concentration- and time-dependent inhibitory effect on cell proliferation rate in all OSCC cell lines. In fact, proliferation was reduced by 50% for treatments with 20µg/ml of Resveratrol at 24 hours (p <0.0001). However, from the data obtained on the lines treated with Polydatin, it has not been possible to observe a statistically significant cell viability decrease compared to control even at concentrations above 20µg/ml. In fact, at concentrations of 20µg/ml we can observe reduction around of 25% cell viability. At increasing doses, the results are superimposable. Viability tests confirmed a proportional reduction at concentration and at duration of Resveratrol treatments. Even the migration was reduced with high significance (p <0.005). Fluorescence images confirmed these data, showing that after 48h by treatment with Resveratrol there was a considerable

cell viability reduction, directly proportional to the concentration used.

**Conclusion:** Resveratrol, in vitro, has halved cell viability in 24h at 40 µg/ml. It had significantly reduced cell adhesion, proliferation and migration of OSCC cells, suggesting that it might serve as chemopreventive agent for reducing invasion and metastasis of OSCC. Further in vitro and in vivo studies would allow to investigate Resveratrol chemical characteristics and its pharmacological use in oral inflammatory, immune and neoplastic diseases.

### Peri-implantitis and medication osteonecrosis of the jaw: a case series

**Nisi Marco\*\***, **Mariotti Stefania\***, **Karapetsa Dimitra\***, **Gennai Stefano\***, **Ramaglia Luca\*\***, **Graziani Filippo\***, **Gabriele Mario\***

\*Department of Surgical, Medical and Molecular Pathology and Critical Care Medicine, University of Pisa, Pisa, Italy

\*\*Department of Neurosciences, Reproductive and Odontostomatological Sciences, School of Medicine, University "Federico II" of Naples, Via S. Pansini 5, 80131 Napoli, Italy

**Aim:** Medication-related osteonecrosis of the jaw (MRONJ) is a side effect of antiresorptive medications (IV and oral BPs, rank ligand inhibitor like denosumab) and antiangiogenic medications. Dental implants are believed to be a risk factor for developing MRONJ. Aim of the present study was to present a case series of MRONJ around dental implants.

**Methods:** A retrospective study was designed. All patients diagnosed with MRONJ associated with dental implants referring to the Department of Dentistry and Oral Surgery of the University Hospital of Pisa from January 2012 to December 2016 were included. Diagnosis of MRONJ was made according to the 2014 American Association of Oral and Maxillofacial Surgeons (AAOMS) classification. Data concerning demographic and medical background, type and duration of antiresorptive medication and osteonecrosis characteristics were collected. Logistic regression analysis was used to evaluate the influence of various risk factors on the MRONJ staging.

**Results:** Fifteen patients presenting peri-implant bone osteonecrosis were identified and included in the present study (13 females, 2 males; mean age: 61,4 years). Seven patients were receiving intravenous bisphosphonates (Zoledronic acid 4mg IV) for the treatment of oncologic pathologies: metastatic breast cancer (2 patients, 29%); multiple myeloma (3 patients, 42%) and metastatic



lung cancer (2 patients, 29%). Seven patients were taking oral bisphosphonates for the management of osteoporosis (Alendronic Acid). Only one patient received denosumab 120 mg for the treatment of metastatic breast cancer. Six patients were smokers, three patients were affected by diabetes and five patients were suffering from hypertension. Eight patients were taking steroids. Twelve patients suffered from periodontal disease. Characteristics of MRONJ lesions - The most frequent stage of MRONJ was stage II (7 subject, 47%), whereas stage I (3 subject, 20%) and stage III (5 subject, 33%) were less common. MRONJ lesions were mainly symptomatic (14 subject, 93%) and accompanied in the vast majority of the cases by bone exposure (9 subject, 60%) and suppuration (12 subject, 91%). Lesions were prevalently located in the mandible (11 lesion 73%). The dental implants involved in the MRONJ lesions were 24; three patients had dental implants (n = 3) without osteonecrosis. Treatment protocols - All patients who presented with purulent or painful MRONJ associated with dental implants were initially treated with oral antibiotics (Amoxicillin with clavulanic acid 2 g/day for 14 days) and were clinically evaluated two weeks later. There were no signs of complete healing at this stage, only stabilisation of necrosis and alleviation of pain have been reported. Patients were then listed for implants remove and local debridement.

**Conclusion:** Peri-implant MRONJ is undoubtedly an emerging clinical reality. All patients who have dental implants and are about to start receiving antiresorptive therapy should be adequately informed about the increased risk of MRONJ around dental implants.

### Appropriateness of drug prescription and procedure in dentistry: a survey on 200 sicilian practitioners

**Campisi G.\*, Maniscalco L.\*\*\*, Di Fede O.\*, Matranga D.\*\*\*, Marotta M.\*\*\*\*, Oteri G.\*\*\*\***

\*Dept. Surgical, Oncological and Oral Sciences, University of Palermo, Palermo

\*\*Section of Oral Medicine, University Hospital Policlinic "P. Giaccone" Palermo

\*\*\*Dept. of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro", University of Palermo, Palermo

\*\*\*\*Dept. of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Messina

**Aim:** The National Italian project "The more does not mean the better" inspired by the American project "Choosing wisely" has the aim to prevent useless medical tests, treatments and procedures

in all fields of Medicine and Dentistry. The clinical appropriateness of drug prescriptions and procedures performed by Sicilian dental practitioners was evaluated through a questionnaire approved by "Società Italiana di Patologia Orale" (SIPMO) and "Società Italiana di Chirurgia Odontostomatologica" (SIdCO). Aim: to investigate the clinical appropriateness of commonest diagnostic exams, pharmacological treatments and procedures in dental practice in Sicily.

**Methods:** A questionnaire including 10 items with multiple responses on topics of dentistry and oral medicine has been administered to 200 subjects that were: a) Medicine Doctors (MD) with specialty in Dentistry; b) Doctor of Dental Surgery (DDS); c) DDS with specialty in Oral Surgery or in Orthodontics. Information about gender, year of birth, academic degree, graduation year, type of specialty, specialty year and professional activity (i.e. public, private or both) was also collected. Categorical variables were summarized with counts and percentages, whereas continuous variables were specified with mean and standard deviation. Multiple correspondence analysis was applied to identify the fields of dental and oral medicine practice, which need education and training. Data were analyzed using R software (version 3.3.2).

**Results:** The sample includes MD with specialty in dentistry (20%), DDS (70%), DDS with specialty in Oral Surgery (3%), DDS with specialty in Orthodontics (7%). Among the subjects, about 86% answered correctly to questions about biopsy, prescription of topical and systemic antifungal drugs and tooth extraction whereas about 40% answered wrongly to questions on antibiotics and rx OPT exams. Of note, half of the subjects answered incorrectly to the item "Who needs of prevention on tumors of oral cavity?". On average, 23% of specialists made two errors and 19.5% made three errors. Based on multiple correspondence analysis, there is more appropriateness, evaluated as correct prescription, of i) topical and systemic antifungal drugs, ii) teeth extraction, iii) treatment of ulcerative lesions. Conversely, there are deficiencies for antibiotics prescription appropriateness.

**Conclusion:** The most commonly observed deficiencies in dental practice deal with the prescription of antibiotics whereas there was appropriateness in i) prescription of topical and systemic antifungal drugs, ii) in the extraction surgery and iii) in the treatment of ulcerative lesions. The high percentage of mistakes about the prevention of cancer in oral cavity poses some alerts and it should be better investigated. The results suggest that more attention should be paid on the prescription of antibiotics, in the prescription of diagnostic exams and in oral cancer early diagnosis.

## Bilateral palatal swelling as first sign of recurrence of a mantle cell lymphoma: a case report

Mauceri R.\*, Di Fede A.\*, Capocasale G.\*, Dioguardi M., Giannatempo G., Di Lorenzo S.\*\*\*, Tozzo P.\*

\*Dept. of Surgical, Oncological and Oral Sciences, University of Palermo, Palermo

\*\*Dept. of Clinical and Experimental Medicine, University of Foggia, Foggia

\*\*\*Dept. of Surgical, Oncological and Oral Sciences, Plastic and Reconstructive Surgery, University of Palermo, Palermo

**Aim:** Mantle-cell lymphoma (MCL) is a rare and aggressive form of non-Hodgkin lymphoma with a poor prognosis. Usually, such lymphomas occur in the lymph nodes, up to 30% of the cases show an extra-nodal pattern. The oral cavity is rarely affected, showing an incidence of 0.1–5% among patients; however, many MCL lesions in the hard palate have been reported in literature. To achieve a definite diagnosis of MCL, it is necessary to carry out the immunohistochemical staining, with or without the assistance of molecular techniques. Early diagnosis, especially for recurrence, is necessary.

**Case report:** In 2017, a 74-year-old partially edentulous male patient was referred to our sector for a bilateral swelling of the palate. Anamnestically, the patient was affected by bone MCL (stage IV); it was diagnosed in September 2011 and, after 6 cycles of R-BENDA (combination of rituximab and bendamustine), was defined in complete remission. Intraoral examination revealed a firm diffused bilateral swelling on the hard palate, the overlying mucosa presented a corrugated non-ulcerated red-purple surface. Radiographically, a magnetic resonance imaging was requested, imaging signs were unremarkable. An incisional biopsy was carried out to confirm the suspect of recurrence. Microscopically, a proliferation of diffused lymphocytic infiltrate constituted by numerous lymphocytes of small size was observed. The immunohistochemical panel showed a diffuse positivity pattern to cyclin D1+, CD5+. The neoplastic lymphocytes showed negative immunohistochemical staining to: CD3, CD10, CD23. Ki-67 immunoreaction was evident in 15–20% of the malignant cells. Patient was subsequently referred to the Department of Oncology for the management.

**Conclusion:** Non-Hodgkin lymphomas are a heterogeneous group of lymphoproliferative malignancies that are much less predictable than Hodgkin's lymphoma and have a far greater predilection to disseminate to extra-nodal tissues. Occasional indolent multifocal lesions of the oral cavity should be carefully investigated; indeed,

during the differential diagnosis is fundamental to remember that unusual lesions like malignant lymphomas can occur. We have described here a case of bilateral palatal swelling as the first sign of recurrence of MCL; this lesion should be meticulously investigated because it may have an aggressive clinical course. Furthermore, a detailed morphologic evaluation with thorough immunophenotyping and genotyping is mandatory for an accurate diagnosis.

## Innovative approach to the treatment of oral manifestations of GVHD using hemo-components

Miranda Michele, Raffone Carlo, Gargiulo Loren, Gianfreda Francesco, Mirabile Daniele, Liciani Francesca, D'Ovidio Nicolò, Agrestini Corrado, Bollero Patrizio

Department of Dental and Oral Pathology, "Tor Vergata" University of Rome, Italy

**Aim:** GVHD in buccal mucosa, tongue and lips leads to lichenoid lesions, ulcers, atrophy, mucocele, dysgeusia, scleroderma, salivary glands hypofunction, xerostomia, tooth decay and fungal infections. Aim of the study is to evaluate efficacy and safety of topic use of platelet gel in clinical management of oral ulcers as oral manifestations of GVHD. Innovative and quick treatments are possible thanks to CSE transplant center and transfusional center of Rome Tor Vergata Policlinic.

**Methods:** 12 patients treated with allogeneic transplant of hematopoietic stem cells were evaluated. All of them developed oral ulcers as cGVHD manifestations. Median of time between CSE transplant and onset of GVHD ulcers was 4 months and median of time before the lesions were treated with platelets gel was 7 days. Microbiological contamination of lesions and presence of pain were considered. Response to treatment is been evaluated in relation to the reduction of ulcers dimensions, to the involution of pain and presence of granulation tissue after every application of platelet gel. Thanks to Vivostat system, platelets concentrations is 7–10 time higher when compare to blood donor. Final product must be used within 2 hours. Gel is applied on oral and perioral GVHD ulcers every 2 weeks away from meal. Despite isn't possible to cover the mucosal lesions with medications, oral treatment resulted as effective as skin treatment.

**Results:** Complete response is been documented in patients after a median number of applications of 2,5 (range 2–9). No adverse effects have been observed except for mild burning sensation described by few patients during first delivery. During follow up weren't observed relapses of ulcers in earlier treated

regions, except for 1 case after 5 months from the platelet gel delivery. These promising results shows the safety and effectiveness of platelet gels, as valid therapeutical local treatment in oral ulcers managements related with severe cGVHD manifestations.

**Conclusions:** Main advantage is the fast resolution of pain with an improvement of quality of patients' life. Probably there's a correlation between clinical conditions and methods for preparation of platelet gel. Topic application of platelet gel is feasible, safe and effective. Despite the limited number of patients, we can assess that is evident how much PRP and platelet gel can be effective in clinical situation to prevent relapses in patients after CSE transplant. For final validation other studies are needed.

### Use of systemic and in situ lactoferrin in MRONJ surgical management: proposal for a therapeutic solution

**Montori A., Rocchetti F., Tenore G., Gaimari G., Cassoni A., Palaia G., Romeo U.**

Head-Neck Unit, Policlinico Umberto I, Sapienza University of Rome, Italy, Head: Prof Antonella Polimeni

**Aim:** Lactoferrin (Lf) is an antimicrobial and iron chelator glycoprotein contained in exocrine secretion, including saliva, and in secondary granules of neutrophil granulocytes. Lf performs its antibacterial and antiviral action through two pathways: the first deprives bacteria of the iron they need for their reproduction and for biofilm formation; the second contributes to restore the inflammatory homeostasis which is essential for tissue health modulating the level of cytokines, like IL-6. The study intends to propose the use of systemic and in situ Lf effectiveness in the healing of oral mucosa and bone tissue affected by medication-related osteonecrosis of the jaw (MRONJ), after surgical excision or spontaneous elimination of necrotic bone.

**Methods:** We considered 10 patients with clinical and radiological diagnosis of MRONJ according to SICMF-SIPMO classification, of these:

- Stage 1: 3 asymptomatic patients
- Stage 1: 1 symptomatic patient
- Stage 2: 3 asymptomatic patients
- Stage 2: 2 symptomatic patients
- Stage 3: 1 patients

Four of these patients are men, six are women; six patients are non-smokers and four are tobacco smokers also during the treatment. In the run-up to surgery, one patient has taken Bevacizumab; one

has taken Denosumab; 8 have taken Zoledronic Acid. All the patients were subjected to a bone removal by traditional surgical technique or spontaneous sequestrectomy; placement of greasy gauzes with powdered Lf and administration of two Lf tablets a day in the pharmaceutical form of Forhans Gengi-For® right after the surgery. Our protocol has also dictated: seven days of antibiotic therapy (Amoxicillin and Clavulanic Acid 1 gr and Metronidazole 250mg twice a day) before and after the surgery; antiseptic therapy with Chlorhexidine 0.2% rinses; two cycles of 10 days of Lf tablets, with an interruption of 10 days among the cycles. Greasy gauze was removed two days after the surgery instead the sutures were removed after 10 days. Furthermore, post-surgical pain was evaluated through a Numeric Rating Scale (NRS). The average of the collected NRS values is equal to 3.

**Results:** after fourty days from the surgery, out of the ten patients:

- 2 relapsed
- 4 reached the complete healing
- 4 are still recovering

No collateral effects were reported caused by Lf.

**Conclusion:** Lf can be a valuable aid in MRONJ post surgical management since its antibacterial and anti-inflammatory properties, easy to prescribe and no side effects are reported. The minimal number of patients and the absence of a control group, until today, does not allow us to state that Lf has a key role in healing tissue.

### Use of Lixenil® in the management oral lichen planus. A preliminary study

**Moreschi C., Cefis N., Abati S., Cappare P., Gastaldi G., Gherlone E.F.**

Dental School Vita-Salute San Raffaele University, Milan, Italy

**Aim:** Oral lichen planus (OLP) is a relatively common potentially malignant disorder of uncertain etiology with a malignant transformation rate lower than 0.5% per year. Clinical features include reticular white lesions, plaque-like lesions or erosive lesions. Lesions may be asymptomatic or cause pain and burning sensation. The management of the symptomatic forms includes the use of topical corticosteroids or tacrolimus. Other drugs, such as retinoids or cyclosporine have not proved better and they may have adverse effects. The aim of the study is to evaluate the effect of a new local drug, Lixenil®, for the improvement of pain and burning sensation of OLP. In addition we evaluated the reduction in size of the lesions comparing photographs before and after the treatment.

**Methods:** Thirteen patients affected by OLP were selected in the Department of Dentistry of San Raffaele Hospital. The diagnosis of OLP was made both clinically and hysto-pathologically with scalpel biopsy. They were nine female and four male, all patients were no-smokers and the age range of the patients was 40-70 years old. Six patients had white reticular lesions, three patients had erosive and ulcerative lesions, two patients had plaque-like lesions and two patients had desquamative gingivitis. Patients in treatment with topical corticosteroid were excluded. The patients were asked to use Lixenil® for one month, two times a day. We gave to each patient a pain and burning sensation score: 1- no pain reduction; 2- mild pain reduction; 3- high pain reduction. We evaluated clinically both the reduction of the white lesions and the erosive one. The analysis was made comparing photographs before and after the treatment with Lixenil®. Two trained clinicians separately analyzed the cases.

**Results:** Ten out of thirteen patients had an improvement in the burning sensation and pain; two patients had mild pain reduction and one patient had no pain reduction. No patient worsened his/her condition. Clinically we found a reduction of lesions size in a half patients. No differences were found between the white reticular form, the plaque-like form or the erosive one.

**Conclusion:** Lixenil® can be useful in the management of symptomatic Oral Lichen Planus. We obtained a reduction of the pain and the burning sensation and a reduction in size of white and erosive lesions

### An atypical oral ulceration on the buccal mucosa

**Mureddu Maura\*, Mergoni Giovanni\*, Martella Eugenia Marta\*\*, Mancini Cristina\*\*, Meleti Marco\*, Vescovi Paolo\*, Manfredi Maddalena\***

\*Oral Medicine and Oral Surgery Laser Unit; University Center of Dentistry; Department of Medicine and Surgery; University of Parma - Italy

\*\*Pathology, University Hospital of Parma, Parma, Italy, Italy

**Aim:** The aim of this study was to describe a traumatic ulcerative granuloma with stromal eosinophilia (TUGSE) with an atypical lymphoproliferative process of B lymphocytes.

**Methods:** A 72 year-old female patient was referred to the Center of Oral Medicine, Pathology and Laser Surgery of the University of Parma, because of a symptomatic, one week standing ulcer in the right buccal mucosa. Medical history disclosed the presence of diabetes, hypertension and osteoporosis. The lesion measured approximately 4 cm in the

larger size and showed an area of ulceration with well-demarcated margins as well as a mild coverage of fibrin. An origin from dental traumatism was excluded because the patient was edentulous in the right side. Even if on palpation a soft consistence could be detected, clinical appearance could not exclude a malignant infiltrative process. Working diagnosis included ulcerative granuloma with stromal eosinophilia, necrotic ulcer associated with vasculitis, lymphoma and squamous cell carcinoma.

**Results:** Initially antibiotic therapy, antiseptic gel (chlorhexidine) and analgesic were prescribed. Since the lesion persisted, two incisional biopsies with quantic molecular resonance scalpel were performed. The histopathological examination demonstrated an atypical lymphoproliferative process of B lymphocytes. Immunohistochemical analysis showed the presence of CD3+, altogether with large size CD20+, CD79alpha+, MUM1+ and numerous elements CD30+ and EBV- EBER+. To exclude a lymphoma further evaluations including blood count and blood chemistry, protein electrophoresis, immunoglobulin dose, TC scan of facial bones and bone marrow biopsy were requested. All the exams resulted negative and a definitive diagnosis of TUGSE with atypical B lymphocytes proliferation was made. During the 8 weeks following the biopsy we observed a spontaneous and progressive resolution of the lesions.

**Conclusions:** TUGSE can occur anywhere in the oral cavity, and the most common sites include the ventral surface of the tongue, buccal mucosa, vestibule and floor of the mouth. Most cases heal without any intervention. In rare subsets of TUGSE, including our case, the lesion does not appear to be associated with trauma and a predominance of B and/or T lymphocytes are observed histopathologically. Because some of these cases show monoclonality, particularly the recurrent ones, they need immunohistochemical evaluation, long-term follow-up and occasionally systemic evaluation including bone marrow biopsy to rule out the presence of lymphoma. The nature of these atypical eosinophilic ulcerations is not fully elucidated and to establish if they represent a single pathosis or a variety of disorders that share stromal eosinophilia is an area for future research.

### A severe and multidrug-induced gingival overgrowth in a kidney transplant recipient: a case report

**Nagni Matteo, Lissoni Alessandra, Moreschi Chiara, Cappare Paolo, Gastaldi Giorgio, Abati Silvio**

University Vita-Salute San Raffaele, Dental School, Dept. of



Dentistry, Chairman Prof. EF Gherlone, Milano, Italy

**Aim:** The prevalence of drug-induced gingival overgrowth is rising since the increasing use of medications that may exacerbate this condition. A severe overgrowth of the gingiva may cause problems such as difficulty with chewing foods, speaking, swallowing, maintaining good oral hygiene standards and obviously, esthetic problems. Bacterial periodontal disease and teeth dislocation may arise as well as a consequence of the disease. The medications that cause this potentially severe side effect are mainly immunosuppressant (cyclosporine), anticonvulsants (phenytoin), calcium channel blockers (nifedipine), ACE-inhibitors and beta-blockers agents. The gingival enlargement usually affects the mandible more than the maxilla and it involves more the buccal surface than the lingual. The more severe cases affect the young adults than the elderly. If the medication can be discontinued and the overgrowth it is not much prominent, it tends to regress spontaneously. The aim of this report was to present a unique case of an enormous gingival hyperplasia in a kidney transplant recipient patient treated for years with several immunosuppressive and antihypertensive drugs.

**Methods:** A male patient of 57 years old came in the Oral Pathology Unit at the Dental School UniSR, Milano. He complained that he couldn't speak normally or eat or clean his teeth and his physician referred him for a specialty visit with the oral medicine expert in our department. His medical records reported bilateral nephrectomy for polycystic disease with complete renal failure, successively treated with renal dialysis and then underwent kidney transplant about 7 years ago. Since then, the patient began a multidrug immunosuppressive and antihypertensive therapy that included medications as cyclosporine, mycophenolate, nifedipine, ramipril, sotalol and oxybutinine. The clinical examination showed a severe gingival hyperplasia with massive growth of upper and lower gingiva both in the buccal and lingual surfaces. The gingival mucosa was inflamed with localized areas of ulceration and the tooth crowns were almost completely covered by the gingival tissues. In the orthopantomogram he presented many fractured roots in the upper and lower arch with teeth dislocation and periodontal disruption. The oral medicine expert with the oral surgery students decided to treat the patient as soon as possible.

**Results:** Sextant gingivectomy were successfully executed prior to antibiotic coverage and 0.2% chlorhexidine rinses b.i.d.; the treatment of choice was conventional cold-knife surgery assisted with diode laser to remove the overgrowing mass of gingiva. A dental hygienist performed scaling and

root planning intra-procedure. Laser was mainly used to control the bleeding. The gingival tissues removed were sent for histological examination and further investigation. Irrigations with chlorhexidine 0.2% were thoroughly used in the sites of removal and then as rinses before and after the surgical procedure.

**Conclusion:** it is essential to verify the medical history of the patient. If the patient is currently taking medications such as immunosuppressant, calcium channel blockers and ace-inhibitors, and presents gingival hyperplasia, it is highly recommended to send a referral to his physician and to keep his hygiene status at optimal levels to prevent the worsening of the condition that may just worsen the patient's quality of life.

### Application of the accuvein system for the diagnosis and therapeutical approach of intraoral vascular malformations

**Pampaloni L., Cerrato A., Sbricoli L., Bacci C.**

Clinical Dentistry (Head Prof. E. Stellini), Department of Neurosciences (Head Prof. A. Martini), University of Padua

**Aim:** Intraoral vascular malformations are benign vascular lesions, generally asymptomatic, which may occur almost everywhere in the oral mucosa. In most cases these lesions don't require any treatment, but the intervention is recommended in case of high bleeding risk and it may consist in sclerotherapy, laser photocoagulation, embolization, cryotherapy, and surgery. The AccuVeinAV400® is a portable instrument, used by many clinicians to locate superficial veins, so it can be useful in various professional fields (aesthetic, surgical, radiological, cosmetic, cardiological...). It provides a map of the vasculature on the surface of the skin, but no attempt has been done as far as oral mucosa is concerned. The aim of the present case presentation is to report on the application of this device in order to detect and treat intraoral vascular lesions, to quantify the intraoperative bleeding risk and to check the treatment effectiveness.

**Methods:** A healthy 82-year-old female patient was referred to the Dental Clinic, Padua University Hospital in May 2016. At the intraoral examination, the clinicians noted the presence of a roundish red and blue elevation, sized 5x3mm, charged to the left retrocommissural area of the oral mucosa. The lesion had been present for four years, but it had recently grown in size, so the patient complained about recurrent bleeding episodes secondary to masticatory trauma. Vascular origin

of the lesion had been confirmed by compressive contact between a glass lens and the lesion (the lesion disappeared under compression, because there was no more blood supply) and by the use of the AccuVein400 Vein Viewing System®. This tool exploits infrared rays to detect the hemoglobin in the blood vessels and shows a map of these vessels directly on the skin or on the mucosa overlying the veins. After local anesthesia with Mepivacaine Hydrochlorate 3% without vasoconstrictor, the treatment consisted in a 830nm diode laser photocoagulation (1,5 – 2,5W). Lasers emit a precise beam of concentrated light energy, well-absorbed, depending on the wavelength, by hemoglobin. As a result of the interaction between the laser and the vascular malformation, a photo-thermal event occurs, so light is transformed into heat and coagulation begins at about 50°C. The treatment effectiveness was checked immediately after the procedure and at the one-month follow-up visit both with the clinical examination and by the AccuVein400 Vein Viewing System®.

**Results:** A six-month follow-up visit was performed after the procedure, in order to confirm the treatment success. Both the intraoral clinical examination and the AccuVein System confirmed the decrease of the lesion size and of the vascular component in particular. No more bleeding episodes had been referred by the patient, who reported an improvement of her masticatory comfort.

**Conclusion:** The AccuVein400 Vein Viewing System® has been created to perform more effective venipuncture procedures with less patient discomfort, but it could have various applications. One of them could be the diagnosis, the treatment monitoring and the follow-up of intraoral and extraoral vascular lesions. It can be useful also to discriminate between benign vascular lesions and other neoformations, which can be similar at the clinical examination. This tool is easy to use, handheld and lightweight, it doesn't require any contact with the patient's skin nor calibration and it also allows the clinicians to check the deepest part of the lesion, which might not be visible at a clinical examination. It could maybe reduce the recurrence rate.

### The potential role of optical coherence tomography in oral medicine: a systematic review

Romano Antonio, Gentile Enrica, Maio Claudio, Paparella Renato, Salerno Carmen, Laino Luigi, Serpico Rosario, Lucchese Alberta

Multidisciplinary Department of Medical-Surgical and Dental

Specialties, University of Campania – Luigi Vanvitelli, Naples, Italy

**Aim:** The introduction of optical coherence tomography (OCT) in dentistry enabled the integration of already existing clinical and laboratory investigations in the study of the oral cavity. OCT gives the operator the ability to perform real-time non-invasive diagnostic examination at a microscopic level. OCT technology can provide high-resolution microscopic images of biological tissues by measuring the intensity of backscattered and reflected light, with axial and longitudinal resolution, estimated respectively at 10 µm and 2–4 mm. This systematic review, presents an overview of the literature, to evaluate the usefulness of OCT for diagnosing oral cavity lesions and its role in oral medicine, and to identify limitations in prior studies so as to improve OCT applications.

**Methods:** We performed a review of the literature using different search engines (PubMed, ISI Web of Science, and the Cochrane Library) employing MeSH terms such as "optical coherence tomography" and "OCT" in conjunction with other terms. The validity of the studies included in this work was established according to the following selection criteria: OCT studies of the soft tissues of the oral cavity in humans, in vivo, published in English, and available in full text. Reviews were excluded.

**Results:** Initial results were 3155. In conclusion, there were only 27 studies which met our selection criteria. We decided to allocate the 27 selected items into three groups: healthy mucosa; benign, premalignant, and malignant lesions; and oral manifestations of systemic therapies or pathological conditions.

**Conclusions:** The results showed that OCT allowed non-invasive real-time visualisation of tissues at a microscopic level, directly in situ, in a manner that is comfortable for the patient. The images obtained were of good quality, and there was high correspondence between in vivo OCT and histology images. Despite all these advantages, literature on this topic is inadequate. Most of the studies concern hard tissues. The paucity of work conducted in oral soft tissues suggests that more OCT analyses of these tissues are necessary. Although the OCT is an easy way to perform test, and although it offers an attractive diagnostic and monitoring prospect for soft tissues of the oral cavity, further studies are needed to complete the current knowledge of this imaging technique.

### Analysis of medication-related osteonecrosis of the jaw (MRONJ) using the data from the Italian spontaneous reporting system of adverse drug events

Pavanelli Francesca\*,\*\*\*\*, Vecchiattini Renata\*,\*\*\*\*,

**Delfino Marcello\*\***, **Carlo Sgarbanti\***, **Marra Anna\*\***,  
**Fedele Daniela\*\***, **Scanavacca Paola\*\***, **Trombelli  
Leonardo\*,\*\*,\*\***

\*Dental Unit, University Hospital, Ferrara, Italy

\*\*Department of Pharmacy, University Hospital, Ferrara, Italy

\*\*\*Research Centre for the Study of Periodontal and Peri-implant  
Diseases, University of Ferrara, Italy

\*\*\*\*Department of Biomedical and Specialty Surgical Sciences,  
University of Ferrara, Italy

**Aim:** The aim of this study was to evaluate the incidence of Medication-Related Osteonecrosis of the Jaw (MRONJ) lesions using the Italian Drug Network (RNF) Database provided by Italian National Pharmacology Agency (AIFA).

**Methods:** Adverse events recorded (ADR) from January 2008 to December 2010 and from from January 2013 to December 2015 in the Natiolan Pharmacovigilance Network (RNF) database were downloaded from the AIFA website. (<https://www.agenziafarmaco.gov.it/Farmacovigilanza/>). The adverse events in "reac" were coded according to the terminology preferred by the Medical Dictionary for Regulatory Activities (MedDRA). MEDDRA Standardized Question (SMQ) chosen was "Osteonecrosis". Preferred term (PT) chosen was "maxillary/mandibular osteonecrosis" Suspected drugs were extracted and analyzed in this study. For signal detection, general qualitative judgments were used.

**Results:** MRONJ cases were 1,38% of all RNF ADRs between 2008 and 2010, and 0,71% of all RNF ADRs between 2003 and 2015. A total of 1780 ADRs were evaluated, after exclusion of reports submitted from others body sites (i.e. Femur necrosis) and duplicate cases (1799). Overall, patients' gender was available for all ADRs, with 81,4% female between 2008 and 2010, decreased to 71,2% between 2013 and 2015. Between 20008 and 2010, 697 ADRs were included for analysis, compared to those included between 2013 and 2015 (1083). When listed, breast cancer, prostatic cancer, myeloma and osteoporosis were detected as clinical condition in 58,7% of MRONJ cases. 41% ADRs involved patients between 60 and 79 age. Median higher values of MRONJ cases were referred to zoledronate, alendronate, ibandronate, pamidronate and denosumab respectively. Although 42% of ADRs came from "literature" source, 53% ADRs came from Clinicians, but less tha 2% came from Dentists and Pharmacists.

**Conclusion:** MRONJ is a rare but serious complication of cancer treatment or osteoporosis management. The clinical report confirms that antiresorptive medications such as oral or intravenous bisphosphonates and/or denosumab are the most common risk factors for developing MRONJ. The

risks of MRONJ related to antiresorptive therapy is well recognized by Clinicians. Further studies are required to draw conclusions, but nowadays this information about MRONJ will prove beneficial to both patients Pharmacists and Clinicians.

### In vitro effects of curcumin on squamous cell carcinoma of tongue

**Perrone D.\***, **Ardito F.\***, **Panzarella V.\*\***, **Campisi G.\*\***, **Laino  
L.\*\*\***, **Lo Muzio L.\***

\*Department of Clinical and Experimental Medicine, University of  
Foggia, Foggia, Italy

\*\*Department of Surgical, Oncological and Oral Sciences, University  
of Palermo, Palermo, Italy

\*\*\*University of Campania "Luigi Vanvitelli", Napoli, Italy

**Aim:** Squamous cell carcinoma of the tongue (TSCC) is one of the most common malignant tumors in the oral cavity often characterized by poor prognosis, in fact the overall five-year survival rate is less than 50%. The high morbidity and mortality of oral cancers are largely due to rapid tumor growth, frequent tumor recurrence, and metastasis. Therefore, it is important to identify and develop novel agents which could simultaneously target abnormal proliferation, apoptosis, invasion, and metastasis of tongue cancer. Use of nutraceuticals as possible adjuvant in the treatment and prevention of cancer is largely studied. In particular, curcumin, a polyphenolic compound derived from dietary spice turmeric, has been used for decades in Chinese medicine for its many pharmacologic effects, including anti-inflammatory, antioxidant, and antiangiogenic activities. Curcumin showed anti-cancer properties in many tumors; here we aimed to investigate the anti-proliferative, anti-migratory, and anti-invasive properties of curcumin on three TSCC cell lines.

**Methods:** The effects of curcumin on TSCC cells (PE/CA-PJ15, PE/CA-PJ49 and HSC-3) were investigate using different concentrations (1, 5, 10, 20 and 50 µM) and different treatment time points (24, 48 and 72 hours). The cell adhesion and proliferation were evaluated with xCELLigence RTCA-DP system. The cell viability was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). ReadyProbes® Cell Viability Imaging Kit (Invitrogen, CA, USA) was used to fluorescence analysis. We used scratch wound healing assays to quantify cell migration in the absence or presence of Curcumin at various concentrations. After treatments with curcumin, levels of the PAR4 and Oct4 were determined by Western Blotting.



**Results:** Through MTT assay, a reduction of 50% is observed in PE/CA-PJ49 cells treated with 5-10  $\mu\text{M}$  of curcumin at all time points, while for the HSC-3 and PE/CA-PJ15 cells a 50% reduction in cell viability is observed for cells treated with 10-20  $\mu\text{M}$  of curcumin at all time points. This data was confirmed by xCELLigence Analysis and by fluorescence images. Through the scratch wound healing assays, the wound size is showed directly proportional to the treatment with curcumin in manner concentration- and time-dependent, unlike in the untreated control. We noticed an increased expression of PAR4, pro-apoptotic protein, for concentration higher than 10  $\mu\text{M}$  at 24 hours. In addition, there was an inhibition of OCT4 with 50  $\mu\text{M}$  of curcumin at 24 hours, which showed that curcumin can inhibits tumorigenesis.

**Conclusions:** These results encourage the potential use of curcumin as an anti-cancer agent in TSCC. Further in vivo and in vitro studies are needed to confirm these effects; in fact tissue targeting and enhanced bioavailability of curcumin using novel drug-delivery systems with minimum side effects will lead this promising nutraceutical to the forefront of therapy for the treatment of human diseases such as cancer in the near future.

### Oral and periodontal conditions of patients affected by medication-related osteonecrosis of the jaw (MRONJ)

**Petrucci Valentina\***, **Vecchiatini Renata\*\***, **Mobilio Nicola\*\***, **Cuneo Antonio\*\*\*\***, **Frassoldati Antonio\*\*\*\*\***, **Trombelli Leonardo\* \*\* \*\*\*\***

\*Dental Unit, University Hospital, Ferrara, Italy

\*\*Department of Biomedical and Specialty Surgical Sciences, University of Ferrara, Italy

\*\*\*Research Centre for the Study of Periodontal and Peri-implant Diseases, University of Ferrara, Italy

\*\*\*\*Hematology Unit, University Hospital, Ferrara, Italy

\*\*\*\*\*Oncology Unit, University Hospital, Ferrara, Italy

**Aim:** Antiresorptive agents such as bisphosphonate (BPs) effectively reduce skeletal-related events incidence in patients with metastatic bone cancer and multiple myeloma, thereby placing them at potential risk for developing medication-related osteonecrosis of the jaw (MRONJ). Although MRONJ onset and progression is due mainly to BPs drug type and overall dose, many local risk factors, such as periodontal conditions have been recognized. Aim of this study was to evaluate MRONJ patients oral conditions, before and during MRONJ onset and to analyze the association between periodontal disease and MRONJ onset, considering the impact

of non surgical periodontal therapy to reduce risk factors.

**Methods:** This retrospective study included MRONJ patients referred to Dental Clinic, University Hospital, with a history of antiresorptive agents therapy among those treated at Hematology and Oncology Unit of different Hospital of Ferrara district, focusing on both medical and dental databases at single-center. All participants underwent complete oral and radiographical examination and clinical parameters records (PPD, BoP, PII, mobile dentures examination). Then all parameters were merged to assign each patient a comprehensive risk evaluation score for MRONJ.

**Results:** During 36 months observation time 320 patients, eligible for antiresorptive agents therapy, mean age of 67 years (range 32-81yrs), received complete dental examination, non surgical periodontal therapy, dental extraction and prosthesis conditioning. Individual risk for MRONJ was calculated for each patients during first visit and after 3 months at least. 31 patients developed MRONJ (mean drug treatment cycles: 10 [range 4-38]). For those patients developing MRONJ, clinical parameters records showed higher percentage when compared to overall population (+6% mean value). Considering risk scores calculated for all patients, a correlation between HIGH risk score and MRONJ was observed, according to Dental Clinic method with high sensibility (96%).

**Conclusion:** According to literature bad periodontal, dental and oral conditions are major risk factor for MRONJ onset. MRONJ is a clinically significant adverse effect of antiresorptive agents. MRONJ with BP treatment typically occurred within 3 years from the start of the treatment with BPs. Despite the clinical correlation between BPs and MRONJ, a definitive causal relationship has yet to be established. A close and mandatory preventive program for oral health, involving a multidisciplinary approach should be developed for all patients eligible for antiresorptive agents to partially reduce MRONJ incidence.

### Pigmented lesions of the oral mucosa: analysis of dermoscopic features

**Lorenzini G.\***, **Rubegni P.\*\***, **Petti M.**

\*Department of Dentistry, University of Siena, Siena

\*\*Department of Dermatology, University of Siena, Siena

**Aim:** Dermoscopy is a non invasive diagnostic technique; it is widely used in dermatology by clinicians in the study of pigmented lesions of the skin and especially in the early diagnosis of



cutaneous melanoma; its use in dentistry for the analysis of pigmented lesions on the oral mucosa is rather limited and still little studied, it lacks consistent data on dermoscopic semiotics of oral pigmented lesions. Aim of the study is to analyze the dermoscopic features of pigmented lesions of the oral cavity and / or lips, malignant and benign, in order to identify those most indicative of malignancy and avoid in the future unnecessary biopsies, sometimes very annoying for the patient.

**Methods:** We conducted a cross-sectional observational study in patients with pigmented lesions of the oral cavity and / or lips; for each lesion were acquired a clinical image and a dermoscopic image, obtained by a microscopic digital device with polarized light Mic-Fi (ITALECO, Rivoli, TO, Italy); all lesions were subjected to histological examination with the exception of those which were classified as physiological pigmentations considering their clinical characteristics. The lesions were classified according to the anatomical location and according to histological diagnosis. In dermoscopic images, there were identified, for each lesion, the types of pattern present and their number and colour. The data, collected in a table, were analyzed to identify the factors most indicative of malignancy, then we have done the Student t analysis to 95% in a queue to demonstrate the statistical significance of this association.

**Results:** We included in the study 49 pigmented lesions of the oral mucosa and / or lips; the analysis of collected data has shown a correlation between the malignancy of the lesion and the higher number of patterns within it; comparing the average of the number of patterns in benign and malignant lesions using the Student t test we obtained a p-value <0.05 and t- value > 1.7 value.

**Conclusion:** The statistical analysis showed that lesions with a higher number of patterns have a greater probability of being malignant lesions. Although these first data are extremely interesting, we believe it is necessary to continue the study and increase the number of samples, in order to be able to evaluate the association between specific patterns and malignant lesions.

### Salivary biomarkers for the diagnosis of cancers and systemic diseases: a comprehensive review

**Pezzi Margherita, Giovannacci Ilaria, Manfredi Maddalena, Bonanini Mauro, Vescovi Paolo, Meleti Marco**

Unit of Oral Medicine and Oral Laser Surgery  
Academic Center of Dentistry  
Departement of Medicine and Surgery, University of Parma, Italy

**Aim:** Development of reliable biochemical methods to detect limited quantity of molecules or traces of substances in fluids has had a terrific impact on several branches of medicine, including early diagnosis and prognosis of diseases. Most human nonblood fluids (e.g, saliva) contain small concentration of molecules directly or indirectly deriving from acute or chronic pathologies, affecting tissues and organs not in anatomical proximity with the site of fluid production. Such molecules are generically named "markers" or "biomarkers". The aim of the present comprehensive review is to present the state of the art on the topic of salivary biomarkers for the diagnosis of several pathologic processes, including cancers and systemic diseases.

**Methods:** We searched the Medline database using as entry terms "saliva and systemic diseases", "saliva and diagnosis", "salivary markers", "salivary biomarkers", "saliva and cancer", "saliva and carcinoma", "saliva and tumors". Only papers published after 2000 were included. No restrictions about type of study have been applied. Studies not in English have been excluded. The research generated a list of 18672 papers. Titles and abstracts (where available) were carefully evaluated and 101 studies have eventually included and considered for the present review. Full text of all studies were retrieved and carefully read. Data extrapolated were subclassified as follows: name of Authors, year of publication, disease evaluated, type of molecule (biomarker) investigated (classified as 1: "proteins and/or metabolites" and 2: "molecules belonging to the genome" (DNA, RNA)), biochemical or biophysical methods applied for the detection of markers and, where available description of sensor used.

**Results:** Seventy-four out of 101 studies (73%) have been published within the last 5 years (2011-2016), the remaining 27 dating not earlier than 2002. Researches focused on a single disease included chronic pathologies such as degenerative neurologic disorders (Parkinson's disease, Alzheimer's disease): 5 studies; inflammatory or metabolic disorders (systemic erythematous lupus, lichen planus, Sjogren's syndrome, celiac disease, rheumatoid arthritis, diabetes): 7 studies; malignant tumours (gastric, pancreatic, breast, lung, oesophageal, oral, ovarian cancers and leukemia): 36 studies; bone disease (medication induced osteonecrosis of the jaws): 2 studies. The remaining 51 papers dealt with combinations of various biomarkers for several different diseases. Forty-two (41%) and 24 (23%) studies took into consideration "proteins and/or metabolites" and "molecules belonging to the genome", respectively, the rest (35 studies) reporting a combination of these. Biochemical or biophysical methods used were extremely heterogeneous and included Western Blot, ELISA,



mass spectroscopy, liquid chromatography, Surface-enhanced Raman spectroscopy (SERS), surface-enhanced laser desorption/ionization-time-of-flight/mass spectrometry (SELDI-TOF/MS), PCR and many others. Sensors were available in some studies and were mainly based on nanotechnology and graphene.

**Conclusions:** Salivary biomarkers for diagnosis of diseases are gaining increasing interest. Such a topic is an innovative one, as demonstrated by papers appeared in the literature only recently. The perspective of realizing a reliable "lab-on-a-chip" for early diagnosis of systemic diseases through saliva seems to represent a concrete target for future research.

### Role of candida infection in oral potentially malignant disorders

**Porrini M., Bombeccari G.P., Pertile R., Bosotti M., M. Rossi, Spadari F.**

Department of Biomedical, Surgical and Dental Sciences, Unit of Oral Pathology and Medicine, Ospedale Maggiore Policlinico Fondazione Ca' Granda IRCCS, University of Milan, Milan, Italy

**Aim:** Candida spp are common members of the oral microflora and are generally regarded as being commensals. Candida infection has been associated with malignant development in the oral cavity. Several studies have investigated the pathogenic mechanism of Candida involved in carcinogenesis, and have highlighted the ability of some species to convert some pro-carcinogens such as nitrite and nitrate in nitrosamines and other substances to produce acetaldehyde. At present, it needs to analyze the strength of the evidences in literature about the role of Candidal organisms if any, with respect to the degree of dysplasia of oral potentially malignant disorders (OPMDs). The aim of this study is to analyze the evidence in the literature on the role of Candida spp. in the course of potentially malignant oral disorders and cancer of the oral cavity.

**Methods:** Review question has been as follows: "There is a positive correlation between the infection by Candida spp and malignant transformation in the oropharyngeal region in patients with OPMDs?" The review was conducted by consulting Medline/PubMed databases from January 1980 to November 2015. Additional studies were cross-examined in data base of Scopus, Web of Science and Google Scholar from January 2000 to June 2016, by excluding any bibliographic overlap versus Medline/PubMed. The key words, in accordance with the terminology MeSH database National Library of

Medicine (NLM), were: oral potentially malignant disorders (OPMD), precancerous oral lesions, oral leukoplakia, oral lichen planus (OLP). MeSH terms were combined by the boolean operator "AND", with the words: Candida, candidiasis, yeast, fungal infection, mycosis. The collected data were divided into two themes: A) potentially oncogenic mechanisms; B) comparison of epidemiological data. Qualitative analysis has been performed on the studies considered relevant to the question of review: 31 for thematic studies "A", and 51 for thematic studies "B". The quality of evidence was evaluated according to the SIGN methodology (Scottish Intercollegiate Guidelines Network).

**Results:** The majority of the studies have a high risk of bias. The level of evidence of the studies is between 2- and 2+; the level of prevailing global quality is grade C/D. The confounding factors most frequently observed were the following: in topic A, the data heterogeneity from a general methodological standpoint; in topic B, the absence of comparison with the healthy control group and the incomplete description and correspondence of the results in the diagnostic methods used. To notice, the absence of Randomized Clinical Trials (RCTs) on this issue, in the examined literature, and a high prevalence of case-control studies and case-series.

**Conclusions:** To date, no evidence has been found of a direct etiological relationship between infection from Candida spp. and carcinogenesis of the oral cavity. However, one can not overlook the fact that the presence of Candida in the oral potentially malignant disorders significantly increases in proportion to the degree of dysplasia, making reasonable the assessment of the diagnosis of infection and eradication of the fungus in this type of injuries, characterized by a higher risk of malignant transformation. Further prospective controlled studies on large population cohorts are needed to assess this issue.

### A 10% curcumin gel in the treatment of desquamative gingivitis in cases with oral lichen planus: a preliminary study

**Romagnoli C.\*, Cafaro A.\*, Romagnoli E.\*\*\*, Arduino P.G.\*, Gambino A.\*, Broccoletti R.\***

\*Oral Medicine Section, Department of Surgical Sciences, University of Turin, Turin, Italy

\*\*Department of Surgical Sciences and Integrated Diagnostics, Genoa University, Genoa, Italy

**Aim:** To verify if a 10% curcumin gel could be considered as a valid alternative therapy to topical corticosteroids in the treatment of symptomatic

desquamative gingivitis caused by oral lichen planus.

**Methods:** This preliminary study involves 2 female patients with histopathological diagnosis of oral lichen planus, without other related immunological disorders, showing desquamative gingivitis with no topical corticosteroid treatment in the previous 3 months. The study considers DCGS index for the lesions' aspect and VAS scale for reported pain. The produced 10 % curcumin gel (Laboratorio Bandi del Dott. Fabbriconi, Milan, Italy) was applied after meals twice daily for 8 consecutive weeks, without any antimicrobial and antifungal adjunctive therapy. Patients were evaluated after 15 days, 30 days and 60 days from the beginning of therapy, and then 1 and 2 months after treatment interruption (by means of the DCGS index and VAS scale). Removal of plaque and calculus, together with domestic oral hygiene assessment, was also performed.

**Results:** After 2 months of treatment, patients showed an outstanding decrease of pain and DCGS index (patient 1 from 17 to 4 and patient 2 from 13 to 7), with an improvement in the quality of life reported. The clinical oral involvement also remained stable also at the end of the follow up period.

**Conclusions:** The application of 10% curcumin gel twice daily for 8 weeks showed a reduction of pain and of the erythema of the lesions. This treatment could be carefully considered as an alternative and safe therapy to topical steroids for cases of desquamative gingivitis associated with oral lichen planus. This therapy requires a strict adherence to given instructions, in order to obtain positive results, but its interruption is possible without dose reduction and it has no evident side effects. It is logically imperative to enlarge the case sample in order to increase data and to verify if 10% curcumin gel is a real effective alternative therapy to topical steroids.

### Experimental protocol of dental procedures for patients affected by hereditary angioedema: the role of anxiety and use of nitrous oxide

**Rosa A., Benegiamo M., Miranda M., D'Ovidio N., Coniglione F\*, Silvi B\*, Dauri M\*, Franco R., Barlattani A., Bollero P.**

UOSD Diagnosis, Prevention and Oral Hygiene with Day Hospital Medical-Surgical General and Special for vulnerable patients, Polyclinic of Rome Tor Vergata, Rome, Italy

\*Complex Operating Units Anesthesia and Resuscitation Polyclinic of Rome "Tor Vergata", Director Prof. M. Dauri

**Aim:** The Hereditary Angioedema (HAE) is a rare genetic disease that affects a number of people between 1: 10,000 and 1: 50,000. It has an autosomal

dominant trait and is caused by a mutation of the gene encoding the C1 inhibitor. They recognize two types of Hereditary Angioedema: type I is the most common form and is characterized by a quantitative defect; Type II is characterized by a protein that is produced but not functioning. Symptoms include swelling of the face that occurs spontaneously or following trauma. The treatment is twofold: short-term prophylaxis with Berinert in the hours prior to any intervention. Anxiety is related to the above disease. In cases of HAE, careful management of anxiety may be important in preventing the attacks. The protocol is intended to refer the patient to a correct diagnostic and therapeutic procedure.

**Methods:** Records of HAE patients treated at the Department of Pathology Odontostomatology the University Hospital of Tor Vergata from 2013 to 2015 have been six. They were considered for the study, only patients with a confirmed diagnosis of HAE. All patients were included in the Day Surgery. The protocol includes a first part anamnestic, a second clinical-pathological part with the use of a questionnaire designed by our team in order to inquire about the pathology and finally a last part psycho-clinical through the Italian version of the dental Scale of Corah (CDAS) and the visual analogue scale for anxiety (VAS). A score of CDAS 12 and / or a VAS-A score of 50 out of 100 were considered as thresholds of distinction between the levels of low and high anxiety. Conscious sedation and local anesthesia was used in all patients according to the protocol. Patients had an age between 10 and 54, and their average values of CDAS and VAS-A were 16 + 1.56 respectively. All patients were subjected to extractions, and endosseous implants in two patients were also inserted. Three patients were on long-term prophylaxis, also the short-term prophylaxis with C1-INH concentrate (Berinert) 20 U / kg / IV was administered to all patients one hour before surgery. No one has reported acute events during surgery and in the postoperative period.

**Results:** Anxiety is a major cause of perioperative stress, increases the perception of pain and compromise the result. In cases of HAE, careful management of anxiety could prove important in preventing acute attacks. The treatment of HAE with C1-INH in conjunction with the aid of nitrous oxide has proven effective in the management of the disease and the condition of high anxiety. The anxiety level in the pool of treated patients was higher than during the preoperative nell'intraoperatorio. Our protocol is structured as follows: - the first essential step is to make a psycho-physical assessment by clinical-pathological questionnaire. It follows antibiotic prophylaxis according to national drug guidelines. We make a premedication with oral midazolam, followed

by inhalation of N2O always preceded with 100% oxygen and followed by titration of anesthetic in 10% intervals. During analgesia / anxiolysis, the concentration of N2O must not exceed 50% of routine.

**Conclusion:** Our experience suggests that the use of conscious sedation techniques for controlling anxiety were associated with dental treatment is the preferred choice for the prevention of HAE acute events.

### Human Beta2-Defensin in oral lichen planus expresses the degree of inflammation

**Scognamiglio Cristian\***, **Manfredini Mattia\***, **Croveri Fabio\*\***, **Boggio Andrea\*\***, **Farronato Davide\*\*\***, **Azzi Lorenzo\*\*\*\***, **Tettamanti Lucia\*\*\*\*\***, **Tagliabue Angelo\*\*\*\*\***

University of Insubria, department of medicine and surgery, Italy

\*Dental Student

\*\*DDS

\*\*\*Assistant professor

\*\*\*\*DDS, PhD

\*\*\*\*\*Aggregate professor

\*\*\*\*\*Dean, Full Professor

**Aim:** The Antimicrobial peptides (AMPs) are polypeptides found in host defense settings, they have an antimicrobial activity. Other biological effects of AMPs have been described recently: chemotaxis, immunomodulating activity, and angiogenesis. Among AMPs, defensins ( $\alpha$ ,  $\beta$  and  $\theta$ -defensins) occupy a strategic position because of their antiviral, antifungal, antibacterial and anti-parasite activities. A decrease in number or function of defensins could increase the host's susceptibility to infections. On the other hand, in autoimmune or dysregulated immune responses the AMPs' activity seem to be altered. Their roles in the oral cavity and their association with several mucosal and gingival diseases such as Candidiasis, Herpes Labialis and Erythema Migrans are recently under study. Defensins have been found in oral tissues, salivary glands, salivary secretions and crevicular fluid. Up to now, just few researches have investigated HBD-1 or HBD-2 in the saliva of patients with Oral Lichen Planus (OLP) or Burning Mouth Syndrome (BMS).

**Methods:** The current study was conducted on a total of 35 patients, 17 were affected by OLP (11 females and 6 males of age >30), 9 by BMS (women of age > 45) and 9 were included as a control group. Each patient underwent a dental examination where a sample of saliva was collected. Secondly, a sterile paper cone was inserted for 30 seconds into the gingival sulcus nearby a dental element chosen through randomized criteria. The procedure was

carried out 4 times per patient, each time near a different tooth, to collect enough crevicular fluid. In OLP patients were also evaluated the lesions subdividing into red OLP and white OLP. The samples were stored at a temperature of  $-20^{\circ}\text{C}$ . Human  $\beta$ -Defensin 2 Elisa Kit protocol was used for this study, the immunoplate was precoated with anti-HBD-2 Capture Antibody and the non-specific binding sites were blocked. The intensity of the colour was directly proportional to the amount of HBD-2 in the samples. In addition, a comparative statistics among the different groups was performed.

**Results:** There was not any significant difference among the 3 groups with regards to the production of salivary and gingival HBD-2. The distribution of values registered in saliva and crevicular fluid was strictly correlated, they both contain an equivalent quantity of HBD-2 in the same patient. In OLP group, the red OLP subgroup shows high values of HBD-2 in saliva and crevicular fluid ( $>3500$  pg/ml), the white OLP subgroup has values similar to those detected in the control and BMS groups ( $<3500$  pg/ml).

**Conclusion:** In this study the choice was to study the role of HBD-2, which is an inducible and not constitutionally expressed defensin, in the pathogenesis of the most frequent autoimmune disease of the oral cavity. There was not any statistically significant difference between the three groups. Besides, the study highlighted the role of HBD-2 in the maintenance and intensity of the inflammatory component in Oral Lichen Planus. Patients affected by OLP showed a dycotomic distribution of values: while 10 of them with white OLP showed similar values to those found out in the other two groups, 7 patients affected by red OLP expressed high levels of HBD-2 in saliva and crevicular fluid. In conclusion, this study shows that HBD-2, represents an index to assess active inflammation and it is probably linked to the presence of the typical band-like CD8+ infiltrate in Oral Lichen Planus.

### A case of osteonecrosis of the jaws in epidermolysis bullosa patient treated with rituximab

**Seidita F.\***, **Campisi G.\***, **Mauceri R.\***, **Lo Russo L.\*\***, **Giannatempo G.\*\***, **Panzarella V.\***, **Di Fede O.\***

\*Dept. Surgical, Oncological and Oral Sciences, University of Palermo, Palermo

\*\*Dept. Clinical and Experimental Medicine, University of Foggia, Foggia

**Aim:** Osteonecrosis of the jaw (ONJ) is a potentially



severe adverse effect of various medications (bisphosphonates, anti-resorptive, and anti-angiogenic drugs), consisting of the progressive destruction and death of bone, that affects mandible or maxilla. Among anti-angiogenic drugs, target therapy has been recently related to osteonecrosis of the jaw. Rituximab is a monoclonal, chimeric (mouse/human) anti-CD20 antibody. CD20 is a molecule found only in most mature B cells, and it is absent from either bone marrow stem cells or pro-B cells. It is used as an anti-tumoral medication in lymphoproliferative diseases and as an immunomodulator agent in some auto-immune diseases such as Epidermolysis bullosa. In particular, it has been reported to be effective in the adult form of Epidermolysis bullosa acquisita (EBA), a chronic, autoimmune, subepidermal and blistering disease characterized by circulating and tissue-bound autoantibodies targeting type VII collagen, which is the major component of anchoring fibrils. Recent studies have demonstrated that Rituximab can exert part of its antitumoral action, due to its influence on angiogenesis, with a reduction in vascular endothelial growth factor (VEGF) levels in patients. We report a case of a patient with EBA, who developed ONJ after Rituximab treatment.

**Methods:** We describe a case report of a 76 years old woman with EBA who referred to our service for a middle palate ulcer. Intraoral examination revealed a proliferative ulcer on the midline of the hard palate. The patient reported a history of EBA, diagnosed in 2016, and has been treated with Rituximab for two monthly cycles (November 2016 and January 2017). The patient has also taken with Alendronate since 3 months for osteoporosis.

**Results:** The patient has been treated with systemic antibiotic therapy (Ampicillin/Sulbactam 1g IM, 2/die for 7 days + Metronidazole 250 mg OS, 3/die for 7 days) and ozone therapy (3 minutes for each session, once a week for 2 months). After one week of antibiotic therapy, the ulcer healed, revealing exposed necrotic bone. We hypothesize that ONJ has been induced by Rituximab and not by Alendronate that has been administered per os and has not reached an adequate cumulative dose for the onset of ONJ.

**Conclusions:** Osteonecrosis of the jaw has been related to several medications, but the association with Rituximab is nowadays rarely described in literature. Rituximab has a pharmacodynamic path that is similar to other ONJ-related medications and this case report emphasizes the role of this particular drug as a risk factor. Nevertheless, further studies, such as RCT, are necessary to assess the role of this drug in the ONJ pathogenesis.

## Secondary syphilis: challenging diagnosis of first

## clinical manifestation in oral cavity

**Setti G.\*\*\*, Mergoni G.\*, Meleti M.\*, Manfredi M.\*, Vescovi P.\***

\*Center of Oral Medicine and Laser Surgery, Department of Biomedical, Biotechnological and Translational Science, University of Parma..

\*\*Unit of Dentistry and Oral-Maxillofacial Surgery - University of Modena and Reggio Emilia

**Aim:** Syphilis is a systemic infectious disease caused by the filamentous, anaerobic spirochete *Treponema Pallidum*. The disease can be transmitted sexually (acquired syphilis) or vertically via the placenta (congenital syphilis). On the basis of its activity and infectivity phase, acquired syphilis can be classified into four stages: primary, secondary, latent, and tertiary. Oral lesions are principally associated with secondary syphilis, occurring in 30–50% of cases, although all stages can give rise to oral manifestations. This wide array of manifestations and variability in their appearance has given syphilis the reputation as the "great imitator" and represent a diagnostic challenge.

**Methods:** We hereby present two cases of secondary syphilis.

1. A 66 year-old Christian Priest who was spending most of his time in Brazil for religious purposes, was addressed to our consultation by his GP and his dentist due to suspicious "aphthous stomatitis". Several ulcers on ventral tongue and dorsum, lower lip, hard and soft palate were observable since two months. Pharyngodynia and malaise were reported; no cutaneous, genital or ocular manifestations could be disclosed at recent, remote anamnesis and clinical observation. No lymph node enlargement was appreciable. Medical anamnesis was positive for hypertension, episodes of angina, benign prostatic hypertrophy, hypercholesterolemia and atrophic gastritis; drug history consist of antihypertensive, gastroprotectants, and ASA. Complete blood test and specific serology (TPPA, VDRL and FTA-Abs) were requested. Multiple incisional biopsies were performed.

2. A 56 year-old man working as trucker, HIV and HCV positive, was referred for consultation by Infectious Disease Center to investigate a single ulceration of the right lateral tongue, noticed two months before. Meantime, before the specialist examination, a new labial ulceration, similar to "aphthous lesion", arose. In addition to HIV/HCV positivity, remote anamnesis was positive for HCV related cirrhosis, hypertension and heroine abuse history. No history of cutaneous, genital or ocular manifestations was reported. Clinical observation was negative. No lymph node enlargement was

appreciable. Drug history consist of antiviral and antiretroviral therapy and antihypertensive therapy. Complete blood test and specific serology (TPPA, VDRL and FTA-Abs) were requested. Multiple incisional biopsies were performed.

**Results:** Serological titer and histological investigation were positive for *Treponema Pallidum* infection in both cases; intramuscular penicillin therapy was administrated with follow-up until resolution. Information about partner screening and prophylaxis were delivered.

**Conclusion:** These cases are significant for the inability to recover the history of the primary lesion from the patient and the systemic comorbidities disguising syphilis. Secondary syphilis is characterized by mucosal and cutaneous manifestations; the hematogenous dissemination causes the colonization of several organs. It starts within three to five months after infection. However, if not treated, secondary syphilis may recur as late as two years after exposure. It is accompanied by unspecific clinical signs and symptoms, such as general discomfort, headache, low-grade fever, anorexia, weight loss, lymphadenopathy, pharyngitis, myalgia, and arthralgia. These conditions disappear spontaneously, without treatment, during the latent period. Depending on clinical manifestation, differential diagnosis are traumatic ulceration, HSV infection, lichen planus, pemphigus vulgaris, pemphigoid, cheilitis, squamous cell carcinoma, leukoplakia and minor/major recurrent aphthae. Moreover, when the clinician deals with a complex medical history, they must consider lesions caused by systemic disease and altered immunological status. Due to the strong association with sexual behavior and STDs, anamnestic interview must disclose as much information as possible, regarding the limit of patient comfort.

### Immunohistochemical expression of CD56 (NCAM) in odontogenic tumors

**Mascitti Marco\***, **Togni Lucrezia\***, **Rubini Corrado\*\***, **Lo Muzio Lorenzo\*\*\***, **Khrystyna Zhurakivska\*\*\***, **Claudia Arena\*\*\***, **Procaccini Maurizio\***, **Santarelli Andrea\***

\*Department of Clinical Specialistic and Dental Sciences, Marche Polytechnic University, Ancona, Italy

\*\*Department of Biomedical Sciences and Public Health, Marche Polytechnic University, Ancona, Italy

\*\*\*Department of Clinical and Experimental Medicine, Foggia University, Foggia, Italy

**Aim:** The aim of the study was to assess the immunohistochemical expression of CD56 in a series of surgical specimens of Ameloblastomas (AMBs), divided in three main subgroups: multicystic (SMA),

unicystic (UA), and peripheral subtypes (PA); and Keratocystic odontogenic tumors (KCOTs), divided in sporadic (NS-KCOT) and syndromic (S-KCOT) variants, in relation to their clinicopathological features, in order to use it as diagnostic and prognostic marker.

**Methods:** This study included 324 specimens of primary and recurrent odontogenic tumors, over a period of 25 years (1990–2015). In particular, this study focused on 230 cases of KCOTs (195 primary and 35 recurrent) in 186 patients, and 63 cases of AMBs (47 primary and 16 recurrent), relating to 47 patients. Data were retrieved and cataloged from clinical records and from the archive of the Institute of Pathology of the Polytechnic University of Marche by a single operator, in order to ensure the uniformity of the collected data. Serial sections (4 µm) from formalin-fixed, paraffin embedded blocks were cut for each case and mounted on poly-L-lysine-coated glass slides, and 1 section stained with hematoxylin-eosin was used to confirm the histopathologic diagnosis. The sections were incubated for one hour at room temperature, with the anti-CD56 antibody diluted 1:100 (clone 124, DakoCytomation). To confirm the reaction, was used a detection system of the company Dako HRP/Envision Detection Systems (DakoCytomation), while for the development of positive reaction was used a chromogenic substrate solution (DAB). Negative control sections were stained, wherein the specific antibody was substituted with buffer or non-immune serum. CD56 immunostaining was classified as focal (<10% of cells expressing CD56), extensive (10–50% of cells) and diffuse (>50% of cells).

**Results:** All cases of AMBs and KCOTs showed CD56-immunoreactivity. In AMBs the positivity was present only in the peripheral columnar cells, while in KCOTs the positivity was present mainly in basal and para-basal epithelial cells. Primary UAs showed diffuse reactivity (10 cases), extensive reactivity (7 cases) and focal reactivity (4 cases), while recurrences showed 4 cases with diffuse reactivity and 1 case with extensive reactivity. Primary SMAs expressed diffuse reactivity (11 cases), extensive reactivity (10 cases) and 1 case of focal reactivity, while recurrences showed 5 cases with diffuse reactivity and 2 cases with extensive reactivity. Primary PAs showed 2 cases with diffuse reactivity, 1 case with extensive reactivity and 1 case with focal reactivity. The results were the same in the recurrence cases. Regarding NS-KCOTs, the results showed diffuse reactivity in 15 cases, extensive reactivity in 74 cases and focal reactivity in 95 cases, while recurrences expressed diffuse reactivity (6 cases), extensive reactivity (18 cases), and focal reactivity (11 cases). Lastly, 11 cases of

S-KCOTs (primary and recurrent) were found. 2 cases expressed diffuse reactivity, 8 cases extensive reactivity and 1 case focal reactivity.

**Conclusion:** CD56 is considered to be a signaling receptor that influences cellular adhesion, migration, proliferation, apoptosis, differentiation and survival. Its expression is related to aggressiveness and relapse in odontogenic tumors. These findings suggest that it can be used as a marker of infiltration, with the aim to differentiate cystic lesions from tumors. Furthermore, CD56 could be used as a prognostic marker, with the aim to define an appropriate follow-up period.

### An unusual presentation of oral syphilis: a case report

**Toma Fabiola, Lissoni Alessandra, Moreschi Chiara, Mainardi Beatrice, Gastaldi Giorgio, Abati Silvio**

University Vita-Salute San Raffaele, Dental School, Dept. of Dentistry, Chairman Prof. E.F. Gherlone, Milano, Italy

**Aim:** Syphilis is an infectious disease caused by the anaerobic spirochete *Treponema pallidum* that may involve many organs. The route of transmission is mainly sexual (acquired syphilis) through direct contact with the lesions, blood and saliva; occasionally the transmission could be vertical via the placenta (congenital syphilis) if the mother is affected. Syphilis may be asymptomatic and might be associated in approximately 10% of the cases with other STDs. The clinical course has 3 stages: primary, secondary, tertiary and a latency in between, with different clinical appearances and infectivity. The incubation time is around 2-3 weeks then a papular lesion appears at the site of inoculation. Successively the ulceration of the papule produces the typical chancre of the primary syphilis. The chancre is about 1-2 cm and has indurated rolled borders. They may heal in a period that goes from 1 to 2 months; meanwhile *Treponema* spreads in other districts. The aim of this report was to describe an unusual lip presentation of an oral primary lesion of syphilis.

**Methods:** The patient observed was a 62 yrs old businessman, travelling back from Brazil where he stayed for two months for working and leisure purposes. His chief complaint was an indolent herpes-like lesion appeared on the upper lip but did not heal spontaneously after 3 weeks.

**Results:** At the first visit, the lesion appeared as a depressed speckled erythematous area with some whitish patches inside and visible on the inner surface of the upper left lip, just below the vermilion border. The lesion was completely asymptomatic in

the first three weeks; then the patient reported the onset of flu-like symptoms. The oral medicine expert began to treat the lesion just with topical applications of a 1% chlorhexidine gel. After two weeks the lesion did not heal but changed in appearance becoming wider, pinkish and thinner and spreading towards the commissural fornix. The clinical suspect of oral syphilis was confirmed after a deeper investigation of the patient's history and the positivity of the serologic values with a blood test (VDRL;TPHA;FTA-ABS) confirmed the diagnostic hypothesis. Moreover, a smear examination from the oral chancre under the darkfield microscope revealed the presence of spirochetes. The patient underwent a cycle of antibiotic therapy with penicillin G 2.400.000 IU i.m. weekly for 2 weeks. The dermatologist followed the patient as well. At the 6 months follow-up he was clinically and serologically completely healed.

**Conclusion:** dental health providers must be aware of the appearance and the pathway of transmission of STDs' lesions, in particular oral syphilis, since its incidence and recurrence is rising again after a decade of remission (from 1990 to 2000). The diagnosis and consequently the treatment of oral syphilis are of relevance of an oral medicine expert and a referral to a specialist of dermatology or STDs should be promptly fulfilled to assess if the patient may have other clinical manifestations of the disease at distant sites.

### Dysregulation of Long Non-Coding RNAs (LNCRNAs) expression in tongue cancer represent promising clinical biomarkers

**Troiano Giuseppe\*, Zhurakivska Khrystyna\*, Caponio Vito Carlo Alberto\*, Boldrup Linda\*\*, Nylander Karin\*\*, Santarelli Andrea\*\*\*, Campisi Giuseppina\*\*\*\*, Lo Muzio Lorenzo\***

\*Department of clinical and experimental medicine, University of Foggia, Via Rovelli 50, Foggia 71122, Italy

\*\*\*Department of Medical Biosciences/Pathology, Umeå University, Umeå, Sweden

\*\*\*Department of Clinical Specialistic and Dental Sciences, Polytechnic University of Marche, Ancona, Italy

\*\*\*\*Department of Surgical, Oncology and Dental disciplines, University of Palermo, Palermo, Italy

**Aim:** Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that are not translated into proteins. Despite, they were previously defined as "junk DNA", more recently they have shown important role in different biological processes. They result often abnormally expressed in many cancer diseases. The aim of this study is to

analyze the different expression of lncRNAs among samples from tongue cancer (T), normal tumor free tissues adjacent to the tumor (TF) and normal tongue samples from healthy patients (C), in order to detect potential biomarkers for clinical use.

**Methods:** Samples for T and TF groups were collected through biopsies before treatment of the patients, while in the C group samples were taken from healthy volunteers on the lateral border of the tongue. RNA from the biopsies has been extracted using two different methods: RNA only (Trizol) and RNA and protein kit (Norgen). After that quantity and quality evaluation of extracted RNA was measured using nano-drop and Agilent RNA 6000 Nano Kit (Agilent Technologies, USA). Illumina HT-12 bead chip array was performed and data were analyzed using a multivariate regression method. On the basis of array results, from about 47000 genes expressed, we selected only those lncRNAs differentially expressed among groups at a statistical significant level. Array results were subsequently confirmed through Real-time quantitative Polymerase Chain Reaction (qRT-PCR) analysis.

**Results:** Array analysis results, confirmed by qRT-PCR, revealed a group of lncRNAs differentially expressed among T, TF and C samples. C11orf44, NACAP1, FER1L4 and SCARNA9 result to be the most promising molecules for clinical practice as biomarker in patients with tongue squamous cells carcinoma.

**Conclusions:** lncRNAs are differentially expressed among samples from tongue cancer, normal tumor free tissues adjacent to the tumor and healthy tongue tissues. Some of them revealed an interesting pattern of expression that could lead to their use as cancer biomarkers. This study encourages the develop of further powerful and well-standardized studies on patients with tongue cancer.

### Microbiopsy used by general dental practitioners in the assessment of oral mucosal lesions: a prospective study

Val M.\*, Marino R.\*, Navone R.\*\*\*, Gadolfo S.\*, Pentenero M.\*

\*Department of Oncology, Oral Medicine and Oral Oncology Unit, University of Turin, Orbassano (TO), Italy

\*\*Department of Biomedical Sciences and Human Oncology of Pathology Unit, University of Turin, Turin, Italy

**Aim:** General dental practitioners frequently deal with oral mucosal lesions in their clinical practice and are basically requested to be able to recognize which of them need to be referred for an oral

medicine consultation, aiming first to early detect oral squamous cell carcinoma (SCC) and oral potentially malignant disorders (PMDs). When used in oral medicine clinics, microbiopsy is able to obtain tissue fragments suitable for a highly sensitive first-level diagnosis of dysplastic/malignant alterations in oral mucosal lesions. If feasible by general dentists, this sampling technique could reduce the diagnostic delay for oral malignant and premalignant lesions. The present study prospectively assessed the diagnostic accuracy of Microbiopsy in ruling out dysplasia or carcinoma in a general dental practice setting.

**Methods:** From 2008 to 2011 fifty dentists, without specific training in oral medicine, volunteered for enrolment. They were given brief training and asked to prospectively sample any mucosal lesion observed during their routine practice. The sampling must include the entire visible surface of the lesion. The mucosal lesions had to be scraped causing some pinpoint bleeding or light abrasion. Then the sampled tissue fragments were transferred to the fixing solutions in order to proceed with paraffin embedding and Hematoxylin and Eosin staining for morphological assessment. Sampling adequacy was assessed looking for significant differences due to clinical features. In case of dysplasia or SCC detected by Microbiopsy patients were referred for an oral medicine consultation. Otherwise, patients were followed up by dentists (mean follow-up period 7.5 years; range 7-9 years). Aiming at testing the negative predictive value of Microbiopsy the potential development of oral cancer was also ruled out through a cross check provided by the Piedmont Cancer Registry, city of Turin.

**Results:** The dentists sampled 152 lesions from 132 patients (71 men and 61 women; M:F = 1.2:1) 53.7 years (range 25-88). Smoking and alcohol habit were observed, respectively, in 50/132 (37.9%) and 20/132 (15.2%) patients; almost a quarter of them had both smoking and alcohol habits (12/132; 9.1%). The sampled lesions were representative of all oral mucosal sites, average age; there were 92.1% of adequate samples (140/152), and the BMZ was visible in 78.6% of these (110/140). Neither the clinical aspect nor lesion site affected either the adequacy or the presence of basal membrane. Clinical follow-up provided information on 48 lesions. The clinical follow-up revealed a complete healing in 21/140 (15%) lesions, while 27/140 were unchanged. The cross check provided by the Piedmont Cancer Registry will be able in a very next future to clarify the status of 92/140 lesions lost to follow-up.

**Conclusions:** The high adequacy rate observed and the advantages histological specimens have over cytological ones go to support the feasibility of



micro-biopsy taken by general dentists for the characterization of oral mucosal lesions and in selecting those requiring further assessment in specialised oral medicine centres. The cross check provided by the Piedmont Cancer Registry will provide reliable data in order to assess the negative predictive value on Microbiopsy in ruling out dysplasia or carcinoma.

### A gingival non-Hodgkin lymphoma as a secondary new manifestation: case report and literature review

**Valpreda L.\*, Cabras M.\*, Broccoletti R.\*, Gambino A.\*\*,  
Carbone M.\*, Arduino P.G.\***

\*Department of surgical sciences, C.I.R - Dental School, University of Turin, Turin, Italy

\*\*Department of mechanical and aerospace engineering, Politecnico di Torino, Turin, Italy

**Aim:** to describe a second event of a diffuse gingival large B-cell Non-Hodgkin lymphoma (NHL) in a patient with a previous history of left intra-parotid NHL diagnosed 5 years earlier; the new case presented as a mere gingival swelling of the right lower jaw, which was initially mistaken as the consequence of an odontogenic infection of the contiguous mandibular teeth. Moreover, a search on the major digital scientific databases was performed (Medline, Pubmed, Google Scholar) with the aim to assess the rate of head and neck NHL.

**Case Report:** A 69-years old female patient was referred to the Oral Medicine Unit of the University of Turin, for the assessment of an approximately six weeks mandibular right side swelling, unresponsive to therapy with antibiotics, and of a non-healing of post extraction socket. Patient, otherwise well, reported a previous episode of large B-cell intra-parotid NHL treated by surgery, chemotherapy and rituximab. Biopsy of the gingival lesion was promptly performed. Based on the histopathological features and immunostaining (CD20 + and co-expression of CD10, BCL-2, BCL-6, Ki67), the patient was diagnosed with a diffuse large B cell lymphoma. She was then referred to the hematology division for staging (stage IIE) and the setting of chemotherapy applicable (4 cycles according to the CHOP-R diagram). Treatment was satisfactory, and a complete regression of the lesion was assessed after 4 months.

**Conclusion:** More than 40% of NHL can manifest as extra-nodal (mainly in the gastro-intestinal tract). Head and neck NHL account for almost 20% of the total cases, usually manifesting as painless fixed swellings, which can develop late ulcerations.

In the oral cavity, the main problem could be the difficulty of an early diagnosis, especially for gingival onset, due to the non-specific clinical presentation. Regarding the etiology, infections have been reported to have some role in the onset of NHL, especially in genetic predisposed patients. Differential diagnosis with benign conditions is fundamental (such as odontogenic abscesses, high-flow vascular lesions, pyogenic granuloma, osteomyelitis); leukemia, metastases and squamous cell carcinoma must also be considered. Early detection of NHL is critical, because chemotherapy could be much more tolerable and successful. This case stresses the importance of an accurate medical history in dentistry, and of a proper clinical and histological differential diagnosis, when facing an otherwise non-specific oral swelling; this is essential in order to avoid therapeutic delay or inappropriate therapies.

### Auto-fluorescence (AF) correlates to histopathological features of oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC): a case series

**Veneri Federica, Giovannacci Ilaria, Meleti Marco, Magnoni Cristina, Manfredi Maddalena, Vescovi Paolo**

Unit of Oral Medicine and Oral Laser Surgery

Academic Center of Dentistry

Department of Medicine and Surgery, University of Parma, Italy

**Aim:** The auto-fluorescence (AF) phenomenon depends on the optical properties of some molecules (fluorophores) naturally located within epithelial and connective tissues. Fluorophores are excited when irradiated with ultraviolet or blue light (375-460 nm). AF could be a somewhat useful tool as aid in the diagnostic pathway of several diseases because of its direct dependence on the nature, amount and microenvironment of endogenous fluorophores such as keratin, collagen, NAD(P)H, flavins, and other proteins, strictly involved in metabolic processes and structural organization of cells and tissues. Normal, unaltered tissue fluorophores will emit a pale green AF (normo-fluorescence), when viewed through a selective narrow-band filter, whereas areas of reduced AF (hypo-fluorescence), will appear dark, being suspicious for oral epithelial dysplasia (OED) or oral squamous cell carcinoma (OSCC). Bright green AF (hyper-fluorescence) is also considered as an AF alteration, often due to hyperkeratosis. The aim of this pilot study is to evaluate the usefulness of Auto-Fluorescence (AF) as aid in the diagnostic pathway of suspicious oral lesions and to assess its correlation with histopathological features.

**Methods:** Twenty-three lesions suspicious for OED and OSCC in 14 patients were investigated at the Oral Medicine and Laser Surgery Unit of the University of Parma. The VELscope system (LED Medical Diagnostics, Inc, Barnaby, BC, Canada) was used to assess AF in each case. Such a tool consists of a non-invasive device developed to visualise early mucosal changes using the principles of tissue AF by emitting a violet-blue light (400-460 nm). Incisional or excisional biopsies were performed in all cases, taking into account both AF and conventional white light examination. Histological, AF and white light features were compared.

**Results:** All lesions could be classified as clinically suspicious after white light inspection and palpation. At the clinical examination 8 appeared as white lesions, 10 as red lesions such as erosive, atrophic or ulcerated areas and 5 had lichenoid features. Similarly, all lesion showed alteration of AF. Eight out of 23 cases (34,8%) were hyper-fluorescent. Among these, 2 (25%) were histologically diagnosed as non-dysplastic, 3 (37,5%) as affected by OED and 3 (37,5%) as OSCC. Seven (87,5%) of

the hyper-fluorescent lesions were also related to histopathological findings of hyperkeratosis. Fifteen out of 23 cases (65,2%) were hypo-fluorescent, of which 4 (26,7%) were histologically diagnosed with OED and 11 (73,3%) with OSCC. Except 2 out of 23, most cases (91,3%) showed concordance between AF alteration and histological diagnosis of OED or OSCC.

**Conclusions:** The histopathological analysis of these cases confirms that both hypo and hyper-fluorescence represent an alteration of AF, which is highly probable of being related to OED or OSCC diagnosis. Therefore, lesions showing hyper-fluorescence, if associated with suspicious clinical and anamnestic features, should be investigated as well. It should also be considered that hyperkeratosis is often easily detectable at the clinical examination and it is exalted by AF. Hypo-fluorescence, instead, can point out erosive, atrophic or erythroplasic lesions which are not always clinically noticeable. Hence hypo-fluorescence has a potential role as an indicator for areas suspicious for OED or OSCC that would need further investigations.