

# Implant borne prosthesis in Head&Neck cancer patients after radiotherapy: treatment outcomes and quality of life

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**Introduction.** Head&Neck (H&N) cancers are important health threat in Italy; 100.000 people survive with this diagnosis. Radiotherapy is necessary in 60% of cases (1). The analysis of quality of life (QoL) is a new important instrument to understand what are principal values which influence the daily life of survivors in order to make a cost-benefit analysis of therapies employed (2).

**Aim:** To assess QoL, implant survival, peri-implant health & hygiene status in H&N cancer patients who underwent radiotherapy and prosthetic rehabilitation with dental implants.

**Materials and methods.** Recall program in our Department included H&N cancer patients treated with radiotherapy and prosthetic rehabilitation after implants' insertion. Retrospective collection of data regarded oncology disease, therapies, risk factors, implant survival rate and incidence of perimplantitis. Prospective clinical and radiological (ortopantomography) data regarded peri-implant health & hygiene status, implant survival and success (3) in survivors and presentation of EORTC H&N 35 test for evaluation of QoL. Statistical analysis checked if demographics, medical and treatment-related variables were significantly different between unrecall and recall group.

**Results.** 21 patients (19♂, 2♀) were included in the retrospective analysis from 1999 to-date. Mean radiation dose was 61 Gy, 55 dental implants were placed, 47 in native mandible, most frequent rehabilitation was removable overdenture. The 96% of implants were present at the last follow-up visit recorded. Mean follow-up period was 38 months. At the time of the recall program a total of 9 (9♂, 0♀) out of 21 eligible patients could be contacted. Main reason of dropout was death. In this subgroup 18 implants had been placed in native mandible. We observed an implant survival rate of 100%, implant success was 83%. Mean peri-implant probing depth was 3.8 mm, mean peri-implant recession was 1.0 mm, mean clinical attachment loss was 4 mm, mean plaque and bleeding on probing indexes were 95% and 86% respectively. Mean marginal bone loss evaluated on orthopantomography was 3.2 mm. Perimplantitis was diagnosed in 2 patients (3 implants). Assessment of QoL revealed most patients complain with salivary alterations (>50% patients), senses and speech problems (>20%). Demographics, cancer stadiation, oncologic therapies, risk factors distribution, mean radiation dose between recalled and unrecalled patients were not significantly different ( $p < 0.05$ ).

**Discussion.** We present a wide timespan collection of data regarding implant survival, implant success and peri-implant health and hygiene in a highly selected population of patients from 1999 to-date. Our findings sustain the good prognosis of dental implants in H&N cancer patients despite radiotherapy. Despite our patients became partially or totally edentulous due to oncologic therapies; assesment of QoL revealed that complaints in our cohort were more likely to be related to previous oncologic treatments (especially radiotherapy) according to literature (2). Advances in conformational and intensity modulated radiotherapy will help in reducing complications and permitting safer and more predictable implant insertion, resulting in better QoL.

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# Eight cases of medication-related-osteonecrosis of the jaw treated with Er: YAG laser resection guided by Auto-Fluorescence

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**Introduction.** Fluorescence is a specific property of some substances to emit light of frequency different from that of an exciting radiation. At a molecular level, such substances, which can be endogenous or exogenous, are called fluorophores. Some evidence exists that fluorophores in oral tissues can be proteins (collagen, elastin, co- at keratin) and co-enzymes involved in cellular metabolism (nicotinamide adenine dinucleotide - NADH, flavin adenine dinucleotide - FAD). Such molecules are stimulated by wavelengths between blue and violet/ultraviolet. Healthy oral mucosa emits fluorescence, detectable as a green light. In many cases of dysplastic and malignant lesions, fluorescence intensity decreases until disappearance, apparently according to the progression of histopathological changes. Tissues losing the ability to emit fluorescence appear dark brown/black. AF of healthy bone tissue was recently described and a Loss of Autofluorescence (LAF) has been reported in necrotic bone. Medication-related osteonecrosis of the jaws (MRONJ) is a well-known adverse side effect of several anti-resorptive drug therapy. Precise identification of necrotic bone margins during osteonecrosis removal is a major difficulty for surgeons being mainly based on subjective parameters.

Ristow suggested that when irradiated with a 400-460 nm wavelength vital bone could be highlighted on the basis of its strong Auto-Fluorescence (AF) whereas necrotic bone lost such an AF appearing very dark.

The aim is to describe a new surgical approach for Medication Related Osteonecrosis of the Jaw (MRONJ) using AF in highlighting surgical margins and Er:YAG laser for bone evaporation. In particular, we detail 8 cases of MRONJ treated with this approach altogether with a histopathologic description of hypo-fluorescent and hyper-fluorescent bone.

**Methods.** Surgical treatment, consisting in resection of necrotic bone, was performed under local anaesthesia and without preoperative tetracycline labelling. After bone exposure, VELscope™ system (LED Medical Diagnostics Inc., Barnaby, Canada) was used to induce and visualize bone AF. Necrotic bone showed no or only pale AF. After removal of necrotic bone block, AF was used to guide marginal osteoplasty. According to AF image obtained after osteoplasty, Er:YAG laser (Fidelis Plus®, Fotona-Slovenia) was used for evaporation of necrotic bone, up to the detection of strongly hyper-fluorescent bone. Further samples of hyper-fluorescent bone were taken from adjacent areas in every patients. Histopathological evaluation was carried out in order to confirm the correlation between bone vitality and presence or absence of fluorescence.

**Results.** Histopathological evaluation revealed in all cases (8 specimens; 100%) necrotic bone tissue. One to 3 (depending on the size of the surgical area) specimens of hyper-fluorescent bone were collected from surgical field and analyzed in order to compare histopathological features with those of the hypo-fluorescent bone.

Histopathological evaluation revealed in all cases (17 specimens; 100%) viable bone tissue with changes typical of BPs therapy. No recurrences were observed in an admittedly short mean follow-up of 14 months.

**Conclusion.** Further clinical trials and basic science studies are necessary to validate the new technique proposed here. AF seems to be a very promising property in differentiating necrotic from vital bone during surgery. Up to now, such a differentiation has been considered an important operative difficulty for the surgeon in removal of MRONJ.

AF could provide important additional information to supplement the clinical appearance of bone as well as preoperative radiographic investigations.

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# Management of patients with Medication Related Osteonecrosis of the Jaws with emphasis on the use of Laser: a series of 276 cases

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**Introduction.** Medication-Related Osteonecrosis of the Jaws (MRONJ), is an adverse effect of several medications (e.g. bisphosphonates, anti-resorptive and anti-angiogenic monoclonal antibodies) used to treat bone metastases of solid tumors, multiple myeloma and other metabolic disorders.

Several clinical approaches have been proposed to treat MRONJ. The use of laser (light amplification of stimulated emission of radiation) technologies to improve bone and mucosal healing provides advantages both for clinicians and patients.

A non-surgical use of laser, based on the principle of low-level laser therapy (LLLT), seems to have a role in the prevention of necrosis; furthermore LLLT seems to reduce pain and decrease suppuration in case of MRONJ. LLLT can also be used as adjuvant therapy after surgical removal of necrotic bone.

Surgical use of laser (cut and vaporization of soft and hard tissues) can improve the healing process with absence of temperature increase, an antibacterial effect and a cleaner surgical area due to a faster and better revascularization.

In the present analysis we compared 5 different therapeutic approaches performed to treat 276 sites of MRONJ.

**Methods.** Two-hundred and seventy-six sites of MRONJ [in 189 females (68.4%) and 87 males (31.6%); mean age 69 years (min. 37, max. 88)] were treated between January 2004 and February 2017 at the Center of Oral Medicine and Laser Surgery of the University of Parma. Ninety-one sites were localized in the maxilla (32.9%) and 185 in the mandible (67.1%); 73 sites were (26.45%) Stage I, 177 (64.13%) Stage II and 26 (9.42%) Stage III (according to the staging system proposed by the AAOMFS in 2014). Five different therapeutic approaches were compared. Group 1: 35 sites treated with medical therapy alone (amoxicillin + clavulanic acid, 1 g every 12 hours and metronidazole, 500 mg every 12 hours). Group 2: 69 sites treated with medical approach and LLLT application performed with Nd:YAG laser (1064 nm FidelisPlus, Fotona-Slovenia, 1.25 W, 15 Hz), 1 session a week for 5 weeks. Group 3: 16 sites treated with medical therapy and traditional rotary surgery. Group 4: 45 sites treated with medical therapy, traditional rotary surgery and LLLT. Group 5: 111 sites treated with medical therapy, Er:YAG Laser surgery (2940 nm FidelisPlus, Fotona-Slovenia, 250 mJ - 20 Hz - VSP - Fluence 50 J/cm<sup>2</sup>) and LLLT. For the statistical evaluation two outcomes were considered: clinical improvement from a higher stage to a lower one (clinical improvement - CI) and clinical regression to Stage 0 (complete healing - CH). The statistical analysis was performed using the Pearson's  $\chi^2$  test. The probability to commit an alpha-type error of less than 5% was considered significant.

**Results.** LLLT associated to medical therapy is more effective in inducing clinical improvement when compared to medical therapy alone ( $p=0.0001$ ) (CI of G1=25.71%, CH of G1=20%, CI of G2=71.01%, CH of G2=39.13%). Surgical approaches give better results than medical therapy (CI of G1+G2=55.77%, CH of G1+G2=32.69%, CI of G3+G4+G5=91.86%, CH of G3+G4+G5=86.05%). Er:YAG laser + LLLT showed the best results in inducing complete healing ( $p=0.0510$ ) (CI of G5=96.40%, CH of G5=91.89%).

**Conclusions.** Both outcomes, clinical improvement and complete healing, are more frequent in groups where LLLT was performed. Er:YAG Laser surgery appears to be the best therapy for all the stages, leading to clinical healing of all Stage III sites in the present series.

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## Plaque control in patients with gingival manifestations of oral lichen planus: preliminary results of a randomized controlled study

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**Introduction.** Oral lichen planus (OLP) is a chronic, inflammatory, immune-mediated disorder whose aetiology is still unknown (1). Gingival lesions are present in 48% of cases, and in up to 10% of patients OLP is confined to the gingiva (2). The role of dental plaque in OLP-related gingival lesions has not been fully clarified (3).

The aim of this randomized, controlled, parallel-arm clinical trial was to evaluate the impact of tailored oral hygiene instructions on clinical and patient-centred outcomes in patients with gingival manifestations of OLP.

**Methods.** Patients with a biopsy proven diagnosis of OLP and symptomatic gingival lesions were randomly divided in 2 groups: treatment group (T) and control group (C). The oral health impact profile (OHIP-14), pain (VAS), plaque index (modified Quigley & Hein Plaque index) and mucosal disease score (modified Escudier index) were recorded and all the patients underwent supragingival debridement at the time of enrolment. Group T patients received tailored oral hygiene instructions, while group C patients were asked to continue with their normal plaque control regimen and did not receive any specific advice. Follow-up was carried out at 4 and 20 weeks and recorded clinical data were compared using a mixed-model regression analysis.

**Results.** At the moment we collected data from 53 patients (group T: n=26, 88% of female sex, mean age = 59.1±17.6 years; group C: n=27, 78% of female sex, mean age=65.2±11.7 years). The regression analysis showed a significant difference for OHIP-14, plaque index and mucosal disease score in group T compared to group C (P <0.001; P <0.001 and P <0.001, respectively). The post-hoc analysis showed significant difference in the 0-4 weeks interval, with a similar trend in the 2 groups in the 4-20 weeks interval. VAS score did not result significantly different (P=0.616).

**Conclusions.** Our data showed that tailored oral hygiene instructions were effective in improving the oral health-related quality of life and clinically observed gingival lesions, particularly in the first 4 weeks. Oral hygienists should be involved in the multidisciplinary management of patients affected by OLP.

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## Head and neck squamous cell carcinoma following hematopoietic cell transplantation

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**Introduction.** The development of Secondary Solid Tumours (SST) is a potential long-term complication after haematopoietic cell transplantation (HCT). In particular, different Authors reported isolated cases of solid squa-

mous cell carcinoma of the head and neck region secondary to HCT 1-2, hypothesizing a relationship between development of carcinoma and chronic GvHD, a common complication related to HCT. The aim of this retrospective study was to determine the incidence and the clinical outcome of head and neck carcinoma and to assess potential risk factors in a large cohort of patients who received HCT in the Pescara Bone Marrow Transplant Center between 1977 and 2013.

**Patients and methods.** 836 consecutive patients (459 males, 377 females) were transplanted either for a malignant hematologic disease (n=654) or non-malignant hematologic disease (n=182). The conditioning regimen was myeloablative for 695 patients and at reduced intensity for 141. Most patients received cyclosporine and methotrexate for graft-versus-host disease (GvHD) prophylaxis. Full engraftment of donor cells was evident in 794 patients. Acute and chronic GvHD affected 337 and 174 patients, respectively. Transplant related mortality was 22% (183 patients). The mortality rate due to recurrence of original disease was 20% (165 patients). Moreover, 71 patients (8%) died for late non-transplant related causes. At time of this report, 417 patients (50%) are living with a median follow-up of 19 years (4-35). Probability of appearance of a squamous cell carcinoma of head and neck region after HCT was the outcome of interest of the present study and it was estimated with the cumulative incidence (CI) method. The curves of various subgroups were compared using the Fine and Gray model.

**Results.** During follow-up, 42 patients showed the occurrence of SST. Of them, 9 patients developed a squamous cell carcinoma of oral cavity (6 tongue and 3 oral mucosa) and 2 of larynx from 3.8 to 32.6 years (median 16.5 years) after HCT. The 30-yr cumulative incidence (CI) of developing SST was 2+0.005%. 8 patients were affected by chronic GvHD with buccal cavity involvement in 7. 6 patients died of tumor progression and 5 are living. The 25-yr overall Kaplan Meyer survival of 11 patients with STT was 51%. In univariate analysis, factors associated with an increased CI of SST were recipient male gender (3%, P=0.03), non-malignant hematologic disease (5%, P=0.03), reduced intensity conditioning (9.5%, P<0.001), chronic GvHD (7%, P <0.001), and oral chronic GvHD (10%, P<0.001). In the Fine and Gray model of multivariate analysis, 3 factors maintained the significance: reduced intensity conditioning (HR 4.953, P=0.0130), non-malignant hematologic disease (HR 2.939, P=0.0498), and oral chronic GvHD (HR 9.512, P=0.0015).

**Conclusions.** This study demonstrates that oral chronic GvHD is the strongest risk factor in the development of a squamous cell carcinoma of head and neck region. Patients with cGvHD, especially those with involvement of the oral cavity, must receive a very long careful monitoring and surveillance in order to prevent the development of secondary cancers.

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Case series

## Prevalence of oral and maxillofacial diseases in an Italian population: retrospective study on clinical and pathological features

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**Introduction.** Oral and maxillofacial lesions are frequently reported among the diseases affecting oral cavity; among these, potentially malignant and malignant disorders affect an increasing number of patients every year, with a poor prognosis in case of late diagnosis. Data on prevalence of oral diseases in Italy are particularly scarce. The aim of our study was to retrospectively investigate over a 6-year period the frequency and spectrum of oral and maxillofacial lesions biopsied in a tertiary referral Italian hospital, in order to give an insight on the prevalence of potentially malignant and malignant oral lesions.

**Materials and methods.** The histopathological reports retrieved from the Stomatology and Oral Surgery Department at the University Hospital in Pisa between 2010 and 2016 were analysed. The reports were divided into categories considering several variables (type of lesion, site of affection, gender and age of the patient). A separate

analysis was conducted for oral potentially malignant and malignant oral lesions. A statistical analysis was performed to evaluate the prevalence for each cluster of disease and its relationship with the other identified variables, and to compare the frequencies of disease in the different groups.

**Results.** We retrieved 3.173 pathological reports in total. In our sample, the most common lesions were periapical inflammatory diseases (30.97%) and non-neoplastic proliferative diseases (30.54%). The most common location of the lesions was bone, followed by cheek lining and gingiva. Precancerous lesions represented 9.11% of the sample, while malignant tumors were 5.70%. Males represented 46.2% of the sample, while females were 53.8%. The most affected age group was from 60 to 69 years, while patients of extreme age-groups (under 10 years old and over 90 years old) were less affected. The mean age for development of malignant lesions was significantly higher than the mean age for potentially malignant lesions ( $p < 0.01$ ); the seventh decade was the most affected by potentially malignant lesions, while for malignant lesions the mean age varied depending on gender. In 26.6% of cases the site of affection was tongue.

**Conclusion.** Many of our findings are consistent with those reported in literature, suggesting that the study of demographic characteristics and their association with occurrence of lesions should be considered in performing differential diagnoses. Our results confirm a higher prevalence of potentially malignant and malignant lesions in patients over 50 years of age, mainly in male patients. Further studies are needed to evaluate the percentage of transformation for potentially malignant lesions.

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#### Case report/Case series

## Auto-fluorescence, clinical and histological evaluation of potentially malignant lesions and carcinoma of the oral mucosa: 50 cases

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**Introduction.** Oral carcinoma is the seventh most common malignant tumour worldwide. The main prognostic factor influencing the 5-year survival rate is tumour stage at diagnosis; therefore, early diagnosis is of paramount importance.

Several visual diagnostic aids, such as auto-fluorescence (AF) analysis, have been developed as adjunctive tools to increase the diagnostic accuracy of the traditional diagnostic pathway (white light conventional oral examination (COE) followed by biopsy) (1). AF directly depends on the nature, amount and microenvironment of endogenous fluorophores such as keratin, collagen, NAD(P)H, flavins, and other proteins, mainly involved in metabolic processes and structural organization of tissues (2). Fluorophores are excited by ultraviolet or blue light ( $\lambda$ : 375-460 nm) and they emit green AF in normal conditions.

The aim of this study is to evaluate the correlation between clinical, histopathological and AF features of oral lesions suspicious for oral epithelial dysplasia (OED) and oral carcinoma (OC).

**Methods.** 50 lesions were investigated at the Oral Medicine and Laser Surgery Unit of the University of Parma, Italy. A suitable system emitting 400-460 nm light (VELscopeVx - LED Medical Diagnostics Inc., Barnaby, Canada) was used to assess AF. Incisional or excisional biopsies, followed by histological examination, were performed in each case.

Concerning their clinical appearance, the lesions were classified as red (erosive, ulcerated or atrophic areas), white (hyperkeratosis) or combined (lichenoid or leuco-erythroplastic lesions).

Regarding the histological diagnosis they were graded as no dysplasia, dysplasia (mild or moderate) and carcinoma (in situ, micro-invasive, verrucous or invasive). White light and fluorescence images were acquired and a

digital elaboration was performed with the software ImageJ (National Institute of Health - NIH, USA), to indirectly measure the AF intensity. We thus considered both a visual and a numerical assessment of AF, which was graded as hypo-fluorescence, normo-fluorescence and hyper-fluorescence.

**Results.** Considering the AF grading attribution, there was a mild discrepancy between visual and numerical evaluation, probably due to confounding factors during the observation.

Red lesions were related to hypo-fluorescence, whereas white lesions were mostly related to hyper-fluorescence due to keratin. There was a statistically significant association between histological alteration (OED or carcinoma) and AF alteration considering both hypo- and hyper-fluorescence ( $p < 0.05$  for visual AF,  $p < 0.001$  for numerical AF). OED and carcinoma are particularly associated with hypo-fluorescence, excluding verrucous carcinomas, which appear hyper-fluorescent because of intense keratinization. It was also found a statistically significant difference in the AF numerical value distribution related to diagnosis, with decreasing values progressing from no dysplasia to dysplasia and carcinoma.

**Conclusions.** AF alteration is statistically related to histological alteration. Note that not only hypo-fluorescence, but also hyper-fluorescence should be investigated, especially if it is associated with anamnestic and clinical suspicious features. Since this correlation is stronger with numerical AF than with visual AF, it could be useful adding an AF simultaneous quantification method to the mere observation. It seems that dysplastic and neoplastic progression induces important modifications to endogenous fluorophores, which result in an alteration of the normal AF pattern of the oral mucosa.

Hence AF analysis is a valid adjunctive technique, when associated to the clinician experience and knowledge.

AF analysis may be used to spot lesions at risk, to identify suitable sites for incisional biopsies and to define excision margins of the lesions.

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#### Case report/experimental study

## miRNA profiling expression from oral brushing in patients with potentially malignant disorders

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**Introduction.** MicroRNAs are short non-coding RNAs that regulate gene expression and are crucial to oral tumorigenesis; miRNAs possess unique properties that make them promising markers to be used in screening tests associated with non invasive collecting procedures. So far, few studies have described the profile of miRNAs in Oral Potentially Malignant Disorders (OPMD). Aim of the present study was to analyze the expression of a panel of miRNAs in epithelial cells sampled by oral brushing from patients with Oral Leukoplakia (OL) and Lichen Planus (LP).

**Methods.** Oral brushing specimens were collected from 30 patients with diagnosis of OL and 17 patients with LP. All cases underwent histological analysis; oral brushing specimens from 20 patients with a histological diagnosis of OSCC were used as positive controls, while oral brushing specimens from 24 healthy donors were used as negative controls. In all groups the expression levels of miRNAs were evaluated by real time PCR. Seven target were evaluated (miR-21, miR-375, miR-345, miR-181b, miR146a, miR-649, miR-518b). The miRNA expression levels across groups were assessed by ANOVA statistics and Tahmane *post-hoc* test.

**Results.** ANOVA analysis showed a between-group significant difference as regards miR146a ( $F=15.048$ ,  $p < .01$ ), miR649 ( $F=9.995$ ,  $p < .01$ ), miR181b ( $F=50.038$ ,  $p < .01$ ) and miR518 ( $F=9.290$ ,  $p < .01$ ). *Post-hoc* test did

not show any significant difference between OSCC and OL, and between LP and healthy donors, while a significant difference was found between OL and LP.

**Conclusions.** The similar miRNA expression in patients with Oral Leukoplakia and OSCC suggests a potential role of these miRNAs in the early phases of oral carcinogenesis. The presence of a different miRNA profile expression in Oral Leukoplakia with respect to Lichen Planus may reflect their different risk of malignant transformation. These preliminary results suggest that microRNAs sampled with a non invasive method can be reliable biomarkers in oral tumorigenesis.

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#### Case report/experimental study

## miRNA profiling expression from oral brushing in oscc patients and in patients surgically treated for oscc

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**Introduction.** MicroRNAs are short non-coding RNAs that regulate gene expression and are crucial to tumorigenesis. miRNAs possess unique properties that make them promising markers to be used in screening tests associated with non invasive collecting procedures: they are abundantly expressed in lesions and in control tissues and their isolation and quantification seems to be easy, convenient and reproducible also in body fluids or in exfoliated cells (1-2).

Aim of the present study was to analyze the expression of a panel of miRNAs in epithelial cells collected by oral brushing from OSCCs, from regenerative areas after OSCC surgical resection and from their respective normal distant mucosa.

**Methods.** Oral brushing specimens were collected from: 20 OSCC and their respective normal mucosa in distant areas, 14 samples from regenerative areas after OSCC surgical resection and their respective normal mucosa in distant areas. Finally oral brushing specimens from 24 healthy donors were collected as control. In all different groups the expression levels of miRNAs were evaluated by real time PCR. Seven target were evaluated (miR-21, miR-375, miR-345, miR-181b, miR146a, miR-649, miR-518b). RNU44 was used as endogenous reference for data normalization. The miRNA expression levels across groups were assessed by ANOVA statistics, Tahnane *post-hoc* test and t-student for paired samples.

**Results.** A between-group significant difference was found for miR181 (F=22.99 p<.000), miR649 (F=8.16, p<.000) and miR146a (F=11.19, p<.000). miR181 and miR649 showed a significant different expression in OSCC areas with respect to samples from healthy donors and from regenerative areas following OSCC resection. Further, normal distant mucosa from OSCC patient showed a significant altered expression with respect to normal distant mucosa from OSCC treated patients. Interestingly, miR146a showed an altered expression in surgically treated patients either considering regenerative areas or their respective normal distant mucosa.

**Conclusions.** Altered miRNA profiles can be expressed both in OSCC and in distant areas from OSCC, but even in regenerative areas following OSCC resection. These preliminary results suggest that microRNAs sampled with a non invasive method can be reliable biomarkers in oral tumorigenesis.

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## Case series

# Influence of VELscope™ in the decision making protocol for detection of oral mucosal lesions

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**Introduction.** VELscope™ is a non invasive device that, using the principles of tissue auto-fluorescence facilitates early recognition and management of clinically not visible lesions. This technology is designed to help detect potentially malignant lesions of the oral mucosa. VELscope™ is based on evidence that, irradiating oral tissues with a high intensity light (400-460 nm) is stimulated a red-green fluorescence dyes endogenous tissue allowing it to highlight the local structural/metabolic changes analyzed. In particular, cells and healthy tissues will appear bright and green when stimulated by this light, while structural alterations occur with loss of natural tissue fluorescence, then modified tissues will be dark and uneven. The loss or decrease in the natural fluorescence reflects structural or biochemical alterations that can guide the diagnosis of mucosal lesions. Previous research indicates a high rate of false positives using this device. The aim of this study is to evaluate the influence of auto-fluorescence using VELscope™ in the decision making protocol for detection of oral mucosal lesions.

**Materials and methods.** 56 patients presenting for oral mucosal lesions were examined by a non specialist practitioner (general dental practitioner - GDP) of the Odontology Unit of UMG. Using conventional oral examination (COE) followed by the VELscope™ and then by correlating the findings from these two examinations. A decision making protocol was followed after the COE and another one after the COE with VELscope™. Patients were either reviewed or referred to an Oral Medicine specialist (OMS) for consultation, and biopsy was undertaken as required for definitive diagnosis.

**Results.** 56 patients presented with a least one oral mucosal lesion, and 71 oral lesions were detected. The conventional oral consulting alone detected 52 oral mucosal lesions and an additional of 19 lesions were detected using the COE with VELscope™. Out of these 19 lesions which underwent biopsy, only one proved to have dysplasia. COE alone showed a sensitivity of 73.5% and specificity of 88.9% while using the decision making protocol, the sensitivity and specificity were 84.1% and 81.5% respectively.

**Conclusion.** In the present study the combined protocol can aid in the detection of oral mucosal lesion, which may not be identified by COE alone, some of whom might present dysplasia. However the use of VELscope™ can increase the number of lesions referred or biopsies, in fact we recorded a decrease in the specificity using the decision making protocol.

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# Malignant gingival involvement: a descriptive analysis of oral squamous cell carcinoma in a Northern Italian population

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Oral squamous cell carcinoma (OSCC) is the most common epithelial malignant neoplasm affecting the oral cavity. Carcinomas of the gingiva are a quite rare and unique subset of OSCC, constituting approximately less than 10% of total cases.

This study describes clinical features, risks' factors, grade of invasiveness, relapses, metastasis, multifocality and precancerous lesions of a large series of gingival OSCC.

The medical case records of 85 patients with previously untreated squamous cell carcinoma of the gingiva, followed up between 1988 to 2017 at the Oral Medicine Section, CIR - Dental School (Azienda Ospedaliero - Univer-sitaria, Città della salute e della Scienza di Torino, Turin, Italy) were reviewed. We obtained personal data, clinical characteristics and potential etiologic factors from ward's database and medical records.

This case comprises 39 males (46%) and 46 females (54%) who ranged in age from 42±90 years (mean age 70 years). Tobacco was used regularly by 10 patients. 9 patients consumed alcohol daily. Tobacco and alcohol were used concurrently in 3 patients. Irritation from prosthesis trauma was present in 15 patients. These tumors commonly arose in the edentulous areas (in 42 patients in our study), although they also developed at dentate areas (n° 27) and at alveolar mucosa (n° 16). Carcinomas of the mandibular gingiva are more common than those of the maxillary gingiva, respectively 59 and 26 cases. We also collected others gingival malignant lesions: there were 5 patients with gingival melanomas, 8 patients with gingival manifestation of leukemia, 2 patients with gingival metastasis and two patients with lymphomatous lesions. In our database, we also found: verrucous carcinoma of the gingiva, which was identified in 10 patients, paved-cement cell carcinoma in 4 patients, others minorities, such as adenoid cystic carcinoma and mucoepidermoid carcinoma, in single case each.

OSCC is the most common malignant lesion in the gingiva. Patients with OSCC have a varied etiology, some of which are established while a few of the cases do not elicit classical risk factors. The coincidence with alcohol and tobacco consumption is not as close as in other series. The 5-year survival rate of gingival SCC is considerably less as compared to SCC developing at other sites, suggesting a poor prognosis. Hence, OSCC of the gingiva should be considered in the differential diagnosis when dealing with gingival lesions, especially those of inflammatory origin and, particularly, in elderly subjects. It is of paramount importance that the lesion must be diagnosed early to initiate treatment as soon as possible and thereby possibly improving the initial prognosis.

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# Oral stomatitis and mTOR inhibitors. A complete analysis of 21225 cases reported in literature

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**Aims.** Traditional treatment of malignancies with chemotherapeutic agents is often affected by the damages inflicted on normal, healthy cells. Toxicities of the oral cavity, such as mucositis and stomatitis, are some of the most significant and unavoidable toxicities associated with anticancer therapies. For these reasons, everyone hoped in the newer targeted agents and their minor effects on normal, healthy cells. Unfortunately, targeted anti-cancer therapy can still cause significant toxicity to non-cancer cells. mTOR inhibitors have some adverse events, such as hyperglycemia, hyperlipidemia, hypophosphatemia, hematologic toxicities and muco-cutaneous eruption, but the most important are still stomatitis and skin rash, often reported as dose-limiting side effect.

**Methods.** The Authors performed a search of PubMed online database using 'sirolimus' OR 'everolimus' OR 'temsirolimus' OR 'deferolimus' OR 'ridaforolimus' combined with the terms 'stomatitis' OR 'mucositis' OR 'oral pain' as search keywords. The research yielded 382 potentially relevant studies, but 114 studies were excluded due to the lack of data on side effects or because were not in English language and 131 studies were excluded due to the absence of specific data on mucositis type. 137 studies were included in the review: 97 on everolimus, 16 on ridaforolimus and 24 on temsirolimus.

**Results and Discussion.** The rate of stomatitis in patients treated with everolimus is 40.9% with a percentage of 74.27% cases of stomatitis grade 1-2. The prevalence of stomatitis grade 1-2 in patients treated with ridaforolimus is 47.76% while the percentage of stomatitis grade 3-4 is 5.87%. In patients treated with temsirolimus stomatitis grade 1-2 is 25.89% while grade 3-4 is 4.34%.

**Conclusions.** Analysis of the reports with patients treated with everolimus, temsirolimus and ridaforolimus showed a clear prevalence of stomatitis grade 1 or 2. These data differ from that of patients treated with conventional chemotherapy in which mucositis are predominantly of grade 3 or 4.

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# Concept therapy: Interdisciplinary tumor board for the diagnosis and treatments of head and neck cancer

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Given the nature of their illness, cancer patients receive care from multiple health professionals, working in a variety of settings. They are particularly susceptible to problems of communication failures and poorly coordinated care. Interdisciplinary teamwork (ITW) is designed to promote the active participation of several disciplines in delivering comprehensive cancer care to patients. ITW provides mechanisms to support continuous communication among care providers, optimize professionals' participation in clinical decision-making within and across disciplines, and foster care coordination along the cancer trajectory. Since 2009, a clinical project named "GOTeC" (Gruppo oncologico testa e collo) started at Policlinico "P. Giaccone" of Palermo. This project was born from the collaboration of different professionals from different disciplines: Oral Medicine, Plastic and Reconstructive Surgery, Medical Oncology, Pathology, Radiology and Microbiology (<http://www.sipmo.it/wp-content/uploads/2015/03/Depliant-GOTeC-2.04.15.pdf>) GOTeC is the first group in Sicily that has developed as an oncologic research team and as an help to the cancer patients to reach dedicated assistance, taking responsibility for the entire diagnostic-therapeutic iter, from the early diagnostic suspect of the lesion to the eventual confirmation of the diagnosis itself. Then, GOTeC provides the most efficient therapeutic protocol, follow-up and management of the related effects of the surgical and medical treatments – both in short or long term – up to the functional rehabilitation of an anatomic district that is of vital importance. Interdisciplinary nature is strongly connected to the advent of e-health. New software and mobile apps came as a novel concept in all fields of social life, including medicine. Considering this, we had the need to create a WebApp responsive GOTeC, based on framework AngularJS, HTML5 and Bootstrap library. For his creation we started from the draw up of a relational database and as language SQL has been chosen. As many tables as departments are added in the database, and they are each other related by the ID of the patient. From the "Home" of the Webapp it's possible to refer to the patients's list and thus to consult the clinical diaries made in every department. It's also possible to 'virtually' create a GOTeC meeting choosing patients who will be take under investigation, and then to add a report of what was discussed on. GOTeC is a promising system, whether used as a communication tool between different professionals from different disciplines.

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# Optical Coherence Tomography as a new device for the evaluation of desquamative gingivitis: preliminary study

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**Introduction.** “Desquamative gingivitis” refers to oral clinical manifestation associated with several mucocutaneous disorders. The most common diseases are mucous membrane pemphigoid, pemphigus vulgaris and lichen planus. Their specific diagnosis is established by histopathological and immunofluorescence evaluation. However, in some instances, a rapid, non-invasive and real-time tool could greatly assist diagnosis procedures and follow-up. Indeed, in certain cases, the medical history is not definitive and, thus, the use of an easy, real-time and non-invasive method would considerably assist the clinician. One of the most new device investigated is Optical coherence tomography (OCT), a new non-invasive biomedical optical technology that provides high resolution of the cross-sectional tissue images already applied in ophthalmology for diagnosis of retinal macular diseases. The aim of this study is to compare OCT findings in desquamative gingivitis with conventional histopathological section of the biopsied lesions.

**Material and methods.** A total of 5 cases of suspected mucous membrane pemphigoid, pemphigus vulgaris and lichen planus were included. We evaluated after informed consent for all procedures by means VivoSight® OCT (Michelson Diagnosis). After a standardized protocol consisting of clinical examination and an OCT analysis of the lesions and then an incisional biopsy. 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.** Clinical examination revealed multiple, painful, gingival erosions. In cases #1 and #3 OCT examination showed hyperkeratosis, with more reflective keratin cell layer and histopathological findings of Oral Lichen Planus. In two cases (#2 and #5) Optical Coherence Tomography examination showed a separation at the level of the dermal-epidermal junction, with fluid-filled bullæ appearing dark, with a significant greyish aspect. Histopathological and indirect immunofluorescence findings showed diagnosis of mucous membrane pemphigoid, and in case #4, OCT features were intraepithelial cleft. In this case was confirmed the diagnosis of pemphigus.

**Conclusion.** Viewing OCT images with an optical microscope is of the utmost importance since different diseases share similar, clinical features, however indicating different treatment and management. OCT could be a new non-invasive 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 oral diseases: further researches are needed.

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# True leukoplakia vaporization methods by CO<sub>2</sub> laser: clinical trial

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True Leukoplakia (TL) is the most common potentially malignant epithelial disorder of oral cavity, defined by the World Health Organization in 2005 as a white plaque of questionable risk having excluded other known diseases or disorders that carry no increased risk for cancer. There is no evidence, in literature, of the existence of a treatment that can significantly reduce the recurrence rate and risk of neoplastic evolution of TL. For this reason the aims of this clinical trial are to investigate the efficacy of laser vaporization in the management of TLs without epithelial dysplasia and to evaluate the need to extend or not, by vaporization, beyond the clinical margin of the lesion, according to the concept of field cancerization (FC). FC considers TL a complex lesion, characterized by the presence of genetically modified cells even beyond the area with alterations evidenced by clinical or histopathological examination.

Thirty-six TLs, diagnosed through a cold blade incisional biopsy as non-dysplastic, were included in the study and were divided into 3 groups: group A (11 TLs vaporized by CO<sub>2</sub> laser, without extension or with extension up to 1 mm beyond the clinical margin of the lesion); group B (9 TLs vaporized by CO<sub>2</sub> laser, with a 3 mm extension); control group (16 TLs, not treated but monitored for 6 months after the sole removal of risk factors, achieved during the diagnostic phase for all the 36 lesions analyzed). Groups A and B were treated by CO<sub>2</sub> laser, (SmartXide®, DEKA, Florence, Italy, 10600nm), 4.5 Watt power in pulsed wave (80 Hz, fluence 44,78 J/cm<sup>2</sup>), 400 µm spot diameter. In group B TLs, before vaporization, a 3 mm widening margin was registered by a periodontal probe and demarcated using a sterile dermatographic pencil. Controls at 1 week, 3 weeks, 3 months and 6 months after therapy were performed for both groups of vaporized lesions. Photos were taken always using the same device (Nikon D200, Nikon Corporation, Tokyo, Japan) and statistical data processing was performed through the Analysis of Variance ANOVA.

Sixteen of the 36 lesions healed completely after 6 months: 6 of group A (55%), 6 of group B (67%) and 4 of control group (25%).

Concerning recurrence data, 5 recurrences of group A (2 partial and 3 complete) and 3 of group B (3 partial) were recorded.

According to the results of this study, it is preferable to adopt an interventionist approach in the management of TLs without epithelial dysplasia and it is advisable to adopt 3 mm safety margins in vaporization procedures.

It is important to emphasize that TL laser vaporization is a specialist treatment that can be adopted only by professionals able to discriminate when a surgical approach is needed.

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# Treatments of Oral Lichen Planus: a systematical review and meta-analysis study

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Oral Lichen Planus (OLP) is a chronic inflammatory condition that affects mucous membranes into the oral cavity. OLP may appear as reticular form, the most common, the plaque and erythematous or atrophic forms; rare is the bullous variant. These lesions may cause burning, pain, bleeding or other complaints. The severity and subsequent disability caused by OLP varies from poor to severe. The real cause of OLP is not completely understood, but genetics and immunity seem to be involved. Because of this uncertain etiology, treatment strategies are focused on relief of pain and healing of oral lesions. Although many options for treating symptomatic OLP are available, no therapy is curative. The aim of this study is to evaluate the efficacy of different treatment protocols through a systematic review and meta-analysis study.

The PICO (Patient and problem; Intervention; Comparison; Outcome) model was used to structure the meta-analysis. Randomized clinical trial with crossover tests about different treatment strategies were included in the review of the literature. Selected papers had to be entirely available on the most important medical databases, such as MEDLINE, PUBMED, SCOPUS. Only studies regarding adult patients with clinical and histopathological manifestations were included.

The total amount of detected studies for the systematic review was 576. The duplicate studies were removed (286), while 216 were excluded because they did not respect the selected criteria. The remaining 74 studies analysed: different drug therapies (steroids, topic and systemic immunosuppressants, retinoids, biological agents) and non-pharmacological ones (surgery, low level laser therapy and photodynamic therapy). After the identification of all the proposed therapies, a statistical meta-analysis study was realized among 11 of the 74 papers selected, to estimate the efficacy of the most used therapeutic protocols. In particular, clobetasol (topic corticosteroid) and tacrolimus (calcineurin inhibitor) were compared. The considered outcome of the analysis was the clinical improvement of the lesions, in size, frequency of appearance, duration, severity and number.

For the statistical analysis, the Cochran's Q test was used. The results identified a higher efficacy of Tacrolimus (4.29% more than other therapies) compared to Clobetasol (1.21% more than other therapies). These results are compatible with Chamali et al. meta-analysis study (2015). Since many studies reported that Clobetasol may cause oral candidiasis, Tacrolimus could be preferred in either diabetic or immunodeficient patients. However, the most important Tacrolimus contraindication is the risk of cancerization. For this reason, a prolonged use of topic tacrolimus is strongly discouraged. Apparently, calcineurin inhibitors are highly effective in the treatment of lichen planus lesions, but more clinical randomized trials are needed to support a greater diffusion of this protocol of therapy.

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# Oral and periodontal conditions of patients affected by Medication-related osteonecrosis of the jaw (MRONJ): University Hospital case series

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**Introduction.** It is well-documented that Antiresorptive Drugs (ARDs) such as bisphosphonate (BPs) could lead patients, both oncologic or osteoporotic ones, to medication-related osteonecrosis of the jaw (MRONJ). Although MRONJ onset and progression is due mainly to ARDs drug type and overall dose, many local risk factors, such as periodontal conditions have been recognized. Aim of this study was to evaluate MRONJ patient's oral conditions, before and during MRONJ onset and to analyze the association between periodontal disease and MRONJ onset.

**Material and methods.** This retrospective study included MRONJ patients referred to Dental Clinic, University Hospital, with a history of ARDs 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). All parameters were merged to assign each patient a comprehensive risk evaluation score for MRONJ, "HIGH" or "LOW" score.

**Results.** During 36 months' observation time 361 patients, eligible/treated with ARDs, such as Zoledronate, Ibandronate, Sunitinib and Denosumab, mean age of 67 years (range 33-92 yrs), received complete dental examination, non-surgical periodontal therapy, dental extraction and prosthesis conditioning. Individual risk for MRONJ was calculated for each patient during first visit and after 3 months at least. Among LOW risk patients (60.39% of overall population) 3 developed MRONJ, compared to 31 within HIGH risk group (39.61% of overall population). For those patients developing MRONJ, clinical parameters records showed higher percentage when compared to overall population (PPD >4 mm +8%, BoP +15%, PII +4% mean value). Considering risk scores calculated for all patients, a correlation between HIGH risk score and MRONJ onset was observed, according to risk method with high sensibility (94%).

**Conclusion.** According to literature periodontal, dental and implant conditions are risk factor for MRONJ onset. MRONJ with ARDs treatment typically occurred within 2 years from the start of the treatment with BPs and within 1 year with Denosumab. Despite the clinical correlation between ARDs and MRONJ, a definitive causal relationship has yet to be established. Routine and thorough oral examinations, hygiene, and periodontal maintenance procedures are mandatory to lessen the likelihood of the development of recurrent or new areas of MRONJ all life long.

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# Psychiatric disturbances and cumulative steroid therapy in Pemphigus Vulgaris patients

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**Introduction.** Pemphigus Vulgaris (PV) is a severe autoimmune blistering disease affecting skin and mucous membranes (1). Although a variety of systemic side effects of conventional high-dose corticosteroids are well known, adverse neuropsychiatric effects, as depression, anxiety, sleep and related neuropathogenetic mechanisms, still remain not clarified (2).

**Aim.** To assess psychiatric disturbances and quality of life (QoL) in PV patients on conventional high-dose corticosteroids therapy.

**Methods.** 30 PV patients and 30 healthy participants were enrolled; Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale, Hamilton Anxiety (HAM-A) and Depression Scales (HAM-D), Health Status Questionnaire Short Form-36 items (SF-36) were used to investigate sleep, anxiety, depression and QoL in both groups.

**Results.** PV patients had an average score higher than healthy patients indicative of a poorer quality of sleep (PSQI p-value 0.004), higher levels of depression and anxiety (HAM-A and HAM-D p-values 0.000). Different scores were also found for some items of SF-36: "physical role limitations" (p-value 0.000) and "general health" (p-value 0.005), "physical pain" (p-value 0.057), "vitality" (p-value 0.044) and "emotional role limitations" (p-value 0.021) indicative of a general impairment of QoL in patients. Three subgroups of patients divided according to the cumulative time of conventional high-dose corticosteroids therapy, one of 8 (26.7%) patients who had been on therapy for less than 1 year, a second one of 13 (43.3%) patients for a time between 1-3 years, and a third one of 9 (30%) patients for more than 3 years, were compared. No statistically significant differences were found on psychiatric disturbances among these groups. Further more, 13 patients on therapy were compared to 17 patients off therapy (3). Even in this case there were no statistically significant differences between the two subgroups.

**Conclusions.** PV patients might be at risk of psychiatric disturbances and poor QoL. Cumulative time of conventional high-dose corticosteroids seems to not influence the incidence of psychiatric disturbances. There are probably several factors to consider that might attempt to explain why patients have psychiatric alterations: side effects and iatrogenic comorbidities that could last for many years after therapy; the interactions between steroids and Hypothalamic-Pituitary-Adrenal axis and Central Nervous System the possible correlation between autoimmune disease and depression-like symptoms or, ultimately, the patients' experience of the disease itself. However more clinical research is needed to expand the study group and better evaluate iatrogenic psychiatric comorbidities/disturbances.

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# Treatments and outcomes: a retrospective analysis of patients affected from MRONJ among 2009 and 2016 at Turin University

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**Introduction.** To analyse the outcomes of the patients affected from MRONJ at Turin University from 2009 to 2016, and to assess the weight of some variables about patients, the staging of MRONJ, and the type of treatment for MRONJ.

**Materials and methods.** Among 1389 at risk for MRONJ from 2009 to March 2016, 153 were selected whose had developed MRONJ. Patient with a follow-up after treatment inferior to 6 months were excluded. A group of demographical variables (age and sex), clinical variables (base pathology, medicine, pharmacological associations, steroid therapy, stadium) and therapeutic variables (surgical vs conservative; LLLT, plasma rich in growth factor) were analysed.

The outcomes were divided in 2 groups: healed/improved (resolution or positive change of stadium after 6 months post-treatment); stable/worsened (stability or negative change of stadium after 6 months post-treatment).

**Results.** 147 patients were included in the study. 109 female (74.15%) and 38 males (25.85%); the mean age was 70.12 years; the most frequent base pathology was breast cancer (36.73%) followed by myeloma (21.09%), osteoporosis (17.01%), prostate cancer (10.88%) and others. 74 (51.39%) patients were treated at stadium I, 52 (36.11%) at stadium II and 18 (12.50%) at stadium III using SIPMO/SICMF staging. Zoledronic acid was used for 107 (72.79%) patients, Alendronate for 24 (16.33%), Denosumab for 5 (3.4%), Ibandronate and Pamidronate for 3 (2.04%) respectively, Bevacizumab and Sunitinib for 2 (1.36%) respectively and Risedronate for 1 (0.68%). Tooth extraction was the principal cause of development of MRONJ (52.38%), while in 33.33% of cases it was spontaneous.

In 109 (74.15%) patients the mandible was involved in MRONJ, in 35 (23.81%) was involved the maxilla and in only 3 cases (2.04%) were both involved. 70.7% of patients underwent to surgery, instead 29.93% were treated conservatively. 74.15% bettered after the treatment.

12.24% had a relapse. Fisher exact test; Kruskal-Wallis test and chi-square test were used for the subsequent results to assess what were the factors that conditioned the outcome of MRONJ. The improvement or the resolution was better in the maxilla than in the jaw ( $p=0.01$ ) with a probability of healing 2.04 greater for the maxilla. Surgical therapy at early stages (small surgery) is 3.62 (OR) times more effective than conservative therapy ( $p=0.03$ ). Even if not statistically significant ( $p=0.11$ ), early stages heal better than tardive stages. Association with other medicines is a worse factor in the outcome (OR=0.48), but the previous use of an oral bisphosphonate doesn't change the outcome (OR=1.03).

**Conclusions.** The knowledge and the study of the outcomes is useful in order to assess the predictability of some therapies that are daily used on patients affected from MRONJ and to compare results among Centers with the aim to find the better strategy to face up to MRONJ.

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# Validation of a non invasive procedure based on Bisulfite sequencing of a 13-gene panel to early detect Oral Squamous Cell Carcinoma in brushing samples

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**Introduction.** 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. Recently epigenetic alterations such as DNA methylation alterations have been associated with tumor aggressiveness, invasiveness and with the malignant transformation of High Grade Squamous Intraepithelial Lesion (HG SIL) (1). To improve early diagnosis, different Authors have proposed to analyze the methylation status starting from saliva and/or brushing specimens (2, 3).

Aim of the present study was to propose and assess the feasibility of a non invasive method for early OSCC detection based on an original method to evaluate DNA methylation of 13 genes in parallel, starting from a minimally invasive oral brushing specimen.

**Methods.** Oral brushing specimens were collected from 31 patients with an histological diagnosis of OSCC and 7 patients with an HG SIL; samples were collected either from the lesions or from clinically normal distant mucosa (opposite cheek). Oral brushing specimens were also collected from clinically normal mucosa of 105 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 a linear discriminant analysis (LDA) 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.** 30/31 OSCC and all HG-SIL were detected as positive, while none of healthy donor showed false positive results (ROC analysis: AUC=0.981). 4/37 (10.8%) samples from normal distant mucosa in OSCC patients showed higher values with respect to the cut off value. The mean values obtained by gene combination in OSCC and HG SIL samples were significantly different from that in normal mucosa of healthy donors. Further, the mean value in the group of normal distant mucosa of OSCC patients significantly ( $P<0.01$ ) differed from that in normal mucosa of healthy donors.

**Conclusions.** The results of the present study confirm the presence of epigenetic alterations in OSCC, but additionally show that these alterations may be also present in normal mucosa distant from OSCC areas. The novel assay based on quantitative bisulfite - NGS analysis could be a highly sensitive and specific method to early detect OSCC starting from not invasive, easy-to-perform, type of sampling.

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# Osteonecrosis of Jaw (ONJ) in osteoporotic patients: increasing incidence in northwestern Italy

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**Introduction.** Osteonecrosis of Jaw (ONJ) in patients suffering from metabolic bone disease (osteoporosis, etc) is reported as rare but increasing. Drugs proposed for prevention and/or treatment of osteoporosis include: oral alendronate; oral risedronate, iv ibandronate (3 mg q3months), oral ibandronate (150 mg monthly), biannual iv zoledronic acid (4 mg q6months), yearly iv zoledronic acid (5 mg q12months), sc denosumab (60 mg q6months), occasionally also pamidronate and clodronate are administered. All these agents showed possible induction of ONJ, described by some Authors as “rare” (1/1.000-1/10.000, according to WHO) or “very rare” (<1/10.000). Actually, evaluations about the individual ONJ risk are uncertain, due to bias in old trials (conducted when ONJ was not yet known, with short-term follow-up, etc.) and in recent trials (adopting a very restricted ONJ definition, with limited observation time). Vice versa, real life reports of ONJ cases after bisphosphonate (BP) and/or denosumab treatment in osteoporosis patients are not so rare (even if probably underdiagnosed), exceeding the ONJ cases in metastatic cancer patients in some countries (eg, Korea).

Since 2005 a multidisciplinary study group collected data of cases of ONJ in patients treated with BP in oncology and hematology centers of a regional network (Rete Oncologica Piemonte-Valle d’Aosta), and among patients followed in the main dental care and maxillofacial surgery centers of the area. Between 2004 and December 2008, out of 241 total ONJ registered cases, 20 pts (8.3%) had ONJ diagnosis after BP therapy of bone disease different from bone metastases or myeloma (i.e. osteoporosis, osteopenia, Rheumatoid Arthritis, Paget’s disease, etc.).

**Patients and methods.** The survey was repeated, asking for ONJ cases observed between 2009 and 2016. We identified cases after cross-checking reports from medical oncology, haematology and oral care centers to avoid double count and to integrate data.

**Results.** At July 2017, we received partial data about 440 cases: 335 advanced cancer patients (76%) and 105 patients treated for other diseases (24%). The median number of cases per year was clearly increased in years 2009-2015, especially in osteoporosis patients. Local visits to collect complete data of all cases (duration and doses of therapy, concomitant treatments and diseases, oral health risk factors) are ongoing.

**Conclusion.** Preliminary data show increase of ONJ cases in patients receiving BP or denosumab for osteoporosis. Further studies are necessary to evaluate the need of oral care measures to reduce the ONJ risk in patients receiving antiresorptive drugs to treat or prevent osteoporosis as well as it is already recommended for cancer and myeloma patients.

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# Tumoral heterogeneity in oral squamous cell carcinoma and related adverse events: a study of 5 cases

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**Introduction.** According to evolutionary models, tumour cells may evolve following different mutational pathways. The formation of distinct subclones is defined as Tumour Heterogeneity (TH) and has been related to tumour aggressiveness and resistance to therapy. So far, only few studies have investigated TH in oral squamous cell carcinoma. Aim of this study is to investigate molecular profiles of different regions of primary tumour and related adverse events. To the best of our knowledge, genetic analysis of TH in subsequent manifestations has never been performed before. A better understanding of TH could be helpful for future individualized therapies for OSCC.

**Methods.** 5 patients with primary OSCC were studied. Samples from different regions including diagnostic biopsy and tumor surrounding mucosa were selected and manually dissected from 10 µm thick sections of formalin fixed paraffin embedded tissue blocs. Similarly, samples from secondary manifestations were collected. DNA sequencing was performed using next generation sequencing (NGS) for a panel of 10 tumour specific genes. mtDNA analysis was also performed while the degree of TH was measured calculating the ratio of unshared mutations over the total amount of mutations.

**Results.** Genetic landscapes of studied tumours showed a high level of heterogeneity. In particular TP53 was the only gene found mutated in all tumours. In addition, as far as the mutations and not the gene were considered, only PIK3CA p.W1051\* and PIK3CA p.G1050S were mutually shared by more than one patient. A high degree of intratumoral heterogeneity was also noted. Indeed, even if in 4 out of 5 patients the same mutated gene was found in more than one sample, the same mutation was never found to be shared by multiple samples of the same tumour (TH value was 100% for all the 5 patients). In non-neoplastic tissue, mutations were found in 2 out of 4 patients. Interestingly in patient 2, where 2 samples were collected, differences in the mutations were observed. Secondary events were studied in patients 2 and 5. Patient 2 developed six secondary tumours while patient 5 developed three tumours after the primary OSCC. Interestingly in patient 2 the same mutation (KRAS p.A130Q) was found in all tumours after the first neoplastic event and was also mutually shared by all the different samples. Consequently, TH values of secondary events were in all cases lower than primary tumour TH value. By contrasts, in patient 5, single mutations, despite occurring in the same gene, were unique in all samples, suggesting different patterns of recurrences in patients 2 and 5.

**Conclusions.** Genetic discrepancies among OSCCs support the idea that tumours of the same subtype may be histologically similar but genetically distinct, reflecting in different clinical behaviors. Furthermore, given the high values found for intratumour heterogeneity among samples, both preoperative biopsy and a single sample cannot be informative of the genetical profile of the entire tumor. Noteworthy, heterogeneity observed in tumour surrounding mucosa suggests that a field heterogeneity may exist. Moreover, in some cases genetic instability in tumour surrounding mucosa may foster the occurrence of aggressive mutations that can "drive" the carcinogenesis of subsequent neoplastic events. These mutations could be used as target for future tailored therapies.

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# Osteonecrosis of Jaw (ONJ) in osteoporotic patients: comparison of stage according to AAOMS and sipmo-sicmf staging systems

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**Introduction.** Osteonecrosis of the Jaws (ONJ), or Medication-Related ONJ (MRONJ), in osteoporosis patients has been defined as rare, but the number of reported cases is increasing. Controversies on ONJ definition induce underestimation of ONJ incidence and/or diagnosis delay. The definition of the American Association Oral Maxillofacial Surgery (AAOMS) task force is based on clinical features), whereas the Italian proposal, supported by Società Italiana Patologia Medicina Orale (SIPMO) and Società Italiana Chirurgia Maxillofacciale (SICMF), imply the use of imaging tools (mainly Computed Tomography, CT) together with clinical features. These definition differences are important in order to make earlier diagnosis and improve treatment effectiveness, implying also different ONJ stage systems.

**Aim.** The aim of this study is to compare the stage of ONJ cases at the first observation time in patients receiving antiresorptive therapy (bisphosphonates, denosumab) for metabolic bone diseases, according to two different staging systems (AAOMS and SIPMO-SICMF).

**Methods.** We reviewed data for patients with osteoporosis and other non malignant disease (Rheumatoid Arthritis-RA, Paget's disease, etc) treated with bisphosphonates and /or denosumab with signs of ONJ. Cases were observed at two northern Italy centers: the hospital ONJ Multidisciplinary Team, in Alessandria, and the City of Health and Science - CIR Dental School, University of Turin. Collected data were: age, sex, date of first ONJ manifestation, baseline disease, type of antiresorptive treatment(s), ONJ site(s) and staging according to both AAOMS and SIPMO - SICMF systems.

**Results.** We collected data about 43 patients: 2 M/41 F; mean age 71.2 years; 40 patients with osteoporosis, 3 with RA.

The AAOMS stage was 0/1/2/3 respectively in 4/13/19/7 cases. The SIPMO-SICMF stage was IA/IB/IIA/IIB/III respectively in 9/1/9/15/9 cases. The 4 AAOMS stage 0 cases (without bone exposure) were reclassified as stage IIA (2 cases), IIB (1), III (1) respectively, according to the Italian system. Out of 13 AAOMS stage 1 (exposed bone, asymptomatic at the observation time) cases, 9 were reclassified as stage IA, and 4 as stage IIA. The 19 AAOMS stage 2 (exposed bone, with pain or purulent discharge) cases were so reclassified: 1 as IB, 17 as IIB, 1 as III. The 7 AAOMS stage 3 (advanced/complicated) cases were all classified as stage III according to the Italian system.

**Discussion and conclusions.** There is no unique ONJ definition and staging system, even if the AAOMS one is mostly reported. In our hands, even in ONJ cases in osteoporotic patients (often incorrectly reported as cases mostly with limited and not severe disease), the AAOMS definition and staging system are insufficient, exposing patients to delayed diagnosis. It would be advisable to establish the diagnosis not only on the basis of the clinical data but also on the findings of the CT scan, since the latter technique offers greater information on the extent and severity of the disorder.

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# Clinical evaluation of chlorhexidine digluconate use in oral mucosal surgery: preliminary results

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The oral biopsy procedures are usually reserved for patients with clinically detectable lesions, in order to figure out their histopathological features. These are actually very common and secure measures, usually quite painless but they do require some healing time. Certain types of mouth washes with particular ingredients, such as chlorhexidine, could help participants to heal faster, prevent pain and improve quality of life after the procedure. The aim of this study was to examine and to differentiate the clinical utility of two chlorhexidine mouth-rinses, dissimilar in concentration, after oral mucosal biopsies.

Participants underwent the standard technique in an Oral Medicine Unit. They were randomly allocated to one of two groups: those in the first group were given a 0.12% chlorhexidine mouth-rinse to take twice daily for six days (starting one day after the procedure); those in the second group were given a 0.20% chlorhexidine mouth-rinse to take twice daily for six days (starting one day after the procedure). Outcome variables statistically evaluated were: age, gender, site and size of investigated lesions, visual analogue score (VAS) of pain, the Oral Health Impact Profile questionnaire (OHIP-14), healing aspects, and number of analgesic drugs taken in the first week after surgery.

Sample size was challenging to estimate, based on the lack of any previously reported changes in patients treated with this protocol; the power of the study was then calculated for a range of values at 0.95 confidence, for a 100x2 sample size (with software PASS13). Each group will have to be composed of at least 100 patients. Descriptive analyses were performed using the t-test or Mann-Whitney test for continuous variables and the Chi square test or Fischer exact test for categorical variables when indicated. Analysis was stratified by gender, age, smoking habits, size of lesions and duration of surgery. Statistical significance level was set at 0.05; SPSS (SPSS for windows, version 19, SPSS Inc, Chicago, IL, USA) statistical software was utilized.

The results now reported are preliminary because the protocol described is still ongoing. To present, 27 patients were treated in group A (0.12% chlorhexidine) and 26 in group B (0.20% chlorhexidine). No statistical differences were found between the two groups regarding gender, age and smoking habits. Patients in group A reported a worse quality of life (with a medium OHIP increase of 3.215) when compared with those of group B (with a medium OHIP increase of 0.6023) and this difference was statistically significant ( $p=0.0186$ ). No statistically significant differences in terms of reported pain, healing aspects and analgesic taken, have been detected between the 2 groups.

To the best of our knowledge, not so many data are available regarding the use of chlorhexidine mouth-rinse after oral mucosal surgery in terms of alleviating reported pain and quality of life. At this point, both concentrations seemed to be useful after oral biopsy procedures in terms of reducing reported pain and fasting healing time; 0.20% chlorhexidine seemed to be better in improving reported quality of life. Our preliminary data seem to underscore the importance of this medication in our everyday clinical practice in oral medicine; however, it would be interesting to know if these preliminary results will be the same at the end of the recruitment, thus dealing with a larger sample of patients and different clinical situations.

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## Estimating cost of illness of oral lichen planus: an out-patients based study

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**Introduction.** To estimate the economic cost of oral lichen planus (OLP) in a sample of Italian population.

**Materials and methods.** The economic burden of OLP was estimated through a retrospective study of a cohort patients with OLP, followed in the Oral Medicine department of the Catholic University of the Sacred Heart in Rome. Data were collected from medical records of the patients and the cost of OLP was carried out.

**Results.** Sixty-two patients with histological diagnosis of OLP (43 females and 19 male), with a mean age of 64.9 years, were enrolled in this study. In our cohort the anatomical site involved were the buccal mucosa (64%), followed by the tongue (28%), with only the 7% of other muco-cutaneous lesions reported. The mean follow-up was 48.4 months with an average of 10 visits per patients (2.5 per years). The study population was divided in two groups according to the need of therapy: 35 subjects took medications (group 1) and 27 did not (group 2). group 1, in a mean follow-up time of 68 months (5.6 years), received an average of 14 visits (range 3-36; 2.5 per year) and 1.6 biopsies (range 1-3) with 417 applications of clobetasol dipropionate (range 120-2610; 74.5 administrations per year) and 192 administrations of anti-mycotic (range 21-1512; 34 administrations per year). group 2, in a mean follow-up time of 24 months (2 years), received an average of 5.7 visits (range 1-17; 2.8 mean per year) and 1 biopsy (range 1-3). group 1 had a mean direct cost of 458 euros, whereas group 2 had a mean direct cost of 230 euros.

**Conclusion.** This study highlighted the economic burden of OLP in a group of Italian patients showing that OLP patients who undergo therapy have a direct cost two times greater than patients who do not undergo therapy.

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## A preliminary correlation study between skull structures and OSAs in adults

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**Introduction.** The Obstructive Sleep Apnea Syndrome is a common disease that is estimated to affect 2% of middle-aged women and 4% of middle aged men. Its incidence and gravity increase in the range of 30-60 years age, and in the overweight and sedentary life patients. The purpose of this study is to verify the presence of a correlation between gravity OSAs and skeletal parameters, and in the specific field if the cranial base angle can be considered an inductive factor of OSAs.

**Methods.** 100 patients (42 males and 58 females) with symptomatic mild to severe OSA (Apnea and Hypopnea Index AHI >10 for hour of sleep during diagnostic polysomnography) were recruited. Their mean age was 51.3 years, their mean body mass index (BMI) was 29 and their minimal oxygen saturation (SaO<sub>2</sub>) was 78.8%. Each patient underwent overnight polysomnography and cephalometric study, to analyse and compare cardiorespiratory parameters and skeletal measurements. In the specific field, we focused on the following values: a) AHI (Ap-

nea Hypopnea Index); b) SaPO2 Nadir, minimum saturation value; c) CBA (Cranial Base Angle), reference angle for the statistical valuation of skull.

**Results and discussion.** The correlation data shows: the positive correlation between AHI-CBA with a p-value of 0.2146605\*; a negative correlation with CBA-Nadir with a p-value of 0.24040\*. This results explains how post-rotation of the occipital-sphenoidal suture, generates the stretching of the upper constrictor pharyngeal muscle, reducing the pharyngeal channel size. In addition, a negative correlation between the minimum saturation SaPO2 Nadir and CBA, explains as an increased value of the width of cranial base can be considered a risk factor of desaturation of the patients.

**Conclusions.** The values of this study confirm the centrality of the skull structures such as a inductive factor of OSAs, suggesting to perform specific controls in patients with and increased cranial base angle.

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#### Case series

## Metabolic syndrome incidence in cases of oral lichen planus: a prospective case-control study in a Northern Italian population

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The term metabolic syndrome (MS) is usually referred to a group of risk factors able to increase the chance for heart disorders and other health problems. The term “metabolic” is interconnected to the biochemical processes of the body’s normal functioning. Risk factors associated could be different: 1) abdominal obesity; 2) high triglyceride level (or medicine to treat high triglycerides); 3) low HDL cholesterol level (or medicine to treat low HDL cholesterol); 4) high blood pressure (or medicine to treat high blood pressure); 5) high fasting blood sugar (or medicine to treat high blood sugar).

Recently, some Authors have reported a potential association between oral lichen planus (OLP) and MS. The aim of this study is to evaluate this possible relationship in a cohort of selected patients.

A prospective case-control analysis was conducted on a comprehensive sample collected from the Caucasian population resident in Piedmont, North-west Italy; only patients older than 18 years-old were selected, while pregnant or breast-feeding women were excluded. The case group consisted of 325 patients attending the Oral Medicine Unit, C.I.R. Dental School, who had a histopathological confirmed diagnosis of OLP in the period between January 2015 and May 2017. A sample of 250 healthy controls with no clinically detectable oral lesions, unrelated to the cases, were recruited from the population attending two dental private practices in need of an oral implant rehabilitation.

Anamnestic data were collected thoroughly in both groups, focusing on pre-existing or coexisting systemic diseases and daily medications. In particular, the prevalence of diabetes, Hepatitis C (HCV) infection, hypertension, use of lipid modifying agents and their serum levels of Fasting Plasma Glucose (FPG).

Quantitative variables were described via means, standard deviations, medians, first and third quartiles; qualitative ones via frequencies and percentages. Due to the non-Gaussian distribution of the medians, their differences were tested using Kruskal-Wallis tests. Chi squared tests were performed to evaluate differences in qualitative variables; meanwhile if the values were expected to be less than 5, Fisher’s exact tests were performed. Then, odds ratios (ORs) and their 95% confidence intervals (95% CIs) were obtained computing two multivariable logistic regression models. The first one was adjusted for age, gender, body mass index (BMI) and smoking status; the second one was adjusted also for diabetes, hypertension and HCV infection. Statistical analyses were performed using SAS ver.9.3 and a 2-tails p-value less than .05 was considered statistically significant.

The two groups were similar for sex, age, habit of smoking and HCV. There was a statistically significant difference

rence between OLP patients and the control group for body mass index (BMI) ( $P=.0001$ ), hypertension ( $P=.005$ ) and diabetes ( $P=.02$ ). Obese patients were associated with an almost 2.15-fold increased odds of having OLP (odds ratio 2.14, 95%CI: 1.17-3.94); if we consider patients with atrophic and erosive types this risk increase up to 3.17-fold (odds ratio 3.17, 95%CI: 1.47-6.84).

We observed a statistically significant association between OLP and MS. Patients with OLP statistically have high blood pressure levels, diabetes and BMI. Physician must be aware of this association, also considering that we recently reported that patients with atrophic or erosive OLP could possibly have a higher risk of developing cardiovascular diseases. Further analysis on larger groups are however warranted.

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#### Case series

## The geographic tongue: clinical and medical features in a cohort of affected patients

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**Introduction.** The aim of this study is to describe the clinical and medical features of a cohort of patients affected by geographic tongue (GT) and identified in the students' clinical training department of the school of dental hygiene of the UniSR - Milano. Geographic tongue, 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 (ectopic geographic tongue). GT is a common oral condition affecting about 3% of population and it occurs more frequently below their fifties. The etiology is still unknown and no risks or severe consequences have been related, although it has been demonstrated an association with psoriasis and/or atopic allergies, probably with a genetic origin. GT could be associated with fissured tongue. The typical lesions of GT are 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. Patients usually refer to GT as asymptomatic, and just in few cases it has been reported a mild irritation due to increased sensitivity to spicy and acidic foods in the affected areas. This condition alternates periods of remission and aggravation in severity of the signs and symptoms.

**Methods.** Among 421 patients consecutively treated in the clinical department of dental hygiene, 23 patients affected by GT (5.46%) were identified on the basis of the clinical aspect of the dorsal surface of the tongue and the referred symptoms. Demographic, medical and oral clinical data were collected and recorded in a customized questionnaire, then digitized and analyzed with JMP 9.0 software. Statistics of the demographic and medical features and correlations were obtained.

**Results.** Patients with GT were 13 females and 10 males, aged from 8 to 82 yrs, mean age=40.0. 5/23 were smokers and 10/23 were moderate regular drinkers. 15 patients referred to be affected by atopic and/or acquired allergies to environmental, chemical, pharmacological or food allergens. Particularly 11/23 referred recurrent or seasonal symptoms of hay fever, allergic conjunctivitis, or cutaneous manifestations. Three patients were diagnosed with psoriasis from several years. Oral hygiene status was adequate in the entire cohort: dental floss was regularly used by 12/23 patients while mouthwashes only by four patients. 15/23 received habitually a six months oral check-up. 15/23 noted a weird aspect of the dorsum of the tongue, significantly related to systemic allergies ( $p<0.05$ , chi square and Fisher test) while only 12/23 reported mild to moderate symptoms like stinging and/or frank burning of the mucosal tongue. 15/23 showed a fissured tongue. Some foods were uncomfortable to 12/23 patients. The VAS scale was indicative for affecting the QOL of the patients with a mild to moderate positivity for 12/23.

**Conclusion.** Although the causes and etiology of GT are still unknown, it is important to investigate the medical history for any possible information and data that might be related to GT, such as allergies and dermatological conditions.

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#### Case series/experimental studies

## Role of tumor stroma myofibroblasts in lymph node involvement in OSCC: analysis of gene expression signature

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Oral squamous cell carcinoma (OSCC) is the most common form of head and neck cancer, and is responsible of approximately 3% of cancers in men and 2% in women in the Western World, with increasing incidence rates in developing countries. Early detection by screening is necessary to prevent fatal disease because early, curable lesions are rarely symptomatic. The overall 5-year survival rate is approximately 50% when surgery, radiotherapy, or both are employed as treatment options, but lymph node involvement outstandingly influences this estimates decreasing the survival rate by about 50%.

Here, we aimed at finding genetic signatures associated with lymph node metastasis in OSCC patients. We addressed this issue by whole transcriptome analysis through microarray expression profiling of a set of OSCC specimens of patients without lymph node involvement (10 patients, mean age  $\pm$  SD 61.2 $\pm$ 13.8, male 7, female 3) and with lymph node involvement (11 patients, mean age  $\pm$  SD 62.1 $\pm$ 15.1, male 8, female 3).

Total RNA was extracted from frozen tissue specimens, and was reverse transcribed in cDNA. Whole genome gene expression profiling experiments was performed by using Affymetrix GeneChip1 Human Gene 1.0 ST Arrays. Statistical analysis of gene expression values was performed by using Partek Genomic Suite, using as significance cutoff a p value < 0.05, FDR < 0.05 and at least a  $\pm$  two-fold change in gene level expression. Subsequently, gene set enrichment analysis and expression data comparison were performed by DAVID ver. 6.8, Web-Gestalt, GeneCodis3 and Genevestigator 4.0. Finally, Ingenuity Pathway Analysis software was used to determine relationships among differentially expressed genes.

A total of 41 genes were differentially expressed: 19 genes were expressed at higher levels and 22 gene were expressed at a lower level in tumor specimens from OSCC patients with lymph node involvement. We evidenced a gene expression signature associated to muscle contraction-related genes in specimens obtained from OSCC patients with lymph node involvement. The expression of muscle tissue associated genes in samples from lymph node positive patients but are generally under-express in OSCC cell lines, suggesting that such gene signature may derive from myofibroblasts, which are absent in normal oral mucosa and constitute the OSCC stroma. Finally, Ingenuity Pathway Analysis identified 3 networks affected by this genetic pattern, involved in cell death, cellular and tissue organization, and muscle components disorders.

This gene signature suggests the presence of myofibroblasts in tumor stoma of patients with lymph node involvement and emphasizes the decisive role played by myofibroblasts probably through their secretome in determining OSCC invasiveness.

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# Salivary substitute use in xerostomic patients after hematopoietic stem cell transplantation: a case series

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**Introduction.** Hematopoietic stem cell transplantation (HSCT) is widely used as a potential curative treatment for a number of malignant and non-malignant diseases. Allogeneic transplantation may also be accompanied by graft-versus-host disease (GVHD). Oral complications may develop during the different phases of HSCT. The complications have a considerable impact on the quality of life. For example, a dry mouth may affect taste, speaking and swallowing, and increase the risk to dental caries and oral mucosal infection. Chronic oral GVHD may compromise salivary gland function. HSCT patients have significantly higher levels of xerostomia as well as several other oral complaints including painful oral mucosal surfaces, altered taste, limited opening of the mouth and difficulties with tooth brushing. HSCT recipients have markedly lower unstimulated and stimulated salivary flow rates than the donors and sicca symptoms are clearly associated with poorer quality of life.

**Aim.** Objective of this study was to evaluate the short-term impact, on xerostomia and quality of life, in patients with HSCT after the use of a salivary substitute (Oral Dry Gel® - Healman srl) containing water, coenzyme Q10, lactoferrin, lysozyme, aloe and sodium hyaluronate.

**Methods.** Xerostomic patients were recruited from those affected by oral chronic GVHD after HSCT for leukaemia. At baseline (T0), subjects reported the VAS (reported pain) values (0-10) and performed a complete oral examination in order to assess the presence or absence of lesions from GVHD (reporting the score active if present, or inactive if absent). The patients have been instructed to use the saliva substitute after their normal oral hygiene procedures (3 times daily), and then they have been re-evaluated after one month (T1).

**Results.** Five patients have been selected in a period from May to July 2017. The study group comprised 4 females and 1 males (average 68 years). No oral GVHD lesions were present in all patients. Only one patients reported an improvement in referred symptoms after 30 days of application. The other 4 subjects remained stable with no improvement neither worsening of oral pain ( $P>0.05$ ) during the follow-up period.

**Discussion and conclusion.** Generally, there is a significant decline in quality of life in early post-transplant period, with gradual improvement thereafter, and generally returned to a pre-transplant level at 1 year. Ongoing research is focused on better understanding of quality of life issues and prediction of post-transplant long-term recovery.

This preliminary data suggested that the use of this product, over a 1-month-period, did give a very feeble improvement for the oral referred status of the patients affected by xerostomia, in terms of pain (20% of patients). Further studies are needed to better evaluate the effectiveness of this product in comparison with a placebo and considering other index of oral health, and also over a longer-term period.

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# Oral health assessment in palliative patients: an exploratory preliminary survey

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**Introduction.** Generally, the multi-disciplinary team of palliative Units does not include any oral health professional (i.e dentist, hygienist). As a consequence, oral care is entrusted to nurses or caregivers who don't have appropriate experience to assess and manage oral problems.

**Aim.** The aim of this preliminary study was to explore the reliability of a specific screening tool created to easily assess the oral health status in terminal patients.

**Material and methods.** For this purpose, we used the Oral Health Assessment Tool (OHAT) among six patients resident in U.O. Hospice A.O.O.R. Villa Sofia-Cervello of Palermo. The OHAT consists of eight categories ('lips', 'tongue', 'gums and tissues', 'saliva', 'natural teeth', 'dentures', 'oral cleanliness', and 'dental pain') with three possible scores (0: healthy, 1: some changes present and 2: unhealthy condition). The scoring of each category is based on a structured oral examination of each patient according to standard clinical practice. The total score is the sum of the various sub-scores (from a minimum of 0 to a maximum of 16). Recording a total score between 8 and 16 indicates the need to refer to an oral health professionals. The materials required to perform the screening include only clean gloves, gauzes, tongue depressors and an adequate light source (daylight or artificial).

**Results.** All six patients (M/F=2/4, mean age 76.3), two with oncological and four with non-oncological terminal diseases, were underwent to oral clinical examination. The total score obtained for 50% of patients were < 5/16; one patient scored 12/16. The 66.6% of the subjects scored 0 in the categories 'denture' and the 83,33% of the patients scored 1 or 2 in the categories 'saliva', showing that dry mouth and salivary gland dysfunction are very important problems in terminal illness, both in oncological and non-oncological terminal patients. Only one patient scored 0 in the categories "oral cleanliness", three patients scored 2 and two patients scored 1.

**Conclusions.** Patients at the end of life are susceptible to a range of oral complications including pain, salivary gland dysfunction, dysphagia, and oromucosal infections. Therefore, it is essential that the right tools are made available, for use also by non-dental professionals, to perform an appropriate assessment of oral status. This study demonstrates that the OHAT is a feasible instrument and is quick and simple to administer (with no need for special equipment). This tool can be used to assess the oral health status of a patient and to identify the need of examination by a dentist. Moreover, it can also be used as an intervention tool to establish a patient's oral health status at baseline, prior to initiating an individualized oral hygiene care plan, and to monitor progress of the intervention.

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## Use of allogeneic platelet concentrates for the surgical treatment of osteonecrosis of the jaw: case series

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**Introduction.** ONJ management is controversial. There are no evidence-based guidelines associated with good results in long-term follow-up, in particular with regard to surgical procedures. The use of PRP in surgery promotes soft tissue healing and in particular neoangiogenesis, helping to minimize the negative effects of BPs<sup>1</sup>. The aim of this study was to assess the positive effects of the application of allogeneic PRP in association with conservative surgery in the treatment of BRONJ.

**Methods.** The cohort consisted of 19 patients [7 males, 12 females, aged 41-87 (mean 68.4) years] with a diagnosis of BRONJ. All surgical procedures took place in the same unit under general anesthesia. Preparation of a mucoperiosteal flap and removal of all infected and necrotic bone using surgical drills. The resection of bone margins was determined by the clinical appearance of bleeding bone and compared with radiological images. After bone curettage, homologous PRP, previously prepared by the Transfusion Unit, S. Orsola, Malpighi Hospital, was applied topically. The platelets used for the preparation of PRP gel were derived from a platelet apheresis procedure.

**Results.** No intraoperative or postoperative complication was observed. At the time of suture removal, only four patients showed minimal bone exposure with no sign of acute infection. These patients continued to show bone exposure in the absence of pain or other signs of acute infection at the 6-month follow-up. The remaining patients presented complete mucosal healing and no sign of exposed necrotic bone during the follow-up period of 6-18 (mean 9) months.

**Conclusion.** In conclusion, although there is a need for further studies, surgical treatment with topic application of allogeneic PRP was effective for soft tissue healing after surgery for osteonecrosis in the most of the cases. The use of PRP from healthy donors provides, in the future, a standardized product and allows analysis of the different cytokines and growth factors present to define a product with specific features.

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## Peri-implant conditions of patients at risk for Medication-related osteonecrosis of the Jaw (MRONJ): University Hospital case series

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**Aim.** Despite an abundance of data exploring the significance of dentoalveolar surgical procedures and dental infection on the etiology of medication-related osteonecrosis of the jaw (MRONJ), scientific information about the associa-

tion between implants and is inconsistent and incomplete. Most reported cases of peri-implant MRONJ were not associated with surgical implant placement but became manifest as a consequence of a peri-implant lesion.

These data raise the question whether the implant itself or any other peri-implant factors might predispose to implant-associated MRONJ. The Aim of this study was to evaluate the peri-implant condition in a population at risk for MRONJ and to assess the impact of non surgical periodontal treatment on peri-implant clinical parameters at 3 and 6 months.

**Material and Methods.** In collaboration with the Hematology and Oncology Unit of the University Hospital of Ferrara, Dental Unit developed a program focused on primary prevention of MRONJ onset. All participants underwent complete oral and radiographical examination and clinical parameters records, including mobile dentures examination and Probing Depth (PD), Bleeding on Probing (BoP), and Plaque Index (PII). Then all parameters were merged to assign each patient a comprehensive risk evaluation score for MRONJ, "HIGH" or "LOW" score.

**Results.** During 36 months observation time 361 patients, eligible/undergoing for antiresorptive drugs (ARDs) therapy, such as Zoledronate, Ibandronate, Sunitinib and Denosumab, received complete dental examination, non surgical periodontal therapy, dental/implant extraction and prosthesis conditioning. Individual risk for MRONJ was checked for each patients during first visit and after 3 months. 34 patients, mean age of 69 years (range 51-85 yrs), presented implant supported prosthesis (ISP) (1 with overdenture ISP, 27 with partially-fixed ISP, 5 with single crown ISP, 1 with complete fixed ISP). After first visit 5 patients eligible for ARDs, presented signs and symptoms of severe peri-implant lesions. According to Oncologist and Hematologist 5 patients underwent implant removal. 2 of them resulted with no implants after surgery. For these patients ARDs therapy was started 8 weeks after surgery at least. Considering all patients with implants (32/34) in unadjusted analyses, baseline and 6 months data did differ in terms of average BoP (-7% mean value), PII score (-17% mean value) and n. of sites with probing depth >4 mm (from 5.05 sites for each patient to 3.08 mean value).

**Conclusion.** This study seems to indicate that non surgical periodontal treatment of moderate peri-implantitis is efficacious in decreasing the level of inflammation as well as pocket depth in patients eligible/undergoing for ARDs therapy.

A more radical approach consisting in surgical explantation was followed for implants affected by severe peri-implant lesions prior to initiation of ARDs Therapy.

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#### Case series

## Dental implants in patients with oral mucosal diseases: a case series and literature review

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**Introduction.** Oral rehabilitation with dental implants supporting fixed or removable prostheses has been shown to be reliable with impressive success rates exceeding 95% in patients without any oral or systemic complications. While some systemic diseases and oral mucosal disorders commonly have been regarded as contraindications or risk factors for the placement of implants the benefits of such treatments in these patients seem to outweigh the risks. In recent years, the spectrum of indications for dental implants has been widened; thus, a number of patients with oral mucosal disorders have been selected for dental implantology. At present, no treatment guidelines or official recommendations exist concerning implant therapy in patients with oral mucosal diseases. The aim of this study was to evaluate the success of dental implants in a cohort of patients with oral mucosal diseases.

**Methods.** A systematic literature search was conducted using PubMed/Medline, Scopus and Cochrane databases for articles published between 1990 and May 2017. Oral Lichen Planus, Sjögren syndrome, Pemphigus Vulgaris and M M Pemphigoid are the main mucosal diseases studied and discussed. In our study clinical cases documented from 2000 to 2016 have been reviewed; patients with dental implants placed before or after a diagno-

sis of oral mucosal disease completed at the clinical Unit of Oral Pathology were selected. Files were composed by demographic, medical and dental data; a total of 43700 clinical photographs were reviewed. Attention has been paid assessing the aggravation or even partial remission of the local disease, in relation to the health of perimplant tissues. 85 patients were selected evidencing dental implants and a mucosal alteration or history; the subjects were divided in 3 groups: OLP, xerostomia and other pathologies. The evaluation of the three groups has been carried out analyzing two parameters: the peri-implant bone resorption and the presence of a mucosal disease next to the peri-implant tissues.

**Results.** In our patients, survival implant rate are 91.7, 74.2 and 100% for respectively for Sjögren syndrome, Lichen Planus and Pemphigus Vulgaris. Concerning the 85 patients included and evaluated, the analysis of the clinical photographs shows that there were some failment in the implant therapy, but in most of cases has been difficult to find out the causes.

**Conclusions.** Scientific papers concerning oral rehabilitation in patients with oral mucosa diseases are rare and consist, in most cases, of single clinical cases. According to the results of this study, it is not contraindicated to place implants in patients suffering from oral mucosal disease as the implant survival were similar as in subjects without mucosal diseases. Implants survival ratio analysis and the evaluation of the quality and functioning of implant rehabilitation have been able to explain the interference between the oral mucosal diseases and the rehabilitation therapy. The degree of systemic disease control may be far more important than the nature of the disorder itself, and in many of these patients the quality of life and functional benefits from dental implants may outweigh any risk. In the decision-making process, dental implant treatment options should be assessed in terms of pros and cons for the oral and the systemic characteristics and outcomes of every single patient. Well-designed prospective and randomized clinical studies are required to clarify the issues involved.

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Case series/experimental study

## Prognostic predictive value of microRNAs expression in oral squamous cell carcinoma: a systematic review and meta-analysis

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**Introduction.** microRNAs (miRNAs) are small, non-protein-coding RNA molecules that regulate gene expression by complementary binding to the 3' untranslated region of target mRNA. They cause target degradation, translational repression, or gene silencing and thus affect subsequent protein expression. miRNAs exhibit important regulatory functions related to cell differentiation, development, and growth (Meltzer, 2005; Croce, 2008). They have also been shown to be dysregulated in a number of cancers, including oral squamous cell carcinoma (OSCC), by influencing oncogenes and tumor-suppressor genes. Recently, near 8000 human miRNAs are registered in miRBase (<http://www.mirbase.org/>), and they regulate approximately 30% of all gene expression.

**Methods.** Electronic databases (PUBMED, SCOPUS, EBSCOHost Research Databases and Web of Knowledge) were searched for articles related to the analysis of microRNAs expression as prognostic factor in patients with OSCC. First round of research was performed independently by two Authors by screening the abstracts of publications. Subsequently, articles admitted to the second round were read full-text, in case of disagreement final decision was taken after discussion in a joint session.

**Results.** A total of 193 records were retrieved from databases and screened by title and abstract. Only 33 of these papers were considered eligible for the full-text examination. At the end of full-text examination, only seventeen papers met the inclusion criteria and were considered for data extraction.

**Conclusions.** The expression of microRNAs is often dysregulated in patients suffering for OSCC. microRNAs

represent promising clinical biomarker to predict poor prognosis and select personalized treatments for OSCC patients. Further studies are needed in order to confirm such preliminary results.

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#### Case series/experimental study

## A non-invasive procedure based on brush sampling and Bisulfite sequencing of a 13-gene panel to study high risk patients to develop oral cancer

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**Introduction.** A genetic procedure for detection of patients with a significant risk to develop an oral neoplasia is an attractive strategy to reduce the burden of OSCC. Recently our research group developed a non-invasive method to detect high-risk OSCC lesions based on oral brushing as method for collecting samples followed by DNA methylation analysis (1, 2).

The purpose of this study is to apply this non-invasive procedure in three different groups of patients: a group of healthy donors, a group of patients with potentially malignant lesions (OPML) and a group of patients treated for oral cancer. Aim of the study is to evaluate the between-group differences and the epidemiologic, clinical and histological variables that may influence the methylation profile in each group.

**Methods.** Oral brushing samples were collected from 54 healthy donors, 41 patients with OPML (27 Oral leukoplakia "OL" and 14 Lichen Planus "LP") and 22 patients surgically treated for OSCC (ex-OSCC). In all cases DNA methylation analysis was applied as previously described (1). Each sample was considered positive or negative in relationship to a pre-definite cut off value. One way ANOVA analysis with multiple range test and Chi square analysis were used to evaluate the presence of any between-group significant difference and the variables that may influence the methylation profile in each group.

**Results.** None of healthy donors was detected as positive, whereas 19/27 (70.3%) of patients with OL, 2/14 (14.3%) with LP and 7/22 (31.8%) ex-OSCC showed higher values with respect to cut off. OLs showed significant ( $p < .01$ ) higher values with respect to all other groups while healthy donors showed significant ( $p < .01$ ) lower values with respect to all other groups. In patients with OLs, presence of high grade dysplasia was the only variable significantly related to positive results: 7/7 OLs with high grade dysplasia resulted positive with respect to 12/20 OLs with no or mild dysplasia (Chi 3.979  $p < .01$ ).

**Conclusions.** DNA methylation analysis in epithelial cells collected by oral brushing seems to be a promising genetic method to distinguish lesions at high risk of developing OSCC. Larger population studies and an adequate follow-up period are necessary to confirm these preliminary data.

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# Photodynamic Therapy with toluidine blue for leukoplasic lesions topical treatment: a case series

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Photodynamic Therapy (PDT) is a minimally invasive method that determines lesion resolution through selective and localized destruction of dysplastic cells by activating a photosensitizing agent with a light source at a particular wavelength and with activation of apoptotic mechanisms through oxygen-mediated cytotoxicity.

A photosensitizing molecule (also called "fluorophor") is administered, which tends to accumulate in mitochondria, lysosomes and cytoplasmic membranes.

A light source - a laser or a LED - directed on the area to be treated, activates fluorophores that damage the subcellular structures in which they accumulated through the production of free radicals of oxygen.

Toluidine blue is a chromophore of the family of phenothiazines with a strong absorption band in the spectrum region between 620 nm and 660 nm, with a "phototherapeutic window" (600 - 750 nm) in where the penetration of light into the tissue is maximized. Specifically, the highest absorption peak is 630 nm.

For the present study, 10 patients (6 F and 4 M), referring to the Oral Mucosa Pathology ambulatory located at the Multidisciplinary Department of Surgical and Dental Specialities at the University of Campania "Luigi Vanvitelli", were enrolled, after being subjected to an incision biopsy with histologic diagnosis of homogeneous oral cavity leukoplakia, with no signs of dysplasia. Each PDT cycle consisted of a variable number of therapeutic sessions (lasting 2 minutes and 30 seconds for each session, as reported by the literature) based on the persistence of the lesion. Between one session and the other there was an average of 7 days.

Sites to be treated were colored by topical toluidine blue application; Photodynamic Therapy was performed with the FotoSan® 630 device, a LED source.

Considering the exiguity of the sample analyzed, the clinical result obtained has yielded a 70% success rate. Blue toluidine PDT resulted in total disappearance of clinically visible lesions in 7 cases on the 10 treated.

In 2 out of 10 treated cases, patients stopped the treatment cycle for personal reasons due to the distance between the place of residence and the hospital structure.

In 1 subject a partial resolution of the initial lesion was obtained; the lesion reduction percentage was about 50% (T0 = 22 mm; Tf = 12 mm).

Several advantages have been highlighted, such as: selectivity of the dye in identifying cells with greater proliferative capacity; utility in patients who can not undergo to ambulatory surgery; easy and quick execution, well tolerated by the patient.

Preliminary clinical results obtained in patients with small to medium size lesions encourage a future use of this technique for the treatment of more extensive lesions, after some technical disadvantages resolution, such as reduced light spot size. The easy and speed execution, low cost and high comfort for the patient, which characterize this technique, could make it a resource for the early treatment of leukoplasic lesions in the future.

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# Quality of life in erosive oral lichen planus patients treated with Clobetasol mucoadhesive formulations - a pilot study

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Oral lichen planus (OLP) is a common chronic inflammatory disease and involves 0.5-4% of general population. OLP has different clinical forms (reticular, erosive-ulcerative, vesiculo-bullous), but the erosive form is the more commonly associated with long-standing painful ulcerations of the oral mucosa. The latter form can affect quality of life of patients because of its negative impact on daily activities (e.g. eating, swallowing and speaking) and on psychological and social status.

Therefore, management of OLP can be very challenging and the actual "gold standard" is the use of Clobetasol propionate preparations: this is due to reduce OLP symptoms.

The objective of this study is the evaluation of the quality of life in painful OLP patients treated with Clobetasol formulations.

A total of 9 patients (2M + 7F) with a minimum of 18 years (Range 43-70, Average 56.11 ± 9.77), with clinically and histologically confirmed diagnosis of painful variant of OLP, was recruited and treated with daily applications of Clobetasol preparations. Quality of life has been evaluated with a dedicated questionnaire at T0 and after 1 month (T1). The questionnaire evaluates the impact of disease on the perception of the general health, including social and working activities and pain during oral hygiene procedures.

The questions have been answered with a Likert scaling; for the question about general health: "Excellent" = 0, "Very good" = 1, "Good" = 2, "Acceptable" = 3, "Poor" = 4. The questions about social and working activities and pain, used the scale: "Never" = 0, "Barely" = 1, "Sometimes" = 2, "Often" = 3 and "Always" = 4. A higher score is related with the worst quality of life.

The perception of general health at T0 showed the 44% of "Good" answers, while at T1 the results were from "Acceptable" to "Very good". The answers about social activities showed an increase from 22% to 33% in the answer "Never", while the same answer in working activities from 33% to 67%. The pain on oral hygiene procedures showed at T0 the 33% of answers "Sometimes", while at T1 the 33% answered "Never" and the 22% "Barely".

The results of the questionnaire are related with an improvement of the quality of life of the patients treated.

The study shows that a proper treatment in symptomatic erosive OLP patients can improve their quality of life, leading to a treatment that consider the patient as a whole, considering also the psychological and social well-being. Due to the small size of the sample, more studies with larger samples should be performed.

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## Evaluation of the AJCC Cancer Staging Manual 8th edition in patients with OSCC

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The American Joint Committee on Cancer (AJCC) published in 2016 the 8th edition of the Cancer Staging Manual. Two new parameters have been added: "Depth of invasion" (DOI) and "Extra nodal extension" (ENE), involving the pT and pN classification systems, respectively. More precisely, tumors up to 2 cm and DOI up to 5 mm are staged as pT1, those up to 2 cm and DOI between 5 and 10 mm or tumors between 2 and 4 cm and DOI up to 10 mm are staged as pT2, while patients with tumors greater than 4 cm or DOI greater than 10 mm are staged as pT3. Regarding ENE positive tumors, these patients are staged as pN2a if a single node up to 3 cm is present, while all other patients are staged as pN3b. Furthermore, the "Worse pattern of invasion" (WPOI) has been proposed as more strongly predictive of disease evolution. The aim of the present study was to evaluate the new AJCC classification in a cohort of patients surgically treated for OSCC, to evaluate if the upstaging of the pT and pN classification was indicative of a worse prognosis.

A cohort of 73 patients (46 males and 27 females) with OSCC of different oral cavity regions was retrospectively evaluated. These patients were operated at Maxillofacial Surgery Department, Ospedali Riuniti, Ancona, and the pathology report was made to the Institute of Pathology of the same hospital.

The distribution of cases by stage was as follows: 6% Stage I, 23% Stage II, 23% Stage III, 36% Stage IVA, and 12% Stage IVB. The presence of WPOI was found in 22 cases (30.1%): the largest number of cases was found in Stage IVA (10 cases). Regarding ENE, 10 cases (13.7%) showed a macroscopic extension, while 7 cases (9.6%) showed a microscopic involvement. The results showed a change in the staging parameters in 25 patients (34.2%). Of these patients, 17 (23.3%) received an upstaging when DOI and ENE were included into the pT and pN classification, while 8 cases (10.9%), although pT and pN have changed, showed no staging modification. Among the 17 aforementioned patients, 3 cases were upstaged from Stage II to III, and 7 cases from Stage IVA to IVB.

In conclusion, in the 8th edition of the Cancer Staging Manual new morphological parameters and a new staging system have been added, that seems to provide a better stratification of OSCC patients.

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