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Oral Scientific Papers

O1

Follow up of TP53-mutated and non-mutated oral lesions

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Predicting progression of oral mucosal lesions to malignancy or recurrence of oral squamous carcinoma (OSCC) is an important clinical problem. Several molecular markers are associated with disease progression but none have been adopted in clinical practice. The TP53 gene is involved in OSCC. No clear association with prognosis has emerged but few studies have analysed protein expression and mutation. This follow-up study used samples from a group of 146 patients: 54 with OSCC, 46 with oral lichen planus (OLP) and 46 with hyperkeratosis (clinically leukoplakia). Samples were analysed by immunohistochemistry for TP53 protein expression and many were subjected to mutation analysis. Follow-up was 11–17 years for OSCC (mean 13.3), 12–22 years for OLP (mean 15.9) and 12–17 years for hyperkeratosis (mean 14.5). 27/54 OSCC patients experienced recurrent disease, 20 died of OSCC, 25 died of other causes. OSCC recurred in 8/14 (57%) with definite ($n = 11$) or possibly ($n = 3$) mutated TP53 and 19/39 (49%) without mutation. The proportion of TP53-mutated cases was higher among OLP patients than OSCC, nine definite and four possible of 31 tested. One OLP patient with TP53 mutation developed OSCC, but in a different site from the original OLP. TP53 mutations were rarer in patients with hyperkeratosis: three definite and two possible, of 22 tested. One hyperkeratosis patient (non-mutated) developed OSCC in the original site.

Conclusion: TP53 mutations can exist in benign oral mucosal lesions for many years without progression to malignancy. No association was found between TP53 protein expression or TP53 mutation and recurrence of OSCC.

O2

Real-time PCR evaluation of IFN-γ, TNF-α and IL-27 in oral lichen planus patients with and without hepatitis-C virus infection

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Purpose: Th1 cytokines are apparently over expressed in oral lichen planus (OLP) and OLP has been frequently associated with hepatitis C virus (HCV) infection. IL-27 can potentiate the early phases of the Th1 response and it is a potent adjuvant for induction of hepatitis C virus-specific cytotoxic T lymphocytes in transgenic mice. The aim of the study was to analyze, using the real-time PCR technique, the differential expression of IFN-γ,TNF-α and IL-27 in OLP patients with and without HCV infection.

Methods: Thirty-seven patients with histologically proven OLP (23 OLP-HCV-ve and 14 OLP-HCV + ve) and 21 healthy controls undergoing tooth extractions were recruited. Total RNA from the oral bioptic samples were extracted using a standardized system. Gene expression of IFN-γ,TNF-α ,IL-27 and CD14 were evaluated by means of real time PCR (ABI Prism 7700, Applied Biosystems, Foster City, CA, USA) using SYBR®Green as the detection method. Comparisons of means were carried out by the Student's *t*-test. A *P*-value $< 0{\cdot}05$ was considered statistically significant.

Results: TNF-α gene expression was enhanced in OLP-HCV-ve and OLP + ve ($P = 0.049$ and $P = 0.020$, respectively) but there was no any significant difference between the two OLP groups. IL-27 gene expression was significantly increased in OLP-HCV-ve and OLP-HCV + ve ($P = 0.011$ and $P = 0.020$) and there was a significant over expression of IL-27 in OLP-HCV + ve compared to OLP-HCV-ve ($P = 0.036$).

Conclusions: Gene expression of IL-27 is significantly increased in OLP but mainly in patients having HCV infection.

Relevance: This study suggests that different pathogenic mechanisms are causing OLP in patients with and without HCV infection

O3

Loss of heterozygosity on chromosome 3p, 9p, and 17p on oral scrapings of patients with oral lichen planus

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Genetic instability on chromosome arms has been considered to be a common and early event in oral carcinogenesis. In oral malignancies and premalignancies, loss of heterozygosity (LOH) has been widely used to assess genetic instability.

Purpose: The aim of our study was to determine the genetic instability on 3p, 9p and 17p chromosomal regions on oral scrapings of patients with oral lichen planus (OLP).

Methods: We have examined LOH at three chromosomal regions (3p14.2, 9p21.3 and 17p13) located on three chromosomal arms (3p, 9p and 17p). 45 cytological samples of patients with OLP (55.7 years mean age, 30 women and 15 men) have been studied using two multiplexed panels of three microsatellite markers. Descriptive statistical analyses were carried out with the results.

Results: Overall, 62.2% of the samples showed LOH in at least one of the chromosomal arms analysed. 35.6%, 26.7% and 22.2% of the samples showed LOH on 3p, 9p and 17p regions respectively.

Conclusions: Loss of heterozygosity on chromosome arms 3p, 9p and 17p seems to be a relative common event found in oral scrapings of patients with OLP.

Relevance: The assessment of genetic instability using oral scrapings is an easy methodology which maybe helpful in the follow-up of patients with oral lichenoid diseases.

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O4

Cyclooxygenase-2 expression in oral lichen planus

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Oral lichen planus (OLP) is a frequent immunologic disease that shows different diagnostic and prognostic controversial aspects. Cyclooxygenase-2 (COX-2) is an inducible enzyme by growth factor, inflammatory stimuli, oncogenes and tumor promoters that has been related with human carcinogenesis.

Purpose: To analyze the COX-2 expression in biopsies clinically compatible with OLP and related with the clinicopathological data.

Methods: We studied 44 biopsies of lesions clinically compatible with OLP. Immunostaining standard method was performed with rabbit monoclonal antibody COX-2 (1:100 Clone SP21, NeoMarkers®). The main clinicopathological data was collected according to a previous protocol and compared with COX-2 immunoexpression according to intensity and localization.

Results: Twenty-three cases (52.3%) were classified as typical of OLP (HPT) and 21 (47.7%) as compatible (HPC). COX-2 was detected in the epithelium in 24 (54.5%) cases, 9 (39.1%) in HPT group and 15 (71.4%) in the HPC group ($P = 0.032$). COX-2 was detected in the inflammatory cells in 14 cases (31.8%), 10 (47.6%) in HPC group and 4 (17.4%) in the HPT group ($P = 0.032$).

Conclusion: We found differences in COX-2 expression in OLP related with his histopathological aspects.

Relevance: COX-2 expression could be a marker to differentiate OLP lesions to predict the malignant transformation. This preliminary study needs further analysis.

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O5

Lichen planus and hepatitis-C virus infection: a meta-analysis of epidemiological data

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Purpose: The possible link between hepatitis C virus infection and LP is still controversial. One meta-analysis on HCV and LP has been published in 2004 (Lodi et al, 2004) and 20 case-control and three cohort studies were published thereafter. Moreover, no one study has ever reviewed systematically the frequency of LP in patients with HCV infection. The aim of our study was to systematically review epidemiological data on the association between HCV infection in LP.

Methods: In a systematic review, studies were identified by searching different databases (PubMed, EMBASE, CINAHL and SCOPUS) in December 2007. Inclusion criteria were: (i) analytical study design; (ii) clinical and histological diagnosis of LP; and (iii) serological test for anti-HCV antibodies as main outcome. Pooled data were analysed by calculating odds ratios (OR's), using a random effects model.

Results: There was a statistically significant difference in the proportion of HCV-seropositive subjects among patients with LP, compared with controls (OR 4.85; 95% CI 3.58–6.56). The risk is mainly high in Mediterranean countries and USA but decreases in Northern Europe. Notably, in patients with chronic HCV infection the risk of having LP was significantly increased (OR 4.47; 95% CI 1.84–10.86).

Conclusions: This meta-analysis confirms that LP may be significantly associated with HCV infections especially in Southern Europe.

Relevance: HCV may be involved in the pathogenesis of the LP, probably via an immunological pathway still to be defined.

O6

The efficacy of topical hyaluronic acid in the management of oral lichen planus

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Purpose: The aim of this study was to evaluate the efficacy of a topical hyaluronic acid (HA) preparation (0.2%) in the management of oral lichen planus (OLP).
Methods: 120 patients with erosive OLP participated in a randomized, placebo-controlled, double-blind trial to evaluate the efficacy of a topical HA preparation. Outcome measures included soreness relief following immediate application, oral function and size of erosive/ulcerative area. Patients were medicated for 28 days and completed a log diary recording oral function and soreness scores.
Results: Application of topical HA produces a significant reduction ($P < 0.05$) in soreness scores when compared to placebo. This reduction in discomfort was maintained for 4 h. There was no difference between treatment groups ($P > 0.05$) with respect to oral function. Patients treated with HA 0.2% showed a significant reduction ($P < 0.05$) in the size of the erosive/ulcerated area after 28 days of treatment but without any significant difference compared with the placebo group.
Conclusions: Topical HA 0.2% is of benefit in the management of painful erosive lichen planus.
Relevance: It can be offered as a safe first line treatment for OLP, thereby potentially reducing the use of immunosuppressant treatment over the treatment period of a patient's OLP.

O7

Biologic agents and oral diseases: therapeutic prospects and restrictions

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Introduction: Biological agents are therapeutic and diagnostic agents synthesized from the products of living organisms and are widely used in the treatment of inflammatory and neoplastic conditions with favorable results.
Purpose: To provide an up-date of the biologic agents: infliximab, etanercept, adalimumab, rituximab, efalizumab, epratuzumab and alefacept, their mode of action, their current use in diseases that affect the oral mucosa, their therapeutic outcomes and their side effects.
Method: Identification of scientific papers referring to the use of biologic agents in patients with oral diseases, by reviewing the medical literature via Medline (Pubmed).
Results: Reports were identified referring to the use of biological agents in the treatment of 237 patients with Sjogren syndrome (including patients with MALT-lymphomas), 38 patients with pemphigus (vulgaris, foliaceous, paraneoplastic), 7 patients with mucous membrane pemphigoid, 5 patients with oral lichen planus, 4 patients with Behcet's disease, 1 patient with orofacial granulomatosis, and 1 patient with apthous ulcers. The above mentioned diseases are off-label indications of the biological agents, the patients in whom they were administered suffered from refractory forms of the diseases and the results were in general encouraging with side effects being also reported (infections mainly).
Conclusion and relevance to oral medicine: Biological agents can be a therapeutic choice in patients with persisting oral lesions in immune mediated conditions but clinicians should be careful in their use as more studies are required in order to evaluate their safety.

O8

A clinical trial of a new treatment for cold sores

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Cold sores are usually treated with topical antiviral drugs requiring early administration to be effective. Several free fatty acids and monoglycerides are known to inactivate the causative virus, HSV-1, on contact. Monocaprin, a 1-monoglyceride of capric acid, shows high virucidal activity *in vitro*
Objective: To investigate the antiviral and healing effect of a hydrogel containing (i) monocaprin alone and (ii) monocaprin with low-dose doxycycline in an *in vivo* clinical study against herpes labialis.
Methods: Two groups of subjects were tested: Group I had prodromal symptoms of cold sore; Group II had vesicular lesions. Both groups used a test ointment five times a day for 5 days. Three formulations were tested (i) hydrogel containing monocaprin and doxycycline (MCD), (ii) hydrogel containing only monocaprin (MC) and (iii) placebo hydrogel. Clinical evaluation of treatment was made by determining the time to healing of the cold sore and an analog score for pain was recorded in a diary.
Results: For the MCD group mean number of days to healing was 5.5 days (prodromal) and 5.1 days (vesicles/sores), a significantly shorter time than for the placebo groups (7.25 and 7.5 days respectively for the two test groups) ($P < 0.05$). Recorded pain relief was significantly greater in the MCD group (combining prodromal and vesicle groups) ($P = 0.0114$).
Conclusion: Monocaprin and low-dose doxycycline in combination were effective in treating cold sores: significantly reducing pain and the time to healing and preventing the development of prodromal lesions, than were either the placebo or the formulation containing monocaprin alone.

O9

Therapeutic efficacy of a new mouth wash containing clobetasol, ketoconazole & amitryptiline on atrophic-erosive oral lichen planus

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Oral Lichen Planus (OLP) is a chronic inflammatory disease of oral mucosa with immunological origin. Atrophic/erosive OLP need appropriate treatment because of pain and malignancy potential. Topical corticosteroids are the most effective drug therapy and mouthwashes are more effective topical dosage forms, but there are no corticosteroid mouthwashes available in Iran. In this study, the efficacy of a new mouthwash containing clobetasol, ketoconasole and amitriptyline was evaluated in comparison to the common treatment. In this double blind randomized clinical trial study, 50 patients who had inclusion criteria were grouped randomly. Experimental group were treated using 5 ml of mouthwash four times a day for 5 min, while control group was treated by Dexanethasone tablet, Nystatin drop and Diphenhydramine syrup. Severity of the lesions and pain were followed as the chief complain and recorded in the initial, 1, 2, 4, 8 and 12 weeks. There were significant differences in the pain reduction in weeks of 1($P < 0.001$), 2 ($P = 0.01$) and 12 ($P = 0.025$) between 2 groups. The lesion reduction was significantly higher in experimental group ($P < 0.001$). Complete resolutions of lesions were occurred averagely in 2.65 and 10.75 weeks for experimental and control groups, respectively. Also most patients in experimental group (70.6%) had complete subjective satisfaction (75–100%) of treatment. Survival analysis showed that the possibility of existence of lesions after 3 months in experimental group and control group were 0 and 100% ($P < 0.001$), respectively. In conclusion, the new mouthwash was more effective in short time with more convenience for patients.

O10

Evolution of OLP therapy: our 15 years experience with 260 patients

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Purpose: Numerous therapies were proposed for the treatment of OLP, but right now a standardized therapeutical approach does not exist. This study reviewed the different therapies for the treatment of OLP used in our section. Particularly we analyzed, in a retrospective way, the efficacy of the treatment, the incidence of side effects, the duration of a support therapy and the duration of the lesion-free periods.
Methods: From 1990 to 2005 we made seven clinical trials, three open trials using fluocinonide, clobetasol and prednisone, one controlled comparative trial between clobetasol, fluocinonide and placebo, one comparative trial between clobetasol and prednisone, two double-blind randomized comparative trials- clobetasol *vs* cyclosporine and 0.025% clobetasol *vs* 0.05% clobetasol-. They included 260 patients with histological diagnosis of OLP, with atrophic or erosive lesions and symptomatic. We excluded patients with histological signs of dysplasia, patients who had therapy in the previous 6 months or had lichenoid lesions. Clinical signs and symptoms were evaluated with the Thongprasom scale or the Visual Analogue Scale while the efficacy of the treatment was classified into: complete remission, great improvement, mild improvement, no improvement.
Results and discussion: We found 0.025% clobetasol is more effective than 0.05% fluocinonide and placebo while the doubling of its concentration (0.05% clobetasol) showed no significant improvement in therapeutical goals. No significant differences were also found between clobetasol and prednisone while one third of the patients who used prednisone showed side effects. Cyclosporine showed less side effects and more constant results but the high cost of the drug and the lower clinical efficacy make its use not advisable.
Conclusions: Clobetasol showed to be the most effective therapy considering its low cost, its manageability and the low side effects. No therapy guarantees a complete and definitive remission of OLP lesions. Every therapeutical approach should be considered only a symptomatic approach to the disease, helping the patients to get trough the most painful periods.

O11

Randomised controlled trial of the efficacy of HybenX for the symptomatic treatment of recurrent aphthous stomatitis

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Purpose: The treatment of recurrent aphthous stomatitis (RAS) is principally directed towards reducing the pain and duration of each episode of ulceration, however there remain few agents for which there are definitive evidence of benefit. This study determined the efficacy of HybenX (Epien Medical Inc, Minneapolis, US) *vs* another device used for the treatment of RAS (Salicept, Carrington Laboratories Inc, Texas US) to reduce the symptoms and duration of RAS, and determined the safety of HybenX for this application.
Methods: 53 out of 63 individuals (19 male, group median age 25.3 years, range 17.8–53.4 years) were evaluated in a randomised control trial of HybenX *vs* an occlusive covering device (Salicept oral patches).
Results: Painful symptoms over a 5 day post-treatment period were reduced by both agents although HybenX was statistically more effective at day 2 than Salicept. There was a trend for HybenX to induce greater pain reduction than Salicept over this 5 day period. Both agents gave rise to few adverse side effects. HybenX was only re-applied on one occasion to the HybenX group, while individuals in the Salicept group required frequent applications of the device.
Conclusion: HybenX safely and effectively reduces the painful symptoms of RAS.
Relevance: HybenX offers effective relief of painful symptoms of RAS following a single application.

O12

Topical dexamethasone and tacrolimus elixirs for the treatment of oral chronic graft-versus-host disease

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Purpose: Oral chronic graft-versus-host disease (cGVHD) is a common complication of allogeneic stem cell transplantation that results in significant morbidity. While topical therapy is commonly prescribed, its clinical efficacy is unclear. The study objective was to estimate the efficacy of dexamethasone rinses alone and in conjunction with tacrolimus rinses in the treatment of oral cGVHD.
Methods: Case-records were reviewed of 53 patients with symptomatic oral cGVHD that were treated with either dexamethasone alone (D+/T-) or in combination with tacrolimus (D+/T+) elixirs. Tacrolimus was added when initial response was poor. NIH and BWH objective and symptom scores were assessed at each visit and retrospectively confirmed from photographs.
Results: Fifty-three patients (96% D+/T−, 4% D+/T+) had one follow-up appointment (FU1), 26 (70% D+/T−, 30% D+/T+) were evaluated twice (FU2), and 17 (65% D+/T−, 35% D+/T+) were evaluated a third time (FU3). All median measures stabilized or improved at FU1 ($P < 0.05$); sensitivity scores improved by one point (0–10 scale). D+/T+ median sensitivity scores improved from FU2 (6.5) to FU3 (3.5). The difference in NIH score improved with time (FU1:0, $P < 0.05$; FU2:1, $P < 0.1$; FU3:2, $P < 0.001$). NIH and BWH scores demonstrated strong correlation ($r = 0.63$–0.8, $P < 0.01$).
Conclusions: Dexamethasone with or without tacrolimus rinses improved signs and symptoms of oral cGVHD. Both scoring systems demonstrated utility. Prospective, randomized studies of these therapies alone and in combination are indicated.
Relevance: Dexamethasone rinses alone or in combination with tacrolimus should be prescribed for patients with symptomatic oral cGVHD.

O13

Oral ulcerations due to cytomegalovirus infection in renal transplant recipients

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Purpose: The aim of this study was to analyze the incidence, clinical characteristics, treatment, and outcome of patients with oral lesions due to CMV after renal transplant.
Methods: The records of 453 patients who underwent kidney transplantation between February 1989 and March 2007 at the Hospital 12 de Octubre of Madrid were analyzed. Incident cases and characteristics of CMV oral lesions were ascertained retrospectively in the post-renal out patients' records.
Results: The cumulative incidence of six cases with oral CMV lesions was 1.32%. The median follow-up ($n = 453$) was 61.84 ± 50.68 months. The interval for the incidence of CMV oral ulcers after renal transplant was 12.83 ± 23.51 months. The oral locations affected by CMV comprised the tongue, buccal mucosa, hard palate, soft palate and floor of the mouth. CMV cases showed no significant differences with regard to gender distribution, age at renal transplant, renal transplant indication, type of immunosuppressive treatment and results donor/recipient CMV serological status before transplant. The number of acute rejection episodes was significantly higher and time since transplant was significantly lower in CMV cases.
Conclusion: CMV infection, which is common in renal transplant patients, only rarely affects the mouth. We report six patients that suffered oral ulcers due to CMV infection.
Relevance: It is very important the early diagnosis of these lesions because CMV infection causes infectious diseases syndromes, increases immunosuppression and is associated with opportunistic infections and allograft rejection.

O14

Lack of association between oral health conditions and adverse pregnancy outcomes in Italian postpartum women

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Purpose: To evaluate the oral health conditions in postpartum women and the relationship to adverse pregnancy outcomes.
Methods: A sample population of 750 postpartum women who had delivered at the University hospitals of three main Italian cities were interviewed and examined bedside within 5 days from delivery. Data collection concerned (i) pregnancy outcomes (low birth weight, prematurity and other obstetric conditions), (ii) demography, education, systemic health, smoking habits, oral hygiene habits, self-reported oral symptoms, (iii) dental (DMFT), periodontal (PPD, CAL, BOP) and oral mucosal conditions. Simple and multiple regressions analysis were performed.
Results: 14.6% ($n = 110$) children were born premature, 11.2% ($n = 84$) low birth weight and 49 (6.5%) were born premature and with low birth weight. In general, adverse outcomes were 230 (30.6%), and controls 520 (69.3%). Cases and controls did not significantly differ in mean age, birthplace, ethnicity, education and smoking habits in DMFT or periodontal indices. Less than 5% of the subjects showed oral mucosal diseases or conditions. Regression analysis indicated that there were no significant relationships between the presence and severity of oral diseases and conditions and adverse pregnancy outcomes.
Conclusions and relevance: No association was found between periodontal diseases in the mother and adverse pregnancy outcomes. However, our study population is different from previously reported case-control populations in both demographic factors and the level of periodontal disease. More data need to be collected in order to exclude the proposed potential link between periodontitis and the risk of pregnancy complications.

O15

Adhesion of *Candida albicans* to endothelial cells under conditions of flow

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Purpose: To cause the tissue damage associated with systemic candidiasis, *Candida albicans* must adhere to the endothelial lining of blood vessels and transmigrate into the tissues. Little is known about the mechanisms involved in this or the role played by yeast, pseudohyphal and hyphal forms of *C. albicans*. Our aim, therefore, was to investigate adhesion of the different forms of *C. albicans* to endothelium under conditions of flow.
Methods: To study this we developed an *in vitro* flow adhesion assay and recorded the number of adherent *C. albicans* (Mean ± SEM).
Results: Using this assay, we found that all three forms bind under conditions of flow with yeast forms (165 ± 1) binding more efficiently than pseudohyphal (111 ± 2) and hyphal (38 ± 5) forms respectively ($n = 3$, $P < 0.001$). The preferential adhesion of yeast forms was also confirmed using a genetically engineered strain of *C. albicans* (SSY50-B) in which morphogenetic change can be controlled.
Conclusions: All three morphogenetic forms adhered to endothelium under flow conditions. However, contrary to previous data using static assays, we found that adhesion was extremely rapid and the adhesion of yeast forms was significantly greater than pseudohyphae which were more adherent than hyphae. These findings are consistent with recent *in vivo* data suggesting that yeast forms are capable of adhesion and migration into the tissues before undergoing morphogenetic change to cause tissue damage.
Relevance: Systemic candidiasis has a high mortality despite availability of effective anti-fungals. Understanding and preventing Candida-endothelial adhesion could help to reduce this mortality.

O16

Towards a multi modal cell analysis of brush biopsies for detection of oral squamous cell carcinoma

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Purpose: This study describes the first clinical application of semi-automated Multimodal Cell Analysis (MMCA), a novel evaluation method of cells for earliest detection of cancer and highly accurate diagnoses using conventional smears from oral brush biopsies.
Method: The MMCA approach is based on repeated staining of identical slide based squamous epithelial cells, resulting in multi-parametric and mono-cellular measurements of cellular and sub-cellular features. The correlation of features and data were extracted from the different stains, related to individual cells, yields a remarkable increase of diagnostic reliability. The implementation of MMCA enables fully automatic, adaptive image pre-processing including registration of multimodal images and segmentation of cell nuclei. Therefore we investigated 47 slides from brush biopsies of suspicious lesions of the oral mucosa. The final diagnoses were, histologically proven, 20 squamous cell carcinomas, seven leukoplakias and 20 lichen planus mucosae.
Results: The stepwise application of different analytical approaches (e.g. DNA-distribution, quantification of the argyrophilic nucleolar organizer regions AgNORs) increased the sensitivity and specificity of our conventional cytological diagnosis from 90% successively up to 100%.
Conclusion: This study proved efficiency, robustness and high accuracy diagnostics of slide based cytological specimens. Brush biopsies of all visible oral lesions are an easily practicable, cheap, minimal-invasive, painless and safe screening method for detection of oral precancerous lesions and squamous cell carcinomas in all stages.
Relevance: MMCA might become a very sensitive and highly specific, objective and reproducible adjuvant tool for identification of neoplastic cells in oral smears in daily clinical routine.

O17

Development of a novel *in vitro* tissue-engineered model of squamous cell carcinoma of the oral mucosa

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Purpose: Worldwide, there are 450 000 new cases of oral squamous cell carcinoma (OSCC) annually and 200 000 deaths. The prognosis for OSCC is poor, with less than 35% 5 year survival for the 67% of patients who present with 'high risk' stage III or IV disease. OSCC evolves from normal epithelium through mild, moderate and severe dysplasia to carcinoma in-situ before invading the basement membrane and connective tissue to form a carcinoma. The aim of this study was to establish a novel *in vitro* model of early OSCC within tissue engineered human oral mucosa.
Methods: The head and neck cell line FaDu cultured as cell suspensions or multicellular spheroids were seeded onto reconstructed human oral mucosa models composed of normal oral keratinocytes and fibroblasts seeded onto a decellularised dermal scaffold and compared histologically with naturally occurring early tumours.
Results: FaDu cells seeded as spheroids produced the best results. FaDu formed spheroids within 18 h and demonstrated a predictable growth in culture with a proliferating outer rim and a hypoxic inner rim encompassing a necrotic central area. Seeded within the reconstructed human oral model they produced a histological picture closely resembling carcinoma in-situ with an island of highly disorganised epithelial architecture and severe cellular atypia juxtaposed to normal keratinised epithelium. Moreover, after 21 days culture some FaDu cells were observed traversing the basement membrane and invading the connective tissue.
Conclusion/Relevance: These preliminary results demonstrate that the addition of tumour spheroids to epithelial models reproduces the *in vivo* appearance of premalignant lesions and early invasive SCC.

O18

Activation of DNA damage response in oral premalignant lesions

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Purpose: Recent evidence suggests that activation of DNA damage response (DDR) is an early event in human carcinogenesis. Here, we analyzed for the first time DDR markers in oral premalignant lesions.
Methods: The study group included nine oral hyperplasias and 29 oral dysplasias (7 mild, 15 moderate, 7 severe), as well as five control cases of normal oral mucosa; moderate and severe dysplasia were grouped together as 'high-grade' dysplasia. To assess DDR activation, immunohistochemistry and immunofluorescence (IF) against phosphorylated H2AX and Chk2 proteins, respectively, were utilized. The percentage of positive cells and their extension in different layers of the epithelium were calculated.
Results: Normal oral epithelium contained rare p-H2AX positive cells. The mean percentage of p-H2AX positive cells in the basal and parabasal layers was 12.8%, 26.8% and 50.9% in hyperplasia, low and high degree of dysplasia, respectively ($P = 0.0005$). Extension of p-H2AX positive cells up to two thirds of the epithelium was more frequent in high-grade dysplasias (55%) than in low-grade dysplasias (14%) and hyperplasias (0%) ($P = 0.007$). DDR was further confirmed in a subset of 5 high-grade dysplasias, showing a typical granular nuclear pattern of p-Chk2 expression in basal and parabasal cells.
Conclusions: Detection of p-Chk2 and p-H2AX expression in oral precancerous lesions, and correlation between H2AX kinase activation and the presence and degree of dysplasia, provide evidence of DDR participation in oral premalignancy.
Relevance: The novel finding of DDR in oral premalignancy may shed light into the early stages of oral cancer development with diagnostic, prognostic and therapeutic implications.

O19

Proteomic analysis of saliva in patients with oral squamous cell carcinoma

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Background: Currently available screening tools for oral squamous cell carcinoma (OSCC) (toluidine blue, brush biopsy, fluorescence imaging) lack high sensitivity and specificity to be useful in screening the general population. As a consequence, early diagnosis of OSCC is still a clinical challenge. In the past years, a number of genetic markers have been used to detect the presence of OSCC with vary decrees of sensitivity and specificity. However, none of these markers universally identifies OSCC. The advent of oncoproteomics has provided the hope of discovering more useful biomarkers for evaluating disease presence, prognosis and response to therapy, since the proteome provides a more realistic view of a biological status compared with the genome.
Aim: We aimed to compare the proteomic profile of saliva from patients with OSCC and healthy subjects by using the surface enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) technology, that allows the generation of an accurate protein profile from minimal amounts of biological samples.
Methods: Whole saliva was collected from 56 patients with OSCC and 30 healthy subjects. Specimens were centrifuged (10 min, 13 000 *g*); the Q10 ProteinChips were prepared according to the manufacturer's instructions and were loaded with the supernatants. A saturated solution of sinapinic acid was used as energy-absorbing matrix. The analysis was performed in a m/z range from 2500 to 25000 Da, and the proteomic profiles were compared by a specific data analysis software.
Results: The average intensities of peaks at m/z 8048, 6239, 8003, 6138, 8037 Da were significantly higher in OSCC patients than healthy controls, while peaks at m/z 4139, 12145 and 13688 Da showed a higher intensity in healthy subjects.
Conclusion: Qualitative differences were noted between patients with OSCC and healthy controls with regard to the salivary proteomic profile obtained using the SELDI-TOF-MS technology. These data suggest that the proteomic analysis of saliva is a promising new tool for a non-invasive screening for OSCC.

O20

Treatment of MALT lymphoma in Sjögren's syndrome: a retrospective clinical study

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Purpose: To retrospectively analyze the clinical course and prognostic factors of patients with mucosa-associated lymphoid tissue (MALT)-type lymphoma of the parotid gland and associated Sjögren's syndrome (SS).
Methods: Retrospective analysis was performed of all consecutive patients diagnosed with SS and MALT lymphoma (MALT-SS) between January 1997 and January 2007 in our department. Response to treatment of SS and MALT lymphoma was evaluated.
Results: Thirty MALT-SS patients were included (median follow-up of 25 months). MALT lymphoma was localized in the parotid gland in all 30 cases, being the only localization in 21 patients. Treatment consisted of 'watchful waiting' ($n = 10$), surgery ($n = 3$), rituximab ($n = 13$), and rituximab with chemotherapy ($n = 4$) leading to complete response or stable disease in 95% of the treated patients. Of the six patients with initial high SS disease activity (M-protein, cryoglobulins, IgM-Rf > 100 KIU/l, severe extraglandular manifestations), five patients developed progression of their MALT lymphoma and/or deterioration of SS disease activity after a median follow-up

of 9 months necessitating retreatment. MALT-SS patients without SS disease activity showed no progression of their lymphoma when left untreated.
Conclusions: Initially high SS disease activity seems to constitute an adverse prognostic factor for progression of lymphoma or SS. These patients may require immunosuppressive therapy for both MALT and SS. In SS patients with localized asymptomatic MALT lymphoma and low or absent SS disease activity, a watch-and-wait strategy seems justified.
Relevance: SS patients are prone to development of MALT lymphoma. The findings provide a tool how to treat these patients.

O21

The international Sjögren's syndrome registry (SICCA): a progress report

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Purpose: Goals of the Sjögren's International Collaborative Clinical Alliance (SICCA) (initiated in 2003) are: (i) develop an international Sjögren's Syndrome (SS) registry and repository, (ii) develop standardized classification criteria, (iii) collect and store clinical data and biospecimens, and (iv) provide these resources for future studies of SS.
Methods: The SICCA sites in San Francisco (USA), Copenhagen (Denmark), Buenos Aires (Argentina), Kanazawa (Japan), Beijing (China) and London (UK) complete uniform baseline evaluations on participants who meet broad inclusion criteria for SS. All participants are systematically characterized for ocular, oral and rheumatologic disease manifestations and nine types of biospecimens are collected using standardized methods. Defined participants are recalled after 2 years to repeat all observations in order to assess changes in disease signs and symptoms.
Results: As of April 2008, SICCA has gathered uniform evaluated and collected clinical data and biospecimens from over 1100 genetically diverse individuals and from over 125 of those in two year follow-up examinations; shown objective measures of SS are strongly associated with each other, but not with sicca symptoms; elucidated two distinct types of keratoconjunctivitis sicca (KCS); and found no measurable overall progression or regression of most SS-related phenotypic characteristics, with notable exceptions.
Conclusions: Sicca symptoms are highly prevalent but not significantly associated with established signs of SS. Preliminary follow-up data mostly shows slowly progressing disease with rapid transition in a few. Two forms of KCS will affect future SS classification criteria.
Relevance: This registry and repository has the capacity to transform our knowledge of SS. Funded by NIDCR/NIH Contract NO1-DE-32636

O22

Sjögren's syndrome: quality of life, employment and disability

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Purpose: To describe health related quality of life (HR-QOL), employment and disability in primary (pSS) and secondary (sSS) Sjögren's syndrome patients.
Methods: In SS patients regularly attending our departments ($n = 235$), HR-QOL, employment and disability were assessed with the Short Form-36 questionnaire (SF-36) and a employment and disability questionnaire. Results were compared to normative data of the general Dutch population. Epidemiological and clinical factors associated with HR-QOL, employment and disability were assessed by regression analyses.
Results: 195 patients (83%) returned the questionnaires (179 women, 16 men; age 55.1 ± 15.0 years; disease duration 9.7 ± 8.8 years). 154 patients were classified as pSS, 41 patients as sSS. SS patients scored lower on all SF-36 scales than the general Dutch population. sSS patients scored lower on physical functioning, bodily pain and general health than pSS patients. Predictive factors for reduced HR-QOL were fatigue, tendomyalgia, articular involvement, artificial saliva use, comorbidity, male sex and receiving disability compensation (DC). Of SS patients of working age, 51% ($n = 69$) were employed and 47% ($n = 63$) received DC. Predictive factors for receiving DC were artificial saliva and anti-malarial drug use, number of extraglandular manifestations, comorbidity, male sex, and high level of education. Recurrent urinary tract infections, use of oral moisturising gel, NSAIDs and oral corticosteroids, comorbidity and age at disease presentation were negatively associated with employment.
Conclusions: SS has a large impact on HR-QOL, employment and disability.
Relevance: The results provide insight in the mental and social status of SS patients and are useful in treatment concepts aimed to increase this status.

O23

Evaluation of the analgesic activity of Pregabalin in burning mouth syndrome

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BMS is a chronic disorder recognized from IASP that can significantly reduce patient's quality of life. An effective treatment for BMS is still lacking. Pregabalin is an antiepileptic drug commonly used for treatment of neuropathic pain from postherpetic neuralgia and diabetic peripheral neuropathy.
Purpose: Demonstrate with prospective open-label study the efficacy of Pregabalin in controlling pain in BMS.
Methods: Patient with a clinical and histopathological diagnosis of BMS and with a VAS > 5 were recruited. Primary endpoint was a reduction of 50% in pain severity at VAS analysis. Secondary efficacy variables were number of responders, patient global impression of change (PGIC), and short sleep quality questionnaire (SQNRS). Pregabalin was given twice a day starting at 150 mg daily and could be increased up to 600 mg die if a satisfactory pain control was not achieved. When pain control was acheived, the Pregabalin dose was manteined for 30 days.
Results: Three patients dropped out the study for side effects (dizzness and daily sleepiness) in the first two weeks. Pregabalin induced an improvement of 51.3% in pain severity at 70 day visit (baseline main VAS = 7, 42-day visit mean VAS = 2.5). Three patients needed to increase the starting dosage from 150 mg up to 300 mg daily and two patients incremented up to 600 mg daily to achieve satisfactory pain control, without increment of side effects. PGIC and SQNRS were slightly improved in all responders.
Conclusions: Pregabalin appears to be effective treatement in patient with BMS.

O24

Temporomandibular disorders (TMD) and co-morbidity

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Purpose: To compare TMD patients, with and without depression, in relation to reported sleep alteration (prevention and disturbance) and other recurrent chronic pains (headache, neck ache, backache and abdominal pain).
Methods: New referrals, to a hospital TMD clinic, with a diagnosis of TMD pain, were categorised into dichotomous groups, depressed and non-depressed, using the Beck Depression Index. Non-depressed (0–9), Depressed (borderline 10–14, mild 15–20 and severe 21 and above). The presence or absence of sleep alteration and other recurrent chronic pains was scored at baseline and compared using nonparametric statistical analysis.
Results: 237 TMD patients, mean age 32.7 years (range 16–55 years), mean duration of pain 3.3 years (range 0.25–32),77% female, 23% male, were categorised into two groups 153/237 non depressed, 84/237 depressed. Depressed individuals with TMD suffered significantly increased sleep alteration ($P < 0.001$). Chronic pain co morbidity was also found to be increased amongst those presenting with TMD and depression; headache ($P = 0.021$), neck ache ($P = 0.007$), backache ($P = 0.049$) and abdominal pain ($P = 0.011$).
Conclusions: A significant difference in reported sleep prevention and disturbance was found amongst patients suffering form depression ($P < 0.001$). Chronic pain co morbidity was also increased in depressed patients. Previous research suggests patients with TMD and multiple co-morbid pain conditions are more psychologically distressed with a greater risk of developing long-term TMD pain and disability.
Relevance: Early recognition of co-morbid depression, other recurrent chronic pain conditions and sleep disturbance in TMD patients is of importance in relation to clinical governance in providing the most effective pain management tailored to the individual patient.

O25

An audit of the effectiveness of group medical hypnosis in burning mouth syndrome patients

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Purpose: This study assessed whether medical hypnosis would benefit burning mouth syndrome (BMS) patients on a variety of parameters.
Methods: Fourteen patients were referred by the oral medicine department after exclusion of all other causes. Of these, 7 BMS patients underwent 4 weekly 45-min sessions of group medical hypnosis. Five out of seven completed the group medical hypnosis intervention. All were assessed by a clinical psychologist and a specialist in medical hypnosis and completed validated questionnaires Pain Catastrophising Scale (PCS), Brief Illness Perception Scale (IPQ-B), Depression, Anxiety and Positive Outlook Scale (DAPOS), Visual Analogue Scales (VAS) for pain intensity, interference, salivation distress and unpleasant sensation, and a salivary flow test before and after the intervention. Results: Group analysis showed on average,

improvements in PCS, IPQ-B, and VAS of pain intensity, interference, salivation distress and unpleasant sensation. The three components of the DAPOS remained relatively unchanged. The10-min non-stimulated salivation test showed an increase in all patients (pre-hypnosis 1.98 ml ± 1.36; post-hypnosis 3.46 ml ± 1.95).

Conclusion: These preliminary results provide evidence for the psychobiological effect of hypnosis, and support the efficacy of group based medical hypnosis intervention for BMS. Further research with a larger sample and control groups is required to provide further evidence for the efficacy of this intervention.

Relevance: BMS affects up to 15% of the population and can be extremely debilitating. Even though it is a neuropathic pain there are no effective medical therapies and psychological interventions may help patients manage their condition.

O26

Alpha-lipoic acid in burning mouth syndrome: lack of efficacy in a double-blind, randomized, placebo-controlled study

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Purpose: A systematic review from the Cochrane Collaboration stated that alpha-lipoic acid (ALA) may help in the management of burning mouth syndrome (BMS). Because all of the data on ALA came from a single group, it has been stressed that its effectiveness should be reproduced in other populations. A double-blind, randomized, placebo-controlled study, including two test groups (Group A and Group B) and one control group (Group C), was carried out to evaluate the efficacy of systemic ALA (400 mg) and ALA (400 mg) plus vitamins.

Methods: Sixty-six patients (54 females, 12 males) were included in an 8-week trial. Symptoms were evaluated by using a visual analogue scale (VAS) and the McGill Pain Questionnaire (MPQ) at 0, 2, 4, 8, and 16 weeks.

Results: Fifty-two patients (43 females, 9 males; aged 67.3 ± 11.9 years) completed the study. All three groups had significant reductions in the VAS score and in the mixed affective/evaluative subscale of the MPQ; the responders' rate (at least 50% improvement in the VAS score) was about 30%. No significant differences were observed among the groups either in the response rate or in the mean latency of the therapeutic effect.

Conclusions: This study failed to support a role for ALA in the treatment of BMS.

Relevance: BMS represents a disorder with a very poor prognosis in terms of quality of life; unfortunately ALA seems not to be a resolutive therapy so that further investigations are needed to identify the cause of BMS in order to develop efficacious therapies.