Šantić M, Asare R, Škrobonja I, Jones S, Abu Kwaik Y. Acquisition of the vacuolar ATPase proton pump and phagosome acidification are essential for escape of Francisella tularensis into the macrophage cytosol. Infect Immun. 2008;76(6):2671-7.

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The Francisella tularensis-containing phagosome (FCP) matures to a late-endosome-like phagosome prior to bacterial escape into the cytosols of macrophages, where bacterial proliferation occurs. The authors data show that within the first 15 min after infection of primary human monocyte-derived macrophages (hMDMs), approximately 90% of the FCPs acquire the proton vacuolar ATPase (vATPase) pump and the lysomotropic dye LysoTracker, which concentrates in acidic compartments, similar to phagosomes harboring the Listeria monocytogenes control. The acquired proton vATPase pump and lysomotropic dye are gradually lost by 30 to 60 min postinfection, which coincides with bacterial escape into the cytosols of hMDMs. Colocalization of phagosomes harboring the iglD mutant with the vATPase pump and the LysoTracker dye was also transient, and the loss of colocalization was faster than that observed for the wild-type strain, which is consistent with the faster escape of the iglD mutant into the macrophage cytosol. In contrast, colocalization of both makers with phagosomes harboring the iglC mutant was persistent, which is consistent with fusion to the lysosomes and failure of the iglC mutant to escape into the macrophage cytosol. The authors have utilized a fluorescence microscopy-based phagosome integrity assay for differential labeling of vacuolar versus cytosolic bacteria, using antibacterial antibodies loaded into the cytosols of live hMDMs. The authors show that specific inhibition of the proton vATPase pump by bafilomycin A1 (BFA) blocks rapid bacterial escape into the cytosols of hMDMs, but 30% to 50% of the bacteria escape into the cytosol by 6 to 12 h after BFA treatment. The effect of BFA on the blocking of bacterial escape into the cytosol is completely reversible, as the bacteria escape after removal of BFA. The authors also show that the limited fusion of the FCP to lysosomes is not due to failure to recruit the late-endosomal fusion regulator Rab7. Therefore, within few minutes of its biogenesis, the FCP transiently acquires the proton vATPase pump to acidify the phagosome, and this transient acidification is essential for subsequent bacterial escape into the macrophage cytosol.

Jazvinšćak Jembrek M, Svob Štrac D, Vlainić J, Peričić D. The role of transcriptional and translational mechanisms in flumazenil-induced up-regulation of recombinant GABA(A) receptors. Neurosci Res. 2008;61(3):234-41.

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The aim of this study was to further elucidate the mechanisms involved in adaptive changes of GABA(A) receptors following prolonged exposure to flumazenil, the antagonist of benzodiazepine binding sites on GABA(A) receptors. The effects of prolonged flumazenil treatment were studied on recombinant $alpha_1beta_2gamma_{2S}$ GABA(A) receptors stably expressed in human embryonic kidney (HEK 293) cells. Using radioligand binding experiments we found enhancement in the maximum number of [^{3}H]muscimol labeled binding sites in different preparations of HEK 293 cells. The parallel increase of [^{3}H]flunitrazepam binding sites in the membranes was reduced in the presence of actinomycin D and cycloheximide, inhibitors of RNA and protein synthesis, respectively. Chronic flumazenil also raised the steady-state level of mRNA encoding $alpha_{vb\ nm,b\ vb}$ receptor subunit. The results suggest that the up-regulation of GABA(A) receptors, observed after prolonged flumazenil treatment is at least partly due to increased *de novo* synthesis of receptor proteins at both transcriptional and translational level.

Ikeda F, *Đikic I. Atypical ubiquitin chains: new molecular signals. Protein modifications: beyond the usual suspects' review series. EMBO Rep. 2008;9(6):536-42.

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Ubiquitin (Ub) is a small protein modifier that regulates many biological processes, including gene transcription, cell-cycle

progression, DNA repair, apoptosis, virus budding and receptor endocytosis. Ub can be conjugated to target proteins either as a monomer or as Ub chains that vary in length and linkage type. The various types of Ub modification are linked to distinct physiological functions in cells. MonoUb, for example, regulates DNA repair and receptor endocytosis, whereas lysine 48-linked Ub chains label proteins for proteasomal degradation. More recently, the importance of chains conjugated through the other six lysines in Ub, known as atypical Ub chains, has been revealed. Atypical chains can be homotypic, sequentially using the same lysine residue in Ub for conjugation; mixed-linkage, utilizing several distinct lysines to connect consecutive Ub moieties; or heterologous, connecting Ub with other Ub-like modifiers. Here, the authors describe recent progress in the understanding of atypical Ub chain assembly and their recognition by Ub-binding domains and discuss further their functional roles *in vivo*.

Sorta-Bilajac I, Baždarić K, Brozović B, Agich GJ. Croatian physicians' and nurses' experience with ethical issues in clinical practice. J Med Ethics. 2008;34(6):450-5.

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The aim of this study was to assess ethical issues in everyday clinical practice among physicians and nurses of the University Hospital Rijeka, Rijeka, Croatia. The authors surveyed the entire population of internal medicine, oncology and intensive care specialists and associated nurses employed at the (n=532). An anonymous questionnaire was used to explore the type and frequency of ethical dilemmas, rank of their difficulty, access to and use of ethics support services, training in ethics and confidence about knowledge in ethics. Physicians (n=113, 55% female) ranged in age from 27 to 61 years, and nurses (n=251, 95% female), from 20 to 52. The most frequent ethical dilemmas concerned uncertain or impaired decision-making capacity (66% of physicians, 47% of nurses, p=0.008), limitation of treatment at the end of life (60% of physicians, 31% of nurses, p<0.001) and disagreements among family members (47% of physicians, 31% of nurses, p=0.025). The most difficult dilemmas concerned euthanasia and physician-assisted suicide (49% of physicians, 52% of nurses) and limitation of treatment at the end of life (14% of physicians, 18% of nurses). Only a minority reported ever using any kind of ethics support services (12% of physicians, 3% of nurses, p=0.001) or being very confident about knowledge in ethics (5% of physicians, 6% of nurses). In conclusion, similar ethical difficulties are present in the clinical practice of both physicians and nurses, with important differences in access and use of ethics support services. A need for systematic ethics educational activities was identified. Inclusion of individual ethics consultants in Croatian healthcare ethics support services is strongly advised.

Vučetić B, Rogan SA, Hrabač P, Hudorović N, Ćupić H, Lukinac L, Ledinsky M, Matejčić A, Lovričević I, Zekan M. Biological value of melanoma inhibitory activity serum concentration in patients with primary skin melanoma. Melanoma Res. 2008;18(3):201-7.

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Melanoma inhibitory activity (MIA) protein was identified in significant quantities in primary and metastatic malignant melanomas, where it has an important role in promoting tumor development and progression. The authors' hypothesis was that MIA serum level will be elevated in patients with metastases or local spreading of the disease before any symptom of such progression is clinically apparent. The authors compared MIA serum levels in two groups of patients with primary melanoma: those with positive as opposed to those with negative sentinel lymph nodes. In addition, MIA serum levels were studied in two control groups: patients with dysplastic nevi and patients with basal cell carcinoma. A blood sample was obtained from each patient included in the study and MIA levels were assessed using standard enzyme-linked immunosorbent assay method. Patients with histologically positive sentinel lymph nodes, meaning that tumor cells were found in the lymph nodes, had much higher mean MIA values than any other patient group considered in this study. With mean value of 14.53 ng/mL, it was almost twice as high as mean MIA value in patients with histologically negative sentinel lymph nodes (7.32 ng/ml) and more than twice as high than any of the two control groups (p<0.001). However, neither the classification by Clarke nor the classification by Breslow could be used to distinguish patients with positive sentinel lymph nodes from those with negative sentinel lymph nodes. In authors' opinion, MIA serum level is the ideal test for screening the tumor spread to sentinel lymph nodes.

Načinović-Duletić A, Štifter S, Dvornik S, Škunca Z, Jonjić N. Correlation of serum IL-6, IL-8 and IL-10 levels with clinicopathological features and prognosis in patients with diffuse large B-cell lymphoma. Int J Lab Hematol. 2008;30(3):230-9.

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his study aimed to determine the relationship between serum levels of interleukin (IL)-6, IL-8 and IL-10, measured by enzyme-immunoassay, and the clinical characteristics and outcomes in 46 untreated patients with diffuse large B-cell lymphoma (DLBCL). Serum IL-6, IL-8 and IL-10 levels were higher in DLBCL patients than in control subjects. Elevated levels of IL-6, IL-8 and IL-10 correlated with more adverse disease features. Consequently, patients with elevated IL-6, IL-8 and IL-10 levels prior to treatment had a lower response to therapy. Furthermore, those with elevated IL-6 and IL-10 levels had poor median, 3-year and 5-year survival, while elevated serum IL-8 level did not correlate with overall survival. Worse survival was also confirmed in patients with combined elevated pretreatment serum levels of IL-6, IL-8 and IL-10 (none, one, two or three elevated). Multivariate analysis identified elevated values of IL-6 and IL-10 and response to therapy as significant predictors for overall survival. Serum levels of IL-6, IL-8 and IL-10 before treatment of patients with newly diagnosed DLBCL may give some insight into the possible prognosis and thus facilitate the decisions regarding therapeutic approaches for individual patients.

Legović M, Legović I, Brumini G, Vandura I, Cabov T, Ovesnik M, et al. Correlation between the pattern of facial growth and the position of the mandibular third molar. J Oral Maxillofac Surg. 2008;66(6):1218-24.

Department of Orthodontics, Rijeka University School of Medicine, Rijeka, Croatia

The purpose of this study was to examine the correlation between variables that determine the position of the third molar (M3) and correlation between the pattern of facial growth and examined variables. Material for the study consisted of 130 panoramic radiographs and lateral radiographs of subjects' heads in whom orthodontic therapy had been started. Analysis of the position of the mandibular M3 and its relation to the bone and other teeth were determined by 4 variables: evaluation of the space for both M3, spatial relation between the second molar and M3, vertical position of the M3 in relation to the alveolar ridge, and inclination of the germ of the M3. In boys, significant correlation was determined on the right side of the jaw, between the retromolar area and the vertical position of M3. Significant correlation was determined in girls, between the retromolar area and the vertical position of M3 on both sides, retromolar area and M3 inclination on the left side, and retromolar area and spatial relation of M3 on the right side. Analysis of correlation between facial growth pattern and 4 variables that determinate the position of M3 showed positive correlation only for the lower right M3 inclination in subjects with anterior facial rotation. In conclusion, the present study showed that the amount of retromolar space for the position of the M3 is not a sign of its normal development. No significant differences were determined between the position of M3 and type of facial growth.

Nogalo B, Mirić M, Maloča I, Turkalj M, Plavec D. Normal variation of bronchial reactivity in nonasthmatics is associated with the level of mite-specific IgE. J Asthma. 2008;45(4):273-7.

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The aim of this study was to investigate association between non-specific bronchial reactivity (NBR) and level of mite specific IgE amongst mite-sensitized non-asthmatic subjects. Subjects attending occupational check-up were assessed for: respiratory symptoms, atopic status (skin prick testing [SPT], total and specific IgE), spirometry and NBR. Individuals without history of respiratory disease (n=234) were included into analysis. All subjects had normal spirometry and 99% had normal NBR while 41.8% had detectable specific IgE to mites. Lung function parameters and NBR were significantly lower in mite sensitized subjects. Multiple regression analysis controlling for age, gender, smoking, family history, SPT, IgE, and lung function showed that NBR was significantly associated only with mite specific IgE level (beta=0.26; 95% CI, 0.05-0.47; p=0.018). In conclusion, even in subjects without allergic symptoms, IgE-mediated sensitization does not appear to be all or nothing phenomenon influencing the normal variability of underlying airway reactivity.

Laškarin G, Redzović A, Rubeša Z, Mantovani A, Allavena P, Haller H, et al. Decidual natural killer cell tuning by autologous dendritic cells. Am J Reprod Immunol. 2008;59(5):433-45.

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Dendritic cells (DC)/natural killer (NK) cells interactions in the deciduas of early human pregnancies were analyzed *in vitro*. Phenotype, cytokine expression and/or cytolytic mediators' expression were measured by flow cytometry in NK and DC from the freshly isolated decidual mononuclear cells or after their purification and co-culture *in vitro*. Pro-

liferation of 5(6)-Carboxyfluorescein diacetate N-succinimidyl ester (CFSE)-labeled CD56+ cells was analyzed by flow cytometry after the co-culture with CD1a+ or CD83+ DC. Decidual CD1a+ cells show less mature phenotype with no expression of CD197, lower expression of CD80 and CD86 and higher expression of CD206 and CD195 in comparison to CD83+ cells. Interleukin (IL)-15, interferon-gamma and tumor necrosis factor-alpha productions were higher in immature than mature DC, whereas IL-10 and IL-18 were equally produced in both subpopulations. Immature DC increase perforin, FasL and TRAIL protein expression and proliferation of NK cells, but decrease their intracellular IL-15 production. Mature DC caused less efficient proliferation of NK cells, and did not affect cytokine and cytolytic mediator expression. These results suggest that decidual CD1a+ cells regulate and shape NK cell function more profoundly than CD83+ cells in decidua.

Špalj S, Plančak D, Božić D, Kasaj A, Willershausen B, Jelušić D. Periodontal conditions and oral hygiene in rural population of post-war Vukovar region, Croatia in correlation to stress. Eur J Med Res. 2008;13(3):100-6.

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The aim of this study was to assess the periodontal conditions of people in post-war area villages in relation to oral hygiene, habits and war stress. The study was conducted on a sample of 282 subjects (mean age, 41.5±17.8 yr.) in seven Vukovar villages using the questionnaire and clinical periodontal examination. Five years after the war in Croatia the population was still very poor, low educated, and had a low level of periodontal health and oral hygiene habits. The level of periodontal disease and attachment loss tended to increase with age and physical activity and decrease with education level, higher frequency of tooth brushing and toothbrush replacement, dental visits and utilisation of auxiliary devices. Subjects exposed to any kind of war stress had a significantly worse periodontal status and more excluded sextants than those who were not exposed to such stress experience ($p<0.05$). Better periodontal conditions were found in individuals that were refugees, than those who were in war, wounded or lost a dear person ($p<0.05$). Individuals who were in war rarely brushed their teeth, visited dentist and changed toothbrushes, but more frequently drank alcohol and smoked, in comparison to other groups, especially those who have not been exposed to war stress. In conclusion, specific socio-economic and psychological conditions in post-war areas could be significant risk factors for poor periodontal conditions.

Wagner J, Dzijan S, Pavan-Jukić D, Wagner J, Lauc G. Analysis of multiple loci can increase reliability of detection of fetal Y-chromosome DNA in maternal plasma. Prenat Diagn. 2008;28(5):412-6.

DNA Laboratory, Osijek University School of Medicine, Osijek, Croatia

Aiming to develop more reliable methods for determination of fetal gender from maternal plasma the authors compared three different systems of polymerase chain reaction (PCR) detection of Y-chromosome DNA. Cell-free DNA was isolated from 96 samples of maternal plasma and (1) amplified using AmpFLSTR-Identifiler (15 autosomal STR loci and amelogenin) or AmpFLSTR-Yfiler (16 Y-chromosome STR loci) kits and subsequently analyzed on ABI-PRISM 310 Genetic Analyzer, or (2) analyzed using Quantifiler-Y DNA-Quantification kit. Gender of fetuses was confirmed by cytogenetic analysis or phenotypically at birth. AmpFLSTR-Identifiler and Quantifiler-Y Human-Quantification kits were rather reliable in determining fetal gender (92.5 and 98.1%, respectively), but false negatives were still present in both systems. AmpFLSTR-Yfiler was found to be fully reliable as it amplified Y-chromosome in all cases of male fetuses, and was thus 100% correct in determining fetal gender. In addition, it enabled comparison of polymorphic Y-chromosome loci between father and a child, thus further supporting specificity of obtained results.

Ajduk J, Marinić I, Aberle N, Rabatić S, Gagro A. Effect of house dust mite immunotherapy on transforming growth factor beta1-producing T cells in asthmatic children. Ann Allergy Asthma Immunol. 2008;100(4):314-22.

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To evaluate the effect of specific immunotherapy (SIT) on the induction of regulatory T cells (Treg) in house dust mite-allergic children and on the expression of specific Treg cell markers (cytotoxic T-lymphocyte-associated protein 4, [CTLA-4], IL-10, and TGF-BETA1). In this uncontrolled open-label study, the percentage of peripheral blood CD4+ Treg cells and the expression of molecules associated with their functions (CTLA-4, TGF-BETA1, and IL-10) were analyzed using flow cytometry in 16 children allergic to house dust mites before and at 3 and 12 months of subcutaneous SIT. Clinical variables, such as symptom score, medication requirements, forced expiratory volume in 1 second, peak expiratory flow rate, and serum IgE levels, were also de-

termined. Ten healthy children were included as controls. All the clinical variables improved during immunotherapy. The percentage of CD4+CD25+CD69-CD45RO+ Treg cells remained unchanged. The percentage of CTLA-4+ -expressing Treg cells transiently increased after 3 months of immunotherapy, whereas the percentage of FOXP3+ Treg cells did not change after 1 year of immunotherapy. Levels of IL-10+ cells transiently decreased after 3 months of immunotherapy. Four children who required inhaled fluticasone propionate administration for significant symptom worsening had no statistically significant increase in TGF-BETA1-secreting T cells at 12 months of SIT, in contrast to 12 children without inhaled corticosteroid treatment. In conclusion, the increase in TGF-BETA1-positive T cells only in children without significant symptom worsening requiring inhaled corticosteroid treatment limits the usefulness of TGF-BETA1 in monitoring response to allergen immunotherapy.