CROATIAN INTERNATIONAL PUBLICATIONS

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Cepika AM, Bendelja K, Vergles JM, Malenica B, Kapitanović S, Gagro A. Monocyte response to LPS after exposure to corticosteroids and chloroquine with implications for systemic lupus erythematosus. Scand J Immunol. 2010;72:434-43

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Essential part of a response to infection is early pathogen recognition and adequate initiation of innate immunity. One of the hallmarks of systemic lupus erythematosus (SLE) is reduced resistance to infection despite overall hyperactivity of the immune system. Immunosuppressive drugs (high-dose corticosteroids and cytotoxic agents) are independent risk factors for infection in SLE, with bacteria as predominant cause. To investigate whether less aggressive immunomodulatory treatment may still affect recognition and response to Gram-negative bacteria, we measured TLR4 expression in monocytes of untreated SLE patients and patients on chloroquine and low-dose steroid therapy and examined the drugs' influence on monocyte TLR4 expression in peripheral blood mononuclear cell (PBMC) culture. Additionally, we determined whether induction of monocyte NF-κB signalling, TNF-α and IL-6 production with lipopolysaccharide (LPS), a TLR4 ligand, can be altered with dexamethasone, chloroquine or both. There was no statistically significant difference in TLR4 expression between patients with SLE and controls, even though treated SLE patients tended to have lower frequency of TLR4(+) monocytes and TLR4 mean fluorescence intensity than healthy controls. However, neither dexamethasone nor chloroquine had major influence on TLR4 expression in vitro or suppressed LPS-induced NF-κB activation in monocytes, although dexamethasone decreased TNF-α and IL-6 production. Therefore, even if low-dose steroids or chloroquine do not seem to affect TLR4 expression and signalling, steroids might decrease cytokine production in response to LPS.

Dilber D, Malčić I. Spectrum of congenital heart defects in Croatia. Eur J Pediatr. 2010;169:543-50

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The aim of our study was to investigate the incidence of congenital defects in children born in Croatia during a period of 5 years, its association with extracardiac malformations, its treatment, and outcome. Medical information about the patients was obtained from 14 paediatric cardiology centres that cover the whole country. Diagnosis was made by clinical findings, electrocardiography, chest X-ray, echocardiography, catheterisation, or autopsy. Between October 1, 2002 and October 1, 2007, there were 205,051 live births in Croatia, 1,480 of which were patients diagnosed with congenital heart disease, accounting for 0.72% of the live-born children. The distribution was made up of 34.6% children with ventricular septal defect, 15.9% with atrial septal defect, 9.8% with patency of arterial duct, 4.9% with pulmonary valvar stenosis, 3.3% with tetralogy of Fallot, 3.3% with transposed great arteries, 3.3% with aortic stenosis, 3.2% with aortic coarctation, 4.3% with atrioventricular septal defect and common atrioventricular orifice, 2.3% with hypoplastic left heart syndrome, and 8.3% other with severe defects. The average age in the time of diagnoses is 70.41 days (SD, 188.13), with low average time of diagnoses of severe heart defects, 9.6 days (SD, 32.52). Among patients, 14.5% had chromosomal defects, syndromes, and/or other congenital major anomalies. During the study, 57 patients died because of cardiac anomalies or other related problems, 24 who died were operated. The rates of specific cardiac defects and association with extracardiac malformations are generally comparable with those reported in similar studies. In spite of all problems, mortality rate of 3.85% is low but could be improved.

Bišof V, Salihović MP, Narančić NS, Škarić-Jurić T, Jakić-Razumović J, Janićijević B, Turek S, Rudan P. TP53 gene

polymorphisms and breast cancer in Croatian women: a pilot study. Eur J Gynaecol Oncol. 2010;31:539-44

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A case-control retrospective association study was conducted to investigate a possible association of the TP53 polymorphisms, Arg72Pro and PIN3 (+16bp), with sporadic breast cancer in Croatian women. Ninety-five women with breast cancer and 108 age-matched healthy women were analyzed. Arg72Pro polymorphism was detected by TaqMan essay. For designation of PIN3 (+16bp) polymorphism DNA amplification was performed by the polymerase chain reaction (PCR) while the PCR products were detected by capillary electrophoresis. Homozygous genotype of minor allele of the PIN3 (+16bp) polymorphism was associated with sporadic breast cancer (OR = 2.15, 95% confidence interval [CI] 1.80-2.56, p = 0.006). For Arg72 polymorphism, the odds ratio for breast cancer of ArgPro versus reference genotype ArgArg was 0.55 (95% CI 0.30-1.02, p = 0.039) suggesting the protective effect. Although different haplotypes did not influence the susceptibility to the disease, the joint occurrence of genotype combination ProPro/A2A2 frequent in cases, was associated with sporadic breast cancer (OR = 2.20, 95% CI 1.89-2.56, p = 0.021). The study provides evidence of the association of the TP53 gene polymorphisms Arg72Pro and PIN3 (+16bp) with sporadic breast cancer in the Croatian population.

Pučić M, Pinto S, Novokmet M, Knežević A, Gornik O, Polašek O, Vlahoviček K, Wang W, Rudd PM, Wright AF, Campbell H, Rudan I, Lauc G. Common aberrations from the normal human plasma N-glycan profile. Glycobiology. 2010 Aug;20(8):970-5

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After performing hydrophilic interaction and weak anion exchange high-performance liquid chromatography to analyze N-glycans in the plasma of 1991 people, we identified several individuals that differed significantly from the "normal" profile of N-glycans. By performing consensus scoring of pairwise distances between vectors containing measured glycan values, we formed six groups of individuals with specific glyco-phenotypes. Some aberrations from the normal plasma protein patterns were found to be associated with clinical conditions (such as renal problems in people with increased monosialylated biantennary glycans, A2G2S1), while other substantial changes in N-glycan structure, such as the near complete absence of neutral glycans or antennary fucosylated tri- and tetraantennary glycans, were not associated with any observed adverse health outcomes. These results demonstrate the existence of specific altered glyco-phenotypes in some individuals and indicate that in some cases they might represent risk factors for the development of specific diseases.

Maričić A, Katunarić M, Šutalo N, Tomić S, Jurišić D, Petković M, Zamolo G. Primary large-cell neuroendocrine carcinoma of the scrotum. Wien Klin Wochenschr. 2010;122:360-2

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Neuroendocrine tumors (NETs) mostly develop from the neural crest cells but a few arise from neuroectoderm. They are common in the lungs and gastrointestinal tract but rare in the genitourinary tract. A 78-year-old man with no family history of malignant or hereditary diseases presented with a 3-month history of a rapidly growing asymptomatic scrotal nodule and swelling in the groin. He had a negative history of sexually transmitted disease and of trauma, fungal infection or chronic irritation in the scrotal area; there was no history of radiotherapy or exposure to chemicals or arsenic. Both the scrotal and groin lesions were excised with a minimum of 1.2 cm of normal skin. Examination of the specimen revealed a confined poorly differentiated large-cell neuroendocrine carcinoma with a metastasis to the inguinal lymph nodes. Three months after the excision we found a local recurrence. The recurrent tumor revealed tumor tissue concurrent with the primary lesion. To the best of our knowledge, there have been no previously published case reports on neuroendocrine tumor of the scrotum.

Gornik I, Vujaklija-Brajković A, Renar IP, Gašparović V. A prospective observational study of the relationship of critical illness associated hyperglycaemia in medical ICU patients and subsequent development of type 2 diabetes. Crit Care. 2010;14:R130

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INTRODUCTION: Critical illness is commonly complicated by hyperglycaemia caused by mediators of stress and inflammation. Severity of disease is the main risk factor for development of hyperglycaemia, but not all severely ill de-

velop hyperglycemia and some do even in mild disease. We hypothesised that acute disease only exposes a latent disturbance of glucose metabolism which puts those patients at higher risk for developing diabetes.

METHODS: Medical patients with no history of impaired glucose metabolism or other endocrine disorder admitted to an intensive care unit between July 1998 and June 2004 were considered for inclusion. Glucose was measured at least two times a day, and patients were divided into the hyperglycaemia group (glucose ≥7.8 mmol/l) and normoglycaemia group. An oral glucose tolerance test was performed within six weeks after discharge to disclose patients with unknown diabetes or pre-diabetes who were excluded. Patients treated with corticosteroids and those terminally ill were also excluded from the follow-up which lasted for a minimum of five years with annual oral glucose tolerance tests.

RESULTS: A five-year follow-up was completed for 398 patients in the normoglycaemia group, of which 14 (3.5%) developed type 2 diabetes. In the hyperglycaemia group 193 patients finished follow-up and 33 (17.1%) developed type 2 diabetes. The relative risk for type 2 diabetes during five years after the acute illness was 5.6 (95% confidence interval (CI) 3.1 to 10.2).

CONCLUSIONS: Patients with hyperglycaemia during acute illness who are not diagnosed with diabetes before or during the hospitalization should be considered a population at increased risk for developing diabetes. They should, therefore, be followed-up, in order to be timely diagnosed and treated.

Ćustović Z, Žarković K, Cindrić M, Cipak A, Jurković I, Sonicki Z, Uchida K, Žarković N. Lipid peroxidation product acrolein as a predictive biomarker of prostate carcinoma relapse after radical surgery. Free Radic Res. 2010;44:497-504

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Cancer recurrence after radical surgery might happen even in the case of patients with localized prostate carcinoma treated by radical prostatectomy. Therefore, identifying predictive markers of tumour recurrence is very important, so this study evaluated the presence of lipid peroxidation product acrolein in primary prostate carcinomas, assuming that acrolein could be involved in prostate carcinogenesis as was recently shown for colon cancer. Samples obtained by radical prostatectomy of 70 patients were analysed, out of which 27 patients suffered afterwards from tumour recurrence, while 43 patients were disease free. Immunohistochemistry using genuine monoclonal antibodies against acrolein-protein adducts revealed the association of acrolein with progression of carcinoma. The logistic regression combining clinical parameters together with the biochemical markers of disease and acrolein immunohistochemistry has shown that the relapse might be predicted with 90% accuracy if tumour-positive surgical margins, stage of disease and the intensity of acrolein presence in tumour stroma were taken together.

Mrakovčić-Sutić I, Tokmadžić VS, Laškarin G, Mahmutefendić H, Lučin P, Župan Z, Šustić A. Early changes in frequency of peripheral blood lymphocyte subpopulations in severe traumatic brain-injured patients. Scand J Immunol. 2010;72:57-65

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Infections are leading causes of increased morbidity and mortality of severe traumatic brain-injured (STBI) patients. The mechanism underlying the susceptibility to the infections is still unexplained. The purpose of the study was to investigate changes in frequency of leucocytes subpopulations in peripheral blood of patients with STBI during the course of intensive care treatment. Twenty patients with STBI were included in the study. Healthy age- and sex- volunteers served as control. Peripheral blood samples were taken from these patients at day 1, 4 and 7, and peripheral blood mononuclear cells (PBMC) were isolated. The percentage of T, B lymphocyte, NK and NKT cells as well as monocytes was analysed by simultaneous detection of surface antigens using fluorochrome-conjugated monoclonal antibodies. The two major subsets of T lymphocytes (CD3(+)CD56(-)CD4(+) and CD3(+)CD56(-)CD8(+)) and NK cells (CD3(-)CD56(+dim) and CD3(-)CD56(+bright)) were also analysed by flow cytometry. Extracranial infections were presented in 55% patients with STBI. At day 4, the percentage of T lymphocytes with cytotoxic phenotype significantly diminished and their numbers restored at day 7. The frequency of NKT cells showed the identical time-dependent pattern, whereas the percentage of NK cells diminished on day 4 but did not restore after 7 days. The frequency of B lymphocytes did not change significantly during the time investigated, whereas the percentage of monocytes increased immediately after the injury and

gradually diminished. The decrease in cells with cytotoxic phenotype might explain high incidence of susceptibility to infection of patients with STBI.

Boras J, Ljubić S, Car N, Metelko Ž, Petrovečki M, Lovrenčić MV, Reiner Ž. Lipoprotein(a) predicts progression of carotid artery intima-media thickening in patients with type 2 diabetes: A four-year follow-up. Wien Klin Wochenschr. 2010;122:159-64

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BACKGROUND: The aim of the study was to establish whether increased levels of serum lipoprotein(a) significantly contribute to an increase in intima-media thickness and the number of carotid artery plaques, and consequently to cardiovascular risk in patients with type 2 diabetes mellitus.

METHODS: Lipoprotein(a) levels, intima-media thickness and the number of carotid artery plaques were determined at the beginning of the study in 146 patients with type 2 diabetes. Patients were divided into two groups according to serum lipoprotein(a) levels (> or <or=30 mg/dl). Intima-media thickness and the number of plaques were again determined after four years of follow-up. Intima-media thickness was assessed using high-resolution B-mode ultrasound.

RESULTS: The two groups of patients revealed no significant differences in baseline intima-media thickness (P = 0.112) in relation to lipoprotein(a) level. After follow-up, intima-media thickness was significantly greater in patients with higher lipoprotein(a) levels (1.24 ± 0.22 vs. 1.15 ± 0.17 mm, respectively; *P* = 0.005). The mean increase in thickness over four years was 0.12 mm (0.030 mm/year) in the group with low lipoprotein(a) levels and 0.17 mm (0.043 mm/year) in the group with high lipoprotein(a). Multivariate analysis indicated that intima-media thickness depended on lipoprotein(a), and not on triglyceride, HDL-cholesterol levels or waist-to-hip ratio. No significant difference in baseline and follow-up number of plaques was observed between the study groups (P = 0.276 vs. *P* = 0.355, respectively). Although the group with lipoprotein(a) >30 mg/dl had more cardiovascular events, the difference was not statistically significant.

CONCLUSIONS: These results indicate that lipoprotein(a) is an independent, genetically determined risk factor closely associated with progression of intima-media thickness in type 2 diabetes.

Obad A, Marinović J, Ljubković M, Brešković T, Modun D, Boban M, Dujić Ž. Successive deep dives impair endothelial function and enhance oxidative stress in man. Clin Physiol Funct Imaging. 2010;30:432-8

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The aim of this study was to assess the effects of successive deep dives on endothelial function of large conduit arteries and plasma pro-oxidant and antioxidant activity. Seven experienced divers performed six dives in six consecutive days using a compressed mixture of oxygen, helium and nitrogen (trimix) with diving depths ranging from 55 to 80 m. Before and after first, third and sixth dive, venous gas emboli formation and brachial artery function (flow-mediated dilation, FMD) was assessed by ultrasound. In addition, plasma antioxidant capacity (AOC) was measured by ferric reducing antioxidant power, and the level of oxidative stress was assessed by thiobarbituric acid-reactive substances (TBARS) method. Although the FMD was reduced to a similar extent after each dive, the comparison of predive FMD showed a reduction from 8.6% recorded before the first dive to 6.3% before the third (P = 0.03) and 5.7% before the sixth dive (P = 0.003). A gradual shift in baseline was also detected with TBARS assay, with malondialdehyde values increasing from 0.10 ± 0.02 μmol l^{-1} before the first dive to 0.16 ± 0.03 before the sixth (P = 0.005). Predive plasma AOC values also showed a decreasing trend from 0.67 ± 0.20 mmol l^{-1} trolox equivalents (first day) to 0.56 ± 0.12 (sixth day), although statistical significance was not reached (P = 0.08). This is the first documentation of acute endothelial dysfunction in the large conduit arteries occurring after successive deep trimix dives. Both endothelial function and plasma pro-oxidant and antioxidant activity did not return to baseline during the course of repetitive dives, indicating possible cumulative and longer lasting detrimental effects.