

Orešković S, Babić D, Kalafatić D, Barišić D, Beketić-Orešković L. A significance of immunohistochemical determination of steroid receptors, cell proliferation factor Ki-67 and protein p53 in endometrial carcinoma. *Gynecol Oncol.* 2004;93:34-40.

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The aim of the study was to preoperatively predict the biologic behavior of the endometrial carcinoma using immunohistochemical analysis of the p53 protein and Ki-67 expression, and estrogen receptor (ER) and progesterone receptor (PR) status, in the material obtained by fractional curettage. One hundred and thirty-six patients with primary endometrial carcinoma were included in the study. In all 136 patients, the fractional curettage was performed before the hysterectomy, and the diagnosis of endometrial carcinoma was confirmed pathohistologically after the surgical procedure on the hysterectomy specimens. The cutoff values of the percentage of ER, PR, p53, and Ki-67 positive cells in terms of survival probability determination were obtained as the values of the highest chi-square test, using proportional-risk regression method. A multivariate Cox regression analysis was performed to estimate the influence of several clinical, pathohistologic, and immunohistochemical covariates to patients' survival. Survival curves were determined by the Kaplan-Meier product-limit method based on the most recent clinical status. According to the histologic type of the tumor, fractional curettage specimens revealed 111 histologically favorable types (81.6%) and 25 unfavorable types (18.4%). The data indicate that ER, PR, Ki-67, and p53 levels of the hysterectomy specimens and those of the preoperative specimens were in fairly good agreement. The patients with the most favorable tumor grade (G I) had significantly better prognosis when the percentage of p53 positive cells was less than 15%. In the group of patients with histologic grade II, the survival was affected by ER expression (more than 30% of positive cells) and p53 levels (less than 15% of positive cells). None of the parameters was predictive in the group of patients with histologic grade III. The determination of immunohistochemical parameters (ER, PR, and p53) on well-differentiated and moderately differentiated endometrial carcinoma of favorable histologic type obtained by curettage enables the recognition of patients with favorable prognosis, who should not be treated by radical surgery.

Kušec V, Šmalcelj R, Puretić Z, Szekeres T. Interleukin-6, transforming growth factor-beta 1, and bone markers after kidney transplantation. *Calcif Tissue Int.* 2004;75:1-6.

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The aim of this study was to investigate the relationship between interleukin 6 (IL-6), transforming growth factor (TGF)-beta 1, IL-6 soluble receptors, and biochemical parameters of bone turnover after kidney transplantation. Of 64 patients en-

rolled in the study, 19 received the kidney transplant 2 to 12 months before the study, and 45 within the previous 15 to 175 months. The authors measured IL-6, TGF-beta 1, intact parathyroid hormone (PTH) bone alkaline phosphatase (BALP), osteocalcin (OC), and procollagen type I propeptide (P1CP) concentrations in the serum, and deoxypyridinoline crosslinks (DPD) in the urine of the patients. In 16 patients in the first posttransplantation year, the concentrations of IL-6 ($p=0.02$), TGF-beta 1 ($p=0.01$), BALP ($p=0.0002$), OC ($p=0.001$), and DPD ($p=0.01$) were significantly higher than in patients with longer posttransplantation period. Statistically significant negative correlation was found between post-transplantation time and IL-6 ($p=0.04$), BALP ($p=0.003$), OC ($p=0.0009$), P1CP ($p=0.03$), and DPD ($p=0.01$) concentrations. Repeated measurements of the investigated parameters in the first post-transplantation year showed a significant decrease only in TGF-beta 1 level. In all patients, IL-6 correlated positively with PTH ($p=0.0009$) and DPD ($p=0.03$), and IL-6 soluble receptor (IL-6 sR) with DPD ($p=0.03$). A decrease in IL-6 and TGF-beta 1 concentrations that paralleled the decrease in bone turnover markers in the posttransplantation period indicated that IL-6 and TGF-beta 1 were probably involved in the bone turnover after kidney transplantation.

Ivanuša M, Ivanuša Z. Risk factors and in-hospital outcomes in stroke and myocardial infarction patients. *BMC Public Health.* 2004;4:26-32.

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The main objective of this study was to compare vascular risk factor profiles with in-hospital outcomes in acute stroke (AS) and acute myocardial infarction (AMI) patients. The authors evaluated 486 consecutive patients who were admitted to Bjelovar General Hospital with diagnoses of AS (ischemic stroke or intracerebral hemorrhage; $n=380$) or AMI ($n=106$) during a one year period. The frequency of risk factors and in-patient mortality rates were assessed in both groups. AS patients were significantly older than AMI patients: the mean age for AS patients was 68.9 ± 9.1 years, and for AMI patients was 62.8 ± 11.7 years ($p < 0.001$). AMI was significantly more common than AS in patients younger than 65 years; 51% of this group had AMI and 26% had AS ($p < 0.001$). Hypertension was a more common risk factor in AS patients (69% AS patients vs 58% AMI patients; $p=0.042$). Patients who died did not differ significantly in age between the groups. In-patient mortality rates were significantly higher in AS than AMI cases (31% vs 12%, $p < 0.001$ for all patients; 37% vs 5%, $p < 0.001$ for men). Women hospitalized for AMI were more likely to die in hospital than men (28% vs 5%; $p=0.002$). In conclusion, the age at the time of presentation was a significant differentiating factor between patients with AS and AMI. The only exceptions were women, whose ages at the onset of AS and AMI were similar. In contrast, patients who died did not differ significantly in age. In

addition, a significantly higher inpatient mortality for men than for women with AS was observed. The five-fold higher inpatient mortality rate in women than in men with AMI is most likely to have resulted from other factors related to treatment.

Uglešić V, Knežević P, Košutić D, Jokić D. Lag screw fixation for straight midline mandibulotomy: four-year experience. *Ann Plast Surg.* 2004;52:349-53.

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The authors' experience with 2-lag screw technique for the stabilization of a straight midline mandibulotomy approach for oral cavity and oropharyngeal tumors is presented. Fixation is performed with 2.0-mm pretapped screws. A washer is used to prevent sinking of the screw head into spongy bone and to provide equal pressure distribution of the screw head to the bone edges. Lag screw fixation was used in 24 patients, 18 of whom underwent a full dose of irradiation therapy postoperatively. Two patients developed salivary fistula and 1 developed a local infection. In all patients there were no signs of mandibulotomy site instability, no disturbance of the occlusion, and radiography showed excellent bone healing. There were no indications for tooth extraction to complete the osteotomy. Lag screw fixation proved to be an uneventful and straightforward procedure for the stabilization of a straight midline mandibulotomy approach.

Crljen V, Višnjic D, Banfić H. Presence of different phospholipase C isoforms in the nucleus and their activation during compensatory liver growth. *FEBS Lett.* 2004;571:35-42.

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Phospholipase C (PLC) was purified from the membrane-depleted rat liver nuclei. About 60% of the total PLC-activity corresponded to beta1b isoform, 30% to PLC-gamma1 and less than 10% to PLC-delta1. PLC-beta1b and gamma1 were found in the nuclear matrix, while PLC-delta1 was detected in the chromatin. Two peaks of an increase in the total PLC-activity were detected occurring at 6 and 20 h after partial hepatectomy. An early increase in PLC-beta1b activity in the nuclear matrix was associated with serine phosphorylation of the enzyme, while the later increase paralleled the increase in the amount of protein. The increase in the PLC-gamma1 activity measured at 6 and 20 h after partial hepatectomy was associated with tyrosine phosphorylation of the enzyme. The activity of PLC-delta1 and the amount of the protein found in the chromatin was increased only at 20 h after partial hepatectomy.

Mihelić R, Pečina M, Jelić M, Zoričić S, Kušec V, Šimić P, Bobinac D, Lah B, Legović D, Vukičević S. Bone morphogenetic protein-7 (osteogenic protein-1) promotes tendon graft integration in anterior cruciate ligament reconstruction in sheep. *Am J Sports Med.* 2004;32:1619-25.

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Bone morphogenetic proteins induce new bone both in patients with bone defects and at extraskelatal sites in animals. After anterior cruciate ligament rupture, tendon graft fixation into a bone tunnel is a widely used method for anterior cruciate ligament reconstruction. Bone morphogenetic protein-7 applied to the bone-tendon interface enables better integration of a free tendon graft into the surrounding bone. In controlled laboratory study, the anterior cruciate ligament was reconstructed using a free tendon graft in the right rear knees of 30 one-year-old male sheep. Recombinant human bone morphogenetic protein-7 (25 microg) was applied randomly to the bone-tendon interface in 15 animals, and a vehicle was applied in 15 control animals. At 3 weeks, 10 animals from each group were sacrificed, and the remaining sheep were sacrificed at 6 weeks after surgery. Subsequently, histologic analysis and mechanical test-

ing were performed. In another group of 20 sheep, the same procedure was used and mechanical testing was performed after 3 weeks. More new bone was formed at the bone-tendon interface in the knees treated with bone morphogenetic protein-7 as compared histologically with similar areas in control animals, creating areas of dense trabecular network with significantly greater invasion of the tendon fibrous tissue into the bone marrow space. Mechanical testing showed greater strain resistance to force (368 N) in the knees treated with bone morphogenetic protein-7 than in control specimens (214 N). There was no difference between mechanical testing of samples from 3 and 6 weeks after surgery. Bone morphogenetic protein-7 promotes complete tendon graft integration into the newly formed surrounding trabecular bone in the reconstruction of the anterior cruciate ligament. Bone morphogenetic protein-7 in tendon graft integration might be successfully used in reconstructive surgery of ligaments.

Ulovec Z, *Šošić Z, Škrinjarić I, Čatović A, Čivljak M, Szivovica L. Prevalence and significance of minor anomalies in children with impaired development. *Acta Paediatr.* 2004;93:836-40.

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The aim of this study was to compare the prevalence of Waldrop's minor physical anomalies in children with developmental disorders (mental retardation, hearing and visual impairment) and healthy schoolchildren. The study was carried out on a sample of 469 children (223 children with developmental disorders and 246 healthy schoolchildren). Significant differences were found between the children with developmental disorders and the healthy children with regard to the number of minor anomalies and their weighted scores according to Waldrop. Multivariate discriminant analysis with two discriminative functions explained as much as 96.51% of the total variability and significantly distinguished the healthy children from the children with developmental disorders. However, no clear distinction was found between the mentally retarded children and those with visual impairment. Interrelation of the number and sum of the weighted scores of minor anomalies showed similar minor anomalies in the mentally retarded children (mean per person 3.65 and 3.82, respectively), the children with visual impairment (3.24 and 3.50), the children with hearing impairment (3.84 and 3.67) and the control group (1.70 and 1.46), although at different levels. The high prevalence of multiple minor anomalies in children with developmental disorders suggests that during early development, factors which cause the specific developmental disorder and the occurrence of a minor anomaly have a joint effect.

Cvijetić S, Koršić M. Apparent bone mineral density estimated from DXA in healthy men and women. *Osteoporos Int.* 2004;15:295-300.

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The aim of this study was to measure bone mineral density (BMD) in healthy people and examine the influence of age, anthropometry, and postmenopause on calculated bone mineral apparent density (BMAD). The study included 541 healthy subjects (249 men and 292 women), aged 20 to 79 years. Anthropometric measurements included height, weight, and body mass index (BMI). Bone mineral content (BMC) and areal BMD were measured at the lumbar spine and proximal femur, using dual-energy X-ray absorptiometry (DXA). The calculation of volumetric density relied on the formula $BMAD = BMD / \text{square root } BA$ (where $BA = \text{bone area}$). Association between densitometric parameters and age, height, weight, and postmenopause was analyzed with multiple regression. BMC and BMD decreased with age, especially in postmenopausal women. The average annual bone loss in spine was 0.2% in both sexes, whereas femur loss was 0.5% in men and 0.3% in women. Bone area slightly increased with age in both sexes, and BMD loss after the age of 50 could be attributed to bone area increase. To minimize the effect of bone size on bone den-

sity, volumetric density and areal density were regressed to age, anthropometry, and postmenopause. Age and postmenopause were significantly associated with BMD and BMAD in the spine and femur. Furthermore, BMD showed a stronger association with height and weight than BMAD, in both regions. Weaker association of body height and weight with BMAD than with BMD suggests that BMD depends on the bone size and body size and that the different BMDs could be the consequence of the difference in those parameters.

Kaštelan M, Prpić-Massari L, Gruber F, Zamolo G, Zauhar G, Coklo M, et al. Perforin expression is upregulated in the epidermis of psoriatic lesions. *Br J Dermatol*. 2004;151:831-6.

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The aim of this study was to analyze the expression and distribution of perforin, T- and NK-cell subsets in psoriatic lesional and nonlesional skin. Skin biopsy specimens from both lesional and nonlesional skin of 11 patients with chronic plaque psoriasis and eight healthy controls were analyzed by immunohistochemistry. The authors found a significant increase in CD4⁺ and CD8⁺ cells in psoriatic lesions compared with nonlesional and healthy skin. The expression of CD16⁺ NK-cells was significantly lower in lesions compared with healthy skin. Perforin expression was significantly enhanced in the epidermis of psoriatic lesions. In conclusions, perforin expression is upregulated in the epidermis of psoriatic lesions, suggesting a potential role for perforin in the creation of the psoriatic plaque.

Gamulin V, Četković H, Ahel I. Identification of a promoter motif regulating the major DNA damage response mechanism of *Mycobacterium tuberculosis*. *FEMS Microbiol Lett*. 2004;238:57-63.

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The principal response of many bacteria to DNA damage is mediated by a mechanism dependent on the LexA and RecA proteins. However, *Mycobacterium tuberculosis* was recently reported to regulate a majority of DNA repair genes independently of RecA and LexA, suggesting that an unknown RecA/LexA-independent mechanism controls the major DNA damage response pathway in this organism. The authors have identified a motif tTGTCRgtg-8nt-TAnnnT that defines a novel RecA/LexA-independent promoter (RecA-NDp) of *M. tuberculosis*. Furthermore, they show that the RecA-NDp type of promoter precedes DNA repair genes in other Actinomycetales.

Colić-Barić I, Kajfež R, Satalić Z, Cvjetić S. Comparison of dietary habits in the urban and rural Croatian schoolchildren. *Eur J Nutr*. 2004;43:169-74.

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Post-war socio-economic changes in Croatia probably affected dietary habits, and dietary data about schoolchildren after the war are missing. The aim of the study was to compare current nutrient intakes and dietary behavior between urban and rural schoolchildren in Croatia. A completely quantified Food Frequency Questionnaire was used. Subjects were 315 urban and 163 rural schoolchildren. Mean age was 12.5 and 12.6 years in the urban and rural area, respectively. Consumption of fast food, soft drinks and alcohol was more prevalent and more linked with dietary behavior in the urban than in the rural area. In both living areas protein intake was excessive (in the urban area 38.1% of subjects and in the rural 36.2% of subjects had protein intake higher than 200% RDA). Under 75% RDA/DRIs in both living areas was observed for vitamin D, folate, calcium and selenium. Micronutrient intakes negatively correlated with age in both living areas, but were more pronounced in the urban area. In conclusion, the urban sample had more adequate

energy and nutrient intakes which is consistent with prewar findings.

Gamberger D, Lavrac N, Zelezny F, Tolar J. Induction of comprehensible models for gene expression datasets by subgroup discovery methodology. *J Biomed Inform*. 2004;37:269-84.

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Finding disease markers (classifiers) from gene expression data by machine learning algorithms is characterized by a high risk of overfitting the data due to the abundance of attributes (simultaneously measured gene expression values) and shortage of available examples (observations). To avoid this pitfall and achieve predictor robustness, state-of-the-art approaches construct complex classifiers that combine relatively weak contributions of up to thousands of genes (attributes) to classify a disease. The complexity of such classifiers limits their transparency and consequently the biological insights they can provide. The goal of this study was to apply to this domain the methodology of constructing simple yet robust logic-based classifiers amenable to direct expert interpretation. On two well-known, publicly available gene expression classification problems, the paper shows the feasibility of this approach, employing a recently developed subgroup discovery methodology.

Kojić-Katović S, Halbauer M, Tomić-Brzac H. Importance of FNAC in the detection of tumors within multinodular goitre of the thyroid. *Cytopathology*. 2004;15:206-11.

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The primary challenge in the management of a multinodular thyroid gland is to rule out malignancy. The present study was undertaken to assess the value of preoperative ultrasound-guided fine needle aspiration cytology (FNAC) in diagnosing tumors of the thyroid gland. Of the 80 patients operated for multinodular lesions, malignant tumors were found in 29 and benign tumors in 36 patients (81%) and non-tumorous lesions in 15 (19%) patients. Compared with the histopathological postoperative diagnosis, the overall sensitivity of FNAC was 85% and specificity 88%. Current morphological diagnosis of the nodules in multinodular goitre requires thorough preoperative examination, including ultrasound-guided FNAC in order to establish the appropriate management.

Bosnar MH, De Gunzburg J, Bago R, Brečević L, Weber I, et al. Subcellular localization of A and B Nm23/NDPK subunits. *Exp Cell Res*. 2004;298:275-84.

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The human Nm23-H1/NDPK A and Nm23-H2/NDPK B encode for two subunits of nucleoside diphosphate kinase, an ubiquitous enzyme that transfers the terminal phosphates from ATP to (d)NDPs. Although having an 88% amino acid sequence identity and an already assigned biochemical role in the cell, the two subunits appear to have additional and distinctive cell functions. In particular, both subunits have been reported to be involved in tumor progression and metastasis. The aim of this study was to determine the specific, and potentially distinct, localizations of both subunits in tumor cells of different origin and differentiation and therefore to search for a possible link between their localization and the stage of disease. The authors used the GFP reporter system to analyze the ectopic expression of GFP-Nm23 proteins in head and neck tumor cell lines by fluorescent microscopy techniques. The experiments revealed that GFP-fused Nm23-H1 and -H2 proteins display the same localization in transfected cells, regardless of their origin and differentiation status. The proteins are principally found in the cytosol and the endoplasmic reticulum. Moreover, some cells exhibit nuclear staining, which appears to be cell cycle-dependent.

Miličević G, Fort L, Majsec M, Bakula V. Heart rate variability decreased by coronary artery surgery has no prognostic value. Eur J Cardiovasc Prev Rehabil. 2004;11:228-32.

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Decreased heart rate variability (HRV) may predict cardiac death after myocardial infarction (MI). Coronary artery bypass grafting (CABG) strongly decreases HRV, but improves survival. The aim of this study was to determine the prognostic value of HRV decreased by coronary surgery. Four-year follow-up was performed in 175 consecutive patients with HRV decreased by CABG (n = 51) or MI (n = 124). Mortality and secondary events rate were analyzed. Decreased HRV, defined by the standard deviation of mean RR interval (SDNN) < 100 ms,

was detected by a routine 24-h Holter electrocardiogram at admission to stationary rehabilitation 3 weeks to 3 months after acute MI or CABG. Two groups did not differ except by age; CABG patients were younger (56 vs. 64 yrs, $p < 0.01$), but this did not influence differences in survival (NS). HRV was lower among CABG patients than among MI patients (SDNN = 66 ± 20 ms vs. 77 ± 14 ms; $p < 0.001$), but cumulative survival and event-free survival were much better in the CABG group than in the MI group. During a 46 ± 20 months follow-up, there were 10% new events in the CABG and 43% in the MI group ($P < 0.001$). Mortality was 8% in the CABG and 33% in the MI group (log-rank = 3.6; $p < 0.001$). Unlike in the MI group, HRV was not different between survivors and non-survivors in the CABG group. In contrast to the strong prognostic potential of HRV in patients with MI, decreased HRV has no prognostic significance in patients who have undergone CABG surgery.