

**Peričić D, Lazić J, Jembrek MJ, Strac DS, Rajcan I.** Chronic exposure of cells expressing recombinant GABAA receptors to benzodiazepine antagonist flumazenil enhances the maximum number of benzodiazepine binding sites. *Life Sci.* 2004;76:303-17.

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The aim of this study was to better understand the mechanisms that underlie adaptive changes in GABAA receptors following their prolonged exposure to drugs. Exposure (48 h) of human embryonic kidney (HEK) 293 cells stably expressing recombinant alpha1beta2gamma2S GABAA receptors to flumazenil (1 or 5 microM) in the presence of GABA (1 microM) enhanced the maximum number (Bmax) of [<sup>3</sup>H]flunitrazepam binding sites without affecting their affinity (Kd). The flumazenil-induced enhancement in Bmax was not counteracted by diazepam (1 microM). GABA (1 nM - 1 mM) enhanced [<sup>3</sup>H]flunitrazepam binding to membranes obtained from control and flumazenil-pretreated cells in a concentration-dependent manner. No significant differences were observed in either the potency (EC50) or efficacy (Emax) of GABA to potentiate [<sup>3</sup>H]flunitrazepam binding. However, in flumazenil pretreated cells the basal [<sup>3</sup>H]flunitrazepam and [<sup>3</sup>H]TBOB binding were markedly enhanced. GABA produced almost complete inhibition of [<sup>3</sup>H]TBOB binding to membranes obtained from control and flumazenil treated cells. The potencies of GABA to inhibit this binding, as shown by a lack of significant changes in the IC<sub>50</sub> values, were not different between vehicle and drug treated cells. The results suggest that chronic exposure of HEK 293 cells stably expressing recombinant alpha1beta2gamma2S GABAA receptors to flumazenil (in the presence of GABA) up-regulates benzodiazepine and convulsant binding sites, but it does not affect the allosteric interactions between these sites and the GABA binding site. Further studies are needed to elucidate these phenomena.

**Cvijanović O, Bobinac D, Zoričić S, Ostojić Z, Marić I, Crnčević-Orlić Z, et al.** Age- and region-dependent

changes in human lumbar vertebral bone: a histomorphometric study. *Spine.* 2004;29:2370-5.

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The aim of this study was to explore region-dependent changes that occur with aging in trabecular and cortical bone of the human vertebral body. Bone cylinders were drilled with a trephine from three regions (central anterior, central posterior, and lateral) of the third lumbar vertebral bodies of 48 autopsy cases 31 to 76 years old. Two consecutive 5 µm sections obtained 150 µm apart were stained with toluidine blue and Masson trichrome and photographed at 40×. Differences in numerous morphometric factors were evaluated by age and region of the vertebra using repeated-measures analysis of variance and Tukey's Honestly Significant Difference test. Starting at about 50 years of age, significant, linearly progressive decreases occurred in trabecular and cortical bone volume (p<0.005), trabecular surface area (p<0.001), number of trabeculae (p<0.001), and thickness of trabeculae (p<0.001). Space between trabeculae increased from ages 31 to 70 years and then decreased (p<0.001). Trabecular deterioration was significantly more pronounced in central versus lateral regions (p<0.001). Cortical bone thickness decreased significantly with aging in central regions but increased in lateral regions between ages 61 and 70 years (p<0.001). The balance between cortical and trabecular bone maintains the strength of the vertebral body until about the age of 50 years, when irreversible deterioration begins in central regions and subsequently involves lateral regions.

**Begovac I, Rudan V, Begovac B, Vidović V, Majić G.** Self-image, war psychotrauma, and refugee status in adolescents. *Eur Child Adolesc Psychiatry.* 2004;13:381-8.

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The aim of this study was to assess how war psychotrauma, refugee status and other factors relate to self-image. Psychotherapeutic-psychiatric interview, the Offer Self-Image Questionnaire (OSIQ), questionnaires for measuring war stressors, posttraumatic stress reactions (PTS-reactions), depression and general data were ad-

ministered. A total of 322 adolescents from Bosnia-Herzegovina and Croatia were included in the study. In 60.32% of the examinees, more than four war stressors were encountered. In 13.68% of the examinees, high PTS-reactions occurred. The refugees had nearly four times higher odds (aOR=3.66; 95% CI=1.63-8.2;  $p < 0.01$ ) of having a higher Offer score for the sexual attitudes subscale. Lower war stress had 0.28 times lower odds (aOR=0.28; 95% CI=0.11-0.71;  $p < 0.01$ ) of having a higher Offer score for the sexual attitudes subscale. More severe PTS-reactions had six times higher odds (aOR=6.15; 95% CI=1.7-22.2;  $p < 0.01$ ) of reaching a higher Offer score for the emotional tone subscale. War psychotrauma and refugee status are related to poorer adjustment only in some of the OSIQ subscales. Practical measures of joined sexually preventive/therapeutic activities are proposed, as well as educational and preventive/therapeutic psychotrauma models.

**Labar B, Suci S, Zittoun R, Muus P, Marie JP, Fillet G, et al. EORTC Leukemia Group. Allogeneic stem cell transplantation in acute lymphoblastic leukemia and non-Hodgkin's lymphoma for patients  $\leq$  50 years old in first complete remission: results of the EORTC ALL-3 trial. *Haematologica*. 2004;89:809-17.**

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In the EORTC ALL-3 trial, the efficacy of allogeneic transplantation was compared with that of autologous marrow transplantation and maintenance chemotherapy in patients  $\leq$  50 years who reached CR. Among 340 patients who entered the study, 279 were  $\leq$  50 years old. Out of these, 220 reached CR, 184 patients started consolidation and were HLA typed; 68 had a donor and 116 had no sibling donor. The median follow-up was 9.5 years; 93 patients relapsed, 26 died in CR, and overall 116 patients died. Allogeneic transplantation was performed in 47 (68%) patients with a donor while autologous transplantation or maintenance chemotherapy was given to 84 (72%) patients without a sibling donor. The 6-year disease-free survival rate was similar in the groups with and without donor [38.2% (SE=5.9%) vs. 36.8% (SE=4.6%), hazard ratio 1.01, 95% CI 0.67-1.53]. Comparing the donor group with the no donor group, the former had a lower relapse incidence (38.2% vs. 56.3%,  $p=0.001$ ), but a higher cumulative incidence of death in CR (23.5% vs. 6.9%,  $p=0.0004$ ). The 6-year survival rates were similar [41.2% (SE=6.0%) vs. 38.8% (SE=4.6%)]. This trial did not show that allogeneic transplantation, when a sibling donor is available, produces a better outcome than the policy of offering autotransplantation or chemotherapy in the absence of a donor.

**Baričević M, Forčić D, Gulija TK, Jug R, Mažuran R. Determination of the coding and non-coding nucleotide sequences of genuine Edmonston-Zagreb**

**master seed and current working seed lot. *Vaccine*. 2005;23:1072-8.**

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To confirm the genetic stability of the Edmonston-Zagreb vaccine strain, the authors determined and compared the nucleotide sequences of genuine Edmonston-Zagreb master seed (EZ D22) and current working seed lot (EZ D24 2/99). Sequence analysis and comparison of the two sequences confirmed that these two sequences are the same at the molecular level. The obtained sequences were also compared to reference strains, i.e. Edmonston wild-type (Edmonston Wt) AF266288 and Edmonston-Zagreb (EZ) AF266290 vaccine strain. The sequence of EZ D22 differed from the Edmonston Wt in 32 nucleotides. EZ D22 differed from EZ AF266290 in six nucleotides. Coding substitution at position 441 and two silent substitutions at positions 11999 and 14612 in the L gene are unique to EZ D22. The differences found between EZ from different sources can be a good reason for periodical sequence analysis of the same strain in the hands of different manufactures.

**Pivac N, Kozarić-Kovačić D, Muck-Šeler D. Olanzapine versus fluphenazine in an open trial in patients with psychotic combat-related post-traumatic stress disorder. *Psychopharmacology (Berl)*. 2004;175:451-6.**

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The aim of an open, comparative 6-week study was to compare olanzapine and fluphenazine, as a monotherapy, for treating psychotic combat-related PTSD. Fifty-five male war veterans with psychotic PTSD (DSM-IV criteria) were treated for 6 weeks with olanzapine ( $n=28$ ) or fluphenazine ( $n=27$ ) in a 5-10 mg/day dose range, once or twice daily. Patients were evaluated at baseline, and after 3 and 6 weeks of treatment, using Watson's PTSD scale, Positive and Negative Syndrome Scale (PANSS), Clinical Global Impression Severity Scale (CGI-S), Clinical Global Impression Improvement Scale (CGI-I), Patient Global Impression Improvement Scale (PGI-I) and Drug Induced Extra-Pyramidal Symptoms Scale (DIEPSS). RESULTS: At baseline, patient's data (age, duration of combat experience and scores in all measurement instruments) did not differ. After 3 and 6 weeks of treatment, olanzapine was significantly more efficacious than fluphenazine in reducing symptoms in PANSS (negative, general psychopathology subscale, supplementary items), Watson's PTSD (avoidance, increased arousal) subscales, CGI-S, CGI-I, and PGI-I scale. Both treatments affected similarly the symptoms listed in PANSS positive and Watson's trauma re-experiencing subscales. Fluphenazine induced more extrapyramidal symptoms. Prolongation of the treatment for 3 additional weeks did not affect the efficacy of either drug. These data indicate that both fluphenazine and olanzapine were effective for particular symptom

profile in psychotic combat-related PTSD. Olanzapine was better than fluphenazine in reducing most of the psychotic and PTSD symptoms, and was better tolerated in psychotic PTSD patients.

**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 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 is 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. Some of the discovered classifiers allow for novel biological interpretations.

**Danko M, Ilić I, Čepulić M, Orlić D, Giljević JS, Fattorini I, et al. Tumor angiogenesis and outcome in osteosarcoma. *Pediatr Hematol Oncol.* 2004;21:611-9.**

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The aim of this study was to investigate prognostic significance of angiogenesis in osteosarcoma. Thirty-nine patients with osteosarcoma were included in a retrospective immunohistochemical study. Sections from diagnostic biopsies were immunostained using anti-von Willebrand factor antibody and microvessels were counted at 400x magnification on 3 microscopic fields per patient. MVD was correlated with overall and disease-free survival by Kaplan-Meier and log-rank analysis. Correlation between clinicopathological variables and the degree of angiogenesis was tested using a  $\chi^2$  test. Significant statistical difference was found regarding overall survival and disease-free survival between patients with high (> 32.3 vessels/field) and low ( $\leq$  32.3 vessels/field) microvessel counts (log-rank test  $p=0.0196$  and  $p=0.0147$ , respectively). The rate of metastasis was significantly higher in patients with high microvessel counts ( $p=0.042$ ). These findings strongly suggest that angiogenesis quantified by microvessel density is predictive of metastasis and poor prognosis in osteosarcoma.

**Čikeš V, Abaza I, Krželj V, Terzić IM, Tafra R, Trlaja A, et al. Prevalence of factor V Leiden and G6PD 1311 silent mutations in Dalmatian population. *Arch Med Res.* 2004;35:546-8.**

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Factor V Leiden has been described as a common genetic risk factor for venous thromboembolism. The geographic distribution of this abnormality varies greatly, being high in Europe and almost absent in Asia and Africa. Particularly high prevalence is observed in some Mediterranean countries, which suggests the Mediterranean origin of this mutation. Similarly, prevalence of silent mutation 1311 of the G6PD gene seems to be higher among Mediterranean populations. Since the Dalmatian population (of south Croatia) geographically belongs to the Mediterranean populations we analyzed the prevalence of FV-Leiden and silent mutation 1311 in this region. Furthermore, because the coincidence of G6PD deficiency and venous thromboembolism was described earlier, we tested a possible association of FV-Leiden and G6PD deficiency. One hundred sixty-eight healthy blood donors and 55 G6PD deficient individuals originating from the Dalmatian region were tested for the presence of FV-Leiden mutation and silent mutation 1311. Prevalence of FV-Leiden among blood donors was 2.4%, while among G6PD deficient individuals it was significantly higher, 11% ( $p=0.011$ ). Prevalence of silent mutation 1311 among blood donors and G6PD deficient individuals was 21 and 15%, respectively. Observed allele frequencies among individuals originating from the Dalmatian region is similar to the neighboring European and Mediterranean populations. Interestingly, these results indicate the association of the FV-Leiden and G6PD deficiency and warrant further studies.

**Župančić B, Popović IJ, Župančić V, Augustin G. Primary vesicoureteric reflux – our 20 years' experience. *Eur J Pediatr Surg.* 2004;14:339-44.**

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The aim of this study is to compare the Lich-Gregoir procedure and antireflux ureterocystoneostomy at the vertex of the bladder (AUVB) based on 20 years' clinical experience. Over a period of 20 years (1978-1998), a total of 1280 children were operated on, 368 bilaterally, which resulted in 1648 antireflux ureterocystoneostomies being performed. Of the total of 1648 antireflux ureterocystoneostomies, AUVB was performed in 1032 ureteric units and the Lich-Gregoir procedure in 616 ureteric units. Between 1978 and 1992 the authors performed only AUVB, and from 1992, both AUVB and the Lich-Gregoir procedure. The final result was evaluated 2 years after the operation. Satisfactory results were achieved in 93.5 % with AUVB and in 96 % with the Lich-Gregoir procedure. The postoperative failure rate was 6.5 % for the AUVB and 4 % for the Lich-Gregoir

operations. The recurrence rate was higher with AUVB (5 %) than with the Lich-Gregoir procedure (1.5 %), but postoperative stenosis was more frequent with the Lich-Gregoir procedure (2.5 %). The authors recommend the Lich-Gregoir procedure as the preferred operative method. If the results of the Lich-Gregoir procedure are unsatisfactory, the authors recommend the AUVB for the first and second recurrence operation. Finally, in cases of repeated VUR recurrence of postoperative stenosis, as the last operation, the authors perform antireflux ureteroileocystoplasty with an intussuscepted segment of the ileum.

**Kopljar M, Brkljačić B, Doko M, Horžić M. Nature of Doppler perfusion index changes in patients with colorectal cancer liver metastases. J Ultrasound Med. 2004;23:1295-300.**

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Occult hepatic metastases from colorectal cancer result in an increase of the ratio of arterial hepatic blood flow to total hepatic blood flow, described as the Doppler perfusion index. Whether this alteration is due to an increase in arterial blood flow or a decrease in portal venous inflow has not yet been unequivocally determined. The purpose of this study was to analyze changes in hepatic perfusion in patients with liver metastases from colorectal cancer by standardization of hemodynamic parameters to body surface area. Hemodynamic parameters (crosssectional area, blood flow, and congestive index) were measured for the common hepatic artery and portal vein with duplex color Doppler sonography in 20 patients with liver metastases and 20 healthy control subjects and evaluated relative to body surface area. No statistically significant differences in age, body surface area,

cross-sectional area of the common hepatic artery, and congestion index of the common hepatic artery and portal vein were observed between control subjects and patients with liver metastases. Patients with liver metastases had significantly greater arterial hepatic blood flow and Doppler perfusion index and significantly smaller portal cross-sectional area portal blood flow as well as total liver blood flow ( $p < 0.01$ ). This study supports the theory that the primary mechanism of alteration in liver perfusion is the reduction of portal inflow with subsequently increased arterial hepatic blood flow.

**Kojić Katović S, Halbauer M, Tomić-Brzac H. Importance of FNAC in the detection of tumours 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.