

The background of the cover is a photograph of two muscular, classical statues of Atlas, known as the Atlantes, supporting a classical building facade. The statues are positioned on either side of a central archway, with their hands resting on the arch. The building's architecture features ornate columns and arches, creating a sense of grandeur and historical significance. The lighting is warm, highlighting the musculature of the statues and the architectural details.

# **2<sup>nd</sup> ZIMS**

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**2<sup>nd</sup> ZAGREB INTERNATIONAL MEDICAL SUMMIT**

**ABSTRACT BOOK**

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**2<sup>nd</sup> Zagreb International Medical Summit**  
For Medical Students and Young Doctors  
October 25<sup>th</sup> – 27<sup>th</sup>, 2002  
Zagreb, Croatia

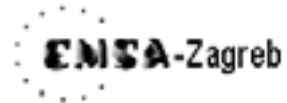
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**2<sup>nd</sup> ZIMS**  
**ABSTRACT BOOK**

Organizers:

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ZIMS is an independent annual student congress, organized by students from Medical school, University of Zagreb. Contributors from all fields of medicine and adjacent sciences are welcome to apply and post their Abstracts for publishing. All participants in ZIMS must be students or young doctors graduated within last year.

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*Old Chinese saying*

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Croatian Medical Association, founded in 1874, acting continuously on this territory for the last 128 years is the oldest independent medical association in our country. Between many professional societies and sections in it, we are extremely proud on our Students sections and all their professional, scientific and social activities.



It is a great honour to host 2<sup>nd</sup> Zagreb International Medical Summit, For Medical Students and Young Doctors. I am sure that ZIMS will be a nice opportunity for gathering and new friendships, and a valuable professional experience for individual young scientists all over the Europe as well as to the member's and non-member's of Croatian Medical Association and all medical community. Let us hope that information from Congress will add to your knowledge base and contribute to a greater understanding of the many challenges that lie ahead our students/scientists. It is important for young medical professionals to be involved in scientific research and presentation of results of theirs research. Most importantly I am sure that you will contribute in future to improving the standard and high quality of healthcare to

all patients throughout Europe when graduate on different Universities.

Many thanks to Organizing Committee and all of its member's for their work and eagerness in organizing this event. Finally, thanks to all of you who have taken the different troubles to contribute to this Congress. And thanks to all of you for coming in Zagreb, in Croatian Medical Association.

Wishing you fruitful Congress with lot of discussions, and the best in professional and private future,



Prof. dr. sc. Dubravko Orlic MD, PhD  
President of Croatian Medical Association

## Messages from the patrons

Education, process of gathering knowledge, is a life long activity in medicine. Each medical worker is obliged to acknowledge this and accept that he/she must continue learning. Therefore, Medical school, University of Zagreb, supports any type of activities associated with learning. In this light, student initiatives are very welcome. It is very important that idea of continuous medical education is planted into students' mind, making awareness on this subject important part of every student's curriculum.



On the other hand, science and scientific orientation also begin during student life. Many great scientist, today professors and known researchers, dived into science during their student days. After all, one day people who are now students will rule the world!

Either way, on behalf of the Medical school staff I wish you a pleasant and educational congress and stay in Zagreb.

A handwritten signature in dark ink, appearing to read 'Boris Labar'.

Prof. dr. sc. Boris Labar MD, PhD  
Dean of Medical school, University of Zagreb



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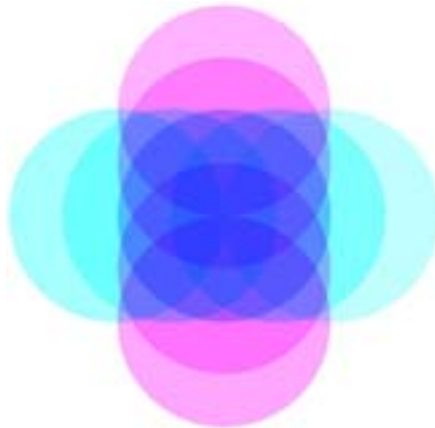
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# **ABSTRACTS**

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## COULD NITRIC OXIDE MEDIATE HAEMODYNAMIC RESPONSE TO FETAL HYPOXIA IN PLACENTA?

Ana Tikvica<sup>1</sup>, Ivana Pintarić\*<sup>1</sup>, Marija Kušan Jukić<sup>2</sup>, Marijana Medić<sup>2</sup>, Goranka Hudiček-Martinčić<sup>3</sup>, Vesna Košec<sup>4</sup>, Aida Salihagić-Kadić<sup>2</sup>

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**Background:** When oxygen delivery to the fetus is compromised, certain circulatory and metabolic mechanisms will be activated. The fetal cardiovascular responses to hypoxia are coordinated to centralise blood flow to organs important for maintenance of fetal life, such as the brain, heart and adrenals. The blood flow centralization, considered as one of the most important adaptive reactions, can be detected by Doppler ultrasound. The modifications of placental haemodynamics, responsible for fetal hypoxia, can be quantified by using the umbilical resistance index (URI), measured on the umbilical arterial Doppler velocity waveforms. The cerebrovascular adaptation (vasodilatation) can be assessed by using the cerebral resistance index (CRI), measured on the middle cerebral artery velocity waveforms. The flow redistribution between the placenta and the brain can be detected and quantified by using the cerebro-umbilical ratio (C/U). If any flow redistribution in favour of the brain occurs, the C/U ratio becomes less than 1. Moreover, it is the most sensitive parameter for the assessment of fetal growth retardation (IUGR) and fetal hypoxia. The aim of our study was to investigate the role of nitric oxide (NO), a potent vasodilator,

in haemodynamic changes in placental insufficiency, IUGR and hypoxia, already detected by Doppler assessment.

**Study design and Results:** The study included pregnant women with normal, in term delivery (n=12) and women with IUGR (n=10) from 33 to 40 weeks of gestation. Doppler indices (CRI and URI) were measured at least twice and C/U was calculated. C/U less than 1, as crucial parameter of fetal hypoxia and blood flow redistribution towards the brain, was recorded in all women with IUGR. After delivery the three samples from each placenta were collected and used for determination of NO metabolites (nitrate and nitrite) by Griese reaction. The mean total concentration of NO metabolites (nitrates and nitrites) measured in the supernatants of homogenized normal term placentas was  $9.47 \pm 1.06 \mu\text{Mol/L}$  (range 5.98-16.17  $\mu\text{Mol/L}$ ). However, the same metabolites measured in samples of placentas from pregnancies with IUGR and hypoxia were statistically higher ( $p < 0.005$ ) than normal, with mean value  $52.97 \pm 13.06 \mu\text{Mol/L}$  (range 25.73-152.74  $\mu\text{Mol/L}$ ).

**Discussion and Conclusions:** Our results suggest that the rather higher concentrations of NO metabolites in samples of placentas from pregnancies with IUGR and hypoxia implicate the activation of compensatory blood flow regulation mechanisms on the placental level. It means that the nitric oxide could be responsible for haemodynamic changes to hypoxia in placenta. Many other factors (drugs, nicotine, hormones) could be involved in induction of NO synthesis on the level of placenta during haemodynamic response to fetal hypoxia and their role also has to be considered.

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## **EFFICIENCY ANALYSIS OF BONE MORPHOGENETIC PROTEIN (BMP), AUTOLOGOUS BONE MARROW AND HOMOLOGOUS SPONGIOSA ON BONE HEALING IN DEFECTS OF RABBITS ULNA**

Janko Szavits Nossan, Nikolina Radakovic\*

Mentor: Slobodan Vukicevic MD, PhD

Medical school, University of Zagreb, Croatia

**Keywords:** bone morphogenic protein, autologous bone marrow, homologous spongiosa, bone healing

Efficiency of autologous bone marrow and BMP-7 (bone morphogenic protein 7) in bone healing was evaluated on bone defects that were done on diaphyses of rabbits ulnae. 30 rabbits were operated and they were separated in groups of 6. Rabbits from control group nothing was implanted, while rabbits from experimental groups were treated with homologous spongiosa, homologous spongiosa combined with autologous bone marrow, homologous spongiosa combined with BMP-7 and only BMP-7. The bone regeneration was monitored using X-ray imaging, taken every two weeks during the ten week period and evaluated using specified criteria. The results have shown that the bone regeneration was faster and more efficient in group of rabbits that were treated with autologous bone marrow combined with homologous spongiosa and in group of rabbits that were treated with BMP-7 combined with homologous spongiosa in comparison with other groups of rabbits.

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## GLUTATHION-DEPENDENT ENZYMES ACTIVITIES DEFINITION IN LYMPHOCYTES WITH SAPONIN APPLICATION

Nataly Podkovka\*, Nataly Skrypnyk, Subodh Kumar, Keyur Radia

Scientific advisor: Zinovij Vorobets PhD

Department of Medical Biology

Danylo Halytsky Lviv State Medical University, Lviv, Ukraine

**Keywords:** glutathione peroxidase, glutathione reductase, glutathione transferase, saponin, lymphocytes

Investigation on the whole cells are specific and have many advantages in understanding of cell function mechanisms, because the total enzymes activity have defined, but not separate isoforms, localizing in different membranes or cytosol. Taking into consideration, that main antioxidant enzymes, such as glutathione peroxidase (GP) and glutathione transferase (GT), localize on the internal side of plasma membrane, and glutathione reductase (GR) presents in cytosolic fraction, cell membranes permeabilization is expedient for their latent activities uncovering. The aim of this work was, using as a permeable agent saponin, to investigate the optimal conditions for glutathione peroxidase, glutathione transferase and glutathione reductase activity definition in human peripheral blood lymphocytes. Saponin was added into the incubation medium for enzymes activity definition in concentration range 0.02-0.2%. It was shown, that GR activity increases according to saponin concentration, reaching 30 nmol NADPH/mg of protein per 1 min at the presence of 2% saponin. GP activity practically didn't changed at the low concentration of this substance (to 0.04%), when enzyme activity was 2 nmol GSH/mg of protein per 1 min. There was observed the increasing of GP activity to 75

nmol GSH/mg of protein at the presence of 1% saponin, its greater content oppressed enzyme activity. For GT latent activity uncovering (to 68 nmol GSH/mg of protein per 1 min) 0.2% saponin concentration was optimal. These experiments suggest that low saponin concentrations (0.02-0.04%) insufficient for GP, GR and GT latent activity uncovering. Since there are mainly the cytosolic enzymes, they activation needs the greater saponin quantity.

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## KINETICS AND MECHANISM OF THE REACTIONS OF AQUAPENTACYANOFERRATE (II) IONS WITH THE BIOLOGICALLY ACTIVE BIS-PYRIDINIUM ALDOXIME (TMB-4)

Blaženka Foretić, Ivana Pogledić\*, Nicoletta Burger

Department of Chemistry and Biochemistry, School of Medicine,  
University of Zagreb, Croatia

The reactions of the aquapentacyanoferrate(II) ions,  $\text{Fe}(\text{CN})_5\text{OH}_2^{3-}$ , with a variety of oximes have been studied in our laboratory for many years. Many of them, especially bis-quaternary pyridinium aldoximes are potent reactivators of the acetylcholinesterase activity inhibited by organophosphorus poisons (insecticides and nerve agents). One of the known antidotes is the 1,1'-bis(pyridinium-4-aldoxime)trimethylene dibromide (TMB-4). TMB-4 reacts with  $\text{Fe}(\text{CN})_5\text{OH}_2^{3-}$  ions, forming a blue pentacyanoferrate(II) complex. According to numerous kinetics studies of the substitution of labile water on the  $\text{Fe}(\text{CN})_5\text{OH}_2^{3-}$  with the series of ligands and formation of  $\text{Fe}(\text{CN})_5\text{L}^{n-}$  complexes follows a dissociative mechanism with a dependence of the specific rate  $k_f$  on the charge of the entering ligand. The reactivity of  $\text{Fe}(\text{CN})_5\text{OH}_2^{3-}$  toward to substitution by oximes has received surprisingly little attention. In this study we have examined the kinetics of the substitution reactions of the  $\text{Fe}(\text{CN})_5\text{OH}_2^{3-}$  ions with TMB-4 in aqueous media in the range pH 5-11,  $I=0.50$  M and 25 °C.

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## KNOWLEDGE AND ATTITUDE REGARDING SLEEP MEDICINE OF MEDICAL STUDENTS AND PHYSICIANS IN SPLIT, CROATIA

Zrnka Kovačić, Mirela Marendić, Martina Soljić\*, Zoran Đogaš  
MD, PhD

Mentor: Zoran Đogaš MD, PhD

Medical School, University of Split, Croatia

**Keywords:** attitude of health personnel; Croatia; hypertension;  
knowledge; sleep; sleep disorder

**Aim.** To assess the knowledge and attitudes regarding sleep medicine among second-year medical students and physicians.

**Methods.** A total of 112 respondents were surveyed for their attitude and knowledge regarding sleep medicine and hypertension. The respondents were divided into three groups: 1) second-year medical students (46 or 41%); 2) physicians at postgraduate study program (26 or 23%); and 3) specialists in different fields (40 or 36%). The questionnaire was composed of the sleep medicine attitude test, sleep medicine knowledge test, and hypertension knowledge test. Differences among the groups were statistically analysed with ANOVA, and differences within each group were analysed with Wilcoxon test.

**Results.** Postgraduate students and specialists showed better knowledge in hypertension than in sleep medicine ( $p=0.001$  and  $p<0.001$ , respectively). In the student group, no difference was found between the knowledge in hypertension and sleep medicine ( $p=0.192$ ). Differences in sleep medicine knowledge were not found between medical students and specialists ( $p=0.228$ ) or between postgraduates and specialists

( $p=0.647$ ). Sleep medicine attitude score was similar in all groups ( $p=0.470$ ). In general, there was a positive correlation between sleep medicine knowledge and attitude ( $p=0.002$ ), and between hypertension knowledge and sleep medicine attitude ( $p=0.019$ ).

Conclusion. Medical students, postgraduate physicians, and specialists showed poor knowledge but positive attitude toward sleep medicine. This finding points to the need for better education in the field of sleep medicine in medical schools.

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## «ONLINE» EDUCATION IN CROATIA – TIGEL PROJECT

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**Keywords:** telemedicine, gastroenterology, continuous education, licence

One of the basic obligations of every medical doctor in order to continuously have licence to practice medicine is to perfect his/her knowledge on a yearly basis – a process called «continuous medical education».

The most frequently used method to achieve this goal - attending congresses - is quite expensive, and one does not always have time to attend them and the same goes for symposia, lectures etc. But there is one resource of information that is available to almost anyone, 24 hours a day, 7 days a week, etc. If you combine previous two paragraphs into one, you get one of objectives of the TIGEL project.

TIGEL stands for «**Tele**interventional **Gastro**enterology». This project was designed and started at the Center of Interventional Gastroenterology in Zagreb University Hospital. Main aim of this project is to develop a web-based (Internet-based) system for continuous medical education for specialists in gastroenterology. When proved to be a successful model, it can be applied virtually to any branch of medicine. It is

supported by the grant from the Ministry of Science and Technology of the Republic of Croatia.

The project is currently being realized on several levels, one of which was creation of a website – currently placed at the server of the Zagreb Medical faculty (<http://www.tigel.net>). The website contains several highly useful features – e-mail based clinical consultation service for practicing gastroenterologists and family physicians in Croatia, educational texts with pictures and movies and an online test following every text. The significance of the first two features doesn't have to be explained, and the significance of the third feature (online test) will become obvious in the beginning of 2003 once when the law on continuous medical education in Croatia is adopted – which will allow every doctor that takes the test, to earn points for re-licencing. Advantages and cost-effectiveness of such a way of earning point are quite obvious.

From students' perspective, this presents a very good introduction on what the future brings (after graduation).

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## **FROM TELEPATHOLOGY TO TELERADIOLOGY NETWORK IN CROATIA**

Iva Popović MD, Anita Mušić, Sanja Masnec-Paškvalin MD  
Mentors: Sven Seiwert MD, PhD, Lovorka Batelja MD

Dept. of Pathology, School of Medicine, Zagreb University, Croatia

Telepathology as a very demanding branch of telemedicine poses real challenge to experts. Introduction of telepathology in emerging countries with poor infrastructure and low health-care budget represents a special task. On the other hand, those countries would mostly benefit by introducing telemedicine/ telepathology. In our experience, it is possible to build an efficient telepathology/teleradiology network using simple plain old telephone network and still image transmission, as well as store and forward mode of operation. It is also important that the applied system has a maximal scalability and support future introduction of faster infrastructure (such as ISDN or ATM). Our experience is based on 7 years of telepathology in Croatia leading to a national teleradiology network. The system used in this development was the ISSA/PHAROS system (Vamstec, Zagreb) integrating patient database with telepathology system. This presentation highlights the ideas, development, and software solutions in the process of establishing a national telepathology and teleradiology network.

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## **EARLY EMBRYONIC EFFECTS OF PHENYTOIN ON A2G MICE DEVELOPMENT**

Peride Ileana\*

Mentor: Chircor Lidia MD, PhD

UMF Carol Davila and Universitatea Ovidius Constanta, Romania

Objective: it has been proposed that human and wildlife suffer adverse effects as a result of environmental exposure to chemicals that interact the development process in early embryo.

Material and method: To evaluate the effects of phenithoin on reproduction, male and female A2G mice were injected i.v. with 25mg/100g body weight day 1 to 5. The uterine contents were examined on day 18 of gestation. Growth and development were examined in phenytoin group and control group. Statistical comparisons were performed between the control and treated groups. The parametric data were evaluated by student's unpaired t-test (the level of significance chosen for all results was  $t < 0,05$ ).

Results: Frequencies of cleft palate ( $t < 0,005$ ), omphalocele ( $t < 0,005$ ) and microphthalmia ( $t < 0,05$ ), in the treated group, were significant statistic higher than those of the control group.

Conclusions: These results suggest the increased susceptibility to induced teratogenesis in offspring derived from A2G maternal-fetal unit treated with the phenytoin: major abnormalities, intrauterine growth retardation with decreased fetal weight and midline malformation such as omphalocele and palatoschizis.

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## FINE STRUCTURE OF SPERMATIDS/SPERMS IN PATIENTS UNDERGOING TESE

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**Keywords:** azoospermia; male infertility; spermatids/sperms; testicular biopsy; testicular sperm extraction

### Introduction and aim

Testicular sperm extraction (TESE) is a well-established method which enables sperm retrieval from the testicular biopsy. The patients show complete azoospermia in their ejaculate because of a defect in their spermatogenetic tissue. Since we know that there may still be areas with a degree of spermatogenetic activity within the testes of these men, TESE is frequently possible. Sperms retrieved by means of TESE could be microinjected into the oocyte/s. The aim of our study was to investigate the electron microscopic properties of late spermatids/sperms in men who underwent TESE.

### Materials and methods

Overall, 60 biopsies were analysed. 8 biopsies from patients with vasectomy reversal were used as a control group. 52 biopsies were assessed from infertile group of patients, suffering from non-obstructive azoospermia. Transmission electron microscopy was performed using Zeiss 902A.

### Results

TESE procedure was successful in 24 cases (54%). Control biopsies displayed regular morphology of testicular tissue, including late spermatids and sperms. In the infertile group, late spermatids and sperms often displayed irregular morphology such as: irregular acrosome region; defect of

nuclear condensation; irregular tail morphology; defects of membranes; non-disjunction of residual body; minor defects of cell organelles.

### Conclusion

Electron microscopy analysis could offer many useful data on the morphology of late spermatids and sperms recovered from the testicular biopsy and then used for intra-cytoplasmic sperm injection (ICSI) to achieve a pregnancy.

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## PIONEERS OF CARDIAC SURGERY: THE GREAT MOMENTS

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**Keywords:** Pioneers of cardiac surgery; History of medicine; Cardiac surgery timeline

**Objective:** The aim of our study is to represent some great individuals and devices that enabled development of modern cardiovascular surgery

**Materials and Methods:** Retrospective analysis, May – July / 2002, of biographic data related to: Williams, D. Hale, Carrel, Alexis, Blalock, Alfred, Lillehei, C. Walton, Gibbon, H. John jr., Barnard, N. Christiaan, Shumway, E. Norman, Cooley, A. Denton and DeBakey, E. Michael. Reprints and transcripts of original Blalock's operative list, Fallot's, Gross's, Gibbon's and DeBakey's scientific papers. Barnard-to-Chief middle of the night phone call reprint. 38 color and black & white illustrations: personal photos, Carrel's 'Apparatus to preserve whole organ', 'Triangulation technique', operative rooms where 'the very first procedures' were performed, 'Blalock-Taussig procedure', Lillehei's devices, Gibbon's cardiopulmonary bypass machine, DeBakey's and Cooley's devices.

**Conclusion:** In this moment of advanced surgical procedures and modern devices we believe that it is a quite right to admonish on some great surgeons and heroes of the 20<sup>th</sup> century.

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## STATISTICAL SIGNAL CLASSIFICATION OF EKG

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**Keywords:** statistical signal classification, EKG

**OBJECTIVE:** analysing periodical signals with statistical methods (EKG in particular).

**INTRODUCTION:** Statistical Signal Characterization involves separating the wave into segments, which are defined as the presence of limits near some extremes of the waves shape. The parameters of the wave's shape by using SSC are: amplitude means and deviation, period means and deviation. This set of parameters is unique for every different shape of wave. There is no redundancy for these parameters. The shape of an EKG is formed from waves, segments and intervals or complexes. The segments of EKG are different of SSC segments. The presence or absence of the waves, positive or negative changes of the waves, duration of the waves, segments or intervals are the basis of the EKG diagnosis.

**METHOD:** The SSC technique used for EKG interpretation is that of the association. The spectral content can be distinguished by using SSC parameters. This method generally involves the following steps: determining the accuracy with which SSC parameters are measured, determining the sampling criteria that will be used, sampling the wave, calculating SSC parameters, doing associations based on differences and similarities for specific applications. In the particular case of the EKG, normalizing the parameters is an important extra step. Normalization is used to exclude

the rhythm - pulse variations influences, when extracting the parameters. Amplitude normalization is not necessary, but that of the temporal cycle is.

**RESULTS:** By measuring a series of EKG cycles for Wolf-Parkinson-white syndrome, Right ventricular hypotrophy, Left branch total block we obtained a series of parameters. We noticed that these parameters show us how many waves coexist on an EKG and offer essential data for diagnosis. In order to analyse the wave, we have created a program that runs under MatLab V.4.2 for Windows.

**CONCLUSIONS:** The SSC method for EKG analysis has various advantages, like: the reduced number of calculations needed, compared to Fourier or correlation analysis, or it being simple and practical.

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## **CROSS-SPECTRAL ANALYSIS OF EKG-EFFECTS OF STRESS ON EKG**

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**Keywords:** cross-spectral analysis, stress, EKG

**THEME AND FUNCTION:** Cross-spectral study of the RR and QT of the EKG during mental stress shows the degree of coherence between 2 signals. The programming language that we used was TurboC. The program has 27.761 Bytes, being portable.

**OBJECTIVE:** Mental stress has a validating potential on the sympatric indexation of the RR and QT spectral variability stimulating ventricular and atrial response.

**PROTOCOL:** We performed the study on 70 students, aged 19-23, average 21. We performed the EKG on 10 of them after placing the electrodes on their body, without exterior stress, but with examination stress. Another 10 were examined in a non-stressful situation, after a reassuring break after setting the electrodes. The 50 left were tested in exterior stress, after a break after the setting of the electrodes.

**METHOD:** We registered EKG-s of our subjects and we also measured their respiratory parameters. We analysed the signals on a computer, on the bases of our program. In order to calculate the QT-LF fraction unaffected by the RR-LF influence, repre-senting the sympatric idioventricular tonus, we modified every QT-LF spectral line with (1-MS) at the line frequency. There were used acceptability criteria for the RR spectrum.

**RESULTS:** All the interventions differ from the relaxed state by shortening the RR and QT means. Mental stress shortens the RR and QT means compared to anterior episodes during which the conversation was present, with the presence of mental stress. RR-LF does not react to postural changes and it increases under interaction stress, but it doesn't distinguish between mental and interaction stress. QT-LF reacts to postural changes. The idioventricular fraction of QT-LF, detached by coherence analysis, differentiates mental from interaction-reassurance stress, while LF/HF differentiates mental and interaction stress-neutral.

**CONCLUSIONS:** The program of cross-spectral analysis of RR and QT variance produces: average values, total and partial variance on high and low frequency series, RR-LF/Hf, corrected QT, average square coherence in LF, the fraction from QT variance independent from RR variance. It shows cardiac repercussions in terms of sympatric idioventricular control, moderate mental stress, examination stress, conversation and recent events. The delay between variance of the RR and QT intervals increases during stress compared to relaxation. In interaction mental stress the delay increases, suggesting the intervention of a mechanism that controls the ventricles independent from the NSA which generates RR variance. Increase in this delay is known to lead to arrhythmic diseases and sudden cardiac death.

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## **CLINICAL AND LABORATORY PROFILE OF PATIENTS TREATED FOR THE ACUTE MYOCARDIAL INFARCTION AT THE UNIVERSITY CLINIC FOR CARDIOVASCULAR DISEASES IN ZAGREB DURING THE YEAR 2001**

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The aim of this study was to analyse and discuss relevant clinical and laboratory data from patients treated for AMI during the year 2001 in the Coronary Care Unit (CCU) of the University Clinic for Cardiovascular Diseases in Zagreb. Following data were analysed from the data base in the CCU during the period from January 1 2001. to December 31 2001: age, gender, previous manifestations of the coronary heart disease (CHD), smoking habit, diabetes, hypertension, heart failure and CHD in family members. Furthermore, following laboratory parameters were analysed: CK on arrival, CK highest, blood glucose, cardiac troponine I (cTn I), LDH, fibrinogen, CRP, total cholesterol, HDL, LDL. Localisation and definition of the infarction (regarding ST-segment elevation as well as Q and nonQ) was performed according to the ECG criteria. The total patient's number with the AMI during the mentioned period was 211. There were 67.3% males and 32.7% females; 20.4% of our patients had previous manifestations of CHD, 6.7% previously had been treated for heart failure, smoking habit was present in 33.6%; there were 18% of patients with diabetes and 60,7% with hypertension, which was the most common among possible risk factors. According to the ECG, anterior AMI was found in 38.4%, inferoposterior in 45,5% and unspecified localisation in 16.1%.

Conclusion. Since there was no similar study of the AMI patients in Croatia, analysing these data and comparing them with similar investigations of several foreign authors, could help to focus on some particularities of the urban AMI patients in Croatia, and innitiate further investitions of AMI patients regarding possible regional differencies.

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## CARDIOPULMONARY BYPASS FLOW TYPE: INFLUENCE ON POSTOPERATIVE RENAL FUNCTION

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**Keywords:** cardiopulmonary bypass; heart-lung machine; pulsatile flow

**Objective:** The aim of our study is to evaluate the influence of cardiopulmonary bypass flow type, pulsatile vs. nonpulsatile, on early postoperative renal function

**Patients and Methods:** Prospective analysis of 24 patients divided into two groups. Group A (pulsatile flow type, n=9; 37.5%, 6 male, mean age 62±7,8) and group B (nonpulsatile flow type, n=15; 62.5%, 10 male, mean age 60±6,2). Patients were operated under same team and technique between May 2001 and January 2002. Demographic data were analysed, (gender, age, weight, height, BSA), p=NS. Preoperative ejection fraction and Morbidity; p=NS. Perfusion parameters: (perfusion duration, ischemia duration); p=NS. Preoperative renal function; p=NS. Group A surgery: aortic valve replacement: n=4, mitral valve replacement: n=2, mitral valve replacement+aortocoronary bypass: n=1, aortic valve replacement+aortocoronary bypass: n=2. Group B surgery: aortic valve replacement: n=5, mitral valve replacement: n=5, mitral valve replacement+aortocoronary bypass: n=2, aortic valve replacement+aortocoronary bypass: n=3; p=NS. Myocardial protection: cold crystalloid Bretschneider HTK 4°C solution; mild hypothermia.

Results: Statistical significant better postoperative renal function in group A; (creatinine  $90.7 \pm 17.5$  vs.  $107.6 \pm 29.4$   $\mu\text{mol/l}$ ,  $p < 0.05$ , diuresis  $400 \pm 230.4$  vs.  $223 \pm 169.8$   $\text{ml/h}$ ,  $p < 0,05$ ).

Conclusion: Better early postoperative renal function parameters in group A; pulsatile flow. Both clinical parameters; diuresis and creatinine within normal clinical values

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## **RELATIONSHIP OF DIABETES MELLITUS TYPES, CHRONIC COMPLICATIONS AND INSULIN THERAPY**

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**Keywords:** Chronic complications

**Objective:** The clinical observations suggest that there is statistically significant correlation between diabetes mellitus (DM) type and the type of treatment on one hand, and the average duration of occurrences of the chronic complications on the other hand.

**Methods:** The studied groups comprised 1315 subjects who represent all the patients suffering from diabetes mellitus DM, under the observation of diabetic centre in Oradea.

**Results:** The studied groups comprised 79 subjects with diabetes mellitus type 1 (DM 1) and 1236 subjects with diabetes mellitus type 2 (DM 2) of which 510 have a combined treatment-insulin + tablets - and 726 only with tablets. The chronic vascular complications of any type appear in average 5.4 years with DM 1 and with DM 2 appear in average of 4.3 years  $p < 0.05$  - statistically slight significant. The chronic metabolic complications of any type appear in average of 7.1 years in the DM 1, and with DM 2 appear in average of 2.6 years  $< p < 0.001$ -statistically significant). For all the chronic complications irrespective of their type the average duration of occurrence in the DM 2 (7.1 years) is statistically significant comparatively with DM 1 (10.3 years –  $p < 0.001$ ).

Conclusions:

1. In the DM 2 with or without insulin comparatively with DM 1 the average duration of occurrence of all types of chronic complications is lower statistically significant.
2. After 15 years of development of DM 1 we witness chronic complications with all patients while with (DM 2) 6.6% don't present any chronic complications.
3. Patients treated with insulin, the chronic complications occur later, statistically significant compared with non-insulin patients.

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## NEUROBORRELIOSIS-CLINICAL DIFFERENTIAL DIAGNOSTIC PROBLEM

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**Keywords:** neuroborreliosis, differential diagnosis

Lyme disease (neuroborreliosis) is not so rare, especially in endemic regions. Clinical manifestations of this disease especially in later stages are differential diagnostic problem in relation to demyelinating diseases, encephalopathies and polyradiculoneuropathies. Some authors call this disease "a great imitator" because of symptom variability. Lyme disease is caused by spirochete *Borrelia burgdorferi* with invasion of CNS through intradermal inoculation after the tick's bite. Neurological manifestations are clinically divided in two syndromes: the early one and the late one. Early syndrome includes meningitis, facial and other cranial nerve palsies and also radiculoneuritis. Late syndrome's manifestations are: sensorimotor polyradiculoneuropathies, encephalopathies and encephalomyelitis.

Purpose of this study is to compare the clinical features of neuroborreliosis to other neurological diseases and syndromes having similar clinical features, by neuroimaging and immunoserology analyses.

The examination included a group of 3 patients with confirmed neuroborreliosis, 3 patients with suspicious clinical features and 4 patients with demyelinating disease and similar clinical symptomatology. The research is done at The Clinic of

Neurology in Podgorica with complete biochemical searches, serology analyses (ELISA test), MR and other.

Medical history, taken in detail, including the fact about contact with the tick and staying at endemic places is the base for making a diagnosis.

Patients whose clinical feature speaks for neuroborreliosis and ELISA test is negative (syndrome encephalopathy) are a diagnostic problem.

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## BACTERIAL RESISTANCE - A NATIONAL PROBLEM

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**Keywords:** germs, resistance, antibiotics

**PURPOSE:** To follow the bacterial resistance by studying the susceptibility tests for germs found in urinary tract infections (UTI). These germs develop a particular resistance to antibiotics.

**METHODS:** A total of 21333 samples of urine have been collected between the 01.07.2000 and the 31.06.2002, from people with symptomatic UTI at the "Carol Davila" Clinical Hospital of Nephrology in Bucharest, Romania. 3819 cultures were found positive and the susceptibility testing was made following the disc diffusion methodology elaborated by the National Committee for Clinical Laboratory Standards of USA (NCCLS). We used a confluent inoculum at a turbidity of 0.5 McFarland and one main sensitivity medium: Mueller-Hinton agar (MHA). We interpreted the results using the Performance Standards for Antimicrobial Disk Susceptibility Tests provided by NCCLS, finding susceptible (S), intermediate (I) and resistant (R) germs for the 22 different antimicrobial agents used.

**RESULTS:** An increased percentage of urinary pathogens are resistant to beta-lactam drugs: an average of 55.6 % for the Gram negative and 49.66 % for the Gram positive germs. For antibiotics like cephalosporins and quinolones, we found resistance average of 18.6 % and 21.3 % respectively.

**CONCLUSIONS:** If the results concerning some drugs like the beta lactams, nitrofurantoin and co-trimoxazole were as expected, those concerning the cephalosporins and quinolones showed that the bacterial resistance is continuously increasing even for drugs thought to be very powerful in the treatment of UTI.

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## SIMPLE CHRONIC GLAUCOMA

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**Keywords:** glaucoma, blinding, therapy

**PURPOSE:** We have studied the Simple Chronic Glaucoma (SCG), a special disease by its delayed symptomatology, its unfavourable prognostic, and its never efficient therapy. This disease is usually diagnosed late and the treatment never cures it (it just delays the blinding).

**METHODS:** Retrospective study on 120 patients from the Clinical Hospital of Ophthalmology of Bucharest, 2000 - 2002 period.

**RESULTS:** Most cases were presented in the advanced stages of the disease, some of them losing one eye because of this disease. For 16 - 20 percent of the cases the surgical indication came too late because:

- 1) Delayed diagnosis
- 2) Refuse of surgery
- 3) Exaggerated extension of the medical treatment.

The surgical treatment, although decreasing the intraocular pressure to normal values, required drug supply to 60 percent of the cases.

**CONCLUSIONS:** This study permits knowledge of a severe ocular trouble, suggesting a destiny sentence that the patient does not know because of the lack of symptomatology. We consider that the management of this disease requires:

- 1) Mass acquaintance
- 2) Screening methods
- 3) Monitoring

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## HISTOPATHOLOGIC FINDINGS IN CHRONIC HEPATITIS C

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**Keywords:** hepatitis C, hepatocellular carcinoma, necroinflammation

**INTRODUCTION:** Infection with the hepatitis C virus/HCV/ leads to chronic liver disease in over 60% of the cases. Although often asymptomatic, chronic hepatitis C can result in cirrhosis. Epidemiological studies have suggested a strong association between chronic HCV infection and the development of hepatocellular carcinoma about 15-30 years after infection.

**AIM:** To estimate the main histological changes, necroinflammatory activity, staging of consecutive fibrosis, assessment of therapeutic effects.

**MATERIAL AND METHODS:** Twenty five patients were followed up in the Department of Internal Medicine in Alexander's University Hospital-Sofia. The diagnosis of HCV infection is based on the identification of HCV antibodies and HCV/RNA. Liver biopsy provides important additional information in the diagnosis.

**RESULTS:** The most characteristic features that were found are: portal lymphoid aggregates often with prominent follicles, some inflammatory bile duct damage with proliferation and mild steatosis. Fibrosis resulting from progressive necroinflammation is a further important element for the histopathologic diagnosis. This histopathological data was observed in the all investigated patients.

**CONCLUSION:** The course of the HCV is slow, with mild inflammatory changes. Nevertheless, even in mild asymptomatic chronic hepatitis episodes of higher inflammatory activity associated with extensive piecemeal necrosis and porto-central bridging necroses can accelerate the course of the disease.

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## **MOLECULAR METHODS IN THE DIAGNOSIS OF SOFT TISSUE TUMORS**

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A significant number of bone and soft tissue tumours are characterized by specific translocation resulting in gene fusions. New chimeric genes often produce new transcription factors with an important role in tumour development. These genetic changes are used as powerful diagnostic tools, because the histopathological classification of mesenchymal neoplasms is still a diagnostic challenge. A number of techniques including cytogenetics, FISH, Southern blotting are developed, but RT PCR techniques is preferable. RT PCR is a specific and sensitive technique which amplifies specific chimeric regions. Snap frozen tissue is more suitable for this type of detection because of easier isolation of RNA which is a first step. The procedure can be also performed on paraffin embedded material if the RNA is of good quality.

Ewing sarcoma, one of two most common malignant bone tumour of children and young adults, is characterized by fusion of EWS to FLI1 or ERG gene. Considerable heterogeneity exists because of different molecular breakpoints so the composition of exons is different. Most common fusion ( 85%) is exon 7 to exon 6 of FLI1. Some studies have shown that distinct variants are associated with better survival. RT PCR technique is a potential tool for monitoring minimal residual disease in patients undergo-ing therapy or in identification of micrometastatic disease.

Synovial sarcomas are specific because of t (X;18) (p11.2;q11.2) translocation and SYT (18) SSX (X) fusion. Molecular studies have shown that 70-90% patients with synovial sarcoma have this fusion gene. Other tumours with specific translocations are alveolar rhabdomyosarcoma, myxoid liposarcoma, clear cell sarcoma.

*Conclusions:* Introducing RT PCR assay for the diagnostics of mentioned translocations in pathology labs will present a useful help in diagnostically problematic cases and for follow up of patients after surgery and chemotherapy.

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## PROGNOSTIC VALUE OF HISTOLOGICAL GRADUS IN OVARIAN CANCER

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**Keywords:** ovarian cancer, histological gradus, survival

**AIM:** To determine the connection between histological gradus defined by Silverberg histological score and survival in patients with ovarian cancer.

**MATERIALS AND METHODS:** In this study, the surgical material of 80 patients with surface epithelial stromal carcinoma of the ovary was examined. Histological gradus was defined by histological score. The tumour architecture, the characteristics of a nucleus and the number of mitoses were analysed on 10 HPF. Survival data were gained from the history of the patients treated in the department of Oncology, Clinical hospital Split. Survival data were analysed by life-tables and graphically presented by Kaplan – Meier curves.

**RESULTS:** Silverberg and collaborators distinguish three histological gradus of ovarian cancer: high (3-5), medium (6-7) and low (8-9). Regarding the tumours with medium histological gradus (histological score 6-7) observed in this study, statistically significant survival difference was found between the patients with histological score 6 and those with histological score 7 ( $p=0.05$ ). Tumours with histological score 7 behave as low gradus tumours. Tumours with histological score 6 behave as high gradus tumours. According to the study results, the patients were divided into two groups: a

group with low (7-9) and a group with high (3-6) histological gradus. Out of 80 patients included in the study, 40 (50%) were placed in high (3-6) and 40 in low (7-9) gradus group. We found statistically significant connection between histological gradus and survival ( $p < 0,0001$ ).

**CONCLUSION:** New grading system is simple, highly reproducible and applicable to all histological types of tumour. To best of our knowledge, we didn't find any data published in the recent literature regarding distinguishment of survival within the medium gradus (prognostically ill defined) group, so we hope that the results of this study which help to distinguish two tumour risk groups, could be encouragement for further research in this field.

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## TRANSFERRIN RECEPTORS ON COLORECTAL CARCINOMA AND VARIOUS CANCER CELLS

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**Keywords:** Feric sorbitol citrate (FSC), colorectal carcinoma, tumour cell lines, transferrin receptor, immunohistochemistry, immunocytochemistry

*Introduction:* Lot of transferrin receptors (TfR) have been identified in many malignant tumours. It has been shown that in lung cancer, lymphoma and breast cancer the expression of TfR correlates with tumour differentiation, probably implying some prognostic value. Iron and iron-containing compounds could act as carcinogenes, but they could also inhibit proliferation of normal or malignant cell. For example, ferric-sorbitol-citrate (FCS), inhibited proliferation of various murin cancer cells in vitro, but it did not affect proliferation of the non-malignant cells.

*Aim:* The aim of this study was to evaluate the expression of TfR on colorectal carcinoma and three cancer cells lines CaCo2 (colon Ca), MiaPaCa2 (pancreatic Ca) and Hep2 (laryngeal Ca), and the influence of different concentrations of iron on the expression of TfR. We also analysed the expression of TfR on colorectal carcinoma at different stages in adult patients.

*Materials and methods:* On monolayer of tumour cell lines the distribution of TfR was detected by immunocytochemistry (PAP method, DAB staining with hematoxyllin contrast staining) applying genuine monoclonal antibodies. For the immuno-histochemical detection of TfR the immunoperoxidase

technique was used. Secondary rabbit – anti – mouse - antibodies (Dako, Denmark) were applied on crio sections of the acetone fixed sections of colorectal carcinoma and colon mucosa. Staining for transferrin receptor was graded according to pattern and intensity and categorised as membrane positive-weak in focal pattern- weak in diffuse pattern- strong cytoplasmic reaction. Clinical information and tissue samples were obtained from the Clinical hospital “Sestre Milosrdnice”, Zagreb. The pathologic stage of each tumour was established according to the Dukes classification.

*Results:* The number of TfR on CaCo2 and Hep2 cells decreases when cells are grown in iron-supplemented media, but it does not affect the expression of TfR on non-malignant cells and MiaPaCa2 cells in vitro. Less transferrin positive cells were observed when CaCo2 cells were incubated with FSC for 24 hours comparing to the positive reaction after incubation of 3 hours. Significant cytoplasmic staining for TfR was revealed in all colorectal carcinomas, while in normal colonic mucose the reaction was positive only on the cell membrane.

*Conclusion:* The results suggest that TfR expression is associated with cellular proliferation. The expression of TfR is dependent on the concentration of the iron supplement, as well as the duration of the supplementation. Iron-containing compound serves as the signal for receptor number regulation. Jectofer serves as the negative signal for TfR expression on tumour cells.

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## **DNA-PLOIDY, DNA-INDEX, S-PHASE FRACTION: PROGNOSTIC SIGNIFICANCE IN OVARIAN SEROUS ADENOCARCINOMA**

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**Keywords:** ovarian serous adenocarcinomas, FIGO stage, DNA  
ploidy, DNA index, S-phase fraction

**OBJECTIVE:** Ovarian cancer is the most lethal of the  
gynaecological malignancies. Thus, it would be very important  
to define prognostic factors for predicting the malignancy  
potential, and to define biologically similar subgroups for  
analysis of therapeutic efficacy.

**MATERIALS AND METHODS:** 106 patients with a diagnosis  
of ovarian serous adenocarcinomas (OSCA) who had been  
treated in Dept. of Gynaecology, Medical School, University of  
Zagreb, by primary surgery in period between 1980 and 1997  
were enrolled in this study. FIGO stage was analysed in  
correlation with DNA ploidy, DNA index and S-phase fraction.  
DNA analysis was performed via DNA flow cytometry through  
paraffin-embedded tissue specimens.

**RESULTS:** Of 106 patients, flow cytometric studies revealed  
61 aneuploidy cases (57.5%). DNA index values ranged from  
1.09 to 1.92 (average 1.59 +/- 0.235). Statistical analysis  
showed significant correlations between overall survival and  
FIGO stage ( $P < 0.001$ ), ploidy ( $P < 0.001$ ) and DNA index  
with cut-off 1,45 ( $P < 0.01$ ). No significance was found for S  
- phase fraction. In advanced (FIGO stage III-IV) OSCA DNA-

ploidy achieved independent prognostic significance ( $P < 0.02$ ).

**CONCLUSION:** DNA ploidy and DNA index are prognosticators for OSCA. DNA ploidy analysis should be incorporated in the routine histopathological evaluation of advanced (FIGO stage III-IV) OSCA.

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## **IS nm23 PROGNOSTIC FACTOR IN LOCAL METASTASING OF BREAST CANCER**

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**Keywords:** nm23-H1 protein, immunohistochemistry, breast cancer

The subject of our study is a correlation between protein nm23 on one side and tumour grade and size, positive armpit lymph nodes, age and steroid receptors on the other side. There is statistically significant correlation between nm23 and positive armpit lymph nodes. Nm23 is one of prognostic factors in breast cancer. 25% of patients with negative armpit lymph nodes have severe prognosis which increases the significance of studying additional prognostic factors in patients with breast cancer. The knowledge of those additional prognostic factors could determine biological behaviour of the tumour, which could bring positive changes in therapeutical approach. Statistically significant correlation between tumour grade on one side and tumour size, positive armpit lymph nodes and positive steroid receptors on the other side was also found. These results are completely congruent with the results from literature.

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## **p53 PROTEIN AS A PROGNOSTIC FACTOR IN PATIENTS WITH PROSTATIC CARCINOMA**

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**Keywords:** p53, prostatic adenocarcinoma

**Objectives:** To establish correlation between over-expression of p53 gene on one side with clinical and pathohistological characteristics of tumour in patients with prostatic cancer and to determine the influence of analysed indicators on survival as well.

**Material and methods:** Surgically obtained material of 50 prostatic adenocarcinomas has been immunohistochemically analysed for expression of p53 gene using p53 specific monoclonal antibodies. The data concerning clinical stage of the disease, differentiation grade of the tumour and the percentage of the resected tissue have been gathered by looking into patients' medical records and pathohistological reports.

**Results:** Negative expression of p53 product has been found in 11 (22%) prostatic carcinomas, mildly positive in 9 (18%), moderately positive in 18 (36%) and strongly positive in 12 (24%) carcinomas. Over-expression of p53 gene product is correlated with percentage of tissue invaded by carcinoma ( $p=0,026$ ), histological differentiation grade ( $p=0,001$ ) and with the clinical stage of the disease ( $p=0,014$ ). Uni-variant analysis has proved that all indicators except the percentage of the invaded tissue by carcinoma have significant influence on



survival. Over- expression of p53 gene product in carcinoma of the prostate has the greatest prognostic value (log rank=17,60) followed by differentiation grade (log rank=12,34) and clinical stage of the disease (log rank=4,08). Multi-variant analysis has proved that only over-expression of p53 gene product in carcinoma of the prostate has retained statistical significance ( $p=0,0003$ ).

Conclusion: Over-expression of p53 gene product is an independent prognostic factor with prostatic carcinoma patients and could be used well to select patients with expected poorer prognosis and poorer biological behaviour of the tumour.

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## INCREASED INCIDENCE OF RENAL ADENOCARCINOMA IN SPLIT-DALMATIA COUNTY

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**Key words:** kidney, renal cell carcinoma, adenocarcinoma, Croatia, incidence.

**Aim:** To investigate the incidence of renal adenocarcinoma in the Split-Dalmatia County in the 1981-2001 period, and to compare the renal neoplasm incidence with the incidence in the Republic of Croatia.

**Methods:** The data were obtained using case records and registries of the Split University Hospital at both Firule and Križine locations. Obtained data were accompanied with pathohistological records, and compared with the records from the Croatian Cancer Registry. Age standardized incidence per 100,000 was calculated from the number of patients and the number of inhabitants.

**Results:** From 1981 to 2001, there were 439 patients (281 men and 146 women) with renal adenocarcinoma living in the Split-Dalmatia County. There was an increase of incidence from 2.07 in 1981 (1.87 and 2.59, men and women, respectively) to 8.84 (10.17 and 7.57) in 2001 per 100,000 inhabitants. Higher incidence was present in the group of patients aged  $\geq 65$  years (16.94) than in the patients of 45–64 (8.63) or 25–44 (1.1) year groups. The data from the Croatian Cancer Registry for the 1987-1998 period showed similar increase in incidence of all renal cell carcinomas in our county (3.27 to 11.47) compared to Croatia as a whole (5.83 to 9.59).

**Conclusion:** In the 1981-2001 period, an increase of renal adenocarcinoma incidence was observed in the Split-Dalmatia County. The relative increase of renal neoplasm incidence was similar in our county as in Croatia as a whole.

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## **DNA CONTENT IN DIFFERENT AREAS OF LARYNGEAL SQUAMOUS CELL CARCINOMA**

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**BACKGROUND:** The heterogeneity of malignant neoplasms is clinically important. To determine an area of prognostic significance, we analysed DNA parameters in different areas of laryngeal squamous cell carcinoma (SCC).

**PATIENTS AND METHODS:** We processed 45 samples obtained by total laryngectomy. Three areas were microscopically defined and analysed: tumour centre, transformation margin (R1), and invasive margin (R2). The nuclei were extracted by pepsin digestion and stained with propidium iodide. The DNA parameters (DNA ploidy, S-phase fraction, and DNA index) were analysed using the flow cytometry.

**RESULTS:** Aneuploidy was more frequent in tumour centre (87%) than in both tumour margins (R1=51%, R2=58%). McNemar test showed significant difference between tumour centre and each margin ( $p \leq 0.001$ ). There was no significant difference between the margins ( $p = 1.000$ ). The centre also had significantly higher average S-phase fraction (11%) compared with the margins (7% each) (Mann-Whitney U-test;  $p < 0.05$ ). The tumour regions did not differ in their DNA indexes. Association between aneuploidy frequency and survival was found for invasive margin only: median survival time in the euploid subgroup was 29 months while the median survival in the aneuploid subgroup was 15 months (log rank test,  $p = 0.029$ ). The DNA content of tumour centre and

transformation margin was not significant predictors of survival (log rank test;  $p=0.738$  and  $p=0.554$ , respectively).

**CONCLUSION:** The ploidy of the invasive margin was significantly associated with the clinical outcome of the tumour. Therefore, it is important to ensure that the invasive margin of the laryngeal SCC will be included in the sample for the pathological evaluation.

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## **DISTRIBUTION OF AgNOR STAINING IN LARYNGEAL SQUAMOUS CELL CARCINOMA**

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**BACKGROUND:** The intratumoral heterogeneity in laryngeal squamous cell carcinoma (SCC) is potential source of highly variable results. To prove that intratumoral heterogeneity is geographically distributed in laryngeal SCC we investigated AgNORs (nucleolar organizer stained with Ag) in different tumour areas.

**PATIENTS AND METHODS:** We analysed 33 specimen obtained by total laryngectomy. The samples went through standard silver impregnation. Two areas were microscopically defined: tumour centre and invasive tumour margin. Using PC based image analysing system (SFORM, VAMSTEC Zagreb, Croatia) the following AgNOR parameters were analysed: mean AgNOR area (A), mean number of AgNOR/nucleus (B) and number of AgNOR/100 nuclei (C). 100 nuclei were analysed per each area per each tumour.

**RESULTS:** Significant difference was obtained between the values in tumour centre and invasive tumour margin for all investigated parameters (A:  $p=0.002$ ; B:  $p=0.007$ ; C:  $p=0.008$ ).

**CONCLUSION:** Different tumour areas show different distribution of heterogeneity. This underlines the importance of consistent material sampling and analysis regarding the macroscopical as well as microscopical areas in studies and in diagnostic approach.

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## **Ki 67 AND p53 AS POSSIBLE PROGNOSTIC PARAMETERS IN INVERTED SINONASAL PAPILLOMAS**

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**BACKGROUND:** Inverted papilloma (IP), a benign tumour of the nose and paranasal sinuses, may be associated with laryngeal squamous cell carcinoma (SCC) in up to 30% of the cases. We investigated the role of p53 and Ki-67 gene products as potential markers of malignant alteration of IP.

**MATERIAL AND METHODS:** We analysed 32 samples obtained from patients with IP. Among those, there were 4 malignant alterations. We performed the immunohistochemistry for p53 and Ki-67 using commercial staining kits and calculated the rate of nuclear staining using image analysis software.

**RESULTS:** The Ki-67 was, on the average, expressed in 11% of the nuclei. The difference in the expression rate between the benign and malignant IPs was not significant (Mann-Whitney U-test;  $p=0.077$ ). All malignant samples were positive on p53. The difference in the p53 expression rate between benign and malignant papillomas was highly significant (Fisher's exact test;  $p<0.001$ ). Used as a diagnostic test, the expression of p53 in IP had the sensitivity of 100% and specificity 89%. However, the false positive rate was 42%. When we combined the positive p53 with the expression of Ki-67 > 11% we got both sensitivity and specificity of 100% and the false positive and negative rate of 0%.

**CONCLUSION:** The combined expression of p53 and Ki-67 could be considered as a valuable test of the malignant alteration of IP. Due to few malignant samples, our results should be interpreted with caution and further confirmatory studies are needed.

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## **GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY IN ERITHROCYTES IN CHILDREN IN NORTH CROATIA**

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Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is a hereditary, sex-linked, enzyme defect that results in the breakdown of red blood cells (haemolysis) when the person is exposed to the stress or infection or certain drugs. G-6-PD deficiency is the most common known human enzyme disease, affecting 10% of the world's populations. The incidence of G-6-PD in the United States is much higher among the Black American population (24 %), and 10-14 % are male. We have found much higher incidence of this disorder in children who are living in continental part of Croatia, then previously recorded.

In period between 1995 and 2002 in Clinic for haematological diseases in Clinical hospital for Paediatrics of Medical school in Zagreb, G-6-PD deficiency was found in 128 children. Gender distribution included 73 boys (57,03%) and 55 girls (42,97%). The age of affected children varied from 2 to 204 months, with median of age of 41 month.

The values of enzyme G-6-PD measured in laboratory KBC Rebro from peripheral blood (normal values are 118-144) varied from 27 to 117 with median of 88,5. In our research we analysed laboratory parameters bound with haemolytic anaemia.

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## **REPORT ON MULTIPLE PREGNANCIES AND THEIR OUTCOMES IN DEPARTMENT OF OBSTETRICS AND GYNECOLOGY, MEDICAL SCHOOL, ZAGREB**

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**Keywords:** multiple pregnancies, twin, neonatal, gestational, caesarian

During 2001 there were 123 multiple pregnancies deliveries in Department of Obstetrics and Gynecology, Medical school, Zagreb, which represent 2,8 % of total deliveries (4329) in that year. There were 115 twin pregnancies, 7 tripple pregnancies and one woman gave birth to quadripples. All together there were 232 liveborn neonates. 8 intrauterine and 15 neonatal deaths were recorded. Distribution by sex was 1,07:1, in favour for females. Gestational age ranged from 22 weeks and 5 days up to 42 weeks. Median gestational age was 35 weeks and 2,4 days. Median birth weight was 2102.9 grams, ranging from 310 grams up to 3930 grams. Median birth length was 43.5 cm. Caesarian section was performed in 141 pregnancies, while 114 pregnancies underwent vaginal delivery.

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## HIGH RISK HUMAN PAPILLOMAVIRUS INFECTION IN SEXUALLY ACTIVE ADOLESCENTS FEMALES

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**Keywords:** cervical dysplasia, human papillomavirus, adolescents

**Study Objective:** To determine the relation between high-risk type human papillomavirus (HPV) infection and cervical dysplasia in an urban Croatian adolescent population using conventional Pap smear combined with risk type HPV testing.

**Design:** Observational study.

**Setting:** Department of Gynaecology and Obstetrics, University of Zagreb, Croatia.

**Participants:** 122 women between 17 and 24 years of age with abnormal Pap smear were enrolled in the study.

**Main Outcome Measures:** A standardized patient file on demographic and sexual history information was compiled and completed by physical examination, including a Pap test with adjunct high-risk type HPV detection.

**Results:** In our study group of 122 specimens with abnormal Pap smear we found 65 (53,3%) with *dysplasia levis* (CIN 1), 39 (32,0%) with *dysplasia media* (CIN 2) and 18 (14,7%) with *dysplasia gravis* (CIN 3). HPV typization was performed in each group. In the CIN 1 group we found 13 (20,0%) patients with high-risk HPV. In the CIN 2 group there were 26 (66,7%) and in the CIN 3 group 17 (94,4%) patients. A significant association ( $p < 0.05$ ) between HPV infection and having had

more than one lifetime sexual partner was found. Abnormal Pap test was significantly related to HPV (odds ratio, 82,1) and inversely, to age at first sexual intercourse (odds ratio, 46,2).

Conclusion: There is well-established association between high-risk human papillomavirus infection and the development of dysplastic (pre-malignant) change of uterine cervix. High-risk type HPV infection is a frequent finding in our study group and is linked to having had more than one lifetime sexual partner.

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## **CIGARETTE SMOKING AS A RISK FOR THE OCCURRENCE OF RESPIRATORY TRACT ALLERGY – A STUDY OF ADOLESCENTS FROM NORTHWEST CROATIA**

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Aim of the study was to investigate correlation between smoking habit and allergic status in young persons.

Methods: Total serum IgE, specific IgE to *Dermatophagoides pteronyssinus* (uniCAP), skin test (prick method) to 9 common inhalation allergens was performed on a sample of 155 adolescents (36 male, 119 female), and history data compiled on the occurrence of allergic manifestations, both in the subjects and among close family members.

Results: In passive smokers, exposed to parental smoking for his/her whole life, total IgE (G.M. IU/ml) was significantly higher compared to non-smokers (76.69 : 47.07,  $p < 0.01$ ), while in active smokers, who had smoked for a shorter period (53.76 : 47.07), this difference was not statistically significant. The level of specific IgE to *Dermatophagoides pteronyssinus* in active and passive smokers compared to non-smokers was not statistically significant (2.21 : 2.71 : 3.10). No significant difference was found in skin reactivity to common inhalation allergens, and no correlation was found between smoking index and the size of the urtica to allergens from the indoor environment. Significant difference was found in the prevalence of allergic diseases of the respiratory system in the group of passive and active smokers compared to non-smokers (53% : 48% : 27%,  $p < 0.01$ ) and in smokers (active+passive) with positive family history of atopy compared with non-smokers (69.76% : 40.74%,  $p < 0.05$ ).

Conclusion: Results of the study indicate that clinical manifestations of allergic diseases are more frequent in smokers than in non-smokers. Indicators of atopy did not differ significantly, apart from total IgE, which was higher in the group of smokers.

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## PROBLEM OF DRINKING AMONG TEENAGERS

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**Keywords:** Alcohol, drinking, teenagers

**Introduction:** Owing to a relatively easy availability of alcoholic drinks, the attitude of society and a more precocious “growing-up”, the problem of drinking among teenagers is ever more acute in our country. By this study we tried to get an insight into the problem of alcohol consumption among secondary school students. The period of secondary school, especially the senior years, being the time when people form attitudes and habits that would affect them for the rest of their lives because of a number of biological (puberty and adolescence) and social changes (socially accepted status of a grown-up major), we wanted to know when, how and how often Zagreb secondary school students drink and what is the role of family in developing such patterns of behaviour.

**Methods:** In pursuit of objectivity, we have made a survey in a Zagreb secondary school. The survey has included one class from every generation, a total of 115 students. The questions were about how frequently alcohol is drunk in their families (every day, occasionally/rarely, three times a week, never), how frequently the students drink themselves, if they drink with their peers, if their friends are acquainted with the fact that they drink or if their parents know, too, and finally, how long they had been drinking up to the moment of this survey.

**Results:** The obtained results show that the number of non-drinking students decreases with their age and that, consequently, the number of students who do drink increases, along with the frequency of alcohol consumption. Consumption of alcoholic drinks in the company of peers

(daily or occasionally) increases accordingly, so that non-drinking is almost non-existent among the senior generations. Among younger generations both friends and parents know about it, but later, as the amount of alcohol and the problems related to it increase, parents are less and less informed and in most cases only friends know about it. The question of how long they had been drinking revealed that students start drinking alcohol at the end of primary and at the beginning of secondary school, and that the survey included the group that has indeed only begun forming their habits and attitudes concerning this issue.

Conclusion: We can conclude that precisely the end of primary and the beginning of secondary school, because of the reasons we mentioned in the introduction, is the time when young people start more intensively to “consume” life and everything that goes with it, alcohol included. Experience with drink, the amount of alcohol drunk, but also problems related to alcohol increase with the age. Only students and their friends know about it, that is, the people who they hang out with, while the majority of the parents are not aware of the problems their children begin to experience. Everything is largely tolerated due to the fact that in most of the families (over 50%) alcohol is drunk often (up to three times a week) or daily.

For the future: The survey providing these data being made in only four classes, we will try, over the next few years, to continue with surveys and research in order to increase the number of the people surveyed, which will provide us with greater objectivity concerning some questions and a better insight into new trends and attitudes of young people regarding this issue.

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