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Mica Todorović (1900-1981), "The Terrace", 1940, oil and tempera on canvas, 670x535 mm. Courtesy of Art Gallery of Bosnia and Herzegovina, Sarajevo.

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The feasibility of topical cocaine use in fiberoptic bronchoscopy

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Objective. To test the hypothesis that the application of 4% cocaine-soaked cotton pledgets to each piriform sinus for one minute represents a safe and efficacious method of providing additional topical anesthesia for fiberoptic bronchoscopy. **Materials and Methods**. We retrospectively reviewed all FBs performed at Mayo Clinic Jacksonville from January 1999 to April 2004. Data abstracted included periprocedural complications and doses of midazolam and fentanyl used in the FBs with or without topical cocaine application in addition to the usual anesthesia with topical xylocaine. The Wilcoxon rank sum test was used for statistical analysis. **Results**. We identified 92 FBs where topical 4% cocaine was used. A sample of 80 FBs without cocaine use served as the control group. There were no periprocedural complications in either group. There was significantly less fentanyl use in the cocaine versus the control group ($P<0.0001$, the median dose 75 vs. 100 mcg, respectively). There was no significance in midazolam use in the cocaine versus the control group ($p = 0.16$). **Conclusions**. Topical application of 4% cocaine to each piriform sinus in addition to standard xylocaine is safe. Its use is associated with significantly less use of fentanyl. **Clinical implications**. The use of topical cocaine may allow FB to be performed with less systemic narcotic use.

Key words: Bronchoscopy, Sedation, Anesthesia, Cocaine.

Introduction

Fiberoptic bronchoscopy (FB) is a common and useful procedure in a variety of clinical settings. There is consensus that FB should be performed in the safest and most comfortable manner possible. Interventions to maximize safety and comfort include appropriate monitoring, topical airway anesthesia and sedative medication use. However, the ideal anesthetic for diagnostic FFB remains undefined (1-4).

Cocaine is an extremely useful drug for various endoscopic procedures, with unrivaled vasoconstrictive and decongestant properties (5, 6). Cocaine has been successfully used topically as an anesthetic in FB (7-9). It was first introduced as a surface an-

esthetic in 1884 in Vienna, by Koler in ophthalmology and by Jelinek in laryngology (10). The "merits" of cocaine made the back cover of the first issue of the Laryngoscope in 1896. In the early 1900's, it was prescribed for colds and hay fever in addition to being sold directly to the public. However, the subsequent development of cocaine abuse resulted in regulation of its use in 1914 with the passage of the Harrison Anti-Narcotic Act (11). Continued medical use of cocaine was limited to topical anesthesia. However, because of concerns with toxicity, abuse potential, and extensive record keeping, even the use of topical cocaine began to decrease in the late 1980s (12).

Intratracheal instillation of cocaine, which results in rapid systemic absorption, has led to cardiovascular toxicity (13, 14). However, different methods of anesthetic application have different absorption profiles (5, 15). We hypothesized that the application of cotton pledgets soaked in topical 4% cocaine to the piriform sinuses may result in potentially less systemic absorption and side effects. The rationale for piriform sinus application is that the superior laryngeal nerve conveys impulses for the laryngeal cough reflex (16, 17) and the laryngeal sensory fibers innervate the caudal end of the piriform sinus (18). Previous reports have confirmed that laryngeal anesthesia with topical cocaine application resulted in a substantial decrease or a complete disappearance of afferent superior laryngeal nerve (SLN) activity (19). Similarly, complete anesthesia of the SLN abolished the cough reflex after inhalation of a nebulized chemoirritant solution of tartaric acid (16).

Although others and we utilize this technique successfully, no study has previously examined its safety or efficacy. In order to study the feasibility of topical cocaine application to the bilateral piriform sinuses as a supplemental anesthesia for FB, we performed a review of all FBs performed at Mayo Clinic Jacksonville from January 1999 to April 2004.

Materials and methods

The study was approved by Mayo Clinic's Institutional Review Board as a minimal risk study. From the medical record data, we identified 1615 bronchoscopies performed at our institution over a period of 5 years. This database included patients undergoing bronchoscopy in the outpatient, inpatient, and intensive care unit settings as well as following lung transplant.

We aimed to identify 100 outpatient FBs performed with the use of topical cocaine in addition to routine anesthesia with xylocaine. The control group was formed by selecting every 16th FB from the medical record list to match the proposed number of one hundred FBs with cocaine use. These two groups, cocaine and control, were compared by required doses of sedation with midazolam and fentanyl. Statistical analysis was performed using the Wilcoxon rank sum test.

Data abstracted included patient characteristics (age, gender, indication for FB), periprocedural complications (arrhythmias, desaturations and intractable cough), and doses of midazolam and fentanyl used in the FBs with or without topical cocaine application. All patients were routinely monitored according to the institutional protocol. Pulse oximetry and a single lead EKG were continuously recorded during the procedure, and automated non-invasive blood pressure was monitored every 5 minutes. All patients received supplemental oxygen at 2 to 5 l/minute via nasal cannula to maintain oxygen saturation above 90%. Conscious sedation was achieved with midazolam and fentanyl in almost all patients, with doses administered initially and during the procedure at the bronchoscopist's discretion.

Anesthesia was achieved with a 2% viscous xylocaine gargle and 4% nebulized xylocaine prior to procedure and aliquots of 2% xylocaine instilled during the procedure. In the cases where cocaine was used, cotton pledgets soaked in 4 ml of 4% cocaine were applied via Jackson laryngeal forceps to the bilateral piriform sinuses for the duration of one minute, in addition to routine anesthesia with xylocaine.

Results

We identified 92 FBs where topical 4% cocaine was applied to the bilateral piriform sinuses for the duration of one minute prior to the procedure. A sample of 80 FBs without cocaine use served as the control group. Twenty cases out of the original 100 cases in the control group were excluded because either they were performed on intubated or post-transplant patients or cocaine use was subsequently documented. No further control cases were included to match the 92 cases with cocaine use due to adherence to the pre-study protocol.

Indications and patient data

The median age of all 172 patients was 69 years. There were 88 males and 84 females. The most common indications for the bronchoscopy were: known or suspected cancer, pulmonary infiltrate and pulmonary nodule. The remainder of bronchoscopies were performed for: hemoptysis, cough or other miscellaneous reasons. The baseline characteristics of patients in both groups are shown in Table 1. There were no significant differences between the cocaine and the control groups in regards to age, gender or indication for FB, except for patients with infiltrates.

Table 1 General characteristics

Characteristics	Cocaine (n = 92)	Control (n = 80)	p
Age, median (1st and 3rd quartile)	70 (64, 75)	67 (59, 75)	NS
Male/Female	51/41	37/43	NS
Indication (n; %)			
Infiltrate	12 (13)	24 (30)	< 0.044
Cancer	44 (48)	31 (39)	NS
Nodule	13 (14)	9 (11)	NS
Hemoptysis	11 (12)	6 (7.5)	NS
Cough	5 (5.5)	6 (7.5)	NS
Other	7 (7.5)	4 (5)	NS

Safety

The application of topical anesthesia with 4% cocaine by the technique previously described did not result in adverse effects. There were no episodes of arrhythmias and no procedure had to be prematurely terminated because of desaturations in either group. There was no intractable cough periprocedurally in either group.

Efficacy

The topical application of cocaine resulted in the use of less conscious sedation. Fentanyl use in the cocaine group was significantly lower than the control group (P<0.0001, the median dose 75 vs. 100 mcg, respectively). There was no significant difference of midazolam use in the cocaine compared to the control group (p = 0.16) (Table 2).

Table 2 Doses of fentanyl and midazolam used in the cocaine and the control group

Median dose (1st, 3rd quartile)	Cocaine	Control	P-value
Fentanyl (mcg)	75 (50, 75)	100 (50, 125)	<0.0001
Midazolam (mg)	2 (2, 3)	2 (2, 3.5)	0.16

Discussion

Bronchoscopic technique is not standardized. Controversies exist with regard to premedication before FB and this depends on the personal experience of individual operators (1, 3, 15). For local anesthesia, a variety of agents have been used (7-9). The preferred sedative medications are opiates and benzodiazepines, alone or in combination (15, 20-21). Combined sedation with a benzodiazepine and an opiate allows a reduction in the dose of supplemental local anesthesia (22). However, the routine use of sedative medications may potentially have untoward side effects (15, 21). Since there are reports of routine diagnostic FB being performed adequately with little or without any sedation (23-25), the risk-benefit considerations become more important.

In order to evaluate the safety and efficacy of topical cocaine, we analyzed the group of patients that received topical anesthesia with 4% cocaine applied to the bilateral piriform sinuses in addition to routine anesthesia with xylocaine. When applied topically with the technique described, cocaine use did not result in any known adverse effects among 92 patients in our cohort.

The main reasons why cocaine use decreased over the years were its toxicity due to systemic absorption, the potential for transfer of infectious agents and extensive record keeping (12-13, 27-28). In our cohort, the dose used for anesthesia was relatively small and with topical administration as described, systemic absorption was likely insignificant. This is supported by the lack of untoward side effects. In the past, a cocaine topical solution had to be prepared by pharmacy personnel, but currently a commercial, 4 ml-vial of 4% cocaine is available. This eliminates the risk of contamination with various microorganisms that was reported in the past (27-28). In contrast to the colorless cocaine solution, which was used in the past and could not be distinguished by the naked eye from other anesthetic solutions, the commercially available solution is dark green in color and no dye needs to be added. Following these improvements in manufacturing and packaging, the requirements for record keeping for medical usage of cocaine are similar to those indicated for any other controlled substance. The results of the study also showed that patients who had additional anesthesia with 4% cocaine required less opiate medication. The proposed mechanism is that the cough reflex is abolished or significantly decreased after anesthesia of the piriform sinuses, thus allowing bronchoscopy to be performed with less systemic sedation, especially with less narcotic medication use. A study on both human and animal subjects demonstrated abolishment of the cough reflex after laryngeal anesthesia with topical cocaine application (19). The nerve responsible for afferent impulses for the cough reflex is the superior laryngeal nerve (16-17), which also innervates piriform sinus (18).

The control of coughing was shown to be of paramount importance for the quality of a bronchoscopy, as this facilitates ease of viewing the bronchial tree and obtaining good biopsy samples (29). Moreover, an excessive cough can increase the risk of complications or prevent the performance of invasive procedures during bronchoscopy. Coughing raises intrathoracic pressure leading to an increased risk of pneumothorax during transbronchial biopsies (30). In a Swiss study, a significant number of patients considered a cough to be the worst side effect of bronchoscopy (31). For all these reasons and to have better control of coughing, bronchoscopists tend to use opiate medications. The primary action of the currently available opiates is on the central cough pathway. The antitussive effect is mediated predominantly by μ receptors with a possible role for κ receptors (32).

Although efficient in controlling the cough, opiate medications were shown to result in more hypoxic complications (29), more unwanted dysphoria (24) and their use prolongs recovery time (33). The results of our study and these observations call for a proper balance between sedation and anesthesia with the goals of increased comfort, safety, and efficacy as well as reduced cost in bronchoscopic procedures.

Potential shortcomings of this study are inherent in its retrospective design, patient sampling, and lack of standardized approach to sedative dosing. However, potential uncontrolled variables were unlikely to affect the major statistical significance that was observed in fentanyl use. Based on our results that suggest improvements in safety and efficacy of cocaine, its use has recently increased at our institution.

Conclusion

In conclusion, our data suggest that topical application of 4% cocaine to each piriform sinus, in addition to standard xylocaine, is safe and feasible. Its use is associated with the use of significantly lower doses of fentanyl. This may allow FB to be performed with less systemic narcotic use. Future prospective studies systematically addressing the impact of reduced narcotic use in the setting of topical cocaine use would be instructive.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organization.

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Lymphocyte profiles and serum antibodies against neurofilaments in preeclamptic Kuwaiti women

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Objective. It is hypothesized that the pathogenesis of (PE) is accompanied by alterations in immunoregulation that may affect normal turnover of peripheral neurons and release of cytoskeletal components (principally neurofilaments). Since this is expected to alter serum levels of antibodies to neurofilament epitopes, the possibility exists to utilize this parameter as a biomarker for severity of the disorder. **Patients and methods**. Peripheral blood of 23 pregnant women in the third trimester; 13 with PE, 10 healthy pregnant women and 10 non-pregnant controls were evaluated by flow cytometry for major lymphocyte populations and for antibodies to neuronal cytoskeletal elements by Western blot analysis. **Results**. The percentages of CD3+CD16+CD56+, CD4+CD25+, CD8+CD25+, and CD8+HLA-DR populations were significantly increased in normal pregnancy and PE compared to non-pregnant women ($p<0.01$), dramatic increase of CD4+CD54+ but not CD4+CD45RA populations was observed in PE. Concentrations of autoantibodies for the 200-kDa neurofilament (NFH) was decreased but for the 160-kDa (NFM) was significantly increased in PE. Autoantibodies against the 70-kDa neurofilament (NFL) was significantly decreased in normal pregnancy compared to non- pregnant women ($p < 0.05$) and further decreased in PE ($p < 0.01$). **Conclusions**. The present study provides preliminary insight into how peripheral blood anti-neurofilament antibody levels and lymphocyte subpopulations correlate with normal and pre-eclamptic pregnancies. As these studies evolve, such correlations may emerge as valuable tools in medical monitoring, therapy and maintenance of healthy pregnancy.

Key words: Kuwait, Neurofilaments, Pre-eclampsia, Th1 Cells, Western Blot.

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Introduction

We have previously shown that serum Butyrylcholinesterase (BuChE) activity in Kuwaiti women afflicted with disorders of pregnancy correlates with immunoregulation in a manner not observed in healthy pregnancies (1). Since BuChE detoxifies pregnancy-threat-

ening neuro - and immunotoxic compounds that are known to have been dispersed into the environment of the Gulf region in 1991 (2, 3, 4) and following the military actions of the 2003 Gulf War. We hypothesize that exposure to these substances may exacerbate pathologic immune processes leading to negative pregnancy outcomes. The present study extends this work in an evaluation of correlations between serum biomarkers of neurodegeneration resulting from organophosphate exposure and lymphocyte subpopulation profiles in women afflicted with pre-eclampsia, a disorder of pregnancy caused by dysregulated maternal immunity (5). It is here hypothesized that pre-eclamptic Kuwaiti women exhibit reduced ability to regulate pregnancy-associated immune activation; and express antibodies to products of pollutant-induced neurodegeneration at levels different from women experiencing normal pregnancies. Autoantibodies may form to cytoskeletal components of pollutant-damaged nerve cells, including: 200-kDa, outer- or high-molecular weight (NFH); 160-kDa, middle or medium-molecular weight (NFM); and 70-kDa, core and low-molecular weight (NFL) neurofilament subunits (6).

Here, serum of pre-eclamptic and healthy pregnant Kuwaiti has been analyzed for content of these antibodies with the objective of establishing parameters for a comprehensive investigation of immunoregulation in a Kuwaiti population.

Patients and methods

Participants in this study included 23 women in the third trimester of pregnancy, 13 afflicted with pre-eclampsia, and 10 experiencing normal pregnancies. 10 healthy, non-pregnant women were enrolled as controls. All were in Kuwait since the 1991 Gulf War. Institutional Ethical Committee Approval was obtained before the commencement of the study and each patient gave her consent before inclusion in the study.

Patient selection criteria

Preeclamptic women had: (a) Blood pressure of 140 mm Hg or higher systolic and 90 mm Hg or higher diastolic on two occasions at least six hours apart on bed rest after 20 weeks of gestation with previously normal blood pressure; (b) proteinuria of 0.3 g or more of protein in a 24-hour urine collection (which usually corresponds with 1+ or greater on a urine dipstick test).

Other features which were signs of severe: (a) Blood pressure of 160 mm Hg or higher systolic and 110 mm Hg or higher diastolic, and proteinuria of 5 g or more in a 24 hour urine collection; (b) oliguria defined as urinary output of less than 500 mL in 24 hours; (c) cerebral or visual disturbances; (d) pulmonary edema or cyanosis; (e) epigastric or right upper quadrant pain; (f) impaired liver function, thrombocytopenia; (g) intrauterine growth restriction. None of the women (patient or control) had evidence of any active infective process such as urinary tract infection or upper respiratory tract infection.

Blood collection and Lymphocyte analysis

Ten ml of venous blood was collected from each patient by venipuncture without use of tourniquet, centrifuged for 10 minutes at 1000 rpm and the serum separated and stored at -70°C until analysis for anti-neurofilament antibodies was carried out. Five ml of peripheral venous blood was collected in EDTA tubes. Fifty µl of blood were incubated for 30 min at room temperature with 5 µl of the flourescein-isothiocyanate (FITC) or phycoerythrin (PE) conjugated monoclonal antibodies (mAb) of interest. FITC- and PE-labeled monoclonal antibodies not reacting with lymphocyte surface antigens were used

as negative controls. After lyses of erythrocytes with Q-prep (Coulter Corporation, Hialeah, FL, USA) followed by fixation with paraformaldehyde, two color fluorescence analysis using an automated flow cytometer (Coulter Altra cell sorter) was performed and analyzed by 3-color flow cytometry for selected lymphocyte subpopulation frequency.

Analysis for Cytoskeletal Proteins

As was reported previously (14), briefly, neurofilament proteins prepared from fresh bovine spinal cord were separated by SDS Gel Electrophoresis, then electrophoretically transferred onto nitrocellulose paper and incubated with serum extracted from each blood sample. Autoradiograms derived from these blots were scanned in a Molecular Dynamics Personal Densitometer SI and quantified, using the gel analysis program IPLab Gel v1.5, to yield serum levels of antibody to each protein.

Statistical analysis

Analysis was performed using multiple comparison analysis of variance (ANOVA) with a post-hoc Tukey test. All statistical analysis were performed using the SPSS for Windows statistical package version 16 (Norusis/SPSS, Inc). A value of $p < 0.05$ was considered statistically significant.

Results

As shown in Table 1, the peripheral blood frequencies of four activated lymphocyte subpopulations (CD4+CD25+, CD8+CD25+, CD8+HLA-DR+, and CD4+CD54+) were observed to be significantly elevated in all pregnant women when compared with their percentages in blood of the non-pregnant group ($p < 0.05$).

The percentages of CD4+HLA-DR+ cells were elevated in PE versus non-pregnant women, but not in healthy pregnant women; and no differences were noted in NK-T (CD3+/CD16+CD56+) cells between all groups. Addi-

Table 1 Lymphocyte subpopulation frequencies in non-pregnant women versus subjects with normal or pre-eclamptic pregnancies

Lymphocyte subpopulation	Non-pregnant (N = 10)	Normal pregnancy (N = 10)	Pre-eclamptic pregnancy (N = 13)
CD3+/CD16+CD56+ [a]	10.4 ± 2.3	22.8 ± 7.4	21.9 ± 6.3
CD4+CD54+ [b]	15.3 ± 2.1	24.9 ± 5.0*	34.9 ± 6.7**
CD4+HLA-DR+ [b]	6.4 ± 1.7	6.8 ± 0.8	9.5 ± 1.7*
CD8+HLA-DR+ [c]	10.6 ± 3.1	26.3 ± 4.5*	27.2 ± 4.1*
CD8+CD25+ [c]	0.7 ± 0.4	6.3 ± 2.1*	7.6 ± 4.8*
CD4+CD25+ [b]	5.3 ± 1.5	14.1 ± 3.8*	19.8 ± 6.9*
CD4+CD45RA+ [b]	38.3 ± 5.2	56.3 ± 4.5*	43.1 ± 4.1¶
CD4+CD45RO+ [b]	47.6 ± 4.1	53.6 ± 5.6	53.0 ± 4.7

Selected activated, memory, and naive lymphocyte species are evaluated by 2-color flow cytometry for percentage representation in CD3+ (a), CD3+CD4+ (b) and CD3+CD8+ (c). Values are given as proportions (%) of lymphocyte subpopulations within each mother population (CD3, CD4 and CD8) ± standard error of the mean (SEM).

* $p < 0.05$ versus non-pregnant subjects

** $p < 0.01$ versus non-pregnant subjects

¶ $p < 0.05$ versus women experiencing normal pregnancy

tionally, the percentages of CD4+CD45RA+ T lymphocytes, which may represent either a naive or suppressor phenotype (7), were significantly elevated in subjects experiencing normal pregnancies ($p < 0.05$), but present at lower frequency in the blood of pre-eclamptic women ($p < 0.05$).

Figure 1 shows no significant differences in serum levels of anti-NFM and NFH between any of the subject groups. However, compared to non-pregnant women, the concentration of anti-NFL was significantly lower for participants experiencing normal pregnancies ($p < 0.05$) and for those with ($p < 0.01$).

Discussion

Maternal immune responses to the feto-placental unit during normal pregnancy include increased percentages of activated T cells in the peripheral blood (7), an effect also observed in the present study (Table 1). If normal immunoregulatory mechanisms are impaired, these cells can cause pre-eclampsia and other disorders of pregnancy (8). This effect may be indicated here by lower CD4+CD45RA+ cells in blood of pre-eclamptic versus healthy pregnant women (Table 1), which confirm

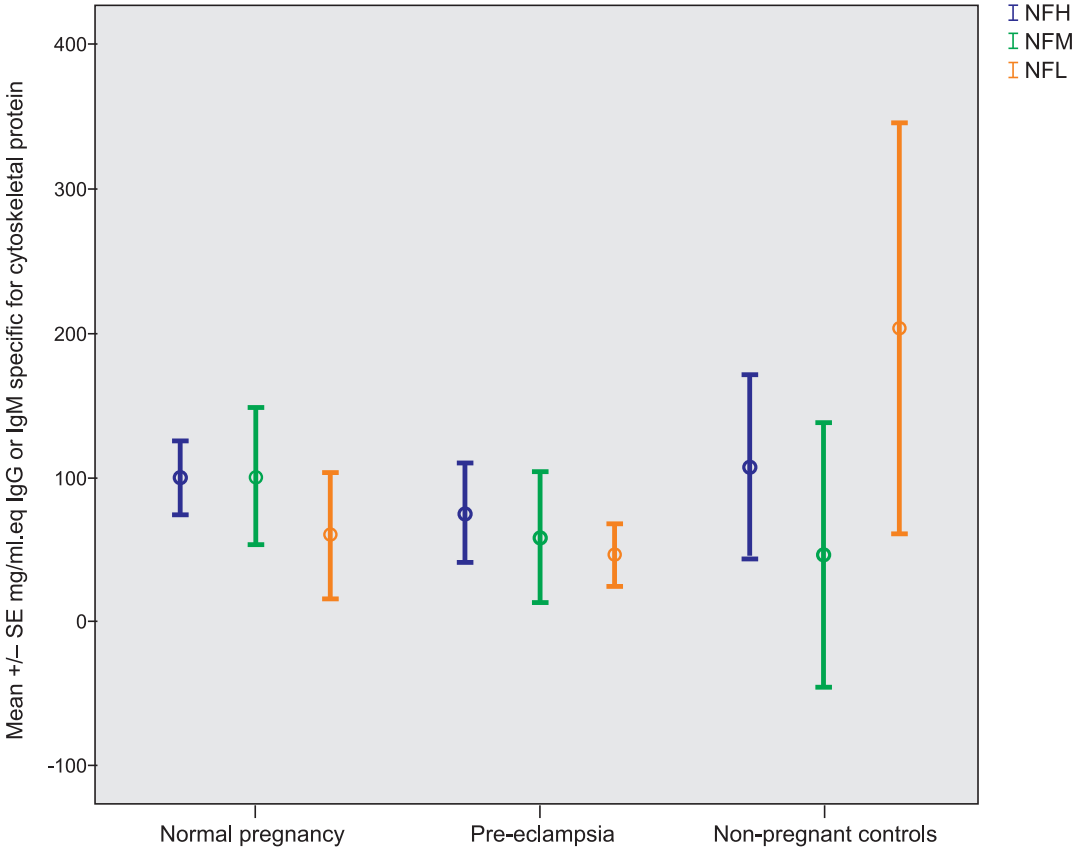

Figure 1 Serum concentration of antibodies to cytoskeletal proteins in non-pregnant women versus subjects with normal or pre-eclamptic pregnancies. Autoantibodies with specificity for 70-kDa (NFL), 160-kDa (NFM), and 200-kDa (NFH) neurofilaments are measured using Western blot analysis. Values are given as ng/ ml equivalence (ng/ ml.eq) IgG or IgM, with specificity for each indicated cytoskeletal protein in serum ± standard error of the mean (SEM). * $p< 0.05$, ** $p< 0.01$ versus non-pregnant subjects.

a previously published study in Poland (9). CD45-related signals have been implicated both directly and indirectly, in a variety of lymphocyte functions (10), as a prerequisite for transmitting activation signals through T cell antigen receptor (TCR). Two T receptor populations are expressed on T cells; one linked to the cytoskeleton via its zeta chain. These cytoskeleton linked receptors which make up 30-40 percent of the total number of TCRs are important in TCR mediated signaling (11). Furthemore, oxidative stress is known to be involved in the heat stress-induced down-regulation of TCR zeta chain expression and TCR/CD3-mediated [Ca^{2+}] response in human T lymphocytes (12). Regardless of the cell type, the regulation of actin cytoskeleton is tightly linked to vital biological properties such as polarity, motility, cell to cell contact, exocytosis and proliferation (13). Exposure to certain environmental pollutants causes nerve cell destruction and formation of antibodies to neurofilaments (14). It may also, as suggested by our previous work, contribute to heightened immune activation and exacerbation of pregnancy disorders (1). A separate investigation by our laboratory demonstrated that serum levels of anti-NFL are lower in a group of Kuwaitis afflicted with a Th1-mediated autoimmune disease (psoriasis) than in those with non-psoriatic controls (15). The present study shows that serum levels of anti-NFL are significantly lower in pregnant subjects than in non-pregnant controls, and lower in pre-eclamptic subjects than in the healthy pregnant group. Although data presented in this report does not allow for a precise mechanistic interpretation of these observations, it is possible that lower trunover of NFL may occur as a result of immunoregulatory processes triggered by normal pregnancy and by PE (1, 5). Nevertheless further characterization of this effect will be required before serum anti-NFL titers may be used as diagnostic or therapeutic biomarkers. Pregnancy is characterized by a predominantly Th1 profile of immune activity (16), which is exacerbated in pre-eclampsia (17). Furthermore, as shown in Figure 1, serum anti-NFL is significantly lower in healthy pregnant than non pregnant subjects and even lower in preeclamptic women, which suggests that increasing Th1 character may inhibit tissue turnover nerve cells. This normally leads to increased serum levels of antibodies to neurofilaments with increasing age. This interpretation, however, is purely speculative in the absence of additional data. The scope of this study does not allow medically useful conclusions to be drawn regarding correlations between neurofilament antibody expression and the occurrence of PE. However, the data presented here suggests strategies for using these biomarkers in characterizing immunoregulatory disorders in a population such as that of Kuwait subjected to a high toxic burden. Studies currently underway by this laboratory will correlate occurrences of immunoregulatory dysfunction with associated changes in lymphocyte activity and neurofilament antibodies in the context of serum activity of detoxification enzymes such as butyrylcholinesterase. This work is expected to result in more focused therapeutic approaches to treating of a wide range of disorders.

There is abundant evidence of modification of the neurons of the gravid uterus. There is functional denervation of the gravid uterus in the latter part of the pregnancy and renervation in the postnatal period (18), suggested by low α-adrenergic receptor density and absence of neuronal nitric oxide synthase (19). In a recent hypothesis, Quinn proposed a connection between preeclampsia and partial uterine innervation, caused by damage to the nerve plexus at the endometrial-myometrial interface which causes impairment of control of a third proliferative, invading trophoblast resulting in the characteristic histological changes (20).

It has been suggested that growth factors like nerve growth factor, vascular endothelial growth factor, and cytoskeleton proteins such as neurofilament produced by nerves and blood vessels may contribute to the process of normal placentation. These processes may be compromised in areas of denervation. Specifically, loss of neural connections between the uterine and renal innervation may cause reduced fetal growth and PE. Neurofilaments are neurone-specific intermediate filament and regulate neuronal cytoskeletons to form the dynamic axonal cytoskeleton. They maintain and regulate neuronal cytoskeletal plasticity through the regulation of neurite outgrowth, axonal caliber and axonal transport (21). It is not yet clear whether there is differential denervation of the gravid uterus in abnormal compared to normal pregnancies, and how they alter the anti-neurofilament levels. In an immunohistochemical study, Khong et al. demonstrated lack of innervation of the spiral arteries, however, nerves were observed in the myometrium in 7 out of 10 normal and 1 in 8 third trimester abnormal placental beds (22).

Cytoskeleton and TCR modification occurs as a result of oxidative stress during pregnancy. Lateral associations between TM helices are also involved in transmission of signals from the activated TCR to downstream effector pathways. Activation of kinases by the phosphatase CD45 appears to be facilitated by CD45-associated protein, which binds kinases with its intracellular domain and CD45 via TM-TM contacts. Recruitment of phosphatidylinositol-3-kinase to the membrane by pp30 (T cell receptor interacting molecule, TRIM) depends on the phosphorylation of intracellular tyrosines on TRIM (23). Stimulation of peripheral blood T cells with phorbol myristate acetate (PMA) or with anti-CD3 monoclonal antibodies resulted in a marked increase in detection of phosphorylated neurofilament on western blotting, thus indicating that T cell activated through the T-cell receptor associated complex express an intermediate filament usually associated with neurally derived cells (24).

Conclusion

The trigger mechanism that results in the production of antibodies to neurofilaments generally is not known. It has been postulated that such antibodies may represent essential homeostatic mechanism for removing damaged or infected cells (25). This protective mechanism may be deficient in pre-eclampsia. If this is true, then anti-neurofilament could be used as a biomarker of pre-eclampsia in early pregnancy. Any relationship between anti-neurofilament and pre-eclampsia certainly needs more extensive evaluation with a larger sample size in a multi-center collaborative effort.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Early nutritional support is associated with improved outcomes in respiratory failure

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Objective. To evaluate early feeding as a predictor of outcome in critically ill patients receiving prolonged mechanical ventilation. **Patients and methods.** A retrospective cohort study in four medical, surgical and multidisciplinary intensive care units (ICU) in a tertiary referral center of adult patients requiring at least 48 hours of mechanical ventilation. Early feeding was defined as any nutritional support (enteral or parenteral) for at least 6 hours, started within 48 hours of mechanical ventilation. The primary endpoint was hospital mortality. The secondary endpoints were length of stay, and duration of mechanical ventilation. Univariate and multivariate analysis were used as appropriate. **Results.** 394 out of 4,546 patients admitted to the ICU were studied. Age (mean: 95% confidence interval was 62 (60-63); female gender 43%; APACHE III 72 (70-75); APACHE III predicted hospital mortality 36 % (33-39); ICU mortality 19%, hospital mortality 28%, ventilation –free days 41 (39-44). Only 11% (3% enteral, 8% parenteral) were fed on day 1, 55% (30% enteral, 25% parenteral) on day 4, and 88% (51% enteral, 37% parenteral) on day 7. Early feeding was associated with a reduced Standardized Mortality Ratio (number of observed hospital deaths/number of expected hospital deaths) of 0.53. When adjusted for various confounding factors such as severity of illness, trauma, route of feeding, post-operative state or the use of vasopressors, early feeding remained independently associated with decreased hospital mortality (Odd Ratio 0.51; 95% confidence interval 0.26-0.98; p = 0.042). **Conclusion.** Early nutrition is associated with decreased hospital mortality in patients receiving prolonged (more than 48 hours) invasive mechanical ventilation.

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Key words: Respiratory insufficiency, Enteral nutrition, Parenteral nutrition, Assessment, Patient outcome.

Introduction

Malnutrition is common in hospitalized patients (1) and contributes to increased morbidity and mortality (2). A low body mass index in critically ill patients is associated with a poor outcome (3). Early nutritional support is essential for malnourished patients, and may also be beneficial in those who are well nourished (4). Indications for

nutritional support of well-nourished patients include inability to eat for more than seven days because of a critical illness, a major surgical procedure or a major trauma (5). Basic requirements include fluids, electrolytes, protein, lipids, carbohydrates, vitamins, and minerals. The tight control of glucose remains controversial (6, 7). The ultimate goal of nutritional support is to improve outcome (8, 9).

A delay in feeding, with or without preexisting malnutrition, may compound the debt patients have to pay (10). In addition, early enteral nutrition (11) may preserve gut integrity, barrier and immune function, reduce infectious complications and ultimately affect outcome (12). The benefit of early nutrition on ventilation days, hospital stay, and hospital mortality however is still not well established (13, 14). Therefore individual practices, including nutrition practice, at Mayo Clinic vary. This variability allowed us to assess the predicting character of early feeding on outcome in critically ill patients who required prolonged invasive mechanical ventilation. Our hypothesis was that early feeding in the critically ill is associated with a higher survival rate.

Patients and methods

The Institutional Review Board of Mayo Clinic College of Medicine approved the study. Subjects or their legal representatives have given written authorization to have their chart reviewed for research purposes. The medical records of all adult patients requiring 48 hours or more of mechanical ventilation in four medical, surgical and multidisciplinary intensive care units (ICU) between January 1, 2001 and December 31, 2001 were reviewed. Patients ventilated for less than 48 hours, those using a ventilator at home; those with neuromuscular disease, pneumonectomy or a terminal illness were excluded. The data of this cohort has been reported elsewhere to study the relationship between mechanical ventilation and ventilator-associated lung injury (15). The relationship between mechanical ventilation and nutrition has not been addressed so far.

Data were collected daily from the first day of mechanical ventilation and included age, gender, body mass index, the presence of acute lung injury, Acute Physiology, Age, Chronic Health Evaluation (APACHE III) score from the day of ICU admission and APACHE III derived predicted hospital mortality. Preexisting conditions included a history of lung, heart, liver, kidney or brain disease, active cancer, and alcohol and tobacco abuse. The reason for admission to the ICU included exacerbation of a chronic lung condition, coma, post-operative state, trauma, congestive heart failure, and sepsis. Postoperative state distinguishes surgically (postoperative) and medically critically ill patients.

Nutritional support was defined as enteral and or parenteral nutrition provided for at least six hours each day and initiated from day 1 to day 7. Early feeding was defined as any nutritional support (enteral or parenteral) started on day 1 or day 2, i.e. within 48 hours of mechanical ventilation. Late feeding was defined as any nutritional support (enteral or parenteral) started on day 3, 4, 5, 6 or 7, i.e. after more than 48 hours of mechanical ventilation. Outcome was evaluated by the mortality rate in the ICU, the mortality rate in hospital, Standardized Mortality Ratio (SMR) defined as the ratio of actual hospital mortality over predicted hospital mortality, the length of stay in the ICU, the length of stay in hospital, the duration of mechanical ventilation and the number of ventilation-free days.

Statistical analysis

Statistical analysis used univariate analysis (Wilcoxon test, Chi-Square) and multivariate logistic regression as appropriate (JMP® 8.0, 2008 SAS Institute Inc., Cary,

NC, 27513). The population was divided between patients who were fed early (less than 48 hours after initiation of the mechanical ventilation) and those fed late (more than 48 hours) without distinction between enteral and parenteral nutrition, usual care at that time being that any patient was started on enteral support whenever feasible, otherwise given parenteral nutrition. Factors that were different by univariate analysis were subsequently plotted together in a multivariate analysis to study their relationship.

Results

Among 4,546 patients admitted to the four ICUs, 441 patients met the study inclusion criteria and 394 patients had complete nutrition data for study purposes. Baseline characteristics are shown in Table 1. In this study, 11% (3% enteral, 8% parenteral) were fed on day 1, 55% (30% enteral, 25% parenteral) on day 4, and 88% (51% enteral, 37% parenteral) on day 7 (Figure 1). The studied population was divided into two groups: those who were fed early, within 48 hours after initiation of mechanical ventilation, and those who where fed late, more than 48 hours after initiation of mechanical ventilation (Table 2). Patients, who were fed early had similar APACHE III scores and predicted hospital mortality rates, were younger, needed fewer vasopressors, more often received parenteral nutrition, and had lower hospital mortality (Figure 2). There was no difference in the number of days of mechanical ventilation or the number of ventilation-free days. When adjusted for confounding factors such as APACHE III, trauma, post-operative state, route of feeding, and vasopressor use, early feeding

Table 1 Baseline characteristics and subgroups of patients fed early (less than 48 hours) and late (more than 48 hours); univariate analysis, mean (95% confidence interval) or number and percent as appropriat

Baseline characteristics	Total N = 394	Early (<48 hours) N = 105	Late (>48 hours) N = 289	P value
Age (years)	62 (60-63)	57 (54-61)	62 (60-64)	0.009
Female gender (%)	170 (43%)	44 (42%)	126 (44%)	0.764
Body Mass Index	27 (27-28)	28 (27-30)	27 (26-28)	0.132
APACHE III*	72 (70-75)	69 (64-74)	73 (69-76)	0.392
APACHE III predicted hospital mortality (%)	36 (33-39)	38 (32-44)	36 (32-39)	0.721
Any vasopressor use (%)	226 (57%)	47 (45%)	179 (62%)	0.002
Post operative state (%)	215 (55%)	53 (51%)	162 (56%)	0.326
Trauma (%)	50 (13%)	18 (17%)	32(11%)	0.110
Route of feeding§				P<0.001
Enteral only	246 (62%)	39 (37%)	207 (72%)	
Parenteral	148 (38%)	66 (63%)	82 (28%	
ICU† length of stay (days)	14 (13-16)	15 (13-18)	14 (13-15)	0.807
ICU mortality (%)	77 (20%)	14 (14%)	63 (22%)	0.066
Hospital length of stay (days)	28 (25-32)	40 (30-50)	26 (23-30)	0.008
Hospital mortality (%)	112 (28%)	21 (20%)	91 (32%)	0.025
Ventilation days	8 (8-9)	9 (7-11)	8 (7-9)	0.674
Ventilation-free days	41 (39-44)	46 (41-51)	40 (37-43)	0.066

*APACHE III: Acute Physiology, Age, Chronic Health Evaluation; †ICU: Intensive Care Unit, § During the first 7 days after ICU admission

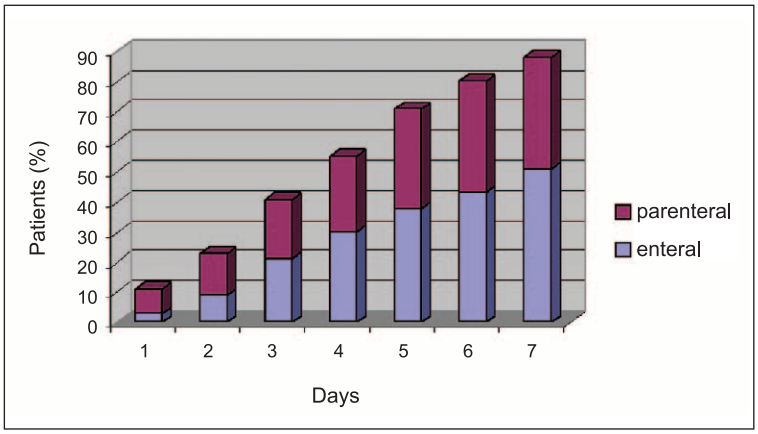

Figure 1 Timing of initiation of nutritional support in mechanically ventilated patients.

Table 2 Comparison between groups of patients fed early (less than 48 hours) and late (more than 48 hours) by multivariate analysis after adjustments for other confounders, early feeding remains a prognostic factor for hospital mortality in mechanically ventilated patients

Variables	Odd ratio (95% confidence interval)	P value
Early feeding	0.51 (0.26-0.98)	0.042
Feeding route*	1.55 (0.87-2.79)	0.135
APACHE III† predicted hospital mortality	1.36§ (1.24-1.49)	<0.001
Any vasopressor use	1.37 (0.79-2.40)	0.262
Post operative state	0.68 (0.39-1.17)	0.162
Trauma	0.33 (0.09-0.94)	0.038

* Enteral or parenteral, during the first 7 days after ICU admission; †APACHE III: Acute Physiology, Age, Chronic Health Evaluation; § by 10th percentile increment

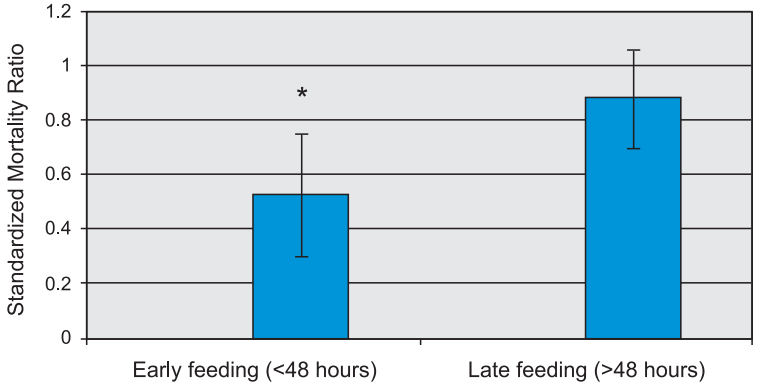

Figure 2 Standardized mortality ratio with 95% confidence interval between groups of patients fed early (less than 48 hours) and late (more than 48 hours): early feeding is associated with significantly less hospital deaths than expected (*), whereas, there is no significant difference between the number of observed hospital deaths and the number of expected hospital deaths in the late feeding group.

remained associated with decreased hospital mortality (Odd Ratio 0.51; 95% confidence interval 0.26-0.98; p = 0.042).

Discussion

The main finding of this retrospective observational study is that initiating feeding within the first 48 hours of mechanical ventilation is associated with lower hospital mortality when compared with delayed initiation of nutritional support. Limitations of this study include its retrospective aspect, the small number of patients initiated with nutritional support (10%) and the heterogeneity of different ICUs.

The large variability of our nutrition practice at a time before the most recent nutrition guidelines were published allowed us to test the association between the timing of nutrition support and the outcome of critically ill patients. In the following sections we will discuss our findings in the context of the evidence based nutrition literature and explore alternative interpretations of the data.

Does the literature support early feeding of critically ill patients?

Current guidelines recommend initiating nutritional support within 24 to 48 hours after admission to ICU, to promote earlier feeding, greater nutritional adequacy, and improve clinical outcome (13) including in well nourished, but critically ill patients. This supports the pretest probability that our observations are valid and should drive a change in practice. In a heterogeneous ICU population, outcome was not different when patients were provided with a full nutritional support from day 1, but this study lacked power (16). In a group of patients requiring prolonged mechanical ventilation, early feeding significantly reduced ICU and hospital mortality; this was true mainly in the sickest patients, and despite an increased risk of ventilator-associated pneumonia (17). When evidence-based recommendations were implemented, stressing early institution of nutritional support, preferably enteral, there were more days of enteral nutrition, a significantly shorter mean stay in hospital and a trend toward reduced mortality when compared to a control arm (18). In other instances however, an excessive intake was associated with excess morbidity and mortality (19). Thus not only the timing but also the amount and likely the composition of nutritional support will determine whether nutrition influences the outcome of critically ill patients. The present study indicates that early feeding is associated with a lower hospital mortality rate than predicted.

Was the decision to initiate feeding within 48 hours of intubation an APACHE III independent predictor of a patient's severity of illness and hence outcome?

Erroneous conclusions due to uncontrolled variables and underappreciated confounders are an inherent danger of all retrospective research. The preceding section dealt with the pretest probability that our findings are real and deserves to shape future nutrition practice. In this section we will examine the alternative, namely, that patient outcomes were independent of nutrition support and reflect variable(s) we did not adjust for. To weigh this possibility we need to examine other aspects of our practice in 2001.

During the year 2001, nearly 10% of patients in four ICU's received invasive mechanical ventilation for at least 48 hours. With the notable exception of trauma patients who required prolonged mechanical ventilation were not fed early. Only half of the patients were given some nutritional support on day 4, either through enteral or parenteral routes. At our Institution, during the study period, nutritional support was implemented at the discretion of the team in charge. Some patients were fed early, other not, according to the practice of the consultant on duty. There was also no clear indica-

tion whether a patient needed to be started on parenteral instead of enteral nutrition. No trial of enteral feed was systematically implemented before considering parenteral support. There was no consideration for combining enteral and parenteral support. Furthermore, glucose management was not intensive since the now controversial tight glucose control recommendation had not yet been published (6, 7). The nutritional service was readily available, with fairly uniform care processes, but was not systematically and/or independently activated.

The reason for the variability in nutrition practice at the time in part reflects the lack of convincing evidence that early feeding was beneficial. The statement of the American College of Chest Physicians from 1997 (8) was vague as when to start nutritional support in well nourished patients. The primary team may have been more focused on stabilizing the patient than feeding him, influenced by the 'seven-day' rule according to which fasting a patient for the first week was not considered harmful (5). There may have been some concern that the adverse effects of lipids or the hyperglycemia induced by the total parenteral nutrition negate any beneficial effect of nonlipid supplementation (12).

Two additional confounders need to be considered: physician preferences and outcome predictors not taken into account by the APACHE III score. In term of physician preferences, there was no link between the propensity to feed and outcome. The situations that would influence a physician to feed or not to feed a patient include the type of patient (medical, post operative or trauma), and the severity of the underlying illness (APACHE III, need for vasopressors). Although trauma patients were fed early more often, they did not bias the result. There was no link between the type of ICU (medical, surgical) and outcome. The APACHE III score is a well-established outcome prediction tool in critically ill patients. The expected hospital mortality rate is usually within 3 percent of the actually observed hospital mortality rate (20). Glucose control is not incorporated into the APACHE III scoring system. Glucose level on day 1 was identical in survivors and non-survivors at hospital discharge as was the albumin level. Ongoing large ARDS-net clinical trial of early full versus trophic nutritional support in mechanically ventilated patients with acute lung injury will further improve our understanding of this important issue.

Conclusion

In a cohort of ventilator dependent patients we observed an association between early nutrition and outcome. Having considered the pretest probability of such a result, and eliminated bias from confounders as far as is possible post hoc, we believe that nutritional support should not be delayed in critically ill patients who require invasive mechanical ventilation.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Assessment of motor function score according to the GMFM-88 in children with cerebral palsy after postoperative rehabilitation

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Objective. To determine the outcome of rehabilitation treatment after orthopedic-surgical treatment of the lower extremities in relation to motor function and degree of disability in children with cerebral palsy. **Subjects and methods.** An historical-prospective study included 44 treated children with CP from May 2000 until June 2009 at the Department of Physical Medicine and Rehabilitation University Clinical Centre Tuzla. The main criteria for entering the study were diagnosed CP and performed orthopedic-surgery of the lower extremities during rehabilitation treatment. Assessment of the motor function score was performed according to the Gross Motor Function Measure 88 (GMFM-88), and classification of the degree of disability was developed based on the scale of the Gross Motor Function Classification System (GMFCS). **Results.** In our study, motor functions were improved, so that the median value and interquartil range (IQR) of the total GMFM score before surgery was 35.7 (IQR from 22.9 to 57.2), and after postoperative rehabilitation 58.6 (IQR from 31.2 to 85.2) with a high statistical significance ($p<0.0001$). Median value GMFCS scores before surgery ranged around 5 (IQR from 4 to 5), and after postoperative rehabilitation ranged around 3 (IQR from 2 to 5), which shows a highly statistically significant reduction in the degree of disability ($p<0.001$). **Conclusion.** Surgical intervention performed on the lower extremities in children with cerebral palsy may improve motor function in all developmental stages and reduce the degree of disability with intensive rehabilitation.

Key words: Cerebral palsy, Operation, Motor function.

Introduction

Cerebral palsy (CP) is usually present as a pediatric neurological problem in physiotherapy. The clinical chart is dominated by motor function disturbances due to abnormal muscle tone that leads to impairment of posture and movement, but there may also be vision, hearing, speech, seizures, behavioral or swallowing disorders (1). The first description of a child with motor disorders resulting from premature childbirth and asphyxia was published in 1862 by Mr. Little (2). In recent years dealing with severe ce-

rebral palsy has intensified, and so has the establishment of special organizations such as the "Surveillance of cerebral palsy in Europe" (SCPE) dealing with standardization, monitoring and registration of children with CP in Europe, and according to its data, the prevalence of CP in Europe is 2-3/1000 live births (3). According to the SCPE due to the volatility of the motor development the final diagnosis and classification of CP is recommended at age 3-5. SCPE proposes division on the basis of neurological disorders such as the spastic (bilateral and unilateral), dyskinetic (dystonic and horeoatetonic) and ataxic form, and the rough division of motor function or the Gross Motor Function Classification System (GMFCS) at five levels of functional disability, so that the first level represents the least and the fifth level represents the highest level of damage to motor functions. In the majority of children with CP, neuroradiological brain damage is proven, and clinical syndromes occur as a result of that, followed mainly by motor disturbances (4). Spasticity and dystonia represent a major problem in children with CP, because the hypertonic muscles shorten and thus lead to contractures and bone-articular deformity. Spasticity increases in children with CP until the age of four, and thereafter gradually decreases until the age of twelve, which is essential for clinical practice and treatment planning (5). Hypotherapy or horseback riding therapy helps postural balance in a sitting position and improves gross motor function in children with neurological disabilities (6).

Application of botulinum toxin, in combination with kinesitherapy reduces spasticity and improves motor function in spastic diplegia (7). Twenty years after selective dorsal rhizotomy, all patients showed improvement of locomotor functions compared to preoperative status (8). In children in whom treatment was delayed or inadequate eventually secondary musculoskletal deformations occurred that could lead to the deterioration of the existing clinical condition. Orthopaedic surgery has an important role in the treatment of children with CP in the sense of passive mobility enhancement, correction of deformity, or in extreme cases, blocking joints in a functional position (9). Improvement of the functional abilities of children who undergo surgery over a short or long period of time after surgery was significantly better than in children who did not undergo surgery (10). Selective percutaneous tendon extension of the lower extremities is a minimally invasive method, it does not create more scarring, and contributes to the improvement of functional status in all operated patients (11). According to Zergollern et al. (12) and Bortona (13) in their research in operated children with CP there was an improvement of motor function, and Mathur et al. (14) reports better mobility and improved activities of daily life in children with spastic diplegia after postoperative rehabilitation combined with orthotics.

The aim of this study was to determine the outcome of rehabilitation treatment after orthopedic-surgical treatment of the lower extremities in children with cerebral palsy, in relation to motor function and the degree of disability.

Subjects and methods

The research was conducted historically-prospectively from May 2000 to June 2009 at the Department of Physical Medicine and Rehabilitation University Clinical Centre Tuzla. The study included 44 (24 males and 20 females) children with CP, at the average age expressed as a median of 4.3 years at the time of surgery (range 1.6 to 9.4). According to the clinical chart 37 children had a bilateral spastic form, 6 had unilateral spastic form and only one child had an ataxic form of CP. Most children (39) had preserved the

ability of understanding spoken or written linguistic information and the possibility of verbal or nonverbal expression, three children had problems with verbal expression, whereas only one child had severe cognitive disorders without the ability to communicate. The main criteria that would satisfy inclusion in the study were that the child was diagnosed with CP and had been subjected to orthopedic-surgery of the lower extremities during rehabilitation treatment.

All subjects underwent rehabilitation of varying duration and intensity prior to the research, and at least six months before surgery rehabilitation treatment was continuous with mandatory parental education for passive and active-assisted stretching of the shortened muscles, relaxation of the spastic muscles and strengthening their antagonists to stimulate the child's functional abilities depending on the degree of disability, while the orthoses were used for proper positioning of the extremities.

Orthopedic-surgical procedures were performed on one or more levels, using various techniques such as elongatio tendinis, tenoplastica, tenotomia subcutanea, trasposition tendinis, desinsertio musculi, fibrotomia and neurectomia.

After postoperative immobilization with or without a plaster splint in the period required by a postoperative protocol, postoperative rehabilitation was conducted for at least 6 months according to an individual program, which depended on the maturity, mental maturity of the child and his functional abilities. Orthoses were used for positioning the operated limb during the night or during the days after exercises. Exercises were relaxing in the beginning, followed by passive and active-assisted exercises in order to improve mobility and strengthen the weaker muscles, such as thigh abductors, lower leg extensors and foot dorsal flexors. After that, exercises in a sitting balance, a four-legged stance, standing, and walking with compliance were applied, and in the end walking with apparatus and if possible independent walking.

Education of parents was required to use the same program of exercises and positioning in order to continue implementing it in the home setting. Evaluation of motor function and degree of disability for each participant was done by the same physiatrists and physical therapist before surgery and one year after the postoperative rehabilitation. Evaluation of specific gross motor function before and after postoperative rehabilitation was performed according to the score of the Gross Motor Function Measure 88 or GMFM-88 (15). It is a questionnaire that examines 88 motor activities, classified into five developmental stages, namely: GMFM-A (lying and turning), GMFM-B (sitting), GMFM-C (crawling and kneeling), GMFM-D (standing) and GMFM-E (walking, running and jumping). Each motor activity is evaluated from 0 to 3. The grade 0 means that the child neither starts or performs the activity, grade 1: the child may begin the activity, grade 2: the child partially executes the activity; grade 3: the child fully executes the activity. GMFM scores can be represented as the total sum of points or according to certain developmental stages, expressed as a percentage, so that the normal score is 100%. The child is tested minimally dressed, without shoes and supplies, so that the person who is doing the test could be considered undisturbed whilst observing the child. The child is allowed a maximum of three attempts to perform any task, except when the child refuses to perform an action that we feel it can at least partially execute, we can put a label "not tested". Necessary equipment in the testing room are two marked straight lines, 6 m long with 20 cm distance between them, then a straight line 2 cm in width and 6 m in length and a circle with a diameter of 60 cm marked on the floor. It requires a small bench up to 1 m in length,

a large bench, stopwatch, a stick, a large ball and five stairs. Assessment of the degree of disability was performed according to the Gross Motor Function Scale Classification System (GMFCS) (16). The scale is based on the ability to perform motor activities divided into five levels of disability, so that the first shows minimal motor deviations, and the fifth shows complete inability to perform motor activities that are variously described by chronological maturity, for the period up to 2 years, 2-4 years, 4 -6 years and 6-12 years.

Statistical analysis

Since no features of normal distribution were found in the groups examined with the Kolmogorov-Smirnov test, data are presented as median and interquartile range and statistical analysis was performed using the non-parametric the Wilcoxon test. Level of significance was defined with $P < 0.05$. In the analysis, we used the statistical package Arcus QuickStat Biomedical version (17).

Results

Specific gross motor function evaluation was performed according to the values of the GMFM 88 score. The median total GMFM score before surgery amounted to 35.7 (interquartile range 22.9 to 57.2), and after postoperative rehabilitation it amounted to 58.6 (interquartile range 31.2 to 85.2) with a high statistical significance of $p < 0.0001$. A graphic representation of the differences is given in Figure 1.

A comparison of individual values of the GMFM score, according to the motor activity at a certain developmental stage, is given in Table 1. As seen, the present value increases in almost all of the developmental stages of the postoperative rehabilitation, which was statistically significant.

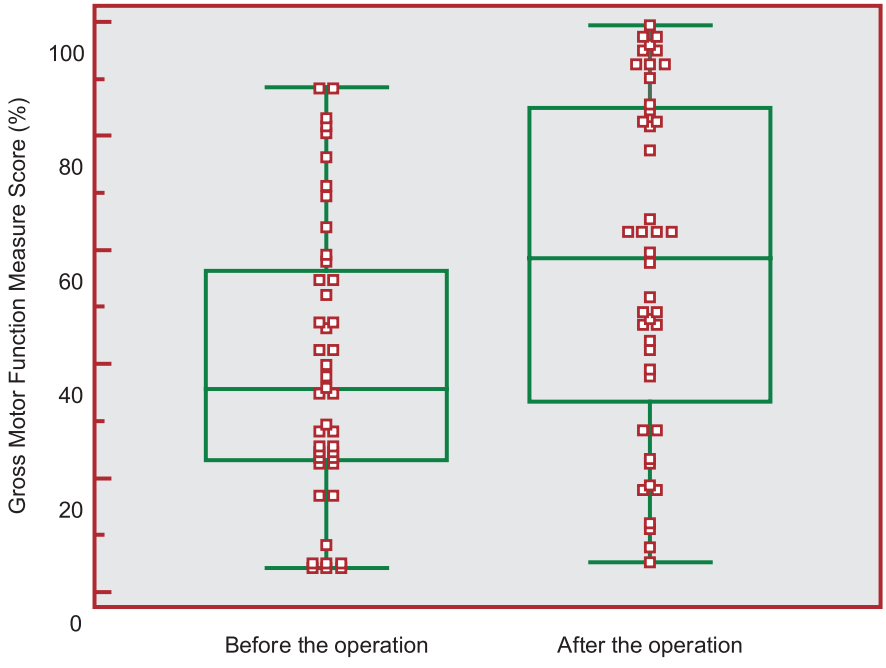

Figure 1 Average values of total motor functions according to GMFM score before and after postoperative rehabilitation

Table 1 Median of motor functions of individual developmental phases according to GMFM score before and after postoperative rehabilitation

Segments of GMFM	Period of measuring		p
	Before operation (Median; IQR*)	After rehabilitation (Median; IQR*)	
GMFM-A (lying and rolling)	84.3 (66.7-92.2)	93.2 (76.5-96.1)	< 0.0001
GMFM-B (sitting)	45.8 (22.1-80)	85.8 (45.4-96.7)	< 0.0001
GMFM-C (crawling and kneeling)	40.5 (0-71.4)	78.6 (35.7-92.8)	< 0.0001
GMFM-D (standing)	2.6 (0-37.8)	23.1 (0-78.8)	< 0.0001
GMFM-E (walking, running, jumping)	0 (0-20.8)	16.7 (0-71.2)	< 0.0001

*IQR= Interquartile Range.

Values of the degree of disability were compared according to the GMFCS scale before and after postoperative rehabilitation. Median values on the GMFCS scale before surgery amounted to 5.0 with an interquartile range from 4.0 to 5.0. Following postoperative rehabilitation, the median value of the GMFCS scale was 3.0 with an interquartile range from 2.0 to 5.0. This decrease in value was highly statistically significant ($p < 0.001$) with 23 (52%) patients who had decreased levels, 21 (48%) patients were without change and no patients with increased levels on the GMFCS scale. The obtained results suggest that prior to operative treatment 75% of respondents had level four or a lower level of disability, while 25% had level five, or the most severe level of disability. After the postoperative rehabilitation the patient's mobility improved, so that 75% of patients had a level two or lower level of disability, and 25% had level five of disability. A graphic representation of this comparison is shown in Figure 2.

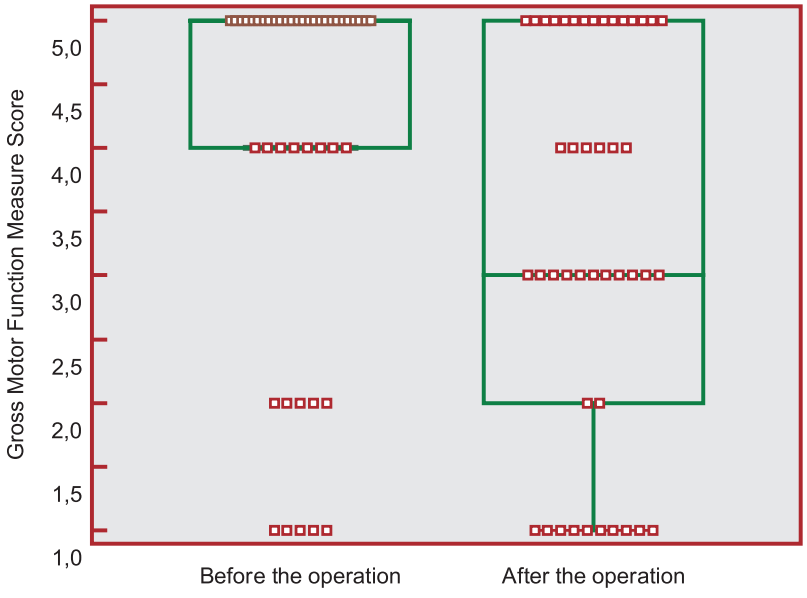

Figure 2 Individual values of disablement level according to GMFCS before and after postoperative rehabilitation

Improvement of motor functions resulted in better mobility and a reduction in the degree of disability. Before surgical treatment 10 patients were able to walk independently with difficulty outside the home and for longer distances (GMFCS I and II), 8 subjects were using a wheelchair to move around (GMFCS IV) and 26 subjects moved with difficultly by using a wheelchair (GMFCS V). The postoperative rehabilitation increased the number of mobile children, so that 12 subjects walked without aids (GMFCS I and II), 11 patients walked with mobility aids (GMFCS III), 6 patients moved with the help of a wheelchair (GMFCS IV), while in 15 cases movement was very limited even with the aid of a wheelchair (GMFCS V).

Discussion

In this study, improvement of motor function and reduction of the degree of disability in children after postoperative rehabilitation was achieved. Motor activity was enhanced after completion of postoperative rehabilitation and the assessment done by GMFM score indicates an increase in the value of the total sum for all the developmental stages as well as the values of certain developmental phases, with a highly significant statistical significance. These changes in motor skills can be explained by the increased range of motion of joints of the lower extremities after surgery, which enabled the further course of continuous and intensive rehabilitation to achieve, in a short period of time, not only improvement of walking, but better stability in sitting and walking on hands and feet.

The validity of the GMFM scores has been demonstrated in a number of studies and has found wide application in clinical practice for assessment of the effect of applied therapy in children with CP, such as botulinum toxin treatment (18). Trahan and Malouin (19) analyzed the effect of applied therapy for eight months for spastic forms of CP showing modifications of GMFM scores, especially with spastic diplegia. GMFM score with a clinical form of CP can be a prognostic indicator of quality of life in this population in adulthood, as Nieuwenhuijsen (20) stated in his research, indicating a high risk of non-active lifestyle in adulthood in children with bilateral spastic CP and low values of GMFM scores.

With proper selection of children for surgery, based on clinical examination and gait analysis, and evaluation of the same children three years later, the operation has been proved to improve spastic muscle function (21). Orthopedic-surgery done on several levels in older children and adolescents who have difficulty moving in a type of squat, brought relief to the knee extensor muscles, reduced pain and improved functional ability and independence (22). Improving the gross motor function resulted in a decrease in the degree of disability estimated according to the GMFCS score before and after postoperative rehabilitation. The median value of the degree of disability according to the GMFCS has been reduced from 5 to 3 which is statistically highly significant, so that in 23 children it reduced the degree of disability, in 21 it remained the same, and no increase in the degree of disability was recorded in any of the examined children. Changes in the degree of disability in the GMFCS score, and functional status after surgical treatment of children with CP as stated by Zorer et al. (23) analyzing 23 operated children, of an average age of 6.3 years, with clinical forms of diplegia, quadriplegia and hemiplegia, in whom a decrease in postoperative contractures was found in all joints of the lower extremities, were improved posture, sitting, walking, and better hygiene, while the degree of disabilities by GMFCS score significantly changed from 3.045 to 1.864. All children with disabilities achieved walk improvement after surgery according to GMFCS I, II and III, while the

functional improvement of 15.7% occurred at GMFCS II, III and IV (24).

The achieved level of motor activity by GMFM score varies with the individual degree of disability, which was investigated by Beckung et al. (25) by testing the achieved GMFM score after five years of life in 317 children with different clinical forms of CP, so that the children achieved the GMFCS I and three quarters of children reached Level II 90%, GMFCS III 80%, 30% GMFCS IV and GMFCS V 20% of GMFM scores. Spinal deformities such as scoliosis in children with CP are correlated with the degree of disability, and the progression of scoliosis is more evident in children with a greater degree of disability, that is in children with GMFCS level V (26). Kinesitherapy has a significant effect on motor function and degree of disability. A significant reduction in the degree of disability according to the GMFCS was noticed after intensive rehabilitation treatment (27). Improvement in gross motor function in children with CP was achieved after applying kinesitherapy treatment for a period of 16 weeks, but significant improvement was noticed in the group of children who exercised five times weekly rather than those who exercised twice a week (28). With intensive and early rehabilitation treatment, we can reduce the degree of disability in children with CP, which means that children with clinical and neuroradiological signs of CP should be included as soon as possible in early rehabilitation (29). Aging of children with CP with a lesser degree of disability leads to reducing the walking speed and increasing knee flexion, while in those with higher levels of disability aging is not associated with a reduction in walking speed and increased knee flexion (30).

As for the operated children, a study that analyzed the operated children with spastic diplegia four years after surgery showed that the walking pace is higher in children that underwent surgery earlier, had fewer surgeries and were walking faster preoperatively, while knee flexion while standing was considerable in patients with multiple surgeries, and lower preoperative knee extension (31). Türker and Lee (32) report increased hip instability, which occurred several years after the aductor tenotomy, while Zwick et al. (33) report improvement in foot mobility, gait speed and support improvement in the phase of movement due to the improved functional abilities of the hip, by analyzing the gait of operated children with diplegia.

Conclusion

Orthopedic-surgical treatment of the lower extremities with the application of kinesitherapy in the complex rehabilitation treatment of children with cerebral palsy, can significantly improve motor function in all developmental stages, such as turning, sitting, crawling, standing and walking, and can significantly reduce the level of disability.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Training for clinical skills in the 20th and 21st centuries: two generations and two worlds apart

Part Two*

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Objective. Here, we compare clinical skills training in the 20th and 21st centuries in two different countries, in order to underline advancements and principal obstacles. **Methods**. The clinical training of medical students in the nineteen-sixties at the Sarajevo School of Medicine, Yugoslavia, and contemporary training at one of Europe's prestigious medical schools at Heidelberg University, Germany were analyzed with respect to the organization of training, teaching tools, methods, and staff. Several issues were defined as unimproved over the course of time, and we suggest that they present the core of the current problem. **Results**. Considerable advances have been made in teaching methodologies, tools and assessment of students. The major remaining obstacles are the institutional value system, poor motivation of teaching staff, curriculum structure, timing, and placement of training in the curriculum, as well as the patients' attitude towards participation in the training. **Conclusions**. In the process of bettering the existing training models we suggest acting along several lines. Increased institutional awareness of obstacles, as well as willingness to develop the ways and means to increase the motivation of the faculty, is imperative. Furthermore, it is necessary to introduce changes in the structure and timing of training and to complement it with a Catalogue, Practicum and Portfolio of Clinical Skills. We believe that recognizing the impediments and employing the proposed solutions could significantly improve the quality of clinical skills training.

Key words: Clinical skills, Medical education, Curriculum reform, Catalogue, Portfolio.

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Results

Comparing the training from the mid of the last century with a contemporary one we tried to identify the improvements that have been made and the major obstacles that are still present. To facilitate this analysis we arranged all components and features we found important in a table and compared the two ways of training in two different systems, with a time-span of forty years (Table 1).

Table 1 Main clinical skills training components in two curricula, 40 years apart

Training component	Sarajevo, ex-Yugoslavia, 1968	Heidelberg, Germany, 2008
Teaching facilities		
Amphitheaters, seminar rooms	Fair	Excellent
Clinical skills labs	Non existent	Poorly equipped
Training in the laboratory	Non existent	Rarely available
Training on small animals	Non existent	Available
Teaching tools		
Standardized patients	Non existent	Available, mostly in development
Mannequins, basic	Non existent	Available
Mannequins, interactive	Non existent	Available, to some extent
Textbooks	Scarce	Abundant
Catalogue of skills	Non existent	Non existent
Portfolio of competencies	Non existent	Non existent
Practicum of skills	Non existent	Non existent
Libraries	Poor	Excellent
Inter-library loans	Non existent	Excellent
Access to journals	Poor	Excellent
Multimedia	Non existent	Abundant
Interactive software	Non existent	Scarce
On-line resources	Non existent	Unlimited
Teaching staff		
Senior	Distanced	Distanced
Junior	Poorly motivated, rarely available	Poorly motivated, available
Teaching methodologies		
Traditional	Yes	Yes
New teaching (PBL, CBL)	No	Yes
Assessment, formative	No	Yes, sporadic
Assessment, summative	Yes	Yes
Bedside practice		
Organization	Chaotic	Well-organized
Practical work	Rare occasions	In some extent
Patients' cooperation	Limited	Restrained
Skills learnt	Small number	Limited number
Skills assessment	Non-existent	Poor
Achieved competencies	Not satisfactory	Not satisfactory

Obviously, considerable advancement was achieved in training of clinical skills. Diligent efforts of hundreds scholars, combined with introduction of high technology, resulted with the substantial changes in this subject (Box 1). Still, when dealing with clinical skills, as well as medicine in general, it is good to permanently bear in mind that technology is almost always a tool, rarely an answer (1-3).

No one can deny that many aspects of clinical skills teaching have been radically changed and that considerable advancement

was achieved over the last 40 years; still, it seems that there is room for further improvements. Based on the overview of data collected in the previous text and summarized in Table 1, we composed a list of obvious advancements and improvements (Box 1), and another list with identified the principal obstacles and impediments (Box 2). These impediments remained the same and did not improve much with the passage of time. We intend to focus our full attention on them, because we believe that some of those impediments can be corrected with carefully planned action.

Box 1 Major advancements in teaching of clinical skills

Teaching facilities
- Clinical skills laboratories

Teaching tools
- Textbooks
- Libraries
- Inter-library loans
- Journals
- CDs and DVDs
- Multimedia
- Traditional software
- Interactive software
- On-line resources
- Standardized patients
- Mannequins

Teaching methodology
- Problem Based Learning
- Case-related Learning

Assessments
- Formative
- Objectively Structured Clinical Examination (OSCE)
- Short answer test (SAT)

Box 2 Major impediments in teaching of clinical skills

Institutional value system
- Non-existent rules of conduct for Faculty
- Insufficient teaching staff motivation
- Non-existent mentorship

Structure and organization of training
- Poor structuring of curriculum
- Fixed schedule of the hands-on practice
- Inappropriate training dynamics

Missing tools
- Catalogue of clinical skills
- Practicum of clinical skills
- Portfolio (logbook) of acquired clinical skills
- Inadequate patients' participation and cooperation

Discussion

At present time, a fair number of students receive their diplomas and licenses to practice still having smaller or larger gaps in their skills arsenal. The teachers are aware of this problem, but they have no adequate response and are prone to neglect it, consoling themselves that the student will master this or the other skill at some later time, during internship or residency. Unfortunately, this "later time" sometimes never comes – and as a consequence we have all witnessed tragic mistakes made by partially competent doctors. We believe that in this part of education rules have to be simple, strict and uncompromising: nobody can receive a diploma and a license to practice, if he or she does not master, to full extent, the essential and most vital clinical skills.

What comprises these *essential skills* could be a matter of discussion for learned men and women, but once a comprehensive list is created there should be no more room for negotiation and compromise – every educated medical doctor must possess this fundament to start building the personal tower of knowledge. In the following text we propose a possible approach to this problem.

Institutional value system

Even in the most distinguished and highly ranked medical schools and teaching hospitals, teaching is handicapped by the institutional value system. Research accomplishments and generation of clinical revenues are rewarded; excellence and innovations in teaching are neglected and underestimated. A recent editorial in New England Journal of Medicine stressed, "*the harsh, commercial atmosphere of the marketplace has permeated many academic medical centers. Students hear institutional leaders speaking more about "throughput," "capture of market share," "units of service," and the financial "bottom line" than about the prevention and relief of suffering. Stu-*

dents learn from this culture that health care as a business may threaten medicine as a calling" (4). As a consequence, the education of medical students was reduced to a byproduct of the operation of academic health centers (4). To quote the advice of a chairman of internal medicine department at a prestigious medical school, "*If you want to teach, do so at lunch – and keep your lunches short*" (5). Fewer and fewer clinical faculty members are willing to serve as teachers and mentors, being under permanent pressure to be '*clinically productive*,' what is just another euphemism referring to the amount of fees generated. Who are the enthusiasts, under these circumstances, to volunteer for an academic career? New times reshape old values and worship new heroes, whether we like it or not.

Rules of conduct for faculty

Substantive reform will be possible only if there is a strong willingness to support the educational mission. Visionary leadership will be needed to change the prevailing culture; institutional values may and must be changed (3). To preserve '*old values*', community of teaching hospitals should reach a mutual agreement and declare solemnly that the teaching of new generations is a noble and sacred task for benefit of entire society, equally important as healing and research. In accordance to such a policy, each of the teaching hospitals should develop an internal set of acts and regulations, which will support the teaching with an adequate financial input and career promotion mechanisms. Same set should clearly state mechanisms for control of the teaching process, regular assessment and evaluation of teaching staff, including the students' surveys.

Teaching staff motivation: mentorship and teaching credits

It seems that in both high-income and low-income countries, the main problem is low interest and the lack of motivation of young medical school graduates to follow an academic career in medicine. A recent international survey of 806 of the 2200 (37%) members of the Association for Medical Education in Europe identified that the main challenges in medical education were lack of academic recognition (40%), funding (36%), faculty development (24%), lack of time for medical education issues (22%), and institutional support (21%) (5). Introduction of well-structured mentorship and 'teaching credits' could motivate the faculty.

Given that most professionals respond to incentives, it is obvious that hospital authorities, who take pride in the management of outstanding teaching hospitals, have to develop means and ways to encourage, support and reward good teaching. In the long run, this novel approach could be considered as a sound investment; without outstanding teaching, one can hardly expect highly competent physicians, on which the flow of hospital's revenues, patients intake and research achievements depend.

There still are a number of enthusiasts who devote their time and energy to teaching, even if the reward and glory are somewhere else, but this is not good enough. Apparently, something has to be changed in the very core of the structure (6).

Mentorship

The essential prerequisite in clinical training is "*a meaningful, ongoing relationship between faculty and students*" (6). Unfortunately, mentorship in the majority of institutions of today is "*either fragile or does not exist, and the progressive advancement of student competencies is not well guided across the curriculum*" (7). Without doubts, mentorship has to be reestablished to ensure adequate observation, supervision, and mentoring of students' professional development (5).

We suggest that students should be attached, at the very beginning of their stud-

ies, to a competent mentor, to instruct and coach them, and to monitor and assess their level of proficiency in clinical arts. We believe nobody would confront or argue this statement, but they will be quick with a substantial question, "*Where to find so many highly competent professionals, willing to be enrolled in such demanding task?*" The answer lies not in the persons but in '*the network,*' in cooperation across clinical specialties, in an "*interdisciplinary ownership of the clinical curriculum*" (8).

In proposed scheme, the mentor would be an experienced clinician–educator, competent to organize and manage a large network composed of preceptors, residents, clinical instructors, technicians and nurses. We are well aware that no single person today is able to demonstrate and instruct all complex skills requested in undergraduate curriculum; still such mentor has to be broadly skilled in the core competencies that transcend all disciplines of medicine. The other members of *the network* will be responsible for specific part of clinical curriculum, and instructs the student in an area of his expertise, from acquisition of basic skills to the most sophisticated ones. The principal role of a mentor is a supervisory one; he gradually and in continuity directs the students, oversees their progress, assesses their acquired competencies, and takes care that nothing of importance is missed or neglected.

An independent assessor should do the final, summative assessment of students' competency. The achieved results would reflect the quality of the mentor's work and serve, beside the teaching credits and other criteria discussed elsewhere in this paper, as a reliable basis for mentor promotion and advancement.

Teaching credits

An alternative (or additive) approach could be sought in *'the credits for good teaching practice*'. Over the past decades, life-long learning (LLL) and continuous medical education (CME) became values which cannot be challenged, and an inseparable part of every health professional life. Why not apply this well-functioning system to the field of medical education? The system could be developed in which every member of the teaching staff would need to collect credits awarded for research and publishing in education theory, through seminars and workshops of advanced teaching and assessment methodologies, etc.

Organization of training

Restructuring of curriculum

In most European and North American medical schools curriculum is artificially separated in two parts, preclinical and clinical. Consequently, the clinical skills are generally taught in senior years of study, and there is a heavy burden on students to master a large number of skills over a short period of time.

This problem could be at least partially solved if the training of simple skills started early in curriculum, even at the very beginning of education. The instructions should start with the simplest tasks of patients' care (such as positioning them in the bed, proper cleaning and skin care, control of antisepsis measures) gradually increasing the level of difficulty of the training, with acquisition of more and more complex skills. Heidelberg Medical School has a similar system, where medical students are engaged as nursing aides in the summer of the first two years. This is useful, but it can be significantly improved by additional structuring and introducing more advanced, *'physicians*' clinical skills at the same time. If medical schools accepted this reasoning, the curriculum would have to be restructured and training in clinical skills organized like a cascade of logical sequences, starting at the very beginning of medical study and ending when

all prerequisite skills are mastered. Final product of such educational practice should be a competent physician. If the suggested changes in curriculum were accepted, students, their mentors and clinical instructors would have more time for clinical training, which should be organized in several stages.

In the first phase, the clinical skills instructor would explain the rationale for the procedure, introduce the equipment, instruments and materials, and present the procedure in depth, gradually and in detail, using drawings and multimedia as well as real instruments and equipment. All instructors don't necessarily have to be doctors – many skills could be learnt by assistance of preceptors, nurses, technicians and other related health professionals, who are proficient in their fields of expertise.

In the second phase, the clinical instructor would practice a skill with students in the Clinical Skills Laboratory on mannequins, models or in virtual reality. At the end of this phase the instructor would inform student's mentor and confirm with signature that the student had mastered the skill well enough to be allowed to practice it in the clinical environment.

In the third phase, the clinical instructor would practice the same skill with students in a clinical setting, first showing them the complete procedure, and finally allowing them to perform under his supervision. When this exercise is performed in a satisfactory way, the instructor would guarantee with his signature that students have fully mastered the skill.

Finally, during the final assessment in all clinical disciplines, the senior assessors would have an opportunity to re-evaluate the students "Portfolio of acquired skills," estimate the students' level of competency and issue the final approval. Only in this way would no single essential skill would be neglected, and no single student would finish his or her studies with the idea that "*this or that skill will be acquired at some latter time*."

In order for the proposed model to succeed, mentoring is the single most decisive factor. Students need regular meetings with mentors to reflect on the information, diagnose the state of their competence, and set further learning goals. Evidence shows that portfolios improve the planning and monitoring of education by combining external assessment and self-assessment with mentoring. They enable the students to develop more challenging learning goals than is customary in traditional medical education (8).

Scheduling the bed-side teaching

In the majority of teaching hospitals, the strict scheduling of training is responsible for poor training outcome. At first sight, scheduling appears to be a trivial and an easy-to-solve matter, but the clinical teaching staff knows well that it is not so. The instructions must not interfere with routine hospital tasks. Besides, often the planned schedule for clinical practice does not match with availability of appropriate clinical cases for demonstration, and even the simplest demonstrations are not possible for myriad of reasons. The standardized patients cannot solve this problem even if they are very talented actors, for obvious reasons.

Another problem can be identified in the partnership between higher education institutions and service providers. Both systems are currently reluctant to devote the required resources and expect the other side to take responsibility for the clinical part of the curriculum Results of these weak and sometimes conflicting relationships result in the poor quality of clinical teaching.

What can at least in part solve this problem is, in our opinion, a flexible schedule able to adapt to given circumstances. As previously discussed, at the beginning of their undergraduate study students should be equipped with a list of skills that must be mastered, along with the name of the tu-

tor/clinical instructor who is responsible for this part of teaching. The task of planning the clinical time of students cannot be the responsibility of an administrative secretary. What we suggest is that students and their instructor plan the in-hospital activities together. Time and date should not be important - the fact that a specific skill has to be learnt should be the primary concern. Weather this happens in the morning or in the afternoon, on a workday or over the weekend, in one hour or over the course of a week – should not matter.

Tools and supplements

Catalogue of the knowledge and the clinical skills

A standard requirement in contemporary literature on medical education is that "*a graduate student should be competent and should posses the adequate clinical skills to examine the patients, to suggest the preliminary diagnosis and to propose the diagnostic plan and strategy*" (1, 4, 6, 7). In such manner, the medical students are uncompromisingly confronted with demand to master a large number of complex clinical skills, which will ensure all competencies necessary to perform in a satisfactory manner during their professional career. Again, there are many definitions of competence in medicine and a good one is a simple one: "*The competence in medicine is the habitual and judicious use of communication, knowledge, technical skills, clinical reasoning, emotions, values, and reflection in daily practice for the benefit of the individuals and communities being served*" (9). This citation is a nice example of flawlessly composed text, result of good and profound thinking. Unfortunately, we are afraid that such outstanding definition would not be of help to young students, who at the very beginning of education need simple and easy understandable answers to their basic dilemmas: what is it that everybody expects of them, where is the clear line between necessary knowledge and decorum and finally, when can one be confident that one is a competent doctor, ready to make decisions with profound impact on people's lives.

With those statements we wish to underline the significance of a *"Catalogue of the Knowledge and the Clinical Skills"*, in which most of the knowledge and skills that should be in possession of a young competent graduate would not only be listed, but also classified in relation to their significance. Apparently, each medical school has to develop its own system – from one part of the world to another the organization of medical care, diagnostic and treatment regimens, as well as pattern of disease, vary greatly. Composing the catalogue in accordance to before mentioned principles we would achieve two important goals: students would know exactly what they have to know and what is expected of them; teachers would know how to plan and structure their teaching.

Recently we started a program of radical curriculum reform (10-13), supported by Trans-European Program for Co-operation in Higher Education in Central and Eastern Europe (Tempus). Within the frame of this program we created the "The Catalogue of Knowledge and Clinical Skills". Thirteen medical schools from eight European countries joined expertise in this enterprise: Vienna, Austria; Gent, Belgium; Aarhus, Denmark; Heidelberg, Germany; Split and Zagreb, Croatia; Chieti, Italy; Ljubljana, Slovenia and 5 schools from Bosnia-Herzegovina (14).

Practicum of clinical skills

Composition of a comprehensive practicum of clinical skills is another important prerequisite for sucessful training of clinical skills. Many would argue that all clinical skills and techniques are already described in hundreds of texts, books and Internet sites. This is undeniably true, still it is surprising that

a detailed and comprehensive practicum, which in one place combines both the essential and the most complex skills, is a rarity on the medical textbook market, in contrary to hundreds existing books on anamnesis and physical examination. One praiseworthy exception is a recent introduction of "*Procedure Consult*", an innovative online clinical procedure reference tool (15).

To execute any given skill, a student should know why it is important, what the indications and contraindications for the procedure are, and which instruments, materials and equipment are necessary for its successful execution. The student needs to know how to explain the procedure to the patient and ask for informed consent, how to position the patient, what kind of anesthesia to apply, and how to handle the specimens for analysis. At first sight all of this can seem trivial and superfluous, but how can a student know if she or he should use the needle number 16 or 20? Moreover, such tiny details can make a crucial distinction between a successful and a failed procedure. This introduction should be followed by a step-by-step description of the procedure itself, with appropriate comments on anatomy and physiology, as well as a warning on possible complications, their prevention and management.

Such a practicum, integrated with the "Catalogue of Clinical Skills", would be a valuable tool. Last year we drafted and published the first edition of our Practicum of Clinical Skills (16), where we applied above mentioned principles. A practicum was organized around different clinical disciplines, and immediately it became clear that there would be plenty of overlapping, since some clinical skills (e.g. airway maintenance or insertion of intravenous line) are omnipresent and students should be able to use them everywhere at any time. We are aware that there is plenty of room for improvement and that a restructuring of our Practicum is necessary.

Portfolio of acquired clinical skills

It has long been observed that assessment drives learning. If we care whether medical students become skillful practitioners and sensitive and compassionate healers, we must employ all instruments we have at disposal today: self-assessment, peer evaluations, written assessments of clinical reasoning, standardized patient examinations, oral examinations, and sophisticated simulations. Most importantly, all results of the learner's work should be duly noted in portfolios. Rigorous assessment has the potential to inspire learning, influence values, reinforce competence, and reassure the public (8).

Permanent follow-up of student's progress during clinical skills acquisition is a prerequisite in the process of building a competent physician. Therefore, we propose that students receive a small booklet at the beginning of their training (tentative denomination could be "*Portfolio of Acquired Clinical Skills*"). In this booklet, all skills that are essential to the practice of contemporary medicine should be listed and classified. Having the *Portfolio* in possession, students will know from day one what to expect and what is *'the must'* if one aspires to become a competent medical doctor. Clear guidelines on the purpose, contents, and organization of the training are essential. Not less importantly, students would be able to plan in advance and set their own pace individually. As previously discussed, every acquired skill should be assessed by clinical instructors, first in the virtual then in the real setting. When a particular skill is mastered, this would be duly noted and acknowledged with the instructor's signature. The concept of combining formative professional development alongside summative assessment is a relatively new one and we believe that, if such approach is applied, nothing of importance would be neglected and the number of medical graduates who start their careers

with considerable gaps in their armamentaria would be significantly reduced (7, 8).

Patients as "the tools"

Cooperation with patients is instrumental in training of clinical skills. Today the market is overflowing with atlases, multimedia and interactive software perfectly able to create any clinical setting in realistic shape of virtual reality. Still, young doctors-to-be have to touch, feel, hear and smell how all this looks like in this real world of ours. Such necessity creates a serious problem, because during the last half century the patients' way of thinking and their attitudes radically changed.

Today the common patient is not a humble, grateful, and obedient one. Doctors are not the God-like creatures anymore and demand for a second (or third) opinion is a standard. Thanks to the Internet, patients believe that they know much more about their illness than any highly specialized expert. Any half-literate person has just to type the question into the machine, and in a few seconds hundreds, if not thousands of answers are readily available. What is the real quality and value of such information? This question is rarely asked. Not less importantly, the Pandora box was opened by greedy barristers, who were quick to advise the patients that the medical profession is full of ignorant and neglectful individuals, who should be brought to curt of law for any reason (21).

As a consequence, the relationship between medical professionals and patients (not rarely addressed in medical jargon as *consumers*) is, euphemistically speaking, tense and one of mutual distrust, in spite of an abundance of sweet words, smiles and warm hand shaking. Under described circumstances, what are the student's chances to palpate a lump in an unfortunate woman breast, or to palpate a breast without the lump at all? We are afraid slim at best: the doctor in charge of clinical teaching should have plenty of courage to suggest this examination, and hope that the lady who posses a lump will not claim sexual harassment and call her lawyers. Better is to forget the idea, students will learn once when they start their own practice. Is it good enough? The question is now.

Over the last years there were quite a few attempts to overcome this problem. Apparently, the use of mannequins, models and plastic parts of human body would serve in part, and would actually be indispensable in introductory lessons. In addition, there is an increasing number of good interactive software which creates a virtual reality and its quality is improving and advancing daily (1, 15). Third track was engagement of patients-actors, as we described in previous paragraphs of this paper.

We also wish to draw the attention of the audience to another possibility, rarely explored in the context of clinical skills training: *basic research*. At the first sight this statement could sound absurd, until we recall that the tedious work with small laboratory animals as mice, rats and cats can beautifully prepare the students for the clinical experience proper. In the laboratory the student can acquire relevant manual dexterity and technical ability, in addition to development of scientific thinking, which will certainly be useful not only in the clinical career but also in everyday life (18). Still, if we want to teach students how to sail, they have to experience the warmth of the sunshine on their skin, feel the blowing of the wind and enjoy the scent of the salt sea air. Only in such a manner they will survive in the storms, when the sea is rough and angry.

The solution to this issue cannot be an administrative one. In spite of the fact that every patient admitted to a teaching hospital understands that such treatment entails active participation in training, they generally dislike being "*the guinea* pigs" and prefer to avoid participation in teaching of students.

Who can blame them? At present, there are two sharply separated worlds - world of pain and suffering, inhabited with patients and their families, and a world of health professionals. Those two worlds need each other and cannot exist independently. A lack of confidence dominates in their communication; they speak different languages and can hardly understand each other. The improvement of a doctor-patient relationship based on compassion and mutual trust is the answer. As suggested in an editorial of Lancet, if the ability to feel compassion is missing and cannot be learned, paying attention to patients, respecting them, and being empathic towards them certainly can be (19, 20).

It would be a good start if we stop to address to our patients as "*clinical material*," "*managed care lives*" or "*consumers*" (21). Our view is that better understanding of patients' needs could arise from an immersion of medical students in patients' world. Obviously, nobody can expect of students to inoculate some germ, become ill and hospitalized. However, it is possible to merge first year students with patients by employing them as nurse aids, orderlies or porters. In such a way students will get a chance to see things from an opposite, more patient centered perspective, to see the pain, misery, confusion, and helplessness, and to give a moral support for people condemned to dependence and suffering (22). There is some hope that the students and doctors-to-be will look at their patients' from a different perspective. If this is achieved most of the patients will know that they are not "*the guinea pigs*" but respectable humans beings and would gladly participate in education of young doctors.

Concluding remarks

As it is possible to see from our recall of '*good old times,*' the problems are not new and they are still in good health. All of them were addressed over the past times by many learned men. To end this article, we will list some of possibilities at disposal for some problems-solving (Box 3).

Box 3

Possible solutions for bettering the training of clinical skills

1. Willingness to reconsider the institution's value structure, with new Rules of Conduct;
2. Re-introduction of the firmly structured mentorship;
3. Cross-departmental ownership of clinical curriculum;
4. Publishing of a set of clear-cut Catalogue, Practicum and Portfolio;
5. Formal relation of teaching staff status and promotion with good teaching;
6. Mix the students with the patients' world; and
7. Independent external assessment of acquired skills and overall competency (on state or national level).

We hope that this paper will be understood as a call for discussion, not as suggestions or ready-made recipes, with hope that after another 40 years one will not see the same problems which are still present today.

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Management of multiple myeloma in the era of novel drugs

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Multiple Myeloma (MM) is a malignancy of terminally differentiated plasma cells and is the second most common hematological neoplasm to Non-Hodgkin's Lymphoma. Generally, it is disease of older patients. Our knowledge about the underlying biological and cytogenetic abnormalities leading to MM is rapidly increasing. Similarly our ability is improving to treat this complex disease. A number of new treatments have been introduced into our armamentarium in the past 10-15 years. Until recently, high rates of complete responses (CR) and other major responses were seen only in patients undergoing treatment with high dose chemotherapy with autologous stem cell support (HD+ASCT). However new regimens, incorporating new agents (thalidomide, lenalidomide, bortezomib) are now offering similar response rates and lower toxicity than HD+ASCT. The new agents seem to combine well with classical chemotherapy agents (melphalan, cyclophosphamide), modern chemotherapy (pegylated liposomal doxorubicin) and steroids (dexamethasone, prednisone). In addition, the novel agents show significant activity when combined with each other in patients with newly diagnosed as well as relapsed/refractory MM patients. Although this is still considered an incurable disease, the life expectancy and quality of life of MM patients is continuously improving. Our hope is that progress in this area of research will continue with the advent of new treatment options and will lead to the ultimate goal: a cure.

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Introduction

Multiple myeloma (MM) results from the clonal proliferation of plasma cells arising in the lymph nodes and "homing" to the bone marrow where these cells localize and proliferate. It represents the second most common hematological malignancy. Multiple myeloma is a neoplastic disorder of plasma cells that accounts for 10% of all hematologic cancers in Caucasians and 20% in African Americans (1). Annually, this malignant disease causes over 19,000 deaths in Europe. Approximately 19,920 new cases of MM

were diagnosed in the United States in 2008, with 10,690 deaths, representing almost 2% of all cancer deaths (2). The median survival of patients with multiple myeloma is 3 to 5 years (3). Persons affected by MM are often elderly, with a median age at diagnosis of 65 years; 80% of patients are older than 60 years and less than 3% are younger than 40 years (4, 5). The disease is twice as common in African Americans as in Caucasians. MM is one of the leading causes of cancer death in African Americans. MM is one of three cancers that show increased mortality rates for both men and women in the 1990's (5.6% and 3.8%, respectively) (6).

The disease is characterized by overproduction of a patient-specific intact monoclonal immunoglobulin (Ig) heavy and/or light chain (paraprotein or M-protein). IgG is detected in about 53% of MM cases and IgA in about 25%; 40% of these IgG and IgA patients also have Bence Jones proteinuria. Light chain MM is found in 15 to 20% of patients; their plasma cells secrete only free monoclonal light chains (κ or λ) that can be detected by the Serum Freelite Assay or as Bence Jones proteins; serum M-components are usually absent on electrophoresis. IgD MM accounts for about 1% of cases. IgM and IgE as well as non-secretory MM are rare (4, 7, 8). Aside from the serum and urine M-proteins, other features in MM patients include anemia (80% of patients), bone pain (70% of patients) due to lytic lesions, renal dysfunction (25% of patients), hypercalcemia, increased susceptibility to infection, and constitutional symptoms (4). Other less common complications include spinal cord compression due to medullary and extramedullary plasmacytomas or vertebral collapse, peripheral neuropathy, amyloidosis, and hyperviscosity syndrome (4, 7, 8).

Durie and Salmon developed a clinical staging system for MM based on a combination of factors that correlate with myeloma cell mass (9). Most patients (40 - 60%) present with advanced (Stage III) disease (4). Other alternative staging systems have been proposed (10). Recently, an International Staging System using two simple blood tests (beta-2-microglobulin and albumin levels) was developed, based on data from 11,171 patients (11). These staging methods do not take into account newer diagnostic studies such as Serum Freelite Assay, cytogenetics of focal lesions versus bone marrow, or gene expression profiling (GEP), all of which could have potential prognostic significance (12-15). A high plasma cell labeling index (PCLI) and elevated B2-microglobulin predict poor prognosis in an untreated patient, but may not be as important in a previously treated patient. If both markers are low, the median survival is about six years in untreated patients. The correlation between response and survival has been evaluated in multiple studies. Although there is no absolute agreement among all, it seems that achieving complete response (CR) or very good partial response (VGPR) are important prognostic factors for long-term survival (16-20). In the South Western Oncology Group (SWOG) large retrospective analysis of 1,555 MM patients treated with standard-dose chemotherapy, the magnitude of response, as a single variable, did not predict survival duration. However, the best indicator of survival was time to first progression (21). Important prognostic variables for response to therapy and survival include the patient's age, stage, immunoglobulin type, ß-2-microglobulin level, PCLI, and the presence of circulating plasma cells. A poor outcome is associated with chromosome 13 deletion or hypodiploidy on conventional karyotyping, deletion of 17p – or immunoglobulin heavy chain translocation t (4:14) or t (14:16) on molecular genetic studies and plasma cell labeling index of 3% or higher (15, 22).

MM is almost always preceded by monoclonal gammopathy of undetermined significance (MGUS), an asymptomatic phase

characterized by a relatively small burden of clonal cells and low levels of monoclonal protein (23). The diagnosis of active symptomatic MM requiring therapy should be based on the end-organ effects of the disease (elevation of ***C***alcium, ***R***enal insufficiency, ***A***nemia or ***B***one disease).

Therapeutic approach

The role of high-dose therapy and peripheral stem cell transplant (HD+ASCT) continues to be controversial, with overall survival (OS) only minimally improved if any (24, 25). In a recent review and meta-analysis of 9 randomized controlled studies involving 2,411 patients, single HD+ASCT was compared with conventional chemotherapy and was found to benefit progression free survival (PFS), but not OS (26). The risk of treatment-related mortality (TRM) was increased three-fold with HD+ASCT. It is worth mentioning that in this analysis HD+ASCT was compared with classical chemotherapy, not new agents or their combinations. Patients with progressive disease can achieve a 50-75% response rate to salvage regimens such as vincristine, doxorubicin, and dexamethasone (VAD) (27, 28); however, these responses are often short-lived. Even in transplant-eligible and willing patients initial therapy has undergone a sea change in the past decade (29). Before the advent of drugs such as thalidomide and lenalidomide (IMiDs) or bortezomib, single agent dexamethasone (dex) and VAD were the most commonly used treatments (28,30,31). From 2005 to 2009 numerous Phase 2 and 3 trials compared different combinations of drugs used as induction therapies prior to HD+ASCT (32). These clinical trials showed the clear advantage of newer agents over classical chemotherapy in improving PFS and response rates, but a mixed picture regarding OS, particularly in clinical trials incorporating high dose melphalan as a consolidated approach (33-35). The first study ever to show the survival advantage of a chemotherapy combination over HD+ASCT was a French study (IFM 99-06) using a combination of a new agent (thalidomide) with classical melphalan and prednisone (MTD), published in 2007 by Thierri Facon and collaborators (36). In this study MM patients treated with MTD had longer OS when compared with Mel-100 HD+ASCT. It is worth mentioning that Mel-100 HD+ASCT is not considered standard care for MM patients by most transplant physicians and could be inferior to standard Mel-200. Despite this caveat, IFM 99-06 opened the door for very intensive investigation into the role of new agents as a primary treatment or cytoreductive therapy prior to HD+ASCT.

Proteosome inhibitors

The ubiquitin-proteasome pathway is the principal pathway for intracellular protein degradation (37, 39) (Figure 1).

This pathway selectively degrades an extensive number of short-lived regulatory proteins involved in the control of normal cellular processes. In order to be degraded, proteins targeted by the ubiquitin-proteasome pathway are covalently tagged by polyubiquitination, via a three-step enzymatic process, which ultimately leads to their recognition and degradation, by the 26S proteasome in a highly specific and regulated manner. This process is accomplished by the sequential action of three enzymes: an ATP-dependent ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2) and an ubiquitin-protein ligase (E3) (39). This cascade covalently links the C terminus of ubiquitin to a free amino group on the target protein, usually the ε-amino of a lysine residue.

The 26S proteasome is comprised of a catalytic proteolytic core (20S) and an activator (19S) (Figure 2).

It plays a vital role in degrading regulatory proteins that govern many signaling pathways, including the cell cycle, transcription factor activation, apoptosis, and pathways that regulate the expression of proteins, which direct angiogenesis, cell trafficking,

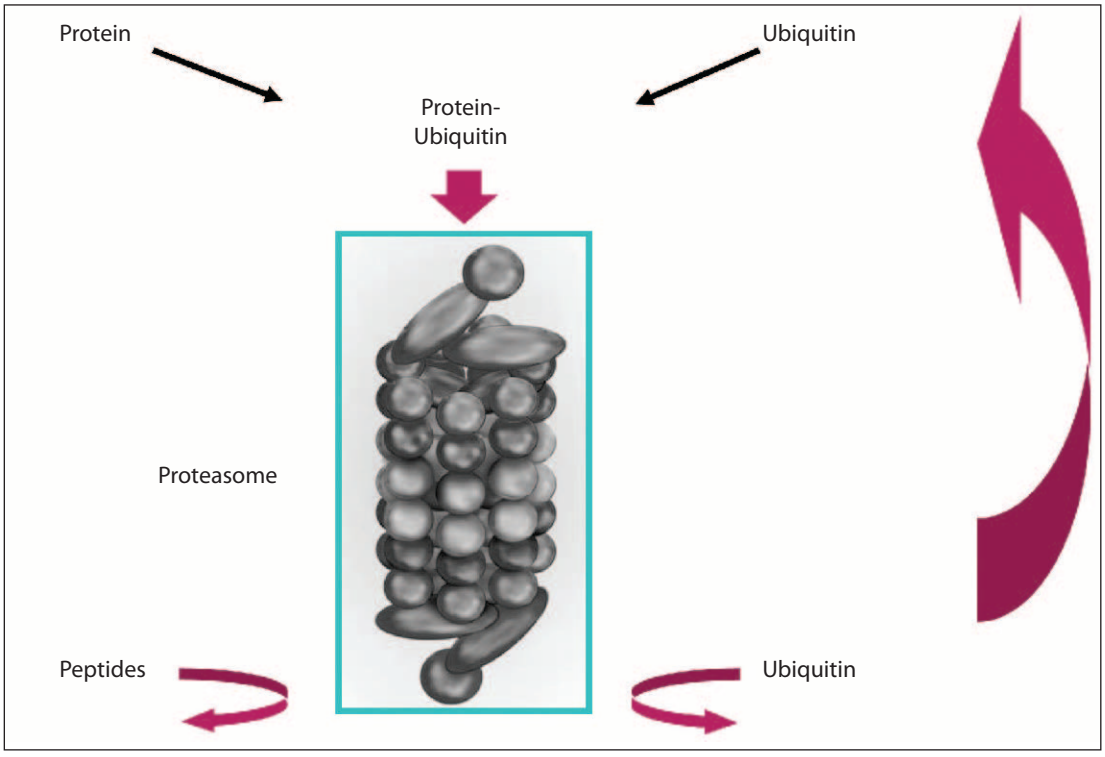

Figure 1 The Ubiquitin-Proteasome degradation pathway

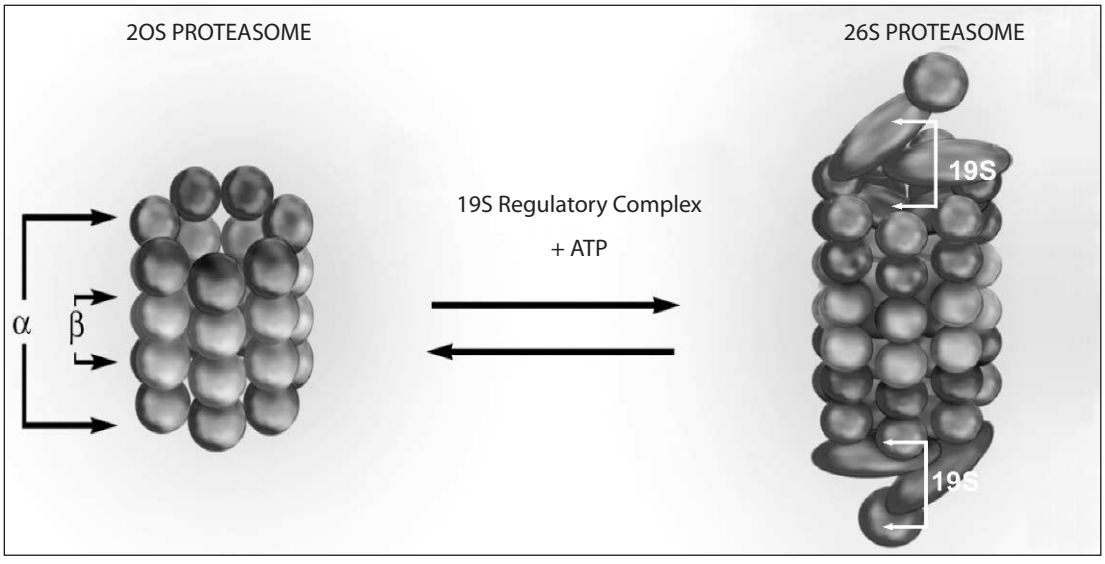

Figure 2 The structure of the proteasome. The 20S core (left) is capped by two 19S regulatory units to form the 26 proteasome (right)

and metastasis (38, 40). Polyubiquinated proteins cannot be degraded directly by the active catalytic proteolytic core (20S). Rather, proteolysis requires another protein, known alternatively as PA700, ball, 19 S cap or μ-particle (39). It is the 700,000 dalton, 20-subunit complex that binds to one or both of the terminal rings of the proteasome in a cooperative manner. This integral role of the 26S proteasome in cellular signal transduction has provided a new target for exploring the therapeutic potential of proteasome inhibition in neoplastic diseases. It is known that several key regulatory proteins relevant to cancer initiation and progression are known to be temporally degraded during the cell cycle by the ubiquitin-proteasome pathway. Ordered ubiquitination and degradation of regulatory proteins is required for the cell to progress through the cell cycle, undergo mitosis and proliferate. Similarly, the proper function of specific ubiquitin ligases responsible for the ubiquitination of these same proteins is required for key cell cycle transitions. Aberrant degradation of cell cycle control proteins can result in accelerated and uncontrolled cell division, thereby promoting cancer growth. Recent evidence from studies reveals that expression of the ubiquitin-proteasome pathway enzymes is elevated in tumor samples. The cyclin and the cyclin-dependent kinase inhibitors p21 cip1 and p27 kip1 are an example of growth regulatory proteins degraded by proteasome-dependent proteolysis (41, 42). Both p21 cip1 and p27 kip1 can induce cell cycle arrest through functional inhibition of cyclin D-, E-, and A-dependent kinases (42). In addition, the p53 tumor suppressor required for cell cycle control and initiation of apoptosis induced by cellular damage, including ionizing radiation and chemotherapy, is also a substrate of the the ubiquitin-proteasome pathway (42, 43). Hence, proteasome inhibition has the potential to arrest the cell cycle in cancer cells through the disruption of a large number of growth regulatory pathways.

The ubiquitin-proteasome pathway also plays an important role in the regulation of many transcriptional responses. On the other hand, proteasome function in the cell can be regulated by altering levels of proteasome, proteasome regulatory proteins, or proteins of the ubiquitin conjugation system.

The relationship between proteasome function, gene transcription and potential cancer therapy is best understood for the transcription factor nuclear factor-kappa B (NF-κB) (Figure 3).

NF-κB activation is regulated by 26S proteasome-mediated degradation of the inhibitor protein I-κB (44, 45). NF-κB activation is integral to many aspects of tumorigenesis, such as tissue invasion and metastasis, angiogenesis, evasion of apoptosis, cell growth, and survival (46). Activation of NF-κB can proceed through multiple mechanisms, including autocrine or paracrine extracellular cytokine signaling, upstream oncogenic signaling mutations in NF-κB and/or I-κB, and in response to DNA damage. Cell adhesion molecules such as E-selectin, ICAM-1, and VCAM-1, as well as IL-8, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs) are regulated by NF-κB and have been implicated in tumor metastasis and angiogenesis in vivo (45, 47). Furthermore, NF- κB is required in numerous cell types to maintain and control cell viability via the production of anti-apoptotic survival proteins such as cellular inhibitors of apoptosis (cIAPs), and the B-cell lymphoma-2 (Bcl-2) family of proteins. NF-κB also plays a role in cell proliferation by activating target genes of the cell cycle such as D1-cyclin, and growth factors such as interleukin-6 (IL-6) (46). It has been demonstrated that blocking NF-κB activation by stabilizing its inhibitor, I-κB, sensitizes cells to environmental stressors and cytotoxic agents, ultimately leading to apoptosis (48,

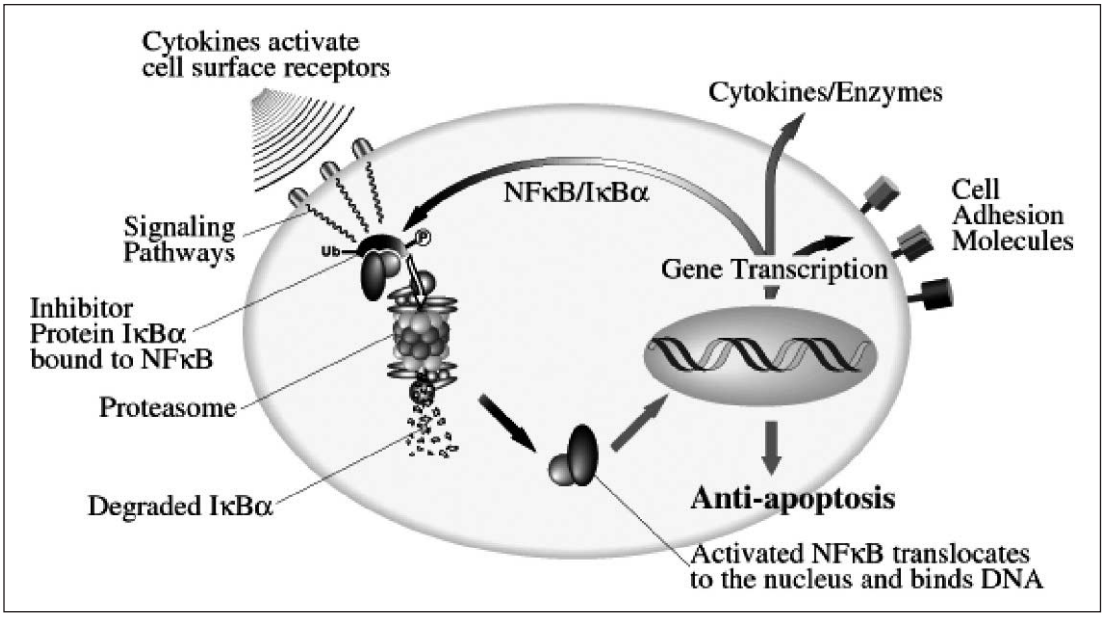

Figure 3 Nuclear factor κB (NF-κB) and its role in the cell.

49). Hence, regulation of NF-κB dependent transcriptional regulation and activation through proteasome inhibition can impact several cancer virulence mechanisms.

Anti tumor activity of proteasome inhibitors

A number of investigators have shown that inhibitors of proteasome, including aldehydes and lactacystin, are growth inhibitory and cytotoxic for cells in culture (50). Boronate proteasome inhibitors have been shown to kill tumor cells in culture as demonstrated in NCI tumor cell line screen (37). The NCI utilizes an *in vitro* screen comprised of 60 human tumor cell lines derived from 9 different cancer types (leukemia, lung, brain, colon, melanoma, ovarian, prostate, renal and breast). Data from the NCI screen showed that proteasome inhibitors have a mechanism of cytotoxicity unlike any other compound in the NCI database of 60000 compounds. Among the large number of proteasome inhibitors, bortezomib was selected for intensive study based on its selectivity and chemical and biological characteristics (37). Bortezomib specifically, selectively and reversibly inhibits the proteasome by tightly binding to the chymotrypsin-like site of the 20S core of the enzyme. By inhibiting a single molecular target, the 26S proteasome, bortezomib has the potential to affect multiple signaling pathways. The anti-neoplastic effects of bortezomib likely involve several distinct cell regulatory mechanisms as discussed above, including inhibition of cell growth and survival pathways, induction of apoptosis, and inhibition of gene expression integral to cellular adhesion, migration, and angiogenesis. Thus, the mechanisms by which bortezomib elicits its anti-tumor activity may vary among tumor types, and the extent to which each affected pathway is critical to the inhibition of tumor growth could also differ.

It has been demonstrated that bortezomib has a novel pattern of cytotoxicity in NCI in vitro and in vivo assays (37) and displays cytotoxic activity in a variety of xenograft tumor models, both as a single agent

and in combination with other chemotherapeutic agents and radiation (37, 51-55).

Numerous published reports show that cancer cells are more sensitive to the pro-apoptotic effects of proteasome inhibition than nontransformed cells (56-62). For example, the toxicity of bortezomib for multiple myeloma (MM) cells was more than 100-fold greater when compared to peripheral blood leukocytes and normal hematopoietic cells (56). Bortezomib has shown direct cytotoxic activity against a variety of MM cell lines and in freshly isolated cells from patients (56, 57). Significantly, these studies have included myeloma cells that are highly resistant to other chemotherapeutic agents. Time-dependent exposure to bortezomib programs MM cells to commit to apoptosis (63). It was shown that this drug directly inhibits proliferation and induces apoptosis of human MM cell lines and freshly isolated patient MM cells, inhibits mitogen-activated protein kinase growth signaling in MM cells, induces apoptosis despite induction of p21 and p27 in both p53 wild-type and p53 mutant MM cells, overcomes drug resistance, adds to the anti-MM activity of dex and overcomes the resistance to apoptosis in MM cells conferred by Interleukine-6 (IL-6) (63). Bortezomib also inhibits the paracrine growth of human MM cells by decreasing their adherence to bone marrow stromal cells and related NF-κB-dependent induction of IL-6 secretion in bone marrow stromal cells, as well as inhibiting proliferation and growth signaling of residual adherent MM cells.

Clinical studies with bortezomib

Bortezomib (Velcade®) is the first proteosome inhibitor approved by FDA for clinical use. Initially, in 2003 it was approved as a third line therapy for MM patients. The present indication is for second line MM as a single agent, first line MM in combination with melphalan-prednisone (MPV) and for patients with relapsed mantle cell lymphoma.

In the fall of 1998, the first human trial with bortezomib was initiated at M.D. Anderson Cancer Center in Houston, Texas. In May 2003 bortezomib was approved by FDA as Velcade for Injection for the treatment of MM patients who had received at least 2 prior therapies and had demonstrated disease progression on the last therapy. Conditional approval was based mostly on the results of the Phase II study of bortezomib in patients with relapsed, refractory MM (SUMMIT) (64). In this study 202 patients were enrolled and 193 could be evaluated. Most (84%) had IgG or IgA MM and advanced disease at diagnosis. Eighty percent had symptoms of peripheral neuropathy at enrollment. Of the 193 patients, 178 (92%) had previously been treated with three or more of the major classes of agents for myeloma. Patients received 1.3 mg of bortezomib per square meter of body-surface area twice weekly for 2 weeks, followed by 1 week without treatment, for up to 8 cycles (24 weeks). In patients with a suboptimal response, oral dexamethasone (20 mg daily on the day of and the day after bortezomib administration) was added to the regimen. Of the 193 patients with measurable disease, 67 (35%) had CR, PR or minimal response (MR) to bortezomib alone. Nineteen patients had CR or near-complete response (NCR). This was first study showing significant complete responses to a single, non-chemotherapeutic antimyeloma agent. The median time to a first response was 1.3 months. The median time to progression of disease among all 202 patients while they were receiving bortezomib alone was 7 months, as compared with 3 months during the last treatment before enrollment. According to a landmark analysis, achievement of CR or PR to bortezomib alone after 2 cycles was associated with significantly longer survival than in other patients ($p = 0.007$). The most common

adverse events (AE) were gastrointestinal symptoms, fatigue, thrombocytopenia and sensory neuropathy. Drug related AE led to discontinuation of bortezomib in 36 patients (18%). The most clinically significant AE was cumulative, dose-related peripheral sensory neuropathy. Overall incidence of clinically relevant neuropathy (Grade 3) was 12 percent. However, complete resolution or improvement of peripheral neuropathy was observed in the majority of patients during the follow-up period. A second study (CREST) compared two different dosages of bortezomib (1 mg/m^2 vs. 1.3 mg/m^2) in relapsed/refractory MM patients. Responses were 33% and 50%, respectively. Median time to progression (TTP) was 10 and 10.9 months (65). Addition of dexamethasone to bortezomib in patients who failed to respond or who relapsed after treatment with bortezomib alone improved responses in 18% of patients in SUMMIT and 33% in CREST trial.

An international, randomized, multicentar phase 3 trial comparing bortezomib with high-dose dexamethasone (APEX) enrolled 669 patients with relapsed/refractory MM (66). Patients treated with bortezomib had significantly higher response rates (38% vs. 18%) and CR (6% vs. < 1%), longer TTP (6.2 vs. 3.5 months) than patients treated with dexamethasone. The one-year OS rate was 80% among bortezomib treated patients and 66% for dexamethasone treated ones (p = 0.003) with hazard ratio (HR) of 0.57. A recent update with extended follow up (22 months) showed again superior OS and overall response rates (ORR) as well as CR in the bortezomib treated group, despite substantial crossover from dexamethasone to bortezomib (67).

Bortezomib is now very successfully combined with other effective therapies for MM. In an international randomized Phase III study Orlowski and collaborators compared a combination of pegylated liposomal doxorubicin and bortezomib with single agent bortezomib in 646 MM patients with relapsed/refractory disease (68). Combination therapy was associated with longer TTP (9.3 vs. 6.5 months, p = 0.000004) and longer 15-months OS (76% vs. 65%, p = 0.03) when compared to bortezomib alone. Grade 3/4 AE were more frequent in the combination arm (80% vs. 64%). The most common side effects were neutropenia, thrombocytopenia asthenia, fatigue, diarrhea and hand-foot syndrome. The significance of this unique drug combination is that steroids were not part of the therapy, allowing use by the treating physician in older patients with intolerance to steroids as well as in hard to control diabetics.

Combination of bortezomib with melphalan and prednisone (VMP) was compared in 682 MM patients with newly diagnosed disease with classical MP in a randomized Phase III clinical trial VISTA (69). Patients treated with VMP had significantly longer TTP (24 vs. 16.6 months; p< 0.001) and median duration of response (DOR) (19.9 vs. 13.1 months). After a median follow-up of 16.3 months, 13% of patients in the VMP and 22% in the MP group had died (HR = 0.61; p = 0.008). At the time of publication, median OS had not been reached in either group. Grade 3 AE occurred in a higher proportion of patients in the VMP than in the MP group (53% vs. 44%; p = 0.02), but there were no significant differences in grade 4 events (285 vs. 27%, respectively) or treatment-related deaths (1% and 2%). At present, VMP could be considered the standard of care therapy for newly diagnosed MM patients who are not candidates for or are refusing HD+ASCT. However, some myeloma specialists would consider other MP combinations, such as one with thalidomide (MPT) or lenalidomide (MPR) as a possible standard of care.

Tables 1 and 2 summarize response rates in selected Phase II and III studies using bortezomibe as a single agent or in combination in newly diagnosed and relapsed/refractory MM patients.

Table 1 Bortezomib combinations in newly diagnosed MM patients

Regimen	Phase	N	PR	CR	Reference
+ melphalan + prednisone	III (VISTA)	344	+ CR 71	30%	San Miguel et al. (69)
+ thalidomide + prednisone	III	128	+ CR 79%	27%	Mateos et al.(117)
+ melphalan + prednisone + thalidomide With maintenance	III	254	+ CR 86%	34%	Palumbo et al. (118)
+ Cytoxan® + dex	II/III	400	+ CR 84%	10%	Einsele et al. (119)

N = number of patients; PR = partial response; CR = complete response; dex = dexamethasone

Table 2 Bortezomib in relapsed/refractory MM patients

Regimen	Phase	N	PR	CR	Reference
Single agent	III (APEX)	333	+ CR 38%	6%	Richardson et al. (66)
+ dex	International III b	208	+ CR 51%	+ VGPR 33%	Mikhael et al. (120)
+ Doxil®	III	324	+ CR 44%	+ nCR 13%	Orlowski et al. (68)
Single agent	II (SUMMIT)	193	+ CR 27%	+ nCR 10%	Richardson et al. (64)
Single agent (1.0 and 1.3 mg/m^2)	II (CREST)	54	33-50%	not reported	Jagannath et al. (65)
+ temsirolimus	II	39	+ CR 36%	+ nCRB 10%	Ghobrial et al. (121)
+ Cytoxan® + dex - intermediate dose	II	64	+ CR 82%	16%	Kropff et al. (122)

N = number of patients; PR = partial response; CR = complete response; VGPR = very good partial response; nCR = near complete response; dex = dexamethasone

Recent studies employed bortezomib as part of cytoreductive regimens prior to autologous stem cell transplants with great success (69-72). New generation proteosome inhibitors (NPI-0052-Salinisporamide A; PX-171-carfilzomib, CEP-18770), as well as IκB inhibitors (PS-1145, MLN120B) are already going through clinical and pre-clinical studies and seem to be very promising.

Immunomodulators (IMiD's)

The IMiDs® are a group of unique, orally bio-available agents that have been refined, using thalidomide as a structural template (Figure 4).

Modification of the thalidomide structure through removal of a carbonyl on the ring formed lenalidomide (CC-5013, Revlimid®), and the addition of an amino group at the 4 position of the phthaloyl ring formed pomalidomide (CC-4047). These IMiDs® were specifically designed to enhance the immunomodulatory and anticancer properties of thalidomide with fewer side effects. Preclinical studies have shown that lenalidomide and pomalidomide are 50,000 times more potent, in vitro, than thalidomide at inhibiting tumor necrosis factor alpha (TNF-α) (73, 74). Studies have revealed that IMiDs® not only inhibit angiogenesis, but also stimulate T-cell proliferation and in-

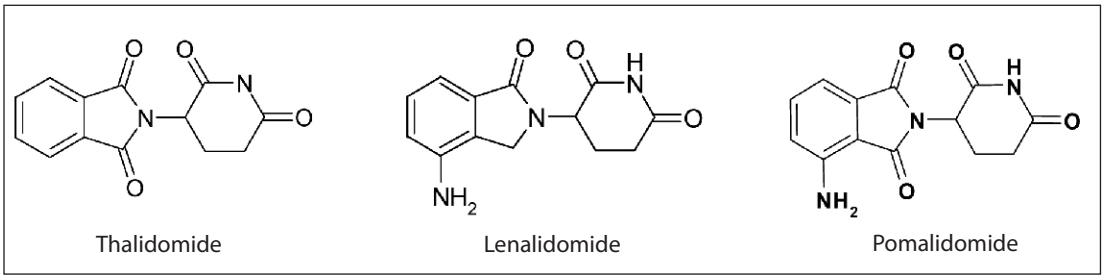

Figure 4 Molecular structure of thalidomide, lenalidomide and pomalidomide

duce apoptosis and growth arrest in resistant myeloma cells (75-77). These compounds also prevent the adhesion of myeloma cells to bone marrow stromal cells, and thereby inhibit the enhanced secretion of migratory factors, such as interleukin (IL)-6, TNF-α, and the vascular endothelial growth factor (VEGF) (78-84). Lenalidomide has more potent activity than thalidomide in the preclinical setting (73, 83), and has also demonstrated impressive clinical activity in both newly diagnosed and relapsed or refractory MM (85-88). Pomalidomide also demonstrates potent activity against TNF-α in vitro, indicating greater synergy than lenalidomide with rituximab in vivo (89). It also promotes T-cell differentiation and cytokine production via the transcription factor T-bet (90), and has demonstrated promising activity in clinical trials (91, 92).

The discovery that thalidomide had anti-angiogenic (93) and T-cell co-stimulatory (94) activity led to the clinical investigation of thalidomide for therapy in MM. In relapsed and refractory MM, thalidomide produced response rates of approximately 30% as a single agent (95). In newly diagnosed patients, thalidomide achieved response rates of 36% alone and 64-72% in combination with dexamethasone (96, 97). As a result, thalidomide in combination with dexamethasone received United States Food and Drug Administration (US FDA) approval for the treatment of newly diagnosed MM in 2006. In addition, recent phase III studies have investigated various thalidomide-containing regimens and reported improvements in quality of response with: thalidomide, adriamycine and dexamethasone compared to VAD (98); bortezomib, melphalan, prednisone and thalidomide (VMPT) compared to bortezomib, melphalan and prednisone (VMP) (99), melphalan, prednisone and thalidomide (MPT) compared to melphalan and prednisone (MP) (100), and bortezomib, thalidomide and dexamethasone (VTD) compared to thalidomide and dexamethasone (TD) (101). However, the encouraging effects of thalidomide are hampered by toxicity, which often compromises the dose or leads to discontinuation of therapy. Common adverse events include fatigue, somnolence, constipation, fluid retention, peripheral neuropathy, venous thromboembolism (VTE), and rash (95, 102, 103). Given the promising activity of thalidomide, synthetic analogs were developed and introduced in an effort to provide equal or greater immunomodulation, but a better tolerability profile. Clinical data indicate that the incidence of peripheral neuropathy, which is common with thalidomide, is low with lenalidomide and pomalidomide, (85, 92, 103-105).

Clinical studies with IMiD's

Studies among patients with relapsed or refractory MM have demonstrated that le-

nalidomide can overcome resistance to prior MM therapy, including thalidomide (106-108). In addition TTP and progression-free survival (PFS) are superior when lenalidomide is given at first relapse rather than later as salvage therapy (108). Two phase I trials of lenalidomide have demonstrated promising activity as well as decreased toxicity in heavily pretreated patients with relapsed or refractory MM (91, 106). These studies established 25 mg/day as the maximum tolerated dose (MTD) for lenalidomide in relapsed or refractory MM, and provided a firm foundation for continuing trials with lenalidomide, either alone or in combination with other active agents in MM.

Two large, randomized, phase III, double-blind, placebo-controlled clinical trials (North American MM-009 and European MM-010) have compared the efficacy and safety of lenalidomide plus dexamethasone (Len+Dex) with placebo plus dexamethasone in patients with relapsed or refractory MM (85, 86). In both trials, lenalidomide 25 mg/day or placebo was administered on days 1-21 of each 28-day cycle and oral dexamethasone 40 mg was administered on days 1-4, 9-12, 17-20 of each 28-day cycle. The MM-009 trial enrolled 353 patients (Len+Dex n = 177; placebo+Dex n = 176) and the MM-010 trial enrolled 351 patients (Len+Dex n = 176; placebo+Dex n = 175). The Len+Dex combination achieved a significantly ORR (MM-009: 61% vs. 20%; MM-010: 60% vs. 24%; both $p < 0.001$) and CR rate (MM-009: 14.1% vs. 0.6%; MM-010: 15.9% vs. 3.4%; both $p < 0.001$), (Figure 5).

The median TTP was significantly prolonged by the addition of lenalidomide to dexamethasone (MM-009: 11.1 months vs. 4.7 months; MM-010: 11.3 months vs. 4.7 months; both $p < 0.001$), (Figure 6) and the median OS was significantly longer in the Len+Dex arm (MM-009: 29.6 months vs. 20.2 months; $p < 0.001$; MM-010: not reached vs. 20.6; $p = 0.03$).

In the MM-009 and MM-010 studies, grade 3/4 hematologic AE were more common with Len+Dex and included neutropenia (41.2% and 29.5% vs. 4.5% and 2.3%, respectively), anemia (13.0% and 8.6% vs. 5.1% and 6.9%), thrombocytopenia (14.7% and 11.4% vs. 6.9% and 5.7%), and febrile neutropenia (3.4% vs. 0%). Other common grade 3/4 AE included infection (21.4% and 11.3% vs. 12.0% and 6.2%, respectively), and fatigue (6.2% and 6.8% vs. 6.3% and 3.4%). The incidence of VTE in the MM-009 and MM-010 studies was higher in the Len+Dex arm (14.7% and 11.4% vs. 3.4% and 4.6%, respectively); however, it was comparable to the incidence of 10% observed for the general MM population in retrospective analyses (109). On the basis of these studies, the US FDA approved lenalidomide in June 2006 and the European Medicines Agency in June 2007 for use in combination with dexamethasone in the treatment of MM in patients who have received at least one prior therapy.

Due to encouraging results in the relapsed or refractory setting, a phase II trial was undertaken to assess the efficacy and safety of the Len+Dex combination therapy in the front-line setting (104). In this phase II trial, lenalidomide (25 mg/day orally on days 1-21 of each 28-day cycle) was combined with dexamethasone (40 mg/day orally on days 1-4, 9-12, and 17-20 of each 28-day cycle) in 34 newly diagnosed, previously untreated MM patients. The ORR was 91%, with CR in 6% and very good partial response (VGPR) and near CR in 32%. Grade 3 or greater non-hematologic AE were reported in 47% of patients and included fatigue (15%), muscle weakness (6%), anxiety (6%), pneumonitis (6%), and rash (6%). Myelosuppression was minimal, most likely reflecting the preserved bone marrow reserve in this group of previously untreated patients. All patients were placed on low dose aspirin prophylaxis, based on the efficacy of

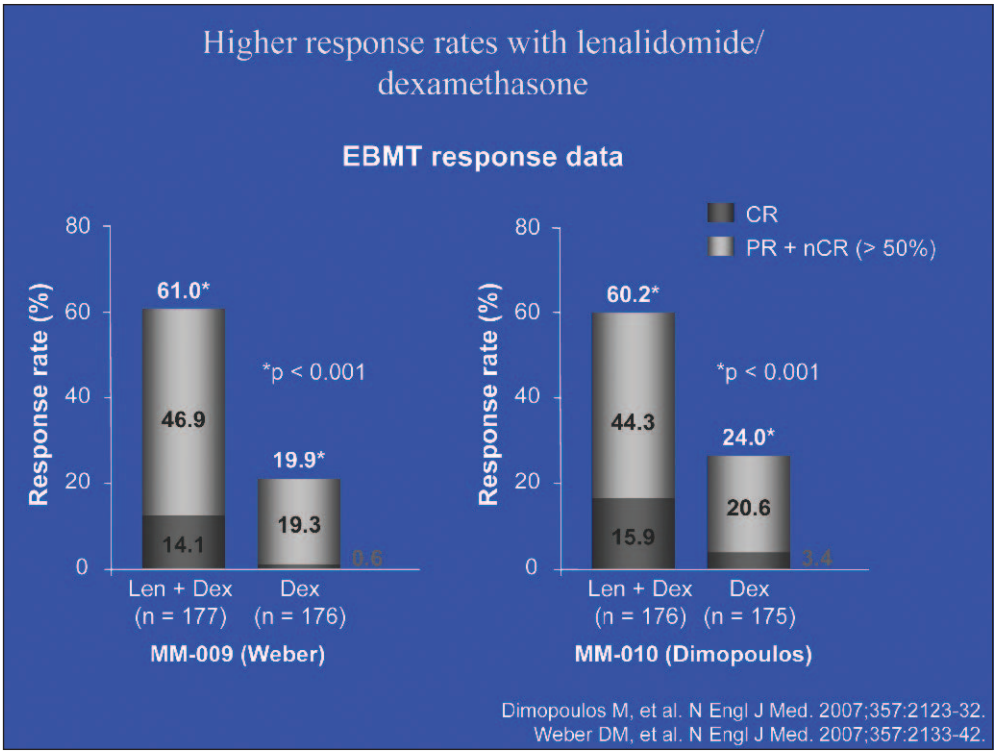

Figure 5 The European Group for Blood & Marrow Transplantation (EBMT) criteria-based response rates in MM-009 and MM-010 studies

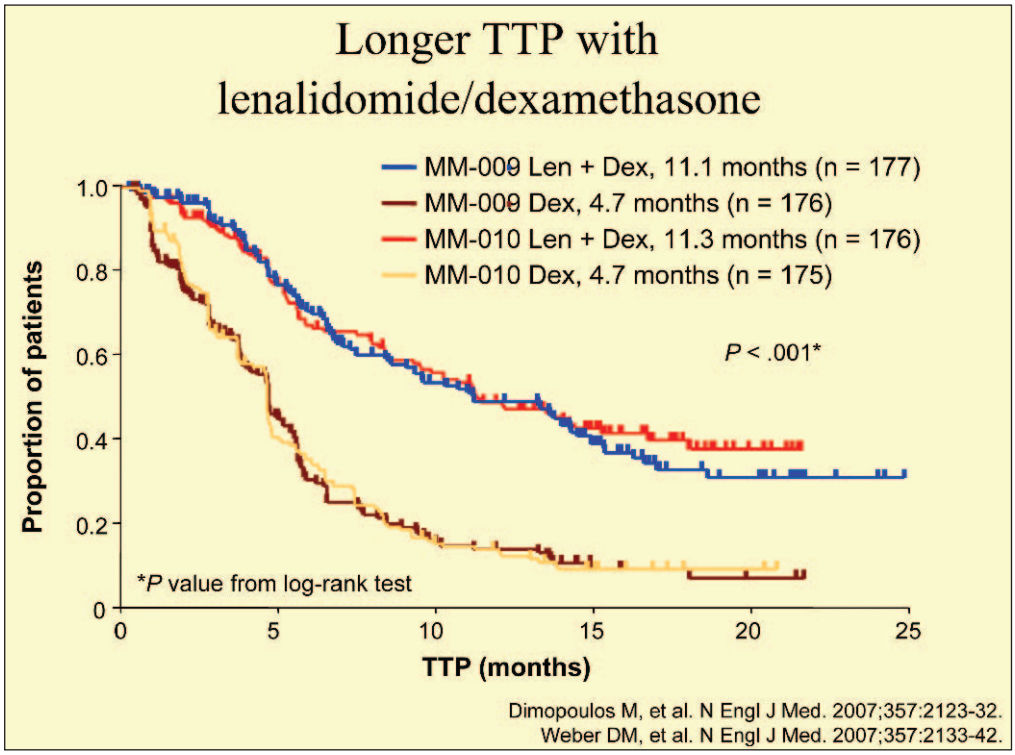

Figure 6 Time to progression (TTP) results in MM-009 and MM-010 studies

low dose aspirin in preventing VTE among patients treated on the thalidomide plus dexamethasone regimen (110), and only one patient developed a VTE. In addition, Len+Dex combination therapy appeared to be a useful pre-transplant conditioning regimen, as there was no adverse effect on stem cell mobilization among these patients.

With successful responses and better tolerability obtained from early trials, lenalidomide is rapidly being incorporated into front-line regimens. The Southwest Oncology Group (SWOG) and Eastern Cooperative Oncology Group (ECOG) performed randomized, phase III trials assessing Len+Dex as primary therapy in the front-line setting. The SWOG trial compared Len+Dex to dex alone in patients with newly diagnosed MM (87). In this study, 198 patients were randomized, 100 received lenalidomide 25 mg/day (28 of 35 days for 3 induction cycles, then 21 of 28 days as maintenance thereafter) plus dex 40 mg/day (days 1-4, 9-12, and 17-20 as induction, then days 1-4, and 15-18 as maintenance) and 98 received dex plus placebo. In 133 patients who were assessable for response, the ORR was significantly higher (85.3% vs. 51.3%; $p = 0.001$) and 1-year PFS was significantly longer (77% vs. 55%, $p = 0.002$) with Len+Dex. The 1-year OS was high and there was no difference between arms (93% vs. 91%). VTE was reported in 25% of patients treated with Len+Dex vs. 7% of patients treated with dex alone. Most patients (81%) who experienced VTE received aspirin as thromboprophylaxis, however it is to be noted that those patients received the full dose of aspirin at 325 mg daily which is known to be thrombogenic as it inhibits prostacyclin activity thus negating its anti-platelet role (111, 112). Patients in the dex arm who progressed were allowed to cross over to the Len+Dex arm. Of 40 patients who crossed over, the ORR in 23 who were assessable for response was 70.4%. These data confirm the superior efficacy with Len+Dex in newly diagnosed patients. Unfortunately, this study was prematurely closed when the results of the ECOG study E4A03 were announced and use of high-dose dex in combination with lenalidomide was no longer considered appropriate.

The ECOG trial compared lenalidomide plus standard-dose dex (RD) to lenalidomide plus low-dose dex (Rd), in an attempt to further diminish adverse events while maintaining the response rate. In this study, patients in the RD arm were treated with lenalidomide 25 mg/day on days 1-21 of each 28-day cycle and dex 40 mg/day on days 1-4, 9-12, and 17–20 of each 28-day cycle, and patients in the Rd arm received dex 40 mg on days 1, 8, 15, and 22 of each 28-day cycle (113). A total of 445 patients were randomized, 223 to RD and 222 to Rd. Grade 3 or higher AE were more common in the RD arm (49% vs. 32%; $p < 0.001$), including neutropenia (10% vs. 19%; $p=0.01$), VTE (25% vs. 9%; $p < 0.001$), and infections (16% vs. 6%; $p < 0.001$). Although response rates during the first 4 cycles were higher with RD (ORR: 82% vs. 70%; $p = 0.007$; CR + VGPR: 52% vs. 42%; $p = 0.06$), OS was significantly higher in the Rd arm, $p = 0.006$, (1-year OS: 96% vs. 88%; 2-year OS: 87% vs. 75%). The 2-year OS rate for the 102 patients who underwent stem cell transplant (94%) was comparable to the 2-year OS for patients in the Rd arm who continued primary therapy beyond 4 cycles (91%). These data demonstrated superior outcome with lenalidomide plus low-dose dex in patients with newly diagnosed MM compared to lenalidomide plus high-dose dex. The dose and schedule of dex will need to be evaluated further in light of the differences between the results of the SWOG and ECOG studies. There are probably groups of patients that would benefit from high dose dex administered according to the SWOG schedule and others that a lower dose will achieve similar disease outcome with less toxicity and mortality.

Baz et al. combined pegylated liposomal doxorubicin, vincristine, and dex (DVd) regimen with lenalidomide (DVd-R) in a phase I/II study among patients with relapsed or refractory MM (114). The study objectives were to determine the MTD and evaluate the safety and efficacy of DVd-R. Lenalidomide was administered orally at doses of 5, 10, and 15 mg/day for 21 days of each 28-day cycle in cohorts of 3-6 patients. Patients were treated for at least 4 cycles, and a maximum of 2 cycles after best response. Maintenance therapy included continuation of lenalidomide with the addition of prednisone 50 mg every other day until disease progression. Low-dose aspirin (81 mg) was administered as VTE prophylaxis. Sixty-two patients were enrolled in the study (40 refractory to prior therapy). The MTD of lenalidomide with DVd chemotherapy was 10 mg. The ORR was 75% with CR or near CR in 29%. After a median follow-up of 7.5 months, the median PFS was 12 months and the median OS had not been reached. Grade 3/4 adverse events included neutropenia (32%), febrile neutropenia (7%), peripheral neuropathy (5%), and VTE (9%). This novel combination appears to be well tolerated, and resulted in a high response rate in the group of patients with MM, most of whom were refractory to prior therapy.

Tables 3 and 4 summarize response rates in selected Phase II and III studies using lenalidomide as a single agent or in combination in newly diagnosed and relapsed/refractory MM patients.

In addition to the ability of lenalidomide to exert effective anti-tumor activity thorough direct anti-malignant plasma cell effects, it also exerts immune modulatory effects. Lenalidomide stimulates the immune cellular system leading to a beneficial impact on infectious complications, especially those that rely on the cellular immune system. One of the major viral infections in patients with multiple myeloma is herpes zoster that occurs in 15% of multiple myeloma patients over the course of the disease. Herpes zoster has high morbidity, especially in this age group, where post herpetic neuralgia could be crippling to the patients. With lenalidomide based therapy the incidence of herpes zoster is less than 5% as compared to other regimens that include proteasome inhibitors, where the incidence ranges from 15-60% (115, 116).

The clinical activity of pomalidomide was first demonstrated in a phase I study in which 24 patients with relapsed or refractory MM were treated with pomalidomide as a single agent (91). The MTD was established at 2 mg/day. The ORR was 54%, including

Table 3 Lenalidomide (Len) in newly diagnosed MM patients

Regimen	Phase	N	PR	CR	Reference
+ standard dose dex	III	223	+ CR 82%	+ VGPR 52%	Rajkumar et al. (113)
+ low dose dex	III	222	+ CR 70%	+ VGPR 42%	Rajkumar et al.113
+ dex	III	133	+ CR 85%	+ nCR 15%	Zonder et al. (87)
+ clarithromycin + dex	II	72	+ CR 90%	+ nCR 39%	Niesvizki et al. (123)
+ melphalan + prednisone	III	153	+ CR 67%	+ VGPR 46%	Palumbo et al. (124)
+ melphalan + prednisone + len maintenance	III	152	+ CR 77%	+ VGPR 50%	Palumbo et al. (124)

N = number of patients; PR = partial response; CR = complete response; VGPR = very good partial response; nCR = near complete response; dex = dexamethasone

Table 4 Lenalidomide (Len) in relapsed/refractory MM patients

Regimen	Phase	N	PR	CR	Reference
+ dex	III	177	+ CR 61%	14%	Weber et al. (85)
+ dex	III	176	+ CR 60%	16%	Dimopoulos et al. (86)
Single agent	II	102	+ CR 17%	+ nCR 4%	Richardson et al. (105)
+ Doxil® + vincristine + dex	II	62	+ CR 75%	+ nCR 29%	Baz et al. (114)
+ melphalan + prednisone + thalidomide + maintenance Len	II	43	+ CR 91%	+ VGPR 45%	Palumbo et al. (125)

N = number of patients; PR = partial response; CR = complete response; VGPR = very good partial response; nCR = near complete response; dex = dexamethasone

CR in 17%. Four patients (17%) experienced VTE. Pomalidomide therapy was associated with significantly elevated serum IL-2 receptor and IL-12 levels, which is consistent with activation of T cells, monocytes and macrophages. Based on these results, a recent phase II study has evaluated the safety and efficacy of pomalidomide (2 mg/day) combined with low-dose dexamethasone (40 mg/day on days 1, 8, 15, and 22 of each 28-day cycle) in 37 patients with relapsed or refractory MM (92). Most patients had received prior ASCT (76%) and prior IMiD® therapy (62%). The ORR was 62%, including VGPR in 24%. Objective responses were also reported 4 of 13 patients (29%) who were refractory to lenalidomide. Grade 3 hematologic AE included neutropenia (31%), thrombocytopenia (3%), and anemia (3%). There was no grade 3 neuropathy, but grade 1/2 neuropathy was reported in 16% of patients. Due to the incidence of VTE in the phase I study, all patients received aspirin as thromboprophylaxis and there were no cases of VTE. Pomalidomide appears to be another promising agent with a role for further studies as an immunostimulatory modality of treatment among patients with relapsed or refractory MM.

New directions

Multiple new therapeutic targets in the treatment of MM have been recently identified. Agents targeting cell surface molecules, specific molecules mediating growth, survival, drug resistance and migration of MM cells, as well as signaling pathways participating in these vital functions of MM cells are already going through pre- and clinical studies. They include agents such as IkB kinase inhibitors, VEGF receptor tyrosine kinase inhibitors, FGFR3 inhibitors, farnesyltransferase inhibitors, histone deacytilase inhibitors, heath shock protein inhibitors, telomerase inhibitors, Smac mimetics, MAPK inhibitors, TGFα inhibitors, TRAIL ligands, IGF-1 receptor inhibitors, HMG-CoA reductase inhibitors, Anti CD40 and Anti CD56 agents. We hope that these new agents in combination with existing ones will lead to the ultimate result for patients and their families: the cure of multiple myeloma.

Conclusions

Treatment options for patients with multiple myeloma have increased significantly in the past 10-15 years. The length and quality of

life have improved due to the greater efficacy and lower toxicity of new treatments. A future challenge for physicians treating patients with this complicated disease is how to use available treatments in a way that best fits a particular patient looking for help, and achieve the goal of individualized therapy.

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Is subjective well-being a measure or the measure of mental health?

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No matter how we conceive subjective well-being, happiness, as a predominantly affective condition, is a key component of it. There is a tendency, mostly within positive psychology, to equate subjective well-being, i.e., happiness and mental health. The question arises as to how well founded it is to equate happiness and mental health. The author challenges the connection between subjective well-being and mental health. He argues that mental health should not be reduced to happiness. In order to back his view he analyses six aspects of the alleged association of happiness and mental health, and shows the mismatch of these two phenomena. **Conclusion**. Mental health comprises inter alia the correct perception of reality, a critical stance towards the given society, and behavioral manifestations. All these occurrences are not the defining characteristics of subjective well-being. Therefore, subjective well-being can be a measure but not the measure of mental health.

Key words: Subjective well-being, Happiness, Mental health.

Introduction

Within the context of positive psychology, there is a strong tendency to equate subjective well-being (henceforth SWB) and mental health. In a good number of cases the most prominent positive psychologists do not express any doubt that SWB and mental health are the same thing. For example, Keyes, one of the leading experts in the field of SWB, quite unequivocally equates SWB and mental health. Thus, he writes: "Mental health may be operationalized as a syndrome of symptoms of an individual's subjective well-being" (1). He writes along the same lines in the same paper: "Mental health is best operationalized as syndrome that combines symptoms of emotional well-being with symptoms of psychological and social well-being" (1). Here is one more citation which shows that Keyes equates SWB and mental health: "Research now supports the hypothesis that health is not merely the absence of illness, it is also the presence of higher levels of subjective well-being" (2).

And what is SWB? SWB comprises a high level of positive affect, a low level of negative affect, and a high degree of satisfaction with

one's life. The dominance of a positive affect is usually called happiness, and it constitutes the core of the *hedonic* stream of well-being, or *hedonic well-being* (3). Life satisfaction involves a cognitive element, and therefore is not strictly focused on happiness. "Viewed as a cognitive component, life satisfaction was seen to complement happiness, the more affective dimension of positive functioning" (4). The *eudaimonic* stream of well-being, or *eudaimonic well-being* refers to living well and actualizing one's human potentials (5).

Irrespective of whether we support more the hedonic or the eudaimonic stream of SWB, happiness is the most important part of SWB. In other words, if there is no happiness, there is no SWB. "Although it is probably not the case that even individuals who focus primarily on happiness view it as the definitive aspect of the good life, it is clear that happiness, positive emotion, and life satisfaction are all typical outcome measures in many studies of well-being" (3). If happiness is a substantial feature of SWB, then it is a key component of mental health as well. Briefly said, no one can be mentally sound if she or he is not happy. And what does it mean to be happy? It means countless things. The common point of numerous definitions of happiness - in an explicit or implicit form – is that happiness is an affective state. Regardless of whether you feel happy because you are well off or because you are not hungry or because you are in love or because you have experienced an epiphany or because you have devoted yourself completely to God or because you made a break-through in science – your happiness is always primarily an affective state. Whenever one feels over the moon emphatically pleasant emotions permeate her or him. There is no such thing as non-affective, non-emotional happiness. The fundamental question relating to associating SWB, i.e. happiness and mental health, is whether such an association is well founded; how much, if at all, it is warranted? In other words, is happiness the key component of mental health?

I have serious reservations about this kind of association. Such an association is, to say the very least, highly debatable. In this paper I will discuss some aspects of reducing mental health to happiness, which render problematic the association of these two phenomena.

I will focus on the following aspects of the association of SWB (happiness) and mental health:

- The tyranny of happiness;
- Is a happy life a real life;
- Happiness and the correct perception of reality;
- Unhappiness as a potential source of creative work;
- Unlike mental health, happiness is a subjective phenomenon, and
- Subjective ill-being rather than subjective well-being as a sign of mental health.

You may have noticed that I have not defined mental health, i.e., that I have not said what I mean by mental health. One way of defining mental health – or anything else, for that matter – is to say what is not meant by mental health. That is what I will do. I do hope that on the basis of what I think mental health is not you could get the idea which notion of mental health is closest to my view of mental health. Let us now see why, in my opinion, happiness is not the defining characteristic of mental health.

The tyranny of happiness

In her recently published book *Smile or Die. How Positive Thinking Fooled America and the World* Ehrenreich claims that in the twentieth century systematic positive thinking and feeling happy about oneself went mainstream in the U.S. "gaining purchase within such powerful belief systems as nationalism and also doing its best to make it-

self indispensable to capitalism" (6). She also says that positivity is not so much something people should seek, it is not so much a preferred condition or mood, either, as it is a part of the dominant ideology in the U.S.

Despite a growing anti-American sentiment across the board it seems that the tyranny of happiness has spread to the rest of the world. Positive thinking and feeling happy has gone global. It has become the *must have* or *must feel*. People are preoccupied not only with how to be happy but also with how to be happier. To paraphrase Mark Twain, to be happy has become as mandatory as paying tax and dying. If you do not see the world in rosy colours, if you are negative about something or somebody, if you are afraid that the worst case scenario could come true, and consequently are more than concerned, something is wrong with you. You should cheer up and see the bright side of the street. The point is: nothing but the bright side of the street.

Not only popular culture has been permeated by the tyranny of positive thinking. As rightly noted by Held, the psychologist, "our professional culture is saturated with the view that we must think positive thoughts, we must cultivate positive emotions and attitudes, and we must play to our strengths to be happy, healthy, and wise" (7). No matter how bad a lot has befallen you, which disease you have been diagnosed with, or how serious the losses you have recently suffered, you should think positive. There is no better remedy for your plight, for the outcome of your disease, for managing hard times caused by the losses, than to think positive. And if you, for whatever reason, are not able to transcend distress, you should feel guilty. And feeling guilty could not help but make your suffering even worse. That is what Held calls "adding insult to injury".

Now, let us go back to the above mentioned association of SWB (happiness) and mental health. Does happiness as something that is mandatory suggest that mental health should also be mandatory? I do not think that mental health should be, officially or unofficially, declared compulsory. Needless to say, it is good to be mentally healthy; in any case, it is better to be mentally healthy than to be mentally disordered. Furthermore, it is not only in your own interest but also in the interest of the society that you are mentally healthy. In that sense it is highly recommended that you undertake activities aimed at preserving or upgrading your mental health. However, mental health should not be perceived as something which is constraining. You should not be, to put it that way, forced into mental health the way people are nowadays compelled to be happy. Mental health is a matter of informed consent. You should be provided information about the benefits of mental health, but it is up to you whether or not you will follow given advice.

Briefly said, preventive measures should not be perceived as the tyranny of prevention. Indeed, one way to make mental health more attractive is to posit that once you are mentally healthy you will be happy or happier. But, such an assertion does not match reality. The truth is that you can be mentally healthy without being happy. One may remark that the tyranny of happiness is of recent date and that wide ranging and overencompassing conclusions should not be drawn on the basis of a recent phenomenon. The tyranny of happiness is of a recent origin, but so is positive psychology's equating of happiness and mental health.

Is a happy life a real life?

Life is not meant to be happy. It can sound like a truism, but it is worth reminding positive psychologists of this truth nevertheless. Life implies sadness and joyfulness, enchantment and disenchantment, despair and elation. The combination of these mental states gives flavour to life. It is the salt of

life. Can you imagine a man or woman who would be happy all her or his life through? Such a happy life might be achieved at the price of turning a blind eye to life as it is, in a cowardly more than courageous manner, which, in fact, means at the price of being in denial. There is another route leading to long-life happiness. You can suffer from chronic mania or hypomania, or your intelligence can be, mildly said, not the best. Either way you will enjoy a distorted outlook on life, which will probably make you feel happy or happier about yourself.

Finally, if you are not keen on burying your head in the sand, or are you not suffering from chronic (hypo) mania, nor is your general cognitive capacity in an unenviable state, there are still two opportunities left to achieve happiness. You can do charity work and approach a psychologist who will treat you by cognitive therapy. These two options (charity work and cognitive therapy) are the key component parts of Seligman's recipe for positive thinking, for (learned) optimism and happiness (8). And Seligman is the leading figure among today's positive psychologists.

The thrust of my argument is that a happy life is a life which is out of step with real life. A happy life is a counterfeit life. Ivo Andric, Nobel prize winner for literature, once said that happiness is something that does not last long. I would add that happiness comes and goes, and it comes when least expected.

One can remark that just because life is not good, it is our duty to eliminate or belittle the bad sides of life and enlarge the good ones, to make the bad days shorter and the good days longer. After all, that is what people from time immemorial have been struggling to achieve; to make more room for happiness; to make positive affect prevail over negative affect, to become satisfied with their own life. However, such a dream – because it is a dream more than anything else – is by and large elusive, as most dreams are. For thousands of years people have been endeavouring to make themselves happy, or less miserable. And what is the result? No one would dare to claim that, today, people are happier than they were for example in ancient Athens or sixteenth century Paris, for they have always been as much happy as unhappy. It does indicate that life is not meant to be either good or bad, and that no matter how noble an exercise it is to be more or less permanently pursuing happiness, to achieve eternal happiness on earth, such an exercise is doomed to fail. And if, hypothetically speaking, long-life happiness was achieved and happy people were somehow generated, would we or should we look at such apparently blissful people as mentally healthy? The answer is in the negative. Such humans would be less than human. Their life would be a parody of life. And most likely they themselves would soon become sick and tired of such a happy life and would start envying those who are not short of bad experiences.

Mental health should not be conceived in such a way that it betrays the complexity and diversity of human nature. And humans – to repeat – are not meant to lead a happy life. Although we now and then curse our destiny, when we put our life in perspective, we cannot help but infer that the life we have had has been quite a savoury combination of happy and unhappy moments.

Happiness and the correct perception of reality

Mentally ill people are said to be ill because, amongst other things, they have a distorted view of reality. Therefore, those who are mentally sound are supposed to have an accurate perception of reality. Jahoda, who was the first to summarize and systematize definitions of mental health, articulated by various scholars, writes that the correct perception of reality is often treated as "the

sine qua non for reality adaptation" (9) and that "many authors present the criterion in an almost axiomatic fashion" (9). If happiness and mental health are the same, as positive psychologists claim, then happy people should have a correct perception of reality. Do they? Taylor and Brown (10, 11) will help us in answering this question. These two scholars start from the widely held belief that mental health and illusions, meaning perceptions that falsify reality, do not go hand in hand. Mentally healthy people are not expected to have illusions either about themselves or the world.

Drawing on the results of a large body of studies, Taylor and Brown (10, 11) ascertain that the greatest majority of people nurture three kinds of illusions: they think they are better than they are; they believe they can master the environment, and they see the future as more rosy than is justified. It is interesting that depressive people (up to the grade of moderately depressive) do not have such illusions. They do not think they have more good than bad sides; and they do not think they are better than average; finally, their self-perception squares with how other people see them. Besides, depressive people do not believe they can control events over which they have no control at all. Nor do they have an unjustified optimistic view of the future.

Seligman could not dodge the association between depression and the correct perception of reality which indicates that depressive realism could be good for mental health. Thus, he found an evasive solution in the notion of *flexible optimism* (8) to correct the insufficiencies of *blind optimism*. Neither the syntagm *flexible optimism* nor the syntagm *benign* illusions can, however, disguise people's inclination to overvalue themselves and their ability.

And what about happy people? Is their perception of reality correct? If the so-called "ordinary" people who are supposed to have a mostly balanced basic mood do not perceive themselves and the world correctly, how could those people whose basic mood is higher than the average have a correct perception of themselves and the world? It is well known that those who are happy are not reliable people, due to their tendency to over-rate their capabilities, to see themselves as more attractive, more praise-worthy than they are, and to ignore everything that could question the accuracy of their self-perception and their perception of the world.So the question arises as to whether we should regard as mentally healthy those who are unreliable and who due to the prevalence of positive over negative affects, nurture an inaccurate view of themselves and the future? I do not think we should.

Unhappiness as a potential source of creative work

It was Stefan Zweig who once said – scolding us a bit for our ignorance – that we are not aware how much we owe to those who have felt crestfallen for most of their lives. Zweig was referring to great creators in general, and great artists in particular. Indeed, if you read the biography of great poets and novelists you easily grasp how many of them were unhappy, the depth of the doubts that tormented their mind, how far they were unable to find peace of mind, how unsuccessful there were in managing their private matters. Even while creating – and the moments of creation are considered to be the only moments of pleasure for them, not to mention happiness – great artists are plagued by discontent, by questions of whether they managed to express what they intended to say, whether they had found the most appropriate form to convey their vision, their nightmares, their specific way of looking at people and the world.

A happy life is not a field in which creative work grows. It does not provide valuable insights. Leo Tolstoy wrote at the very beginning of his novel *Anna Karenina* that

all happy families are happy in the same way and that each unhappy family is unhappy in its unique way. My guess is that if you read Tolstoy's sentence in the following way, you will get it right. Happy families and happy people are not worthy of special attention. Once you meet a happy man – if there is such a creature – you know what all happy people look like. To that extent happy people are much less engaging than unhappy people.

The uniqueness of individual experience, meaning the differences between people in regard to how they feel, how they think, how they relate to themselves and other people – this is what renders life so interesting and exciting. I have in mind not only the matchlessness of my own experience, the awareness that I am unlike any other human being, but also the knowledge that other people are also unique in their own way. And the longer one lives, the more she or he unveils that unhappiness is an unavoidable and at times a dear life companion, a potential source of inspiration, and a royal route to larger-than-life knowledge about life. In the light of what I have just said, I am wondering whether it would be fair and founded to say that unhappy people are mentally ill people or that they are not mentally sound? Or, to put it another way, is it proper to contend that happiness and mental health come down to the same, that the first is the key part of the latter, and that the latter cannot do without the former as its dominant element?

Finally, should we say that, irrespective of how much we owe to great artists, we should not regard them as mentally sound due to the mere fact that, in a good number of cases, and for a good number of years, they felt more unhappy than happy? After all, this question refers not only to artists but also to all those people whose unhappiness led them to do praiseworthy things, which they probably would not have done had they been in a good mood most of their lives.

Unlike mental health, happiness is a subjective phenomenon

Aware of the imperfection of the confinement of SWB to the affective category alone, first Ryff (12) and then Ryff and Keyes (4) expanded the concept of well-being. These authors maintain that when assessing how well they are, people take into account not only the emotional but also the psychological and social aspects of their life. In other words, there are three kinds of well-being: emotional well-being, psychological well-being, and social well-being. Each of these forms of well-being has its own characteristics. *Emotional well-being*: cheerful, in good spirits, extremely happy, calm and peaceful, satisfied, full of life. *Psychological well-being*: self acceptance (I like most parts of my personality), positive relations with others (For me, life has been a continual process of learning, changing and growth), purpose in life (I feel as if I have done all there is to do in life), environmental mastery (I am good at managing the responsibility of daily life), autonomy (I tend not to be influenced by people with strong opinions). *Social well-being*: social acceptance (People care about other people's problems), social actualization (My daily activities are a contribution to my community), social coherence (I can make sense of what is going on in the community), social integration (I feel close to other people in the community). If the concept of SWB is enlarged so as to include the psychological and social spheres, then for example a manic or hypomanic patient or mentally underdeveloped person cannot be considered as having SWB. Persons with these kinds of conditions do not have psychological and social well-being. Their life is not a continual process of learning, changing and growth. They are poor at managing the responsibilities of daily life. They cannot make sense of what is going on in the community, and so on.

Besides, there is no doubt that in its expanded form, that is, as the unity of emotional, psychological and social well-being, SWB seems closer to mental health than is the case when only affective SWB (happiness) is taken into consideration. The problem is that in assessing the state of an individual's mental health, positive psychologists rely completely on self-reporting. Respondents are asked either verbally or in written form to say how they feel and how they evaluate their functioning. No other instrument of assessing respondents' SWB, i.e., mental health is applied. It is quite understandable that respondents' own view of how they feel is the only possible way of learning about how they feel about themselves, which virtually means learning how mentally healthy they are – if we stay within the context of positive psychologists' equation of SWB and mental health.

Yet relying on people saying how they feel about themselves in assessing their mental health can be misleading. Both mentally healthy and mentally unhealthy people may say that they feel bad about themselves. Moreover, neither mental health nor a mental disorder is only a matter of how one feels about oneself. Apart from distress which might or might not have external equivalents, impairment of one or more important areas of function is a substantial element of the definition of mental disorder set out in DSM-IV (13) and in the draft of DSM-V (14). The point I want to make is that in assessing if an individual is mentally healthy or unhealthy we have to rely not only on what people say about how they feel, but also on particular objective, behavioural elements, on how they behave, on what they do, on whether and how impaired one or more of their mental functions are.

The assessment of whether an individual is mental healthy cannot be delegated to the individual whose mental health is the object of assessment, to their estimation of whether or not they for example have a positive attitude toward themselves and their past life and accept various aspects of self, or hold goals and beliefs that affirm a sense of direction in life and feel that life has a purpose and meaning, etc. A serious imperfection of such a subjective or, or more accurately, subjectivist way of assessing one's mental health, is the fallout of equating SWB (happiness) and mental health.

Can subjective ill-being rather than subjective well-being be a sign of mental health?

There are many definitions of mental health. I will mention just a few. According to the clinical-pragmatic definition of mental health, mental health is the absence of mental disorder. This is a negative definition. It does not indicate what mental health is all about. As stated, positive psychologists equate mental health and SWB, i.e., happiness. There is also a humanistic-anthropological definition of mental health. Humanistic psychologists (e.g., Carl Rogers, Abraham Maslow) and the proponents of critical social theory (e.g., Theodor Adorno, Herbert Marcuse, Erich Fromm) argue that a mentally healthy individual is an individual who is critical of the society she or he lives in, and consequently sees not only its countless deficiencies but also its inhuman character. At first sight such a view of a mentally healthy individual may seem far too abstract. However, if we take a closer look at what is, nowadays, meant by a mentally normal person, we will easily comprehend how well founded the cited definition of mental health is.

Our contemporaries are busy with the satisfaction of false rather than real needs. Marcuse makes a clear distinction between true and false needs. False "are those which are superimposed upon the individual by particular social interests in his repression:

the needs which perpetuate toil, aggressiveness, misery, and injustice" (15). In the same way, no matter how much such needs may have become the individual's own, Marcuse continues, "reproduced and fortified by the conditions of his existence; no matter how much he identifies himself with them and finds himself in their satisfaction, they continue to be what they were from the beginning – products of a society whose dominant interest demands repression" (15).

On the other hand true needs are those needs that reinforce individuality and creativity. "But as historical standards, they do not only vary according to area and stage of development, they also can be defined only in (greater or lesser) *contradiction* to the prevailing ones" (15). Today's people are self-centred. They are driven by acquisitiveness and competition. The dominant political and economic model today not only allows but also encourages citizens, writes Singer (16), to make the pursuit of their own interests, understood largely in terms of material wealth, the chief goal of their life. The point is that people strive to achieve such goals because they consider them their own goals. Those who are in power have managed through ideologization and indoctrination to force people to want to do what they have to do, and to make them feel happy about a lifestyle which they should have. In other words, the way they behave, activities they undertake and possessions they have or yearn to have represent the kind of behaviour, of being and of having, which is in the interests of those who hold power. This is the main reason why people are so committed to the satisfaction of false needs and oblivious of their real needs which originate in human nature.

From this kind of distortion of real needs, dubbed "socially patterned defects" comes people's behaviour that is considered normal "the pathology of normalcy". Even though they are regarded as normal those people are not mentally healthy, say humanistic psychologists and the protagonists of critical social theory. Those people are mentally healthy who manage to see through the veil knit of indoctrinations and ideologization. And they feel discontented. They first feel discontented because their real human needs have not been fulfilled, which means that they have not been totally integrated into the system of "euphoria in unhappiness" (Marcuse). Also, they feel discontented and uneasy because they are aware of how strong is society's resistance to create opportunities for the realization of real human needs. Thus, their subjective ill-being rather than subjective well-being would be the indicator of their mental health (17). They are mentally healthy in so far as they comprehend that they cannot be mentally healthy under the given social circumstances, and that actions should be undertaken to humanize the existing social reality.

Conclusion

There are many reasons why SWB, i.e., happiness, and mental health should not be equated. I have elaborated some of them. Indeed, it is more difficult to define mental health than mental disorder, most likely for two main reasons. Unlike mental disorder, mental health is common: we are mentally healthy, mental health is all around us. Being an integral part of the commonest denominator of our daily existence, mental health evades definition. Mental health thwarts attempts to conceptually squeeze it into a definition. The same as life does. On the other hand, mental health could be considered as an ideal. We are never as mentally healthy as we should be. In this case the notion of mental health is closely linked to the nature of the most cherished value, be it human nature, real human needs, the individual's autonomy, one's ability to love and work (Freud), or something else. Difficulties

in defining mental health should not be used as an alibi for resorting to definitional shortcuts, one of which is equating SBM (happiness) and mental health. Finally, answering the question put in the title of this paper, I would say that SWB might be a measure but not the measure of mental health.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Catamenial anaphylaxis in three patients

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Catamenial anaphylaxis, also called cyclical anaphylaxis, describes recurrent episodes of multi-system allergic reactions occurring at the time of menstruation. This case reports demonstrates the management of three women with catamenial anaphylaxis. The first patient is a 38 years old woman who presented with symptoms consistent with anaphylaxis in relation to her menstrual cycle. Her symptoms were controlled with cetirizine at a dose of 20 mg daily. The second patient is 33 years old with similar symptoms coinciding with her menses. We were able to control her symptoms with leuprolide (Lupron), a luteinizing hormone releasing hormone (LHRH) agonist. The third was a 29 years old woman with catamenial anaphylaxis who was successfully treated with Depo-Provera (medroxyprogesterone). Catamenial anaphylaxis is a rare yet an important presentation to the Allergist/Clinical immunologist. The management of the patients we present here represents a spectrum of the different therapies tried in the medical literature. Here, we report the first successful use of medroxyprogesterone for such rare, yet critical medical condition.

Key words: Anaphylaxis, Menstrual period, Allergic reactions.

Introduction

Acute allergic reactions or exacerbations of pre-existing chronic allergies in association with the female menstrual cycle are well documented, especially the premenstrual exacerbation of urticaria (1) and bronchial asthma (2). Several reports have described the uncommon occurrence of peri-menstrual anaphylaxis. Anaphylaxis around the time of the menstrual period may be related to certain drugs used for menstrual cramps or to specific foods ingested around that time. However, in some instances, no obvious etiology is seen in spite of exhaustive investigations. Catamenial anaphylaxis, also called cyclical anaphylaxis, describes recurrent episodes of multi-system allergic reactions occurring at the time of menstruation. The present paper reports 3 patients with recurring life-threatening peri-menstrual allergic reactions.

Case reports

Case one

A 38-year-old woman was first seen in the clinic with a two-year history of episodic re-

actions that occurred temporally in relation to her menstrual period. At the time of consultation, she had had a total of twenty-four reactions, with twelve to fifteen associated with loss of consciousness. Manifestations included a 'draining sensation' in the chest, followed by itching of the palms and palpitations. These were followed by crampy abdominal pain leading to diarrhea, lightheadedness and loss of consciousness. On some occasions, there was associated flushing, urticaria, and chest tightness. Systolic blood pressure was documented to be as low as 70 to 80 by paramedics on several occasions.

All of these episodes were peri-menstrual, starting either the day prior to, the day of, or the day after the onset of menses. Episodes were not related to food, exercise, alcohol, or to use of any medications. Past medical history was only significant for hypothyroidism for which she was controlled on replacement therapy. Investigations done on this patient are shown in Table 1. She was started on cetirizine 10 mg, an antihistamine, daily and prednisone 60 mg daily for 1 week, followed by prednisone 60 mg on alternating days without improvement. Later, prednisone was discontinued and cetirizine was increased to 20 mg daily with near-total suppression in the frequency of such reactions. Follow-up for a period of one year on cetirizine 20 mg daily showed the occurrence of only one mild reaction.

Case two

A 33-year-old woman was first seen in the clinic with an 18-month history of recurrent anaphylactic reactions. Her first reaction consists of generalized flushing, itching with facial swelling, itching of the oropharynx and uterine cramps. There was associated nausea, vomiting, crampy abdominal pain, diarrhea and a sensation of lightheadedness and presyncope. The second reaction happened several months later, but it was more severe, involving respiratory symptoms, syncope, and hypotension documented by paramedics. By the time she was seen in consultation, she had experienced a total of twelve such reactions with ten of them coinciding with her menstrual period. The other two were mid-cycle. She was not on any medications at the time of the reactions, and the reactions were not associated with any specific food, or with alcohol intake or exercise.

Investigations done on this patient are shown in Table 1. Initially, she was treated with a suppressive regimen for idiopathic anaphylaxis using prednisone at a daily dose of 50 mg PO. This failed to suppress her reactions. Prophylactic treatment with cetirizine 10 mg twice daily in combination with ketotifen 4 mg twice daily failed to control her symptoms. Celecoxib, a cyclooxygenase 2 (cox-2) inhibitor was tried, but this caused episodes of angioedema. Finally, leuprolide (Lupron), a luteinizing hormone releasing hormone (LHRH) agonist, was started, with no recurrence of her symptoms during a one-year follow-up period.

Case three

A 29-year-old woman was initially seen in the clinic with a two year history of recurrent episodes of chest pain, generalized urticaria, swelling of her face, lips and neck, cough, vomiting, crampy abdominal pain, dizziness and lightheadedness. These reactions typically started one week prior to the beginning of her menstrual cycle with worsening over the course of the week and peak symptoms on the first day of her menstrual cycle. At the time of her initial evaluation, she had had a total of twelve such reactions, none of which was associated with food, alcohol or exercise. At the time of her first visit she was on rofecoxib with no change in the frequency or severity of her attacks. Past medical history was significant for fibromyalgia for which she was on daily Tylenol number 3 (acetaminophen with codeine). Results of investigations done on this pa-

tient are shown in Table 1. Initially, she was treated with cetirizine 10 mg twice daily and prednisone 40 mg daily from three days premenstrually until two days after the onset of her menstrual cycle. This failed to suppress her reactions. Later, she was started on Depo-Provera (medroxyprogesterone) and over the ensuing three years she had total suppression of her multi-system reactions. Later, she decided to become pregnant. Depo-Provera was stopped, and three months later she had a recurrence of her anaphylactic symptoms. Those were initially mild, but worsened over the course of several months with generalized urticaria associated with nausea, abdominal cramps, and crampy uterine pain. Even more worrisome, were associated symptoms of lip swelling, throat constriction, dysphonia, dysphagia, chest tightness, wheezing, and presyncope. Due to those life-threatening manifestations, Depo-Provera was restarted again with no further recurrence over a follow-up period of two years.

Discussion

Catamenial anaphylaxis is an uncommon clinical entity. It is a diagnosis of exclusion that represents considerable challenge from the standpoint of management. We conducted a literature search through the pubMED database by typing the phrases cyclical AND anaphylaxis, catamenial AND anaphylaxis, and hormonal AND anaphylaxis, and found very few reports describing such reactions (3-7).

Anaphylactic reactions around the time of the menstrual period may be either allergic or pseudo-allergic. Conditions mimicking anaphylaxis include carcinoid syndrome, pheochromocytoma and systemic mastocytosis (8). Allergic reactions may often be due to medications, especially aspirin, non-steroidal anti-inflammatory drugs (9), or foods, amongst other causes. Such etiologies need to be kept in mind while working up a patient presenting with cyclical anaphylactic reactions, and should be ruled out first. Upon excluding extrinsic triggers of anaphylaxis as well as conditions that mimic multisystem allergic reactions, the diagnosis of catamenial anaphylaxis should be considered. The mechanism involved in catamenial anaphylaxis is not clearly understood. Some authors have suggested hypersensitivity to progesterone as an underlying cause (3). This theory was supported in some patients by a positive cutaneous and systemic reaction to intradermal challenge with medroxyprogesterone (3). However, in a report

Table 1 Investigations done for the three patients

Investigations	Case 1	Case 2	Case 3
Skin test to common foods	Negative	Negative	Negative
Skin test to inhalants	Negative	Negative	Negative
Skin test to medroxyprogesterone[1]	Negative	Negative	Negative
Total serum IgE[2]	31.0	19.2	27.0
Serum tryptase[3]	Normal	46.9[A]	Normal
24-hour urine for 5-HIAA	Negative	Negative	Negative
24-hour urine for VMA	Negative	Negative	Negative
24-hour urine for Catecholamine	Negative	Negative	Negative

[1] Done by intradermal injection of 10 mg, ie 0.03 ml, medroxyprogesterone
[2] Normal range is < 300 IU/ml
[3] Reference range is 3.8 – 11.4 ng/ml. Serum tryptase was done within 2 hrs after acute episodes as well as in between attacks
[A] Done after an episode of a systemic reaction. In the same patient, serum tryptase was normal in between the episodes.

of four patients with similar cyclical anaphylactic reactions, two of four patients failed to show a positive skin test to medroxyprogesterone (5). A similar negative skin test was also seen in one other patient (7). Clearly, this phenomenon cannot account for all cases of cyclical anaphylaxis. Furthermore, the use of depot preparations of progesterone (Depo-Provera) would likely exacerbate the condition in patients allergic to endogenous progesterone, in contrast to Case 3 described above.

Another mechanism proposed to account for catamenial anaphylaxis involves a vasoactive constituent of menstrual fluid, the prostaglandins (4, 6, 7). This was supported by the finding that PGF_2-α plays an important role in modulating mediator release in mast cells (10). Another possibility was involvement of PGI_2 (prostacyclin), which act as a powerful vasodilator leading to such systemic reactions in susceptible persons (4). This theory was supported by a positive intradermal skin test to menstrual fluid in one (4), but not another patient (6).

Extensive investigations for anaphylactic triggers, including foods and medications were negative in all three patients presented in this report. In addition, other conditions masquerading as anaphylaxis such as carcinoid syndrome, pheochromocytoma, and systemic mastocytosis were ruled out by history, by manifestations atypical for these conditions, and by appropriate laboratory tests. Skin tests to progesterone were negative in all of our patients. Interestingly, tryptase remained normal at the time of the acute episodes. This is not surprising, as β-tryptase, a hallmark of mast cell activation, is known to remain normal in some patients with anaphylaxis (11).

The management of all three patients represents a spectrum of therapeutic modalities described in the medical literature. For the first patient, we were unable to control her symptoms with cetirizine 10 mg daily, but increasing the dose of cetirizine to 20 mg per day successfully controlled her reactions. Anaphylactic reactions in the second patient improved remarkably after the start of LHRH-analogue, a finding also seen in two of four patients reported by Slater et al. (5). Of interest is the angioedema observed in the second patient after the start of celecoxib and the lack of suppression of the reactions in the third patient while on rofecoxib. This observation is in contrast with the reports of Simpson et al. (6) and Burnstein et al. (7) in which their patients' symptoms improved after initiation of celecoxib and indomethacin respectively.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are amongst the most common drugs known to precipitate anaphylaxis and other allergic reactions (9). There is also compelling evidence that NSAIDs and aspirin can intensify immediate hypersensitivity reactions in patients with a background of anaphylaxis (12, 13). Also, NSAIDs have been shown in vitro to augment histamine release from human leukocytes (14). The same is true for COX-2 inhibitors, with anaphylactic reactions related to such medications (15, 16).

Of interest is our third patient who had complete suppression of her anaphylactic episodes upon initiating treatment with medroxyprogesterone. Such therapy was tried to our knowledge in only one other patient and failed to control her symptoms (6). In all patients, high dose systemic steroids failed to control the anaphylactic reactions, in spite of the fact that this approach is recommended for the control of idiopathic anaphylaxis (17, 18). Moreover, we found that ketotifen, a mast cell stabilizer with antihistaminic activity, did not help to reduce the frequency or severity of attacks, in spite of its reported efficacy in the treatment of idiopathic anaphylaxis (19).

Conclusion

Whether the mechanism causing cyclical anaphylaxis involves hypersensitivity to progesterone or to prostaglandins, cessation of the menstrual cycle by means of induction of medical or surgical menopause reportedly results in control of such anaphylactic reactions (5, 6, 7). Surgical menopause is generally reserved for patients who fail medical treatment either due to breakthrough reactions or due to intolerable side effects (5). However, the variable response to suppressive medications in the three cases described above suggests that catamenial anaphylaxis is a heterogeneous disorder in which a number of mechanisms and mediators may play a role.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Giant hydronephrosis: case report and review of literature

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Giant hydronephrosis (GH) is a rare entity that should be considered in the differential diagnosis of huge intraabdominal cystic masses. A hydronephrotic kidney usually contains 1–2 litres of fluid in the collecting system. We report a case of a 24 year old man with a hydronephrotic left kidney, from which 14 litres of fluid was surgically drained. In this report the importance of computed tomography in the diagnosis and differential diagnosis of giant hydronephrosis to other cystic masses is emphasised. **Conclusion.** GH should be included in the differential diagnosis of huge intraabdominal cystic masses. CT alongside MR should be the method of choice.

Key words: Hydronephrosis, Computed tomography.

Introduction

Giant hydronephrosis (GH) was described by Sterling in 1939 as the presence of more than one litre of urine in the collecting system of the kidney (1, 2). It is seen more often in males than in females (2.4:1) and more often on the left than on the right side (1.8:1) (1). GH usually occurs secondarily due to ureteropelvic junction obstruction (UPJ) (3). The other causes are: stone disease, trauma, renal ectopia and ureterovesical junction obstruction (4, 5). Most patients are asymptomatic because GH develops gradually over a long time but they usually have abdominal enlargement (4). Symptoms that arise are due to compression of the surrounding organs. Usually the first signs of disease are: opstipation, dysuria, obstructive jaundice (6). Possible complications are: hypertension, renal failure, malignant change and rupture of the kidney (7-9).

Case report

A male patient, 24 years old was admitted to the Department of Internal Medicine because of abdominal distension, abdominal pain, opstipation and dysuria over the last two months. The abdomen was tense and above the patient`s chest level, with a palpated mass in the left hemiabdomen. Apart

from 10-15 leucocytes with some bacteria in the urine and mild sedimentation increase, the other laboratory tests, including creatinine and blood urea, were within the normal range. Abdominal ultrasonography revealed a huge cystic mass in the retroperitoneum on the left. Seven litres of clear fluid were drained from the cystic mass under ultrasound control. The cystic mass was continually drained daily for one month. Every day up to two litres of fluid were drained off. The patient was discharged from hospital with the diagnoses: *Cysta per magna abdominis congenita* and *Agenesio renis l. sinistri.* Seven days after removing the drainage catheter, the patient was re-admitted to hospital for the same reasons. Urgent computed tomography (CT) of the abdomen was performed with a 6-row multidetector scanner (MDCT Simens Emotion). Unenhanced and enhanced CT scans with sagittal and coronal reconstruction revealed a huge hypodense, fluid density area, 350x250x200 mm, with a significant peripheral enhancement which occupied the entire left hemiabdomen from the diaphragm to the pelvis (Figure 1, 2, 3).

CT exam showed an extremely enlarged hydronephrotic left kidney with an enormously dilated collecting system and considerably reduced, partly immeasurable parenchyma, although with some signs of secretion. The hydronephrotic kidney displaced the intestine and pancreas to the right with compression on huge abdominal and right renal vascular structures (Figure 2, 4). The ureter of the kidney was not dilated. On coronal CT images of the caudal part of renal pelvis, a structure was shown that could correspond to the ureteropelvic junction with high insertion of the ureter.

After partly evacuation of the urine, an emergency left nephrectomy was performed. From the hydronephrotic kidney 14 litres of urine was evacuated, after the kidney measured 240x170x150 mm (Figure 5). The kidney was adhesed to adjacent organs. The hilum of the kidney was identified as high with high insertion of the ureter and gracile renal hilar vessels. The histopathological examination confirmed hydronephrosis of the left kidney with chronic pyelonephritic changes and microabscesses (Figure 6). There was no

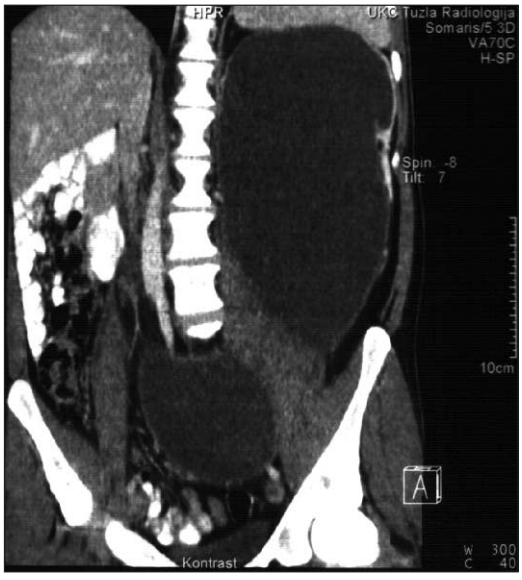

Figure 1 Coronal CT multiplanar image vizualise a cystic mass occupying the left hemiabdomen and crossing the midline on the right. The mass is displacing the intestine to the right.

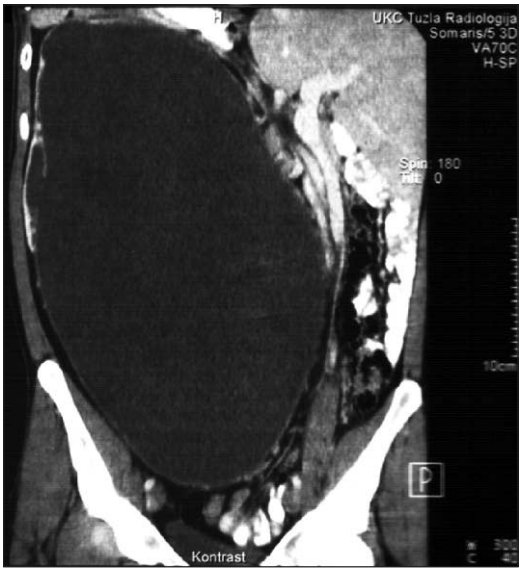

Figure 2 Oblique CT multiplanar image showing a giant cystic mass with compression of vascular structures

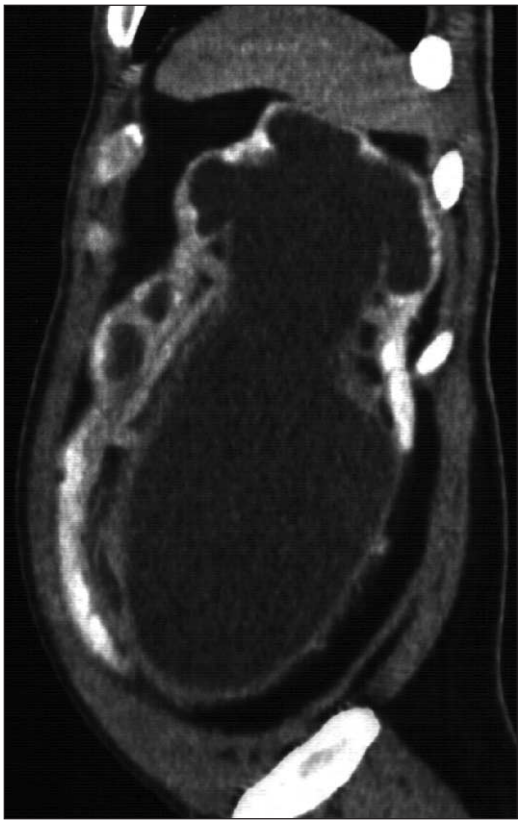

Figure 3 Sagital CT image of extremly enlarged left kidney with enormous dilated collecting system and considerably reduced parenchyma that shows signs of secretion

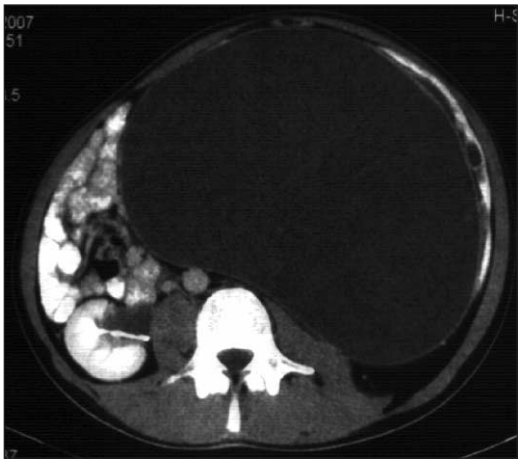

Figure 4 Axial CT image shows cystic mass displacing intestine

evidence of malignant growth. The renal pelvis was 200 mm in diameter. The ureter was not widened nor was stenosis of the ureteropelvic junction found. The histopathological examination of ureter revealed an ureteritis.

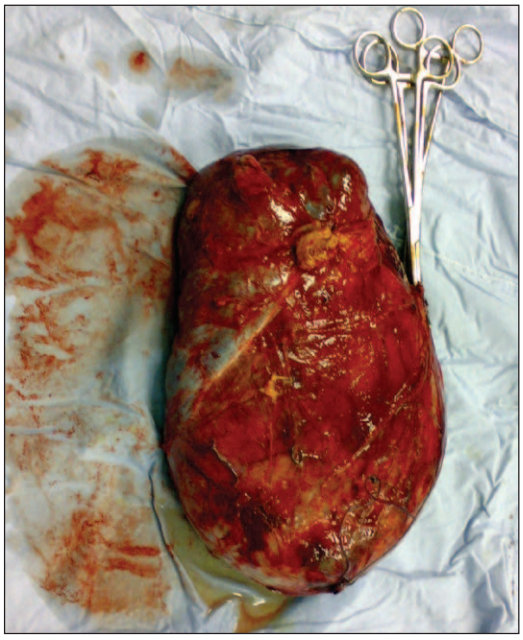

Figure 5 Kidney after 14 litres urine evacuation

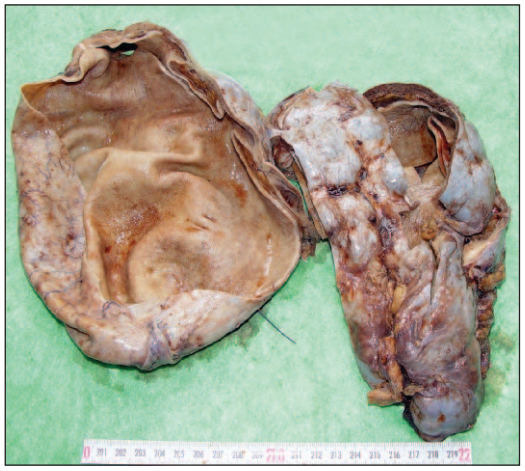

Figure 6 Pathologic specimen

Discussion

Giant hydronephrosis is rarely seen in adults. In literature more than 500 GH cases have been published, and in six cases malignant alteration was proven (1). A hydronephrotic kidney usually contains no more than 1–2 li-

tres of fluid (1, 2, 10). Chiang et al. reported 4 cases of GH containing 1900 ml, 3400 ml, 2100 ml and 3200 ml (1). Turgut et al. 2007 reported a case with 5000 ml of fluid in the collecting system (11). Schrader et al. reported GH with a kidney of more than 15 kg (12). Yilmas et al. reported hydronephrosis in a 12 year-old boy with 13.5 litres of urine in the collecting system (13). As in our case the hydronephrotic kidney contained 14 litres of urine, where previously the largest amount was reported by Schrader et al. (12).

The most common cause of UPJ that usually induces GH is the high insertion of the ureter, and not congenital stenosis (3). In our case, the ureter, which was not dilated, indicated the UPJ obstruction. Some coronal CT multiplanar images and surgical findings proved the high insertion of the ureter while the histopathological finding eliminated the narrowing of the UPJ as the possible reason for GH.

The first radiological method in GH diagnostics is abdominal ultrasonography but in many cases differential diagnosis between GH and another cystic formation is difficult. The list of differential diagnosis is wide and includes: ovarian cysts, retroperitoneal haematoma, hepatobiliary cysts, mesenteric and hepatobiliary cysts, pseudomyxoma, renal tumour, retroperitoneal tumours, ascites and splenomegaly (10).

In most cases, CT and magnetic resonance (MR) are the methods of choice in the differential diagnosis of GH with other intraabdominal cystic masses, especially if renal parenchyma is partially preserved and functional, with contrast enhancement. Even if contrast enhancement is absent due to the atrophy of the renal parenchyma, GH should be included in the differential diagnosis of intraabdominal cystic masses.

Conclusion

GH should be included in the differential diagnosis of huge intraabdominal cystic masses. CT alongside MR should be the method of choice.

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Reversible delayed hypoxic leukoencephalopathy associated with methadone overdose

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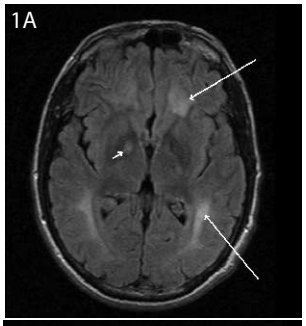

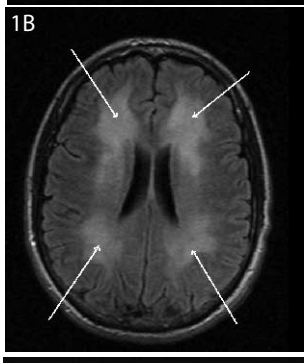

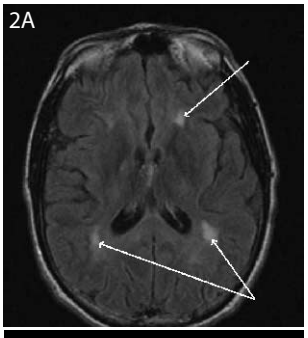

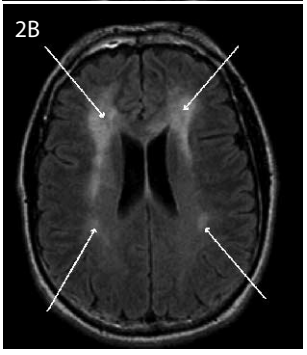

A 42-year-old man developed coma and hypoxemic respiratory arrest after snorting and ingesting methadone and required transient intubation and mechanical ventilation in the emergency department (ED). He was given naloxone intravenously, woke up and was extubated. He was dismissed from the ED but according to the wife later became somnolent and possibly intermittently apneic overnight. He was brought back to the ED where he was reintubated, and admitted to the intensive care unit. He was found to be hypotensive which responded to normal saline. After a couple days he was extubated and was mildly encephalopathic. Over the next month, the patient developed progressive cognitive decline, disinhibited behavior, hypersexuality, poor concentration, and mild parkinsonism. Marked white matter abnormalities were evident on MRI (Figure 1A and B). Results of arylsulfatase and galactocerebroside testing for adult-onset metachromatic leukodystrophy and Krabbe disease were normal. Six months later, the patient's clinical condition and MRI findings improved (Figure 2A and B) but did not completely normalize. The hallmark of narcotic-associated hypoxic leukoencephalopathy ("Chasing the Dragon") is its delayed yet reversible neurologic and MRI appearance (1, 2) as opposed to progressive metabolic leukodystrophy which progressively worsens unless an underlying cause is discovered. The putative mechanisms causing the delayed leukoencephalopathy are 'leukotoxins' or the carrier agents within the opiate drugs. This condition was originally described as "Chasing the Dragon" by heroin users who would inhale the vapors of smoke ("dragon") from burning heroin (1, 2).

Conflict of interest: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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Institute of Oncology, Sarajevo, Bosnia and Herzegovina

BACKGROUND: Treatment options for patients with metastatic breast cancer (MBC) include a rapidly expanding repertoire of medical, surgical and supportive care measures. DESIGN: To provide timely and evidence-based recommendations for the diagnostic workup and treatment of patients with MBC, an international expert panel reviewed and discussed the evidence available from clinical trials regarding diagnostic, therapeutic and supportive measures with emphasis on their impact on the quality of life and overall survival of patients with MBC. RESULTS: Evidence-based recommendations for the diagnostic workup, endocrine therapy, chemotherapy, use of targeted therapies and bisphosphonates, surgical treatment and supportive care measures in the management of patients with MBC were formulated. CONCLUSIONS: The present consensus manuscript updates evidence-based recommendations for state-of-the-art treatment of MBC depending on disease-associated and biological variables.

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Department of Internal Medicine, Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

PURPOSE: The purpose of this study was to investigate the effect of local chemoimmunotherapy and hyperthermal intraperitoneal chemotherapy (HIPEC) in a mouse model of induced peritoneal carcinomatosis. MATERIAL AND METHODS: Peritoneal carcinomatosis in mice was produced by intraperitoneal implantation of MCa cells (5 x 10(3)). Interleukin-2 (4.1 x 10(4) IU/mouse) was injected into the abdominal cavity of mice at day 7 and 3 before implantation of tumour cells. Immediately after implantation of MCa cells mice were treated twice with 2 ml of saline that was heated either at 37 degrees C or 43 degrees C and cytostatics (doxorubicin 20 mg kg(-1), cisplatin 10 mg kg(-1), mitomycin 5 mg kg(-1), or 5-FU 150 mg kg(-1)). We followed the survival of animals and side effects appearing with different forms of treatment. RESULTS: Combined treatment with Interleukin-2 (IL-2) and cytostatics (5-FU, CIS or MIT) significantly affected the development of peritoneal carcinomatosis and increased the survival of mice (ILS% - 37 degrees C = 29.88, 199.32, and 108.52, ILS% - 43 degrees C = 62.69, 260.50, and 178.05, respectively). However, intraperitoneal chemotherapy on survival time of mice with DOX + IL-2 was ineffec-

tive as compared with DOX alone. CONCLUSION: We would like to stress that treatment with IL-2 prior to tumour diagnosis is not clinically practical, rather, the manuscript attempts to describe an experimental proof of principle. Results suggest the synergistic effect of hyperthermia, chemotherapy and immunotherapy; IL-2 significantly increases antitumor activity of hyperthermic chemotherapy and survival rate of mice with peritoneal carcinomatosis.

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School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

The aim of this study was to examine the characteristics of acute tonsillopharyngitis diagnosis and treatment in the family care physicians in Mostar, Bosnia and Herzegovina. All patients diagnosed with acute tonsillopharyngitis in the Center of Family Medicine in Mostar in 2005 and 2006 were included in this study. Data were collected from medical records, including age and sex, month in which they visited their physician, symptoms and signs that they had (McIsaac's clinical score was calculated accordingly) and treatment. Results showed that there were no gender differences regarding diagnosis. Mean age was 32.6 +/- 16.9 years. The most patients were recorded during the January-March period. Total of 65.6% patients received antibiotic treatment. Phenoxymethylpenicillin, considered as the recommended antibiotic was used in 46.3% cases only. In conclusion, this first critical assessment of the existing family practice records on treating patients with tonsillopharyngitis suggested that physicians have to be more critical when treating patients with this diagnosis and that the knowledge and treatment of patients with pharyngitis need to be continuously improved in general medical practice.

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Department for Infectious Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

The aim of this study was to provide an overview of the snakebites in patients hospitalized at the Mostar Clinical Hospital, admitted between 1983 and 2006. A total of 341 patients were recorded, with moderate men predominance (52.8%). Majority of patients were bitten for the first time (99.1%). In 98.8% of patients snakebite occurred to the bare skin, most commonly during June to September period (64.2%). Snakebites were the commonest in agricultural workers (48.1%). Until 2003 all admitted patients were treated according to Russel's scheme (3-anti). As of 2003 new treatment scheme was applied, resulting in the reduction of antidote and supportive treatment use, causing a reduction in the number of clinically apparent allergic reactions. Serum sickness was recorded in only 2 patients, while lethal outcome was recorded in one (0.3%). Overall results indicate that lethality of snakebite is low, and that patients were often administered treatment without medical indication. High number of tourists as well as the presence of the peace keeping troops and other visiting personnel in this region make the snakebites and awareness on snakes not only a local issue, but also more general concern.

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Department of Physiology, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

Prostaglandins (PGs) are lipid compounds that mediate the variety of physiological and pathological functions in almost all body tissues and organs. Prostacyclin (prostaglandin 12, PGI2), which is synthesized by the vascular endothelium, is a potent vasodilator, inhibits the aggregation of platelets in vitro and has cytoprotective effect on gastrointestinal mucosa. The aim of this study was to determine whether PGI2 is playing a role in host defense to toxic effect of acetaminophen (APAP). This was investigated in C57Black/6 mice which were intoxicated with single lethal or high sublethal dose of APAP. APAP was administered to mice by gastric lavage and PGI2 agonists or antagonists were given intraperitoneally (i.p.) 30 minutes before or 2 hours after administration of APAP. The toxicity of APAP was determined by observing the survival of mice during 48 hours, by measuring the concentration of alanine-aminotransferase (ALT) in plasma 20-24 hours after APAP administration, and by liver histology. Mice were given either pure PGI2 (PGI2 sodium salt), its stable agonist (iloprost) or inhibitor of prostacyclin (IP)-receptor (CAY-10441). The results have shown that PGI2 exibits a strong hepatoprotective effect when it was given to mice either before or after APAP (both increase of survival of mice and decrease of plasma ALT levels were statistical significant). Iloprost has not shown a similar effect and CAY-10441 increased toxic effect of APAP if given 2 hours after its administration. Histopathological changes in liver generally support these findings. These investigations support the view that PGI2 is involved in defense of organism to noxious effects of xenobiotics on liver.

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Essential oil of Thymus aureopunctatus (Beck) K. Malý, obtained by hydrodistillation and headspace technique, was subjected to capillary GC-MS analysis, and its volatile composition was compared with essential oil profile of Thymus serpyllum L. and a botanically undetermined thyme species, Thymus spp., all growing wild in the same habitat in Bosnia and Herzegovina. This paper presents the first report on phytochemical analysis of the rare T. aureopunctatus species, and also the first report on headspace analysis of plants belonging to the genus Thymus. One hundred and two volatile constituents were identified. The investigated Thymus species of Bosnian origin clearly belong to the thymol-chemotype with relatively abundant level of this main constituent varying from 30.5% for T. serpyllum, and 34.5% for T. aureopunctatus, to 44.2% for Thymus spp., while their corresponding headspace samples contain very high percentage of p-cymene, thymol biosynthesis precursor, in the range from 29.1% to 68.1%.

7. Ćeremida-Dragišić M, Dragišić V. Characteristics of motor vehicle accidents in the Herzegovina region. Coll Antropol. 2009;33 Suppl 2:193-7.

Department of Physical Medicine and Rehabilitation, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

Epidemiological studies around the world point to motor vehicle accidents as being one of the leading causes of death. The objective of our study was to analyze some characteristics of motor vehicle accidents in the region of Herzegovina. The study included 226 patients treated at the Clinic for Surgical Diseases and Urology of the University Clinical Hospital Mostar in 2005. A total of 78.3% or examinees were men and 21.7% women. The majority of patients of both sexes were aged between 20 and 30 years. Most of the accidents occurred during autumn and winter months, on weekends and in sunny-dry weather conditions. As expected, most of the hospitalized patients were injured either while driving an automobile or in a pedestrian versus automobile collisions. Of the 226 patients 92.5% admitted to the emergency department and 60.2% were hospitalized. Most of the injured (72.1%) were not under the influence of alcohol at time of the accident. 82.7% of injured were examined by medical personnel at the site within 30 minutes from injury but only 43.0% of them arrived at the hospital within 30 minutes of injury. As most of the injured patients were not under the influence of alcohol at the time of the injury we are of the opinion that more attention in preventing motor vehicle accidents should be directed to speeding and the state of the motorways and vehicles themselves. We propose a large-scale epidemiological study in the Herzegovina region and a review of current road management practices and emergency protocols.

8. Damjanović V, Vasilj I, Vlak T, Zelenika D. Prevalence and risk factors of the rheumatoid arthritis in Herzegovina region in 2003-2005. Coll Antropol. 2009;33 Suppl 2:73-7.

School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

In this study we evidenced prevalence of the rheumatoid arthritis (RA) in Herzegovina region of the Bosnia and Herzegovina and studied selected RA risk factors. Sample of subjects comprised RA diagnosed subjects which were compared to randomly selected controls. In diagnosing the RA we used criteria for the classification of rheumatoid arthritis suggested by The American College of Rheumatology. Risk factors of RA included in this investigation were (1) educational status, (2) quality of nutrition, and (3) socioeconomic status. Average prevalence of the RA in our sample was 0.46/100, ranged from 0.36/100 to 0.64/100, which is comparable to other European samples. The RA occurrence is six times more often in females than in males. We have found indices that the Mediterranean diet has to be considered as protective factor against RA. Although RA occurrence is more frequent in the low socioeconomic samples of subjects, because of the methodological reasons we can not undoubtedly support the socioeconomic status as significant risk factor of the RA. Finally, it is interesting that we have found education level as risk factor significantly related to RA occurrence in our sample. All evidenced should be more precisely studied in some future study, while accurately controlling all relevant factors.

9. Dedeić-Ljubović A, Hukić M, Pfeifer Y, Witte W, Padilla E, López-Ramis I, Albertí S. Emergence of CTX-M-15 extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates in Bosnia and Herzegovina. Clin Microbiol Infect. 2010;16(2):152-6.

Institute of Clinical Microbiology, University of Sarajevo Clinics Centre, Sarajevo, Bosnia and Herzegovina

Fifty-seven nosocomial Klebsiella pneumoniae isolates producing extended-spectrum beta-lactamases

(ESBLs) were collected between February 2007 and November 2007 in different wards of the Sarajevo (Bosnia-Herzegovina) reference hospital. These isolates comprise two major epidemic pulsed-field electrophoresis-defined clones plus two minor clones. In addition to the ESBL-mediated resistance, all strains uniformly showed resistance to ciprofloxacin, gentamicin and tobramycin. The beta-lactamases involved in this resistance phenotype were TEM-1, SHV-1, and CTX-M-15, as demonstrated by isoelectric focusing, PCR amplification, and sequencing. TEM-1 and CTX-M-15 beta-lactamases, as well as the aminoglycoside resistance determinants, were encoded in plasmids that could be transferred to Escherichia coli by conjugation. In three of the infected patients with the predominant clone, cefoxitin resistance development (MICs >128 mg/L) was documented. The analysis of the outer membrane proteins of the cefoxitin-susceptible and cefoxitin-resistant isolates revealed that the former expressed only one of the two major porins, OmpK36, whereas in the latter, the expression of Ompk36 was altered or abolished. This is the first report of CTX-M-15-producing K. pneumoniae in Bosnia-Herzegovina. Furthermore, we document and characterize for the first time cefoxitin resistance development in CTX-M-15-producing K. pneumoniae.

10. Definis-Gojanović M, Gugić D, Sutlović D. Suicide and Emo youth subculture--a case analysis. Coll Antropol. 2009;33 Suppl 2:173-5.

Department of Forensic Medicine, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

Depression and suicide present a serious health problem especially for teenagers as they are increasingly diagnosed with mood disorders of different severity, possibly leading to suicidal activity. Reported here is a misfortunate young girl who committed suicide by jumping from high altitude. She left a suicide note which, together with her behavior in the death-preceding period, pointed to her apparently belonging to an Emo subculture. Although few and scarce, most existing articles and reports on Emo subculture found that its members like to focus on negative things, dark premonitions and deprivation of enjoyment, like self harm and suicide but no scientific information are available about the characteristics, trends and possible suicidal tendencies of children and adolescents who belong to this subgroup. It is for the future researches to answer whether this type of behavior and music preference are causal factors for increased suicidal vulnerability, or personal characteristics and anamnesis, upbringing and mental health status are actual sources of the problem.

11. Delibegović S, Matović E. Hem-o-lok plastic clips in securing of the base of the appendix during laparoscopic appendectomy. Surg Endosc. 2009;23(12):2851-2854.

Department of Surgery, University Clinic Center, Tuzla, Bosnia and Herzegovina

BACKGROUND: During laparoscopic appendectomy (LA), the standard technique in securing of the base of the appendix is by endoloop ligatures. However, application of the endoloop demands dexterity and a short training, while hem-o-lok clips may be more advantageous to use due to their simplicity of application and low cost. The objective of this study was to evaluate the technical feasibility and eventual advantages of this way of securing of the base of the appendix. PATIENTS AND METHODS: Prospective study was conducted in the period from August 2006 to August 2008. The patients were divided into two groups; in the first group the base of the appendix was secured by double endoloop ligatures, while in the second group it was done by double nonabsorbable hem-o-lok clips. The data collected included age, gender, operative time, hospital stay, costs, and intra- and postoperative complications. RESULTS: There was no difference in hospital stay between the two groups of patients; mean operative time was 47.1 +/- 6.7 min in the first group where the base was secured by endoloop ligatures, and was 38.7 +/- 5.0 min in the group where the base was secured by hem-o-lok clips. The cost of the three hem-o-lok clips was <euro>76.9, and that of the three endoloop ligatures was <euro>88.5. In hem-o-lok group of patients, one intraoperative complication was observed, involving bleeding of mesoappendix. There were no postoperative complications in either group of patients. CONCLUSION: The simplicity of application, shorter time of operation, and lower cost of hem-o-lok clips are advantages of this way of securing of the base of the appendix in relation to the standard endoloop procedure.

12. Fatušić Z, Hudić I. Incidence of post-operative adhesions following Misgav Ladach caesarean section--a comparative study. J Matern Fetal Neonatal Med. 2009;22(2):157-60.

Clinic for Gynecology and Obstetrics, University Clinical Centre, Tuzla, Bosnia and Herzegovina

AIM: To evaluate the incidence of peritoneal adhesions as a post-operative complication after caesarean section following the Misgav Ladach method and compare it with peritoneal adhesions following traditional caesarean section methods (Pfannenstiel-Dörffler, low midline laparotomy-Dörffler). METHODS: The analysis is retrospective and is based on medical documentation of the Clinic for Gynecology

and Obstetrics, University Clinical Centre, Tuzla, Bosnia and Herzegovina (data from 1 January 2001 to 31 December 2005). We analysed previous caesarean section dependent on caesarean section method (200 by Misgav Ladach method, 100 by Pfannenstiel-Dörffler method and 100 caesarean section by low midline laparotomy-Dörffler). Adhesion scores were assigned using a previously validated scoring system. RESULTS: We found statistically significant difference ($p < 0.05$) in incidence of peritoneal adhesions in second and third caesarean section between Misgav Ladach method and the Pfannestiel-Dörffler and low midline laparotomy-Dörffler method. Difference in incidence of peritoneal adhesions between low midline laparotomy-Dörffler and Pfannenstiel-Dörffler method was not statistically different ($p > 0.05$). The mean pelvic adhesion score was statistically lower in Misgav Ladach group (0.43 +/- 0.79) than the mean score in the Pfannestiel-Dörffler (0.71 +/- 1.27) and low midline laparotomy-Dörffler groups (0.99 +/- 1.49) ($p < 0.05$). CONCLUSIONS: Our study showed that Misgav Ladach method of caesarean section makes possible lower incidence of peritoneal adhesions as post-operative complication of previous caesarean section.

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13. Galić G, Tomić M, Galešić K, Kvesić A, Šoljić M, Možetić V, Lončar Z, Maričić A, Martinović Ž. Hypoalbuminemia and complication incidence in hemodialysed uremic patients. Coll Antropol. 2009;33(2):559-66.

Clinical Hospital Mostar , Mostar, Bosnia and Herzegovina

DISCUSSIONS whether hypoalbuminemia is just a marker for the malnutrition-inflammation syndrome as well as for the increased morbidity and mortality of those patients or is it an etiological factor, are becoming more and more intense. In this research of the relation between hypoalbuminemia and the complications that threaten the vascular access with special reference to infection, and consequently to the life of the patients treated with chronic haemodialysis, we have chosen 120 patients with terminal renal insufficiency (ESRD) treated at the Clinical Hospital Mostar by chronic haemodialysis. The chosen patients for this study were observed throughout a time period of 18 months. Only the patients who, at the moment of starting the research did not exhibit either a local or a systemic infection, as well as no signs of any other complication that might have endangered the vascular access and consequently the life of the patient, were selected. From the 120 (100.0%) patients, 86.8% of them had a serum albumin level below 40.0 g/L. By analysing the research results of the clinical material, it has been established that in patients with serum albumin level below 40.0 g/L, the infection incidence was significantly higher than in those patients with the albumin level above 40.0 g/L ($chi2 = 7.215$ $P = 0.0077$). The complication incidence is significantly higher ($chi2 = 9.92$ $P = 0.0022$) among the patients with serum albumin level below 40.0 g/L, than in those patients with higher serum level. Among the patients with a serum albumin level lower than 40.0 g/L, the sepses incidence was significantly higher ($chi2 = 4.77$ $P = 0.03$), than among those patients with a serum albumin level above this value. However, the difference in incidence of local infection of the vascular access between the group of patients with a serum albumin level below 40.0 g/L and those patients with albumin level above this value is not significant ($chi2 = 0.65$ $P = 0.69$). The total infection incidence in the 120 observed patients was 3.8 episodes per 100 patient months, and within the parameters mentioned by other authors.

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14. Hudić I, Fatušić Z. Progesterone - induced blocking factor (PIBF) and Th(1)/Th(2) cytokine in women with threatened spontaneous abortion. J Perinat Med. 2009;37(4):338-42.

Clinic for Gynecology and Obstetrics, University Clinical Center, Tuzla, Bosnia and Herzegovina

OBJECTIVE: The aim of this prospective study was to compare serum and urine concentrations of progesterone-induced blocking factor (PIBF) and serum concentrations of anti-inflammatory (IL10) and proinflammatory (IL6, TNFalpha, IFNgamma) cytokines of women with threatened spontaneous abortion with normal pregnancy and to evaluate the impact of PIBF on outcome of pregnancy. METHODS: A sample of 30 women with threatened spontaneous abortion (study group) and 20 healthy pregnant women (control group) between 6(th) and 24(th) gestational weeks was studied. Serum and urine PIBF, IL10 and IL6, TNFalpha, IFNgamma cytokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA). RESULTS: Five (16.7%) pregnancies in the study group ended missed abortion vs. none in the control group ($P<0.05$). Five (20%) threatened aborters delivered between 24(th) and 37(th) weeks of gestation, whereas two (10%) preterm deliveries occurred in the controls ($P>0.05$). PIBF concentrations in urine (19.5+/-12.9 ng/mL) and serum (214.4+/-120.6 of patients with threatened abortion were significantly lower than in healthy pregnant women (45.3+/-33.7 ng/mL and 357.3+/-159.9 ng/mL, respectively). Women with threatened abortion had significantly lower serum levels of anti-inflammatory cytokine, but levels of proinflammatory cytokines were higher in this group compared with healthy controls. CONCLUSIONS: Determination of progesteron-induced blocking factor level in body fluids in early pregnancy might be used for the diagnosis and prognosis of threatened abortion.

15. Hudić I, Fatušić Z, Szekeres-Bartho J, Balić D, Polgar B, Ljuca D, Dizdarević-Hudić L. Progesterone-induced blocking factor and cytokine profile in women with threatened pre-term delivery. Am J Reprod Immunol. 2009;61(5):330-7.

Clinic for Gynecology and Obstetrics, University Clinical Center, Trnovac bb, Tuzla, Bosnia and Herzegovina

PROBLEM: The objective of this study was to compare serum concentrations of progesterone-induced blocking factor (PIBF), anti-inflammatory (IL-10),and pro-inflammatory (IL-6, TNFa, and IFNc) cytokines of women with threatened pre-term delivery, with those of women with normal pregnancy and to evaluate the impact of PIBF on the outcome of pregnancy. METHOD OF STUDY: A prospective study was conducted on a sample of 30 women with threatened pre-term delivery (study group) and 20 healthy pregnant women (control group) between the 24th and 37th gestational weeks. Serum PIBF, anti-inflammatory (IL-10), and pro-inflammatory (IL-6, TNFa, and IFNc) cytokine concentrations were measured by enzyme-linked immunosorbent assay (ELISA). RESULTS: Thirteen of 30 patients (43.3%) with symptoms of threatened pre-term delivery, and one of 20 patients (5%) in the control group delivered before the 37th week of gestation. Mean PIBF concentrations in serum samples of patients with threatened pre-term delivery were significantly lower than in those of healthy pregnant women (171.12 +/-162.06 ng/mL versus 272.85 +/-114.87 ng/mL; P < 0.05).Women with symptoms of threatened pre-term delivery had significantly lower serum levels of IL-10, and higher levels of IL-6 as well as IFNc compared with healthy controls. CONCLUSION: Our results indicate that measuring PIBF and cytokine concentrations in serum during pregnancy is feasible and may be important for understanding immunological causes of pre-term delivery.

16. Hukić M, Nikolić J, Valjevac A, Šeremet M, Tešić G, Markotić A. A serosurvey reveals Bosnia and Herzegovina as a Europe's hotspot in hantavirus seroprevalence. Epidemiol Infect. 2009:1-9.

Institute for Clinical Microbiology, Clinical Centre University of Sarajevo, Sarajevo, Bosnia and Herzegovina

SUMMARYThe extent of hantavirus seroprevalence in the healthy population from Bosnia and Herzegovina has not yet been investigated; therefore, the aim of this study was to assess the hantavirus seroprevalence in the population from different regions of Bosnia and Herzegovina and in different risk groups. The serosurvey included 1331 subjects from endemic and non-endemic regions in Bosnia and Herzegovina. All sera samples were examined using IgG ELISA, and Western blot (Bunyavirus IgG) tests. Hantavirus seroprevalence was 7.4% in the endemic region and 2.4% in the non-endemic region (P<0.05). Former soldiers from the endemic region had significantly the highest seroprevalence (16.1%) compared to the general population from the endemic region (6.2%), the occupational risk group from the non-endemic region (5.6%) and the general population from the non-endemic region (0.8%) (P<0.01). No difference in hantavirus seroprevalence between gender or age groups was observed. Hantavirus seroprevalence in different populations in Bosnia and Herzegovina was found to be highest compared to other central European countries.

17. Jandrić S, Manojlović S. Quality of life of men and women with osteoarthritis of the hip and arthroplasty: assessment by WOMAC questionnaire. Am J Phys Med Rehabil. 2009;88(4):328-35.

Department of Physical Medicine, Rehabilitation and Rheumatology, Institute for Rehabilitation Dr Miroslav Zotovic, Banja Luka, Republika Srpska, Bosnia and Herzegovina

OBJECTIVE: The aim of this study was to investigate the differences in quality of life between men and women in preoperative and postoperative period after hip arthroplasty because of severe hip osteoarthritis. DESIGN: This is a prospective study of 160 patients (average age, 61.7 yrs), 92 women and 68 men, with a diagnosis of osteoarthritis of the hip who underwent total hip arthroplasty. All patients completed the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaires that measured health-related quality of life preoperatively, at discharge, and 6-wk postoperatively. To establish the occurrence of differences between men and women in preoperative and postoperative period after arthroplasty, Student's t test and multivariate logistic regression analysis were used. RESULTS: The WOMAC global score was a significant predictor (the better the rather men) preoperatively (P < 0.01) and 6 wks after total hip arthroplasty (P < 0.001). Global WOMAC score was significantly better in men than in women preoperatively (t = 4.02; P < 0.001) and 6 wks after arthroplasty (t = 3.42; P < 0.001). CONCLUSIONS: These results suggest that men with severe osteoarthritis of the hip have better quality of life than do women preoperatively and 6 wks after hip arthroplasty. These findings would be important for improving quality of care of our patients.

18. Jurić M, Čarapina M. A case repot of Madelung's disease in a 69 years old man. Coll Antropol. 2009;33 Suppl 2:169-71.

Department of Maxillofacial Surgery, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

Madelung's disease is an extremely rare disorder of unknown etiology. It is characterized by the huge, bilateral, fatty deposits in regions of the neck, shoulders and the upper extremities. A 69-old patient with developed symptoms of Madelung's disease with a 12-years history is described in this study. The patient was initially considered to have a goiter and chronic heart insufficiency, for which he has been treated for three years. Although the Madelung's disease can be diagnosed right after detailed clinical examination, this study pointed out possible diagnostic and therapeutic mistakes when a physician in a differentiation of symmetrical neck and shoulder swellings doesn't consider the possibility of diagnosing a Madelung's disease.

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19. Klarić M, Letica I, Petrov B, Tomić M, Klarić B, Letica L, Francisković T. Depression and anxiety in patients on chronic hemodialysis in University Clinical Hospital Mostar. Coll Antropol. 2009;33 Suppl 2:153-8.

Psychiatric Clinic, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

Depression and anxiety are prevailing mental problem in patients on chronic hemodialysis and they have great influence on outcome of illness. Additionally, these disorders are rarely identified in that population of patients and they are insufficiently treated. The aim of this study was to assess the prevalence of depression and anxiety in patients on chronic hemodialysis in University Clinical Hospital Mostar and to examine the correlation between the demographic variables and the time spent on dialysis with depression and anxiety levels. The experimental group consisted of 56 patients on chronic hemodialysis in Mostar Clinical Hospital. The control group 1 consisted of 53 patients diagnosed with a chronic illness and treated for at least a year, while the control group 2 consisted of 51 persons who were not diagnosed with any chronic somatic or mental illness. Demographic data were collected using the constructed questionnaire. The Beck Depression Inventory-BDI was used to determine depression, while the Spielberger State-Trait Anxiety Inventory-STAI was used to determine anxiety. We recorded significantly higher prevalence of depression in patients on chronic dialysis (51.8%) than in patients with a chronic illness (41.5%) and persons without chronic illnesses (9.8%; $p < 0.001$). Trait anxiety level was significantly higher in hemodialysed patients compared to the other two groups ($p = 0.006$) but there were no significant differences in state anxiety level. The study has not shown any significant difference in the prevalence of depression and anxiety level regarding the differences in sex, gender and education level, apart from a higher level of state anxiety in patients with a lower education level ($p = 0.032$). These results indicate that patients on hemodialysis have a significantly higher level of depression and a higher level of trait anxiety compared to patients with chronic illnesses and especially compared to general population.

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20. Kozomara D, Galić G, Brekalo Z, Kvesić A, Jonovska S. Abdominal pain patient referrals to emergency surgical service: appropriateness of diagnosis and attitudes of general practitioners. Coll Antropol. 2009;33(4):1239-43.

Department of Surgery, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

This study evaluate the need for general practitioners referrals and self referrals of acute abdominal pain patients to emergency surgical service, the appropriateness of GP referral diagnosis and their attitudes dealing with abdominal pain. In three months period all acute abdominal pain patient referrals to our hospital emergency surgical service were audited. Data on final diagnosis, surgical treatment, admission to hospital and surgery performance were recorded. Self referral or GP referral, referring GP diagnosis, referral letters indicating presenting complaint or history, axillar and rectal temperature measurement, laboratory checking and abdominal radiography checking by GP were recorded as well. Also, GPs examination details as palpation, auscultation and digit-rectal checking were recorded. We calculated sensitivity, specificity, positive and negative predictive value (PV) for referring diagnosis. Self referrals and GP referrals differences were evaluated. During the study 318 patients were admitted. A total of 163 (51.25%) referrals were deemed inappropriate; 102 (52.6% of GP referrals) and 61 (49.2% of self referred) ($p < 0.05$). There were no differences in general treatment, hospital admission and operative treatment in self referred and GP referred groups ($p < 0.05$ for all three categories). Sensitivity, specificity, positive and negative predictive values for most frequent GP referral diagnoses were: abdominal colic/abdomen in observation 0.78; 0.66; 0.74; 0.70; acute appendicitis 0.37; 0.92; 0.44; 0.90; acute abdomen/peritonitis 0.30; 0.97; 0.54; 0.92; constipation 0.95; 0.98; 0.85; 0.99; and ileus 0.83; 0.97; 0.50; 0.99. Data on GP including clinical examination, patient history and running basic diagnostics were poor. Our results suggest that a general agreement within the profession about what constitutes a necessary hospital referral is necessary. GP consultation quality must be improved by booking more time per patient and by giving more medical/technical attention to patients.

21. Lačević A, Pojskić LK, Lojo NK, Ramić J, Bajrović K. Tannerella forsythia detected in infected root canals using nested PCR. Am J Dent. 2009;22(4):211-4.

Department of Dental Pathology and Endodontics, School of Dentistry, Bolnicka 4a, University of Sarajevo, Bosnia and Herzegovina

PURPOSE: This study assessed the occurrence of Tannerella forsythia in patients with acute and chronic primary endodontic infections. METHODS: Clinical samples were collected from 40 patients with acute and chronic periradicular disease. Nested polymerase chain reaction (PCR) assay technique was used to detect the presence of T. forsythia in primary endodontic infections. The first round of PCR amplification used universal primers to detect the 16S rDNA sequence. Product from the first round was then used to amplify T. forsythia specific fragment with species-specific pairs of primers. RESULTS: T. forsythia was found in 12 of 27 chronic and 5 of 13 acute infected patients for an overall occurrence frequency of 42.5%. No significant correlation was found between patients with the T. forsythia positive genotype and the occurrence of clinical symptoms in the primary endodontic infections ($P < 0.05$) ($P = 0.496$). Also, no significant relationship was found between the occurrence of T. forsythia and the patient's age ($P = 0.61$) or gender ($P = 0.239$).

22. Lakičević G, Ostojić L, Splavski B, Roth S, Vlak T, Brekalo Z, Ostojić M. Comparative outcome analyses of differently surgical approaches to lumbar disc herniation. Coll Antropol. 2009;33 Suppl 2:79-84.

School Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

Lumbar disc herniations (LDH) occur in the lower back, most often between the fourth and fifth lumbar vertebral bodies or between the fifth and the sacrum. It is evident lack of studies dealing with comparative analysis of the surgical outcomes of the spine operation techniques. In this paper we analyzed and compared outcomes of the LDH standard techniques (laminectomy and hemilaminectomy), and contemporary operation techniques (interlaminectomy, and micro-discectomy). Adult patients (18-75 years of age) surgically treated on the Neurosurgery Department of the University Clinical Hospital Mostar - Bosnia and Herzegovina between January 1998 and December 2007 were sampled as subjects. We analyzed and compared, number of the LDH surgically treated patients; age, patient's satisfaction with postoperative status, postoperative recurrence of the LDH; incidence of the postoperative complications, and duration of hospitalization. In conclusion, modern operating methods have to be considered as superior over traditional operating types mostly because of smaller violations of forms and integrity of lumbar spine.

23. Lepara O, Alajbegović A, Začiragić A, Nakaš-Ićindić E, Valjevac A, Lepara D, Hadžovic-Džuvo A, Fajkić A, Kulo A, Sofić E. Elevated serum homocysteine level is not associated with serum C-reactive protein in patients with probable Alzheimer's disease. J Neural Transm. 2009;116(12):1651-6.

Institute of Physiology and Biochemistry, School of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Elevated plasma homocysteine (Hcy) levels have been associated with Alzheimer's disease (AD) and cognitive impairment. Studies have shown that Hcy may have direct and indirect neurotoxicity effects. The aim of the study was to investigate serum Hcy concentration in patients with probable AD with age-matched controls and to determine whether there was an association between serum Hcy and C-reactive protein concentration in patients with probable AD. We also aimed to determine whether there was an association between serum tHcy concentration and cognitive impairment in patients with probable AD. Serum concentration of total Hcy was determined by the fluorescence polarization immunoassay on the AxSYM system, and serum C-reactive protein (CRP) concentration was determined by means of particle-enhanced immunonephelometry with the use of BN II analyzer. Cognitive impairment was tested by the MMSE score. Body mass index (BMI) was calculated for each subject included in the study. Age, systolic and diastolic blood pressure and BMI did not differ significantly between the two groups. Mean serum tHcy concentration in the control group of subjects was 12.60 mumol/L, while in patients with probable AD the mean serum tHcy concentration was significantly higher than 16.15 mumol/L ($p < 0.01$). A significant negative association between serum tHcy concentration and cognitive impairment tested by the MMSE score in patients with probable AD was determined ($r = -0.61634$; $p < 0.001$). Positive, although not significant correlation between CRP and serum tHcy concentrations in patients with AD, was observed. Increased tHcy concentration in patients with probable AD, and the established negative correlation between serum tHcy concentration and cognitive damage tested by MMSE score in the same group of patients, suggests the possible independent role of Hcy in the pathogenesis of AD and cognitive impairment associated with this disease.

24. Ljuca F, Gegić A, Salkić NN, Pavlović-Čalić N. Circulating Cytokines Reflect Mucosal Inflammatory Status in Patients with Crohn's Disease. Dig Dis Sci. 2009.

Faculty of Medicine, University of Tuzla, Univerzitetska 1, Tuzla, Bosnia and Herzegovina

BACKGROUND: There is a great need for a simple activity assessment tool that can reliably predict activity in patients with Crohn's disease (CD). AIM: To investigate the relationship between serum cytokines and endoscopic activity of CD using Crohn's Disease Endoscopic Index of Severity (CDEIS) as a gold standard. METHODS: We prospectively evaluated 32 firmly established CD patients using ileocolonoscopy, CDEIS score, and Crohn's Disease Activity Index (CDAI) score. Blood samples for cytokine analysis were obtained 1 day prior to procedure. RESULTS: The correlation between CDEIS and CDAI was moderate ($r = 0.43$; $P = 0.01$); however, the correlation between CDEIS and inflammatory cytokines was excellent, with the highest coefficients for tumor necrosis factor alpha (TNFalpha) and interleukin-6 (IL-6) ($r = 0.96$ and $r = 0.96$, respectively; $P < 0.001$). CDEIS and anti-inflammatory cytokines were correlated nonlinearly (power function). We identified two separate models for predicting CDEIS value, based on the best performing pro-inflammatory [CDEIS = 0.445 x (IL-6) - 5,143] and anti-inflammatory [CDEIS = 27.478 x (IL-10)(-0.71)] cytokines. Both IL-6 and IL-10 models had high adjusted R (2) values (0.916 and 0.954, respectively). IL-6 had excellent diagnostic accuracy for detecting patients with CDEIS >7 (active disease), with area under the receiver operating characteristic (ROC) curve of 1.0 [95% confidence interval (CI) = 0.89-1.0; $P < 0.001$]. CONCLUSION: Serum cytokine levels are excellent predictors of endoscopic activity in patients with CD.

25. Marjanović D, Durmić-Pašić A, Kovačević L, Avdić J, Džehverović M, Haverić S, Ramić J, Kalamujić B, Lukić Bilela L, Škaro V, Projić P, Bajrović K, Drobnić K, Davoren J, Primorac D. Identification of skeletal remains of Communist Armed Forces victims during and after World War II: combined Y-chromosome (STR) and MiniSTR approach. Croat Med J. 2009;50(3):296-304.

Institute for Genetic Engineeringand Biotechnology, Gajev trg 4, Sarajevo, Bosnia and Herzegovina

AIM: To report on the use of STR, Y-STRs, and miniSTRs typing methods in the identification of victims of revolutionary violence and crimes against humanity committed by the Communist Armed Forces during and after World War II in which bodies were exhumed from mass and individual graves in Slovenia. METHODS: Bone fragments and teeth were removed from human remains found in several small and closely located hidden mass graves in the Skofja Loka area (Lovrenska Grapa and Zolsce) and 2 individual graves in the Ljubljana area (Podlipoglav), Slovenia. DNA was isolated using the Qiagen DNA extraction procedure optimized for bone and teeth. Some DNA extracts required additional purification, such as N-buthanol treatment. The QuantifilerTM Human DNA Quantification Kit was used for DNA quantification. Initially, PowerPlex 16 kit was used to simultaneously analyze 15 short tandem repeat (STR) loci. The PowerPlex S5 miniSTR kit and AmpF/STR MiniFiler PCR Amplification Kit was used for additional analysis if preliminary analysis yielded weak partial or no profiles at all. In 2 cases, when the PowerPlex 16 profiles indicated possible relatedness of the remains with reference samples, but there were insufficient probabilities to call the match to possible male paternal relatives, we resorted to an additional analysis of Y-STR markers. PowerPlex Y System was used to simultaneously amplify 12 Y-STR loci. Fragment analysis was performed on an ABI PRISM 310 genetic analyzer. Matching probabilities were estimated using the DNA-View software. RESULTS: Following the Y-STR analysis, 1 of the "weak matches" previously obtained based on autosomal loci, was confirmed while the other 1 was not. Combined standard STR and miniSTR approach applied to bone samples from 2 individual graves resulted in positive identifications. Finally, using the same approach on 11 bone samples from hidden mass grave Zolosce, we were able to obtain 6 useful DNA profiles. CONCLUSION: The results of this study, in combination with previously obtained results, demonstrate that Y-chromosome testing and mini-STR methodology can contribute to the identification of human remains of victims of revolutionary violence from World War II.

26. Markota I, Markota D, Tomić M. Measuring of the heparin leakage into the circulation from central venous catheters--an in vivo study. Nephrol Dial Transplant. 2009;24(5):1550-3.

Division of Nephrology and Dialysis, Department of Medicine, Mostar University Hospital Center, University of Medicine Mostar, 88 000 Mostar, Bosnia and Herzegovina

BACKGROUND: A catheter lock with a highly concentrated heparin solution is often used to maintain its patency. The result of the in vitro study shows a significant catheter leakage that occurs after locking the catheter. The goal of this study is to develop a model to measure the catheter leakage in vivo and test it on various kinds of catheters. METHODS: Twenty-four patients with central venous dialysis catheters

were examined. After the 48-h interdialytic period, we aspirated the contents of the catheter lumen for analysis. We simultaneously took a sample of the peripheral blood for analysis. In the second part of the test, instead of taking the sample after 48 h, we took it after 10 min. Based on the difference in haematocrit in those two samples, we were able to determine the amount of heparin that remained in the catheter, and indirectly, the amount of heparin that leaked out of the catheter. RESULTS: Using the lock volumes indicated on the catheter by the manufacturer, the early leakage is significantly higher in nontunnelled catheters compared to tunnelled Hemoflow and Ash Split catheters (P = 0.05). There is no significant statistical difference in the early leakage between Ash Split and Hemoflow catheters. The late leakage is significantly higher in nontunnelled catheters compared to Hemoflow and Ash Split catheters (P = 0.05). There is no significant statistical difference in the total leakage between Ash Split and Hemoflow catheters. CONCLUSION: We present a model that enables the measurement of the catheter leakage in vivo. We applied the model on three kinds of catheters and concluded that both early and late leakages are significantly higher in nontunnelled catheters compared to Hemoflow and Ash Split tunnelled catheters. Our results show that the so-called early leakage measured in vivo is significantly lower compared to the results from in vitro studies. Further research is necessary to determine the amount of leakage volume for different kinds of catheters and to determine the exact leakage dynamics of lock solutions in vivo.

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27. Markota NP, Markota I, Tomić M, Zelenika A. Inappropriate drug dosage adjustments in patients with renal impairment. J Nephrol. 2009;22(4):497-501.

Department of Family Medicine, DZ Mostar, Mostar, Bosnia and Herzegovina

BACKGROUND: The aim of this study was to determine whether appropriate dosage adjustments were made in patients with renal impairment discharged from the Department of Internal Medicine and to evaluate a possible role for family medicine physicians in reducing the number of inadequate drug dosages. METHODS: The study included all patients discharged from the Department of Internal Medicine. Data regarding serum creatinine levels, age, sex and prescribed drugs and their dosage were collected from the patients' medical records and discharge letters after discharge from hospital. We calculated the estimated glomerular filtration rate (GFR) using the abbreviated MDRD equation. Drug dosage adequacy was controlled in the patients with GFR less than 60 ml/min per 1.73 m2. RESULTS: At the time of discharge from the hospital, 161 of 712 patients (22.6%) had estimated GFR <60 ml/min per 1.73 m2. These patients were prescribed 874 drugs, which amounted to 5.43 per patient. Dosage adjustment according to renal function was necessary for 171 prescriptions (19.6%). This adjustment was performed adequately in 81 cases (47.4%) and inadequately in 90 cases (52.6%). Digoxin metformin and the combination of ACE inhibitors and spironolactone amounted to 65.6% of the inadequate prescriptions. There were significantly more incorrect drug dosages in women. CONCLUSION: Drug dosage in patients with renal impairment can be improved. Since a computerized dynamic alert system is not available in our hospital the role of family medicine physicians is significant in reducing the number of inadequate drug dosages at hospital discharge.

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28. Marković-Peković V, Stoisavljević-Šatara S, Škrbić R. Utilisation of cardiovascular medicines in Republic of Srpska, Bosnia and Herzegovina, 5 years study. Pharmacoepidemiol Drug Saf. 2009;18(4):320-6.

Health Insurance Fund, Republic of Srpska, Banja Luka, Bosnia and Herzegovina.

PURPOSE: The objective of this study was to analyse cardiovascular medicines utilisation patterns in Republic of Srpska (Bosnia and Herzegovina) over the 2002-2006 period. METHODS: A retrospective study was taken to analyse utilisation medicines reimbursed by the Health Insurance Fund (HIF), with a focus on cardiovascular medicines utilisation. ATC/DDD methodology was used to monitor utilisation of medicines. Drug utilisation 90% (DU90%) method was used to determine the prescribing quality of cardiovascular medicines. RESULTS: Utilisation of cardiovascular medicines accounted for more than one half of the total medicines utilisation, with an increasing trend. Most prescribed were antihypertensive medicines i.e. ACE inhibitors, both plain and in combination with thiazide diuretics, and calcium channel blockers (CCB). Utilisation of beta-blockers and thiazide diuretics remains rather low despite an overall increase in utilisation. Re-introduction of statins in 2004 instantly led to high utilisation. The number of cardiovascular medicines within DU90% segment varies from 8 in 2002 to 12 in 2006. CONCLUSIONS: This study showed a constant increase in total medicines utilisation with the cardiovascular medicines as the most prescribed. DU90% is shown as a simple method for assessing general quality of medicines prescribing, which indicated that better adherence to the guidelines is needed in order to practice a more rational use of medicines and a cost-efficient use of all resources. More stratified routinely performed analyses are also needed.

29. Maslov B, Marčinko D, Miličević R, Babić D, Đorđević V, Jakovljević M. Metabolic syndrome, anxiety, depression and suicidal tendencies in post-traumatic stress disorder and schizophrenic patients. Coll Antropol. 2009;33 Suppl 2:7-10.

Department of Psychiatry, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

Persons with schizophrenia and post-traumatic stress disorder (PTSD) tend to have higher psychiatric and somatic morbidity. They typically have higher rates of substances abuse (including smoking), more prevalent obesity, diabetes mellitus, and cardiovascular disease (CVD). This is especially well seen in case of the metabolic syndrome, with a number of published studies on psychiatric patients in the last few years. This study investigated the associations between metabolic syndrome, anxiety, depression and suicidal tendency in schizophrenic and combat-related PTSD patients controlled by healthy controls. Higher rates of anxiety, depression and recent life changes scores in participants with metabolic syndrome were recorded compared to those without metabolic syndrome. Suicidal tendencies were equally present in both groups.

30. Memišević H, Sinanović O. Epilepsy in children with intellectual disability in Bosnia and Herzegovina: effects of sex, level and etiology of intellectual disability. Res Dev Disabil. 2009;30(5):1078-83.

Center for Education and Rehabilitation of Children with Intellectual Disability, Mjedenica, Sarajevo, Bosnia and Herzegovina

The purpose of this study was to examine the occurrence of epilepsy in children with intellectual disability. An additional goal was to determine if there were statistical differences in the occurrence of epilepsy related to the sex, level and etiology of intellectual disability of children. The sample consisted of 167 children with intellectual disability attending two special education schools in Sarajevo, Bosnia and Herzegovina. The method for data collection was the examination of the children's medical records. A chi-square test was performed to determine if there were any significant differences in the occurrence of epilepsy among different categories of children with intellectual disability. Additionally, Phi coefficient and Cramer V coefficient were calculated to determine the strength of association. The occurrence of epilepsy in children with intellectual disability is high and certain etiological categories are associated with an even higher risk of epilepsy. The study confirmed a high occurrence of epilepsy in children with intellectual disability. Some psycho-educational implications of epilepsy were discussed and in the future there should be better cooperation between medical and educational institutions in treating the bio-psycho-social issues of a child with epilepsy.

31. Mikulić I, Petrik J, Galešić K, Ž, Čepelak I, Zeljko-Tomić M. Endothelin-1, big endothelin-1, and nitric oxide in patients with chronic renal disease and hypertension. J Clin Lab Anal. 2009;23(6):347-56.

Mostar University Hospital, Mostar, Bosnia and Herzegovina

The complex pathogenesis of chronic renal disease (CRD) depends on endothelin (ET) axis (ETs and ET receptors) and nitric oxide (NO) because of their vasoactive effects and their role in general modulation of vascular homeostasis. Various renal cells synthesize ETs and NO that play a significant role in renal hemodynamics as well as in water and salt excretion via urine. ET-1 is a strong vasoconstrictor. Besides its vasoactive effects, ET-1 modulates mitosis and apoptosis in a cell type-dependent manner, and may play an important role in CRD pathogenesis. The aims of this study were to emphasize the role and interactions of ET-1, Big ET-1, and NO in CRD. Concentrations of these vasoactive molecules were measured in plasma/serum and/or urine of 57 patients with diabetic nephropathy (subgroup 1), arterial hypertension (subgroup 2) or CRD with chronic renal insufficiency (subgroup 3), and in healthy control subjects (n=18). In comparison with control group, urine concentration of Big ET-1 was significantly increased (13.13 pmol/L vs. 11.34 pmol/L; $P<0.001$) in CRD patients, whereas plasma and urine concentrations of ET-1 did not differ significantly. NO concentrations were also significantly increased in CRD patients (serum, 72.55 micromol/L; $P<0.001$, and urine 141.74 micromol/L; $P<0.05$) as compared to control group. Study results indicated that Big ET-1 and NO could be useful diagnostic parameters in CRD for their diagnostic sensitivity and diagnostic specificity (Big ET-1 in urine: 56.1 and 88.9%, and NO in serum: 66.7 and 83.3%, respectively). In addition, Big ET-1 may prove useful in the differential diagnosis of diabetic nephropathy (78.6% diagnostic sensitivity and 88.9% diagnostic specificity).

32. Mimica M. Management of Helicobacter pylori-associated diseases: survey of attitudes changes among general practitioners in Bosnia and Herzegovina. Coll Antropol. 2009;33 Suppl 2:159-63.

Department of Internal Medicine, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

The aim of the study was to investigate the attitude change of general practitioners (GPs) in Bosnia and

Herzegovina considering the key decision points in the management of treatment of Helicobacter pylori (H. pylori)-associated diseases in 2008, after the four-year period. The first survey was done from February to April 2004. Two hundred and sixty four GPs in B&H responded to questionnaire especially designed for the study. In May 2008, four years after the first survey, data were collected again by same questionnaire from 53 GPs. The most important source of information about the management of treatment of H. pylori-associated diseases in both surveys were symposia sponsored by pharmaceutical companies, but the percent decreased from 53% in 2004 to 34% in 2008. The percent of GPs who named the Internet as the major information source increased from 5% in 2004 to 28% in 2008. Medical journals were used as the most important source of information by every fourth GP in 2008 almost in the same percent as in 2004. In 2008 the percent of GPs who considered that the main obstacle to proper management of H. pylori-related diseases was the patient's low income status which was doubled in relation to 2004 (64%; 31% respectively). Almost all GPs (98%) claimed to eradicate H. pylori in 2008--a significant increase compared to 2004 when 71% of GPs reported eradication. Sixty percent of GP in 2008 claimed confirmation of infection prior to prescribing the eradication therapy which was significant increase in comparison to 2004 when only 9% of GPs confirmed presence of the H. pylori infection before starting eradication. All GPs who claimed to eradicate H. pylori infection used a proton pump inhibitor based on triple drug therapy, while in 2004 18% of GPs chose some other inadequate eradication therapy. As a conclusion, better selection of information sources eliminated management options of questionable value, but scarce economic resources in B&H will probably remain the main obstacle to the comprehensive H. pylori treatment.

33. Naletilić M, Tomić V, Šabić M, Vlak T. Cerebral palsy: early diagnosis, intervention and risk factors. Coll Antropol. 2009;33 Suppl 2:59-65.

School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

Early diagnosis and intervention intensity were suggested to be crucial factor in cerebral palsy (CP) treatment. Herein we observed 347 children diagnosed for CP in Clinical Hospital Mostar, Bosnia and Herzegovina, and studied the relationship between (a) intervention start point and the final motor outcome, (b) intensity of treatment and final outcome, and (c) relationship between documented risk factors and early diagnosis of the CP. Our study suggests that it is possible to relatively accurately diagnose the CP in the first trimester. Previous miscarriages, sepsis and intracerebral haemorrhage were significantly related to early diagnosis, while delivery outcome, RDS, premature birth, intracerebral haemorrhage, sepsis, meningitis, hydrocephalus and convulsions were found as significantly related to final motor CP outcome. We have found no significant influence of the intervention intensity and final diagnosis. Our results support the idea that the intervention start point has to be considered as one of the most important factors for the effective intervention program. In future studies dealing with the CP interventions and risk factors, special attention should be paid to homogeneity and size of the sample, as well as necessity of including the non-treated controls in the investigation.

34. Nežić L, Škrbić R, Dobrić S, Stojiljković MP, Šatara SS, Milovanović ZA, Stojaković N. Effect of simvastatin on proinflammatory cytokines production during lipopolysaccharide-induced inflammation in rats. Gen Physiol Biophys. 2009;28 Spec No:119-26.

Department of Pharmacology, Toxicology and Clinical Pharmacology, Medical Faculty, Save Mrkalja 14, 78000 Banja Luka, Republic of Srpska, Bosnia and Herzegovina

The effect of simvastatin applied in a short-term pretreatment on proinflammatory cytokines production in acute systemic inflammation induced by endotoxin - lipopolysaccharide (LPS) in rats was investigated. Both LPS and simvastatin doses were established in separate experiments in which increasing doses of both compounds were given to obtain the LD(50) LPS and the maximally protective dose of simvastatin against LD(50) LPS. To determine the anti-inflammatory effect, simvastatin was given orally for 5 days, followed by a single intraperitoneal non-lethal dose of LPS (0.25 LD(50)). Plasma concentrations of tumor necrosis factor alpha (TNF-alpha), interleukin (IL)-1beta and IL-6 were measured by enzyme-linked immunosorbent assay. The acute i.p. LD(50) LPS amounted to 22.15 mg/kg. Simvastatin of 20 mg/kg p.o. was maximally protective against LD(50) LPS, and this dose was used for studying its effects on LPS-induced cytokines production. Cytokines concentrations were significantly increased upon challenge of non-lethal dose of LPS. The peak levels of TNF-alpha and IL-1beta were significantly suppressed by simvastatin, compared to control rats only treated with dimethylsulfoxide before LPS. In contrast, simvastatin did not affect IL-6 levels at all timepoints. Simvastatin pretreatment given orally produced acute anti-inflammatory effects by inhibiting TNF-alpha and IL-1beta, but no IL-6 production.

35. Nežić L, Škrbić R, Dobrić S, Stojiljković MP, Jaćević V, Šatara SS, Milovanović ZA, Stojaković

N. Simvastatin and indomethacin have similar anti-inflammatory activity in a rat model of acute local inflammation. Basic Clin Pharmacol Toxicol. 2009;104(3):185-91.

Department of Pharmacology, Toxicology and Clinical Pharmacology, Medical Faculty, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Statins, such as simvastatin, lower circulating cholesterol levels and are widely prescribed for the treatment of hypercholesterolaemia. Several studies have shown unexpected effects of statins on inflammation. We studied the anti-inflammatory effect of simvastatin using a standard model of an acute local inflammation, the carrageenan-induced footpad oedema. Experimental groups (n = 6-8) were given simvastatin in a dose range 5-30 mg/kg, indomethacin 1-8 mg/kg and methylcellulose (control) per os. Footpad volume was measured with a plethysmograph and compared with the pre-injection volume of the same paw. Swelling (in microlitres) was then calculated, and in drug-treated animals, per cent inhibition was derived through comparison with the control group. Histopathological examination of the skin biopsies was performed to examine severity of paw skin lesions and to confirm the simvastatin-induced inhibition of acute inflammation. Both simvastatin and indomethacin administered orally, 1 hr before carrageenan injection, significantly reduced the extent of footpad oedema. Indomethacin dose-dependently blocked the swelling; the maximal effect was obtained with 8 mg/kg by 48.3% ($P < 0.05$). Simvastatin produced a comparable anti-inflammatory activity at a dose of 5 mg/kg (32%), while 10 and 30 mg/kg caused a 47.6% and 51.7% reduction, respectively, with the maximal effect observed at 20 mg/kg by 57.2% ($P < 0.05$). The comparison of the ED(50) of these agents on molar basis showed equipotent anti-inflammatory activity. Histopathological examination of the footpad skin biopsies revealed that simvastatin, dose-dependently and comparablly to indomethacin, reduced polymorphonuclear leucocyte infiltration. These data support the hypothesis that simvastatin has an acute anti-inflammatory activity.

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36. Nikolić J, Kuzman I, Markotić A, Rode OD, Curić I, Ivanković HB, Grgić S. The occurrence of hemorrhagic fever with renal syndrome in southern parts of Bosnia and Herzegovina. Coll Antropol. 2009;33 Suppl 2:37-42.

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Bosnia and Herzegovina (B&H) has been known as an endemic region for hemorrhagic fever with renal syndrome (HFRS) for over 50 years. Multiple epidemics of this disease have been registered so far, especially in endemic parts of Central and Northeastern Bosnia, as well as the Sarajevo region. Seroepidemiological investigations demonstrate naturalization of Hantaviruses and their wide spread in B&H. However, there are no studies from the southern areas of B&H, and endemic foci of this disease are unknown. The aim of this study was to determine the distribution and serologic prevalence of Hantavirus infections by testing for specific IgG antibodies against hantaviruses in the population of Herzegovina. This study included two groups of participants. The target group consisted of 300 participants from exposed professional and population groups, and control group included 100 educators with lower exposure to HFRS. Identification of specific IgG antibodies against hantaviruses in 16 participants confirmed an initial assumption about the presence of Hantavirus infections in the region of interest. Seroprevalence of 5% was registered in the "exposed" and 1% in the "unexposed" group. Simultaneous circulation of Puumala (PUU) and Dobrava (DOB) viruses was discovered. The frequency of positive antibody results was higher in the population above 50 years of age, and three times more prevalent in men then at women. The highest proportion of exposed participants (80%) was registered in the municipalities which geographically belong to high or mountainous Herzegovina.

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37. Novaković M, Babić D, Dedić G, Leposavić L, Milovanović A, Novaković M. Euthanasia of patients with the chronic renal failure. Coll Antropol. 2009;33(1):179-85.

Department of Psychiatry, Faculty of Medicine, Foca, Bosnia and Herzegovina

This study deals with frequency and form of euthanasia in dialysis patients with chronic renal failure (CRF) in Bosnia and Herzegovina (B&H) within the period from 2000 to 2006. Of total number of 2700 patients on dialysis we examined n = 753 of them. Examinees with the Balkan Endemic Nephropathy (BEN) (n = 348) were in the first group, and the Control group was formed of patients with other diseases (n = 405). In this study the following methods were used: adapted Questionnaire from the Renal Registry of B&H, Beck's Anxiety Inventory (BAI), Hamilton's Depression Rating Scale (HDRS) and Mini-Mental Scale of Estimation (MMSE). Age of the BEN group of patients ranged: 64.77 +/- 8.86 and the control one 53.85 +/- 3.60. Multivariate analysis for the BEN group with passive euthanasia was: 0.760 (95%, CI = 0.590-0.710) (p = 0.001) and for the active one was 0.450 (95%, CI = 0.125-0.510 (p = 0.001). Euthanasia is associated with the rural life and renal heredity, and psychological BAI scale-total, HDRS-total and MMSE-total. For

the BEN group passive euthanasia is 3.75% as well as active 0.86%. The findings stressed that euthanasia of dialysis patients requires better nephrological-psychiatric control and social care in B&H as well as complete program for the CRF samples protection too.

38. Omeragić J, Vejzagić N, Zuko A, Jažić A. Culicoides obsoletus (Diptera: Ceratopogonidae) in Bosnia and Herzegovina-first report. Parasitol Res. 2009;105(2):563-5.

Department of Parasitology and Invasive Diseases, Veterinary Faculty of Sarajevo, Zmaja od Bosne 90, 71000, Sarajevo, Bosnia and Herzegovina

The first occurrence of bluetongue disease in Bosnia and Herzegovina was registered in 2002 in the area of Kalesija municipality. Entomological investigation of the presence of Culicoides species in that area was conducted in 2007. The aim of the research was to establish the presence of the main vector of bluetongue virus. Collections and analyses of Culicoides midges were performed in accordance with the protocols of the National Reference Centre for Exotic Diseases (Centro Studi Malattie Esotiche) in Teramo, Italy. Traps for capturing midges were placed next to four sheep farms. During the investigation, a total of 2,256 Culicoides midges were collected and only one species was identified, Culicoides obsoletus Meigen, 1818.

39. Pajević I, Hasanović M, Koprić A. Psychiatry in a battle zone. Bioethics. 2009.

School of Medicine, University of Tuzla, Bosnia and Herzegovina

ABSTRACT The authors describe the arrival and treatment of 164 severe chronic psychiatric patients who were displaced from the Serbian army-controlled Jakes psychiatric hospital and off-loaded on the afternoon of 28th of May, 1992 at the gates of the Psychiatry Clinic in Tuzla. Through analysis of their incomplete medical records, which arrived with the patients in Tuzla, and analysis of their activities during and after the war, they found that 83 of the patients (50%) were males and 147 (89.6%) were admitted to the Psychiatry Clinic in Tuzla. Of the patients, 86 (58.5%) were found to be Serbs. The majority of them were incapable of independent living and required ongoing medical and social care. They were from all regions of Bosnia-Herzegovina, 81.6% had schizophrenia and 70 (47.6%) were over 50 years of age. For its humanitarian work, its contribution to peace and for the maintenance of the multi-ethnic Bosnia-Herzegovina, the Psychiatry Clinic in Tuzla received the Golden Award for Peace from the International Legion of Humanists in May 1998.

40. Pravdić D, Vladić N, Bošnjak ZJ. Intracellular Ca2+ modulation during short exposure to ischemia-mimetic factors in isolated rat ventricular myocytes. Coll Antropol. 2009;33 Suppl 2:121-6.

Department of Physiology, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

We investigated the effects of different ischemia-mimetic factors on intracellular Ca2+ concentration ([Ca2+]i). Ventricular myocytes were isolated from adult Wistar rats, and [Ca2+]i was measured using fluorescent indicator fluo-4 AM by confocal microscopy. Intracellular pH was measured using c5-(and-6)-carboxy SNARF-1 AM, a dual emission pH-sensitive ionophore. Myocytes were exposed to hypoxia, extracellular acidosis (pH(o) 6.8), Na-lactate (10 mM), or to combination of those factors for 25 min. Monitoring of [Ca2+]i using fluo-4 AM fluorescent indicator revealed that [Ca2+]i accumulation increased immediately after exposing the cells to Na-lactate and extracellular acidosis, but not during cell exposure to moderate ischemia. Increase in [Ca2+]i during Na-lactate exposure decreased to control levels at the end of exposure period at extracellular pH 7.4, but not at pH 6.8. When combined, Na-lactate and acidosis had an additive effect on [Ca2+]i increase. After removal of solutions, [Ca2+]i continued to rise only when acidosis, hypoxia, and Na-lactate were applied together. Analysis of intracellular pH revealed that treatment of cells by Na-lactate and acidosis caused intracellular acidification, while short ischemia did not significantly change intracellular pH. Our experiments suggest that increase in [Ca2+]i during short hypoxia does not occur if pH(i) does not fall, while extracellular acidosis is required for sustained rise in [Ca2+]i induced by Na-lactate. Comparing to the effect of Na-lactate, extracellular acidosis induced slower [Ca2+]i elevation, accompanied with slower decrease in intracellular pH. These multiple effects of hypoxia, extracellular acidosis, and Na-lactate are likely to cause [Ca2+]i accumulation after the hypoxic stress.

41. Prohić A. Distribution of Malassezia species in seborrhoeic dermatitis: correlation with patients' cellular immune status. Mycoses. 2009.

Department of Dermatology, University Clinical Center, Sarajevo, Bosnia and Herzegovina

Malassezia species are implicated in the pathogenesis of seborrhoeic dermatitis (SD), but the relationship between each species and the disorder remains unclear. It is hypothesised that the pathogenesis of SD has an immune component, which is supported by the increased incidence in patients with immunosuppressive disorders. The purpose of our study was to analyse the prevalence of Malassezia species in lesional skin

of SD, and to assess the distribution of the species according to severity of the disease and cellular immune status of the patients. Forty SD patients with scalp involvement were included in the study. The samples were obtained by scraping the skin surface of the scalp and then incubated on Sabouraud dextrose agar and modified Dixon agar. The yeasts isolated were identified by their morphological and physiological properties according to the method of Guillot et al. In addition, we performed two-colour flow cytometry analysis to investigate the lymphocyte subpopulations in the peripheral blood. The most commonly isolated species was Malassezia restricta (27.5%), followed by Malassezia globosa (17.5%) and Malassezia slooffiae (15%). We demonstrated low helper/suppressor ratios in 70% patients, because of an increase in the suppressor T-cell population, suggesting an impaired cellular immunity. However, we found no significant difference in the distribution of isolated Malassezia species according to the severity of the scalp involvement and changes in the peripheral blood lymphocyte subpopulations.

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42. Rašić S, Hadžović-Džuvo A, Tomić M, Unčanin S, Ćorić S. Impact of hemoglobin concentration on plasma B-type natriuretic peptide level and left ventricle echocardiographics characteristics in chronic kidney disease patients. Coll Antropol. 2009;33 Suppl 2:141-4.

Clinic of Nephrology, University Clinical Center Sarajevo, Sarajevo, Bosnia and Herzegovina

Anemia is common in patients with chronic kidney disease (CKD) and contributes to cardiovascular alterations. Recent findings suggest that B-type natriuretic peptide (BNP) is a sensitive biomarker for left ventricular dysfunction, but relationship between hemoglobin and BNP in CKD patients is unclear. Hemoglobin, plasma BNP and serum creatinine levels were measured in 49 patients with CKD (without heart failure), divided in two groups according to the hemoglobin status (cut-off point 110 g/L). All patients underwent echocardiography in order to assess left ventricular (LV) morphology and function. The results showed that in the group of patients with hemoglobin levels under 110 g/L BNP levels were significantly elevated ($p < 0.001$), as well as left ventricular mass index ($p < 0.001$). Systolic and diastolic LV function were significantly better in patients with hemoglobin levels above 110 g/L ($p < 0.001$). Hemoglobin levels were inversely related to BNP values ($r = -0.451$, $p < 0.001$). Significantly negative correlation between BNP level and creatinine clearance ($p = 0.009$), and significantly positive correlation between BNP level and left ventricular mass index (LVMI) were established. A similar but positive relationship was observed between hemoglobin levels and creatinine clearance ($p < 0.01$). We established statistically significant negative correlation between hemoglobin levels and LVMI ($r = -0.564$, $p < 0.001$). In conclusion, BNP and hemoglobin levels depend on the renal function. Anemia may contribute to elevated BNP levels in CKD patients, and may represent an important confounder of the relationship between BNP and cardiac alteration in these patients.

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43. Salkić NN, Pavlović-Čalić N, Gegić A, Jovanović P, Bašić M. Ulcerative colitis in the Tuzla region of Bosnia and Herzegovina between 1995 and 2006: epidemiological and clinical characteristics. Eur J Gastroenterol Hepatol. 2009.

Department of Gastroenterology, Internal Medicine Hospital, University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina

BACKGROUND: Bosnia and Herzegovina (B&H) is one of the Eastern European countries that lacks data on the epidemiology of ulcerative colitis (UC). AIMS: We aimed to assess the epidemiological and clinical characteristics of UC in Tuzla Canton of B&H during a 12-year period (1995-2006). PATIENTS AND METHODS: We retrospectively evaluated hospital records of both UC inpatients and outpatients residing in Tuzla Canton of B&H (total of 496 280 inhabitants) between 1995 and 2006. Patients that firmly satisfied the diagnostic criteria for UC were included in the study. Incidence rates were calculated with age standardization using European population standards. Trends in incidence were evaluated as moving 3-year averages. RESULTS: During the observed period, 214 patients met the diagnostic criteria for UC. The average age-standardized incidence was found to be 3.43/10 inhabitants [95% confidence interval (CI) = 2.97-3.89], ranging from 0.22 to 7.44 per 10. The mean annual crude incidence in the last 5 years of study (2002-2006) was 5.55/10 (95% CI = 4.63-6.48). The prevalence of UC during the observed period was found to be 43.1/10 (95% CI = 37.3-48.8). The incidence of UC increased dramatically from the average of 1.01/10 in the period between 1995 and 1997 to 6.04/10 between 2004 and 2006, as did the number of colonoscopies performed, from 29 in 1995 to 850 in 2006. The average yearly incidence of confirmed UC cases detected on colonoscopy was 5.56 per 100 colonoscopies per year (95% CI = 4.81-6.30) and only 3.92 per 100 colonoscopies (95% CI = 3.26-4.57) in the last 5 years of the observed period. CONCLUSION: Tuzla Canton of B&H is a region with an increasing incidence of UC, which is most likely a direct consequence of a wider use of colonoscopy. We believe that in the next few years, the incidence of UC in this region will probably reach the annual incidence rate of 6 per 10 inhabitants.

44. Salkić NN, Zerem E, Zildžić M, Ahmetagić S, Čičkušić E, Ljuca F. Risk factors for intrafamilial spread of hepatitis B in northeastern Bosnia and Herzegovina. Ann Saudi Med. 2009;29(1):41-5.

Department of Gastroenterology, Internal Medicine Hospital, University Clinical Center Tuzla, University of Tuzla, Tuzla, Bosnia and Herzegovina

BACKGROUND: Accurate estimations of hepatitis B virus transmission risk for any region in Bosnia and Herzegovina are not clearly established. We aimed to determine levels of risk associated with intrafamilial transmission of hepatitis B infection within families in our region. PATIENTS AND METHODS: Family members of 81 chronic carriers of hepatitis B surface antigen (>6 months positive and considered as index case) were tested for hepatitis B markers. For family members, we recorded their age, sex, and family relationship to the index case, and vaccination status. RESULTS: The proportion of HBsAg positive family members was 25/207 (12.1%), while the proportion of family members with evidence of exposure to HBV was 80/207 (38.6%). Only 17/207 (8.2%) family members had evidence of past HBV vaccination. Age was found to be a significant predictor of HBV exposure of family members (odds ratio 1.05, 95% CI 1.03-1.07, P<.001). In a multivariate analysis, HBsAg positivity was associated with a female index case (odds ratio 11.31, 95% CI 3.73-34.32, P<.001), HBeAg positivity in the index case (odds ratio 5.56, 95% CI 1.80-17.23, P<.005) and being a mother of the index case (odds ratio 9.82, 95% CI 2.43-39.68, P<.005). A female index case (odds ratio 4.87, 95% CI 2.21-10.72, P<.001), HBeAg positivity in the index case (odds ratio 3.22, 95% CI 1.15-9.00, P<.05) and being a mother of the index case (odds ratio 3.72, 95% CI 1.19-11.64, P<.05) were also risk factors for HBV exposure among family members. The combination of HBeAg positivity and female index case was a significant predictor for HBsAg positivity of family members (odds ratio 70.39, 95% CI 8.20-604.61, P<.001). CONCLUSIONS: Children of HBeAg positive mothers are at highest risk for becoming chronic carriers themselves and generally, the combination of female sex and HBeAg positivity dramatically increases the chances of HBV transmission within the family.

45. Salkić NN, Zildžić M, Zerem E, Smajić M, Gegić A, Alibegović E, Jovanović P. Simple uninvestigated dyspepsia: age threshold for early endoscopy in Bosnia and Herzegovina. Eur J Gastroenterol Hepatol. 2009;21(1):39-44.

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OBJECTIVE: To establish an optimal age threshold for endoscopy referral in patients with simple uninvestigated dyspepsia in the setting of European developing country (Bosnia and Herzegovina) with low availability and high workload of endoscopy units. METHODS: We reviewed patient information on all upper endoscopies performed during a 6-year period (2000-2005). Different age thresholds were evaluated in terms of their predictive power for absence of malignancy. RESULTS: A total of 82 of 4403 (1.86%) dyspeptic patients had upper gastrointestinal (GI) malignancy. Age cutoffs of 40 years for men and 45 years for women had the best predictive power, without any cases of upper GI malignancies below those thresholds. Age cutoffs of 45 years for men and 50 years for women also had excellent negative predictive values (99.7 and 99.9%, respectively) with 1.45 and 0.98 cases of missed upper GI malignancies per 1000 endoscopies, respectively. A total of 1709 of 4403 (38.8%) of endoscopies might have been avoided in men of less than 45 and women of less than 50 with uninvestigated dyspepsia. CONCLUSION: (i) Age thresholds for endoscopy referral are lower than in Western countries and should be different for men and women. (ii) Cutoff values of 40 and 45 years for men and women, respectively, are completely safe to use. (iii) Thresholds of 45 years for males and 50 years for females have a small level of risk of missing upper GI malignancy, but are acceptable to use in areas of low availability of endoscopy.

46. Sarajlić N, Topić B, Brkić H, Alajbeg IZ. Aging quantification on alveolar bone loss. Coll Antropol. 2009;33(4):1165-70.

School of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Objectives were to measure labial alveolar resorption using Lamendin's method; to correlate the measured values of resorption with age at death; to determine whether age influences alveolar resorption, and if so, to quantify this impact. The study was performed during the 1992-1995 period in identified war casualties in Bosnia and Herzegovina. Data on the date, month and year of birth, and on the month and year of death were known in all cases. Measurements were carried out in 845 anterior monoradicular maxillary and mandibular teeth from male bodies (n=198) aged 23-69, divided into five age groups of 23-29, 30-39, 40-49, 50-59 and 60-69 years. Teeth with macroscopic pathologic lesions were excluded from analysis. Lamendin's method was used to measure the alveolar bone level on the labial aspect of the extracted teeth. Results are presented in tables. Regression analysis was used to determine the alveolar resorption increase with age. Results are also presented by the factor of alveolar re-

sorption, where the youngest age group was divided by older age groups. In the total sample of 845 teeth, alveolar bone level of up to 3.49 mm was recorded in 740 (87.76%), of 3.50-5.99 mm in 99 (11.79%), and of > 6.00 mm in 6 (0.71%) teeth. In anterior monoradicular teeth, labial alveolar resorption increased with age and showed a regular age dependent pattern toward older age groups in mandibular but not in maxillary teeth. A > 6-mm pocket was very rarely recorded. Study results contribute to clinical practice, demonstrating that periodontology is a preventive discipline in dental medicine.

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47. Smajlović A, Berbić S, Schiene-Fischer C, Tušek-Žnidarič M, Taler A, Jenko-Kokalj S, Turk D, Žerovnik E. Essential role of Pro 74 in stefin B amyloid-fibril formation: dual action of cyclophilin A on the process. FEBS Lett. 2009;583(7):1114-20.

Department of Biochemistry, Farmaceutical Faculty, University of Tuzla, Tuzla, Bosnia and Herzegovina

We report that Pro74 in human stefin B is critical for fibril formation and that proline isomerization plays an important role. The stefin B P74S mutant did not fibrillate over the time of observation at 25 degrees C, and it exhibited a prolonged lag phase at 30 degrees C and 37 degrees C. The peptidyl prolyl cis/trans isomerase cyclophilin A, when added to the wild-type protein, exerted two effects: it prolonged the lag phase and increased the yield and length of the fibrils. Addition of the inactive cyclophilin A R55A variant still resulted in a prolonged lag phase but did not mediate the increase of the final fibril yield. These results demonstrate that peptidyl prolyl cis/trans isomerism is rate-limiting in stefin B fibril formation.

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48. Šimić D, Šitum M, Letica E, Penavić JZ, Živković MV, Tomić T. Psychological impact of isotretinoin treatment in patients with moderate and severe acne. Coll Antropol. 2009;33 Suppl 2:15-9.

Department for Dermatology and Venerology, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

Acne patients are subject to different degree of psychosocial distress. The emotional impact of acne vulgaris due to disfigurement caused by the disease is undisputed. Most common reactions to the acne are depression and anxiety. The use of isotretinoin, one of the most effective options in acne treatment, increases depression symptoms. The aim of this study was to investigate the psychological status of the patients with moderate to severe acne and to compare patients treated with isotretinoin with patients treated with vitamin C. A total of 85 patients suffering from acne vulgaris were included in the study. The results of this study do not find a significant correlation between the use of isotretinoin and the psychological effects of the drug.

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49. Škrbić R, Igić R. Seven decades of angiotensin (1939-2009). Peptides. 2009;30(10):1945-50.

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Two research groups in both North and South America independently discovered that renin released a novel vasopressor agent. The Argentine group named it hypertensin, and called its plasma protein substrate hypertensinogen. The group from the United States named it angiotonin. In 1958, Braun Menendez and Irvine Page suggested that the peptide should be named angiotensin. The combined name eventually became commonly used to avoid linguistic confusion. Research scientists and physicians today acknowledge that studies of the renin-angiotensin system (RAS) have greatly improved our understanding of several diseases. Certainly, medical practice profited significantly from the synthesis and application of numerous pharmacological agents that antagonize either the biosynthesis or pharmacological responses of endogenously generated angiotensin II. Ultimately, discovery of the renin-angiotensin system led to many studies that resulted in therapies for vascular disease. This article briefly reviews research related to the discovery of angiotensin and indicates the importance of additional studies related to the RAS.

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50. Šumanović-Glamuzina D, Božić T, Brkić V, Robović A, Saraga-Karačić V. Minor malformations: neonatal or anthropological story? Coll Antropol. 2009;33 Suppl 2:31-5.

Clinic for Child Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

Minor malformations (mM) are mild physical deformities that with their incidence, number and evolution may be external indicators of hidden, more serious disorders. Most often these are recognized by the neonatologists. First studies done some forty years ago showed an average incidence of 15% in the general population of newborns and about 50% in children with major malformations (MM). A study done in Maternity Hospital Mostar covering a one-year cohort of the newborns and assessing 38 mM showed an average incidence 23.7% mM in children without MM. Twelve mM have had a frequency above 1%, many of them in the head region. The most frequent specific

mM was a deep sacral dimple (4.6%). Eighteen mM malformations that appeared more often were re-evaluated three months later. A large part (50-80%) disappeared, but a small number (about 17%) were newly discovered. In the newborns with MM, the incidence of mM was 57.5%. 15 of 23 children with MM (65.2%) had more than three associated mM. The highest percentage was in the group of hypotrophic newborns. The connection of mM with MM and specificity of incidence of mM in one population are the reason why the search for mM in the neonatal period could be benefit also for children and whole population.

51. Tahirović E, Begić H, Nurkić M, Tahirović H, Varni JW. Does the severity of congenital heart defects affect disease-specific health-related quality of life in children in Bosnia and Herzegovina? Eur J Pediatr. 2009.

Heart Center Sarajevo, Clinical Center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

The aim of this study was to assess whether the severity of congenital heart defects (CHD) affects disease-specific health-related quality of life (HRQOL) in children after open heart surgery. One hundred and fourteen children with CHD and one of their parents participated in the study. HRQOL was evaluated by the PedsQL 3.0 Cardiac Module. The children were assigned to one of three groups according to severity of CHD. Children with cyanotic CHD (Group 3) reported that their HRQOL on several domains was lower than that of children with anomalies with the left-right shunt and children with obstructive anomalies. Also, by parent proxy-report, patients with anomalies with the left-right shunt had statistically significant, better HRQOL scores for the heart problems and treatment scales, perceived physical appearance, treatment anxiety, cognitive problems, and communication scales in comparison to the children with cyanotic CHD. By self-report, children of Group 1 reported that they had statistically significant, better HRQOL in the heart problems and treatment scales compared with Group 2. Conclusions: The results of the assessment by the PedsQL 3.0 Cardiac Module, a cardiac disease-specific instrument for children with CHD, indicate that HRQOL is poorest in children with complex CHD. Therefore, it is necessary to take the appropriate preventive measures for these patients, which include early (timely) cardiosurgical intervention and active psychological support to limit the negative impact of serious forms of CHD on the quality of life of these children.

52. Tahirović H, Toromanović A, Balić A, Grbić S, Gnat D. Iodine nutrition status of pregnant women in an iodine-sufficient area. Food Nutr Bull. 2009;30:351-354.

Department of Pediatrics, University Clinical Center, Tuzla, Bosnia and Herzegovina

Background. Iodine is necessary for the synthesis of thyroid hormones, which play a decisive role in the development of the brain during fetal and early postnatal life. Objective. To evaluate whether prophylaxis with 20 to30 mg of iodine per kilogram of salt is enough to ensure optimal iodine nutrition during pregnancy in an iodinesufficient area. Methods. A cross-sectional study of pregnant women was conducted in 2007. The urinary iodine concentration (UIC) was measured in 300 randomly selected women in Tuzla, Bosnia, and Herzegovina, in all three trimesters of pregnancy. Results. The median UIC of the pregnant women was 142 μg/L, ranging from 27 to 1,080 μg/L. The median UIC of the pregnant women in each trimester of pregnancy who were not restricting their salt intake was consistent with adequate iodine nutrition, as defined by the World Health Organization Technical Consultation, whereas the median UIC of women who were restricting their salt intake was insufficient. Conclusions. Pregnant women in the urban area of Tuzla had adequate iodine status except for those with restricted salt intake, which presents an increased risk to the mother as well to as the unborn child. Women in the rural area of Tuzla were found to be iodine-deficient, regardless of whether they had restricted their salt intake or not. However, for those pregnant women who have been advised to restrict their salt intake and who thus face the risk of iodine deficiency, the use of salt with higher concentrations of iodine could be advised.

53. Tahirović H, Toromanović A, Grbić S. Higher frequency of screening TSH above 5 mIU/l in infants likely exposed to higher doses of iodine-containing skin antiseptic: implications for assessment of iodine sufficiency. J Pediatr Endocrinol Metab. 2009;22(4):335-8.

Department of Pediatrics, University Clinical Center, Tuzla, Bosnia and Herzegovina

BACKGROUND: Neonatal TSH screening has as its main goal the early detection and treatment of permanent sporadic congenital hypothyroidism. At the same time neonatal TSH is one of the indicators for monitoring progress towards eliminating iodine deficiency disorders (IDD). AIM: To evaluate the usefulness of neonatal TSH screening data as a monitoring tool for IDD evaluation and control in Bosnia and Herzegovina. INFANTS AND METHODS: The study included a total of 8,105 newborns. Neonatal TSH was measured in whole blood drawn between day 3 and day 5 of life, spotted on filter paper using a sensitive fluorometric

assay (Delfia). RESULTS: Levels above 5 mIU/l were found in 282 (5.5%) neonates. Median TSH values and the percentage of samples of neonatal TSH above 5 mIU/l were significantly higher in neonates who were born by Caesarean section (6.4%) compared with neonates born by vaginal delivery (1.4%). CONCLUSION: Our findings suggest that neonatal TSH should not be used as a monitoring tool for IDD evaluation and control if iodinated skin disinfectant is used on a large part of the mother's skin in maternity hospitals.

54. Tahirović H, Toromanović A, Grbić S, Bogdanović G, Fatušić Z, Gnat D. Maternal and neonatal urinary iodine excretion and neonatal TSH in relation to use of antiseptic during caesarean section in an iodine sufficient area. J Pediatr Endocrinol Metab. 2009;22(12):1145-9.

Department of Pediatrics, Division of Endocrinology & Diabetes, University Clinical Center, Tuzla, Bosnia and Herzegovina

AIM: To evaluate the influence of topical iodine-containing antiseptics on neonatal TSH in full-term infants born by Caesarean section in an iodine sufficient area. POPULATION AND METHODS: Urinary iodide excretion (UIE) was estimated in 86 mothers on the second day after delivery by Caesarean section and their 86 full-term neonates. The mothers were divided into two groups according to the use of antiseptic to prepare Cesarean sections: 42 mothers who were prepared with povidone-iodine (Isosept, Bosnalijek) comprised the study group, and 47 mothers who were prepared with alcoholic solution (Skinsept color, Ecolab) formed the control group. Neonatal TSH was measured in whole blood drawn between day 3 and 5 of life, spotted on filter paper using a sensitive fluorometric assay (Delfia). RESULTS: Maternal and neonatal UIE were significantly higher ($p < 0.05$) in the study group compared to the control group. No significant difference was found for neonatal TSH. CONCLUSION: Our data suggest that perinatal iodine exposure of full-term neonates who were born by Caesarean section in an iodine sufficient area did not influence neonatal TSH, although median UIE was higher, suggesting optimal iodine intake during pregnancy. Further research is needed to define a critical value of urinary iodine concentrations in full-term neonates in an iodine sufficient area that may lead to the impairment of thyroid function.

55. Tahirović H, Toromanović A, Grubić M, Grubić Z, Dumić K. Untreated congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Eur J Pediatr. 2009;168(7):847-9.

Department of Pediatrics, University Clinical Center, Tuzla, Bosnia and Herzegovina

Congenital adrenal hyperplasia (CAH) is an inherited metabolic disease caused by the deficiency of one of the enzymes necessary for cortisol synthesis. With carefully supervised medical treatment, CAH patients have the capacity for normal puberty and fertility. We report on a 12.4-year-old female who, because of the early interruption of treatment, developed progressive virilization with reduced final height and altered psycho-social orientation to male. One of the reasons for interrupting replacement therapy in our case was the difficult social and economic status of the family, who lived for many years without basic medical care.

56. Tomić T, Peršić M, Rajić B, Tomić Z. Endoscopic features of gastric mucosa in children having pathohistological evidence of Helicobacter pylori infection. Coll Antropol. 2009;33 Suppl 2:53-7.

Clinic for Children's Diseases, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina.

Infection with Helicobacter pylori (Hp) is common in children from developing countries, particularly in adolescents. It is associated with chronic gastritis and stomach cancer. A characteristic endoscopic finding in children is nodular gastritis. The aim of this study was to assess and confirm association of nodular gastritis, mainly of anthral mucosa, with Hp infection in children. A total of 195 children 1 to 15 years of age were studied during a two-year period (2004-2006). There were 107 girls (54.9%) and 88 boys (45.1%). The patients presented with recurrent epigastric pain, nausea, vomiting, heartburn, sour mouth, regurgitation, bloating or other dyspeptic symptoms. The complaints were recorded by a structured interview with parents and older children. Upper endoscopy was performed in all children. The presence and degree of mucosal granulation was recorded and two samples of mucousa from the antrum and the small curvature were taken. Biopsy material was processed for histology as usual, stained with 2% Giemsa and examined by a pathologist for the presence of Hp. A total of 40 of 195 children (20.5%) have had positive Hp infection and a 27 of 40 (67.5%) have had a granular aspect of anthral mucosa at the endoscopy. Sensitivity of the finding was 87.5%, specificity 93.5%, positive predictive value 73%, negative predictive value 91.8%, $p < 0.05$. Average age of those patients was 11.5 +/- 3.0 years. Subjective symptoms of dyspepsia (as recorded by the questionnaire) were often associated with Hp positivity, but short of statistical significance. No difference between boys and girls was noted. Endoscopic finding of nodular gastritis, especially in areas of antrum and small curvature, showed a highly positive correlation with Hp infection.

57. Tomić V, Petrović O, Petrov B, Bjelanović V, Naletilić M. Hypertensive disorders in pregnancy: a 5-year analysis of the wartime and postwar period in South-Western region of Bosnia and Herzegovina. Coll Antropol. 2009;33 Suppl 2:115-9.

Department of Obstetrics and Gynecology, School of Medicine, University of Mostar, Bosnia and Herzegovina

Hypertensive disorders are among the most common complications in pregnancy and a major cause of perinatal morbidity and mortality. The aim of this study was to investigate the risk factors and adverse perinatal outcomes of pregnancies in mothers with hypertensive disorders, as well as the adequacy of prenatal care during the wartime and postwar period in South-Western region of Bosnia and Herzegovina. This study included a total of 542 pregnancies with hypertensive disorders during 5-year study period (1995-1999) and 1559 randomly selected controls. Data on risk factors, adverse perinatal outcomes (for singleton pregnancies only) and prenatal care on pregnant women were extracted from the medical records and compared with controls. Chi-square test and crude odds ratio (OR) with 95% confidence interval (95% CI) were used in statistical analysis. The average five-year incidence of hypertensive pregnancy disorders was 6.5% and it was significantly higher in 1995, the last year of the war, than in the postwar period (1996-1999) ($p = 0.02$). Factors significantly associated with hypertensive pregnancy disorders were maternal age > 34, nulliparity, multifetal gestation and male newborn ($p < 0.001$; except $p = 0.002$ for male newborn). Severe forms of hypertensive disorders were significantly associated with adverse perinatal outcomes: preterm birth (OR 2.6, 95% CI 1.08-6.3), cesarean delivery (OR 9.2, 95% CI 5.4-15.6), fetal growth restriction (OR 63.8, 95% CI 34.8-117.0), and stillbirth (OR 5.5, 95% CI 2.1-14.1). Women with hypertensive pregnancy disorders had significantly lower number of prenatal care visits than controls ($p < 0.001$). There was a high proportion of normally formed macerated stillbirths in the study (27 out of 30 or 90%) and in the control group (10 out of 12 or 83%). In conclusion, severity of the disorder and adequacy of prenatal care are strongly associated with adverse perinatal outcome related to hypertensive pregnancy disorders.

58. Toromanović A, Tahirović H. Congenital hypothyroidism associated with neonatal tooth, Pierre-Robin syndrome and congenital heart defects. J Pediatr Endocrinol Metab. 2009;22(10):881-2.

Department of Pediatrics, University Clinical Center, Tuzla, Bosnia and Herzegovina

A female infant was born as the second child of healthy unrelated parents at 37 weeks of gestation by Caesarian section at a local hospital with birth weight 2,880 g, length 51 cm, and head circumference 33 cm. The pregnancy was complicated by polyhydramnion and placenta previa. The infant had stigmata of Pierre-Robin syndrome, and on the 5th day of age a tooth eruption in the region of the lower central incisor was noted. She required nasogastric tube feeding. Jaundice occurred during the first week of life. The patient was recalled because of a positive screening test for congenital hypothyroidism. At the time of screening on day 4, thyroid-stimulating hormone (TSH) was 136 mIU/l. On admission to our hospital, on the 13th day of age, physical examination revealed temperature 38.7°C, tachypnoea, peripheral cyanosis, slight oedema of the eyelids, low frontal and posterior hairline, micrognathia, retrognathia, cleft soft palate, muscular hypotonia. In addition, a continuous murmur with weak radial and femoral pulses were noted. An ultrasound examination of the thyroid gland was carried out confirming the absence of any thyroid tissue in the thyroid area at the base of the neck. At the same time, an ultrasound examination of the knee was done. She had evidence of delayed bone maturation based on absence of the distal femoral epiphyses. She was immediately started on 25 μg/day of L-thyroxine before awaiting biochemical confirmation of suspected diagnosis. Thyroid function tests done on the 13th day of life revealed TSH >100 mIU/l (normal range: 0.15-3.2), free thyroxine (fT4) 0.10 pmol/l (normal range: 10-25), total thyroxine (T4) 0.0 nmol/l (normal range: 65-160), total triiodothyronine (T3) 0.5 nmol/l (normal range: 1.04-2.5), and thyroglobulin (Tg) 6.1 ng/ml (normal range: 2-70). Roentgenography of the knee demonstrated absence of distal femoral epiphyses. Thyroid scintigraphy was not done. Echocardiography showed coarctation of the aorta, patent ductus arteriosus and patent foramen ovale with aneurysma of interatrial septum. Cytogenetic analysis demonstrated normal female karyotype. Because of a high degree of mobility, the tooth was extracted on the 20th day of life. Her clinical condition markedly improved and she was discharged from hospital on the 36th day of life. During the following weeks she demonstrated signs of airway obstruction and failure to thrive. Unfortunately the child died from cardiorespiratory insufficiency on the 65th day of age at a local hospital. Higher incidence of congenital extrathyroid anomalies in infants with congenital hypothyroidism than in the general population has been reported and especially congenital heart diseases[1,2]. However, neonatal teeth with congenital hypothyroidism, as in our patient, has not to our knowledge been previously published. Further studies on congenital malformations in the general population and those associated

with congenital hypothyroidism are still needed to help us understand the role of local genetic and environmental factors.

59. Toromanović A, Tahirović H. Transient neonatal hypothyroidism manifested at birth. J Pediatr Endocrinol Metab. 2009;22(1):11.

Department of Paediatrics, University Clinical Center, Tuzla, Bosnia and Herzegovina

A 2 day-old male infant was referred to the Department of Paediatrics for a large goiter. The boy was born as the third child of healthy unrelated parents at 40 weeks of gestation with birth weight 4,950 g and length 55 cm. Antiseptics containing iodine were not used in the mother, nor in the infant. Physical examination revealed myxedema of the face, protruding tongue, dry skin, hoarse cry, large goiter and hypotonia. An ultrasound examination revealed enlarged thyroid gland. Thyroid function tests at 2 days of age were as follows: total triidothyronine (T3) 3.0 nmol/l (normal range: 1.04-2.5), total thyroxine (T4) 23.2 nmol/l (normal: 65-160), free thyroxine (fT4) 2.6 pmol/l (normal range: 10-25), thyroid-stimulating hormone (TSH) 165.1 mIU/l (normal range: 0.15-3.2), and thyroglobulin 2,093 ng/ml. He was immediately started on 50 μg/day of L-thyroxine. Roentgenography of the knee demonstrated dysgenesis of the distal femoral epiphysis, while the proximal tibial epiphysis were not visualized. Molecular genetic analysis of the TPO gene was done. In the examined regions of the TPO gene, all 17 exons except exon 14, no mutations were detected. His mother's serum T4 and TSH levels were normal, and thyroglobulin and thyroperoxidase antibody tests were negative. There was no family history of thyroid diseases. No maternal use of medication was reported. Weaning off the L-thyroxine was started on the 23rd day of life due to elevated T4 level. At the age of 4 months he was completely weaned off L-thyroxine and has not required any since. The patient is now 3.8 years old, thyroid enlargement is still present, and physical and neurological development are normal. Iodine overload, iodine deficiency, and maternal thyroid antibodies are common causes of transient congenital hypothyroidism. The etiology of transient hypothyroidism in this newborn is unknown. More research is required in order to evaluate the frequency, causes and optimal therapy of transient neonatal hypothyroidism.

60. Toromanović A, Tahirović H, Milenković T, Koehler K, Kind B, Zdravković D, Hasanhodžić M, Huebner A. Clinical and molecular genetic findings in a 6-year-old Bosnian boy with triple A syndrome. Eur J Pediatr. 2009;168(3):317-20.

Department of Pediatrics, University Clinical Center, Trnovac bb, 75000, Tuzla, Bosnia and Herzegovina

The triple A syndrome is a rare autosomal recessive disease that is characterised by the triad of adrenocorticotropin (ACTH)-resistant adrenal insufficiency, achalasia and alacrima. In most patients, neurological and dermatological abnormalities are associated features. We report on the first Bosnian patient with triple A syndrome. Endocrine investigation confirmed primary adrenal insufficiency at the age of 5.8 years. Two months later, achalasia was diagnosed, and in the presence of alacrima, the patient satisfies the diagnostic criteria of triple A syndrome. In addition, a large number of associated neurological and dermatological features were present in this patient. Moreover, he has dysmorphic facial features, which have not been previously described in triple A syndrome. Triple A syndrome was confirmed by molecular analysis, revealing a nonsense mutation p.W84X in the AAAS gene. The parents are both heterozygous carriers of the mutation. The affected twin brother unfortunately died from hypoglycaemic shock, despite a normal cortisol rise in an ACTH stimulation test. Further, triple A syndrome patients carrying the identical homozygous p.W84X mutation have to be studied to assess a genotype-phenotype relationship for this mutation.

61. Trninić Z, Vidačak A, Vrhovac J, Petrov B, Šetka V. Quality of life after colorectal cancer surgery in patients from University Clinical Hospital Mostar, Bosnia and Herzegovina. Coll Antropol. 2009;33 Suppl 2:1-5.

Surgery Clinic, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

Quality of life (QoL) has become an important outcome measure for patients with cancer, but results from population-based studies are infrequently published. The objective of this study was to asses QoL in patients who underwent the colorectal cancer (CRC) surgery and to compare it to the QoL of general population. The patients who were admitted from January 2004 until May 2006 at the Department of Gastrointestinal Surgery at the Clinical Hospital Mostar, Bosnia and Herzegovina were divided in three groups: group of CRC patients who had received surgery and as a result of surgical treatment have colostomy, group of CRC patients who had received surgery in the same period and don't have colostomy and the third group that consisted of controls. QLQ-C30 and QLQ-CR38 questionnaires by the European Organization for Cancer Research and Treatment (EORTC) were used. A total of 67 patients were included in this study, supplemented by the thirty healthy examinees. Healthy group had significantly better results in physical

functioning compared with colorectal cancer patients and better results in cognitive and social functioning. Also, they reported symptoms of diarrhea and constipation less frequently than the group with colostomy and. The group with colostomy had poorer results in emotional functioning than the group without colostomy, and also reported significantly poorer results for domain "body image". Healthy group showed better results in sexual enjoinment than the patient with colorectal cancer. Patients without colostomy reported more micturition and defecation problems and female sexual problems compared to the healthy group. Generally we found that healthy population had better results than the CRC patients, while the patients with stoma had worse results than the nonstoma patients. The results presented here suggest that psychological treatment should be an integral part of the CRC treatment plan.

62. Vasilj I, Pilav A, Maslov B, Polašek O. Cardiovascular risk factors research in Bosnia and Herzegovina. Coll Antropol. 2009;33 Suppl 2:185-8.

Institute for Epidemiology and Biostatistics, School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

This study describes the current situation of cardiovascular risk factors research in the Bosnia and Herzegovina, with special emphasis on the Herzegovina region. The available data for the analysis includes various secondary sources, including project reports, official vital statistics data and other sources. Currently, there is a substantial lack of relevant information, which is available from occasional surveys or isolated studies. One of the main problems in detailed analysis is the lack of detailed and reliable census data, which causes problems in calculation of various rates and disables the creation of representative population samples for the field work and subsequent analysis. Comparison of the available information with neighbouring Croatia indicates interesting mixture of relatively high prevalence of some risk factors and rather low prevalence of others; almost 50% of men reported smoking on a daily basis, while only 16.5% of men were obese, while 40% of them had blood pressure over 140/90 mmHg. The results provide useful but incomplete information for the policy, thus suggesting that broader scope of public health research is needed in the region coupled with the census data, in order to provide better information for health policy and ultimately delivery of the optimal health care to the entire population.

63. Vlatković V, Trbojević-Stanković J, Stojimirović B. Malnutrition-inflammation complex syndrome and hepatitis C in maintenance hemodialysis patients. Ther Apher Dial. 2009;13(2):113-20.

International Dialysis Center, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Protein-energy malnutrition and inflammation are among the leading causes of poor outcome in hemodialysis patients. Hepatitis C virus (HCV) infection is accompanied by elevated proinflammatory mediators, also found in dialysis patients with malnutrition-inflammation complex syndrome. We aimed to study the rate and characteristics of malnutrition-inflammation complex syndrome (MICS) in hemodialysis patients, especially those with hepatitis C. The study included 147 patients (mean age 55.1 +/- 12.9 years), 24.5% of whom were HCV-positive, undergoing adequate hemodialysis three times a week for the last 52.7 +/- 52.5 months. Parameters of nutrition and inflammation were investigated to evaluate MICS. HCV-positive vs. HCV-negative patients had significantly higher hematocrit (29.6 +/- 4.5 g/dL vs. 28.1 +/- 4.3, $P < 0.05$), uric acid (345.8 +/- 96.5 vs. 321.3 +/- 118.8 micromol/mL, $P < 0.05$), aspartate aminotransferase (AST, also known as serum glutamic oxaloacetic transaminase [SGOT]) (23.3 +/- 14.9 vs. 17.8 +/- 9 U/L, $P < 0.008$), alanine aminotransferase (ALT, also known as serum glutamic pyruvic transaminase [SGPT]) (41.2 +/- 28.7 vs. 26.6 +/- 17.1 U/L, $P < 0.0003$), serum creatinine (980.4 +/- 219.1 vs. 888.4 +/- 202.9 micromol/mL, $P < 0.022$), intact parathyroid hormone (329.7 +/- 630.5 vs. 110.2 +/- 145.3 pg/mL, $P < 0.002$), malnutrition-inflammation score (7.4 +/- 5.2 vs. 5.6 +/- 4.1, $P < 0.038$), and Charlson comorbidity index (4.5 +/- 1.5 vs. 4 +/- 1.4, $P < 0.05$). MICS had a prevalence of 20-40% in our study. HCV-positive patients had a significantly higher prevalence of MICS than HCV-negative patients (30-40% vs. 20-30%).

64. Vukojević M, Soldo I, Granić D. Risk factors associated with cerebral palsy in newborns. Coll Antropol. 2009;33 Suppl 2:199-201.

School of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina

The aim of this study was to investigate the risk factors associated with cerebral palsy (CP). For this purpose, a total of 55 newborns were investigated in the case control design study, with a total of 55 additional newborns that were matched to the cases. All patients were recruited in University Clinical Hospital Mostar and other institutions in the region between 1997-2005. The comparison of the Apgar score did not seem to show significant differences between cases and controls (odds ratio [OR] = 1.15, 95% confidence intervals [CI] 0.36-3.69). Hypoxia was more common in the CP group (36.3% vs. 5.4% in the control group;

p < 0.001). Additionally, cases were more frequently exposed to the infections (p < 0.001), intracranial hemorrhage (p = 0.002), premature delivery, before the 28th gestation week (p = 0.027), as well as the premature delivery during the 28-34 gestation week (p = 0.001), and 34-38 gestation week (p = 0.018). Accordingly, small birth weight was associated with cases more often than controls (p = 0.003). Bleeding during pregnancy was also more common in cases than controls (p = 0.032), while the breech presentation, emergency cesarean section, hydrocephalus, placenta disorders and pre-eclampsia were not associated with CP. The results suggest that CP cases were more commonly exposed to numerous risks, which all seem to contribute to the increased chances of PF. Traditional indicator, poor Apgar score was not found to be significantly associated with the CP.

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65. Zelenika D, Karanović N. Diffusion lung capacity of patients with arterial hypertension. Coll Antropol. 2009;33 Suppl 2:165-7.

Department of Internal Medicine, University Clinical Hospital Mostar, Mostar, Bosnia and Herzegovina

The aim of this study was to investigate the diffusion lung capacity in patients with untreated hypertension. For this purpose, a total of 30 cases and 30 controls were included in the present study, which was based on several spirometry indicators in the analysis. The measurements were based on "single breath approach". The results indicated that the two groups differed in several spirometry results, including vital capacity, maximum willing ventilation, but the overall lung diffusion capacity did not seem to be significantly different between cases and controls. The results suggest that although there is a strong link between respiratory and circulatory system, the overall lung diffusion capacity is not altered by the increased arterial pressure and hypertension. Furthermore, the results of this suggest the need for creation of the population-specific spirometry standards for the population of Herzegovina in order to provide more meaningful results of spirometry.

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66. Zerem E, Imamović G, Omerović S. Percutaneous drainage without sclerotherapy for benign ovarian cysts. J Vasc Interv Radiol. 2009;20(7):921-5.

Interventional Ultrasonography Department, University Clinical Center, Trnovac bb, Tuzla, Bosnia and Herzegovina

PURPOSE: To evaluate percutaneous short-term catheter drainage in the management of benign ovarian cysts in patients at increased surgical risk. MATERIALS AND METHODS: Thirty-eight patients with simple ovarian cysts were treated with drainage of fluid content by catheters until output stopped. All patients were poor candidates for surgery. All procedures were performed under ultrasonographic (US) control and local anesthesia. Cytologic examination was performed in all cases. The patients were followed up monthly with color Doppler US for 12 months. Outcome measure was the recurrence of a cyst. RESULTS: During the 12-month follow-up period, 10 of 38 cysts recurred. Seven of the 10 cysts required further intervention, and three were followed up without intervention. Four of the seven patients who required further intervention underwent repeat transabdominal aspiration and three declined repeat aspiration and subsequently underwent surgery. After repeated aspirations, two of four cysts disappeared, one necessitated follow-up only, and one necessitated surgical intervention. Cyst volume (P = .009) and diameter (P = .001) were significantly larger in the cysts that recurred. No evidence of malignancy was reported in the cytologic examination in any patient. No patients developed malignancy during follow-up. No major complications were observed. The hospital stay was 1 day for all patients. The median duration of drainage in the groups with resolved and recurrent cysts was 1 day (interquartile range, 1-1) and 2 days (interquartile range, 1-3), respectively (P = .04). CONCLUSIONS: In patients considered poor candidates for open surgery or laparoscopy, percutaneous treatment of ovarian cysts with short-term catheter drainage without sclerotherapy appears to be a safe and effective alternative, with low recurrence rates.

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67. Zerem E, Imamović G, Omerović S. Simple renal cysts and arterial hypertension: does their evacuation decrease the blood pressure? J Hypertens. 2009;27(10):2074-8.

The University Clinical Center Tuzla, Trnovac bb, Tuzla, Bosnia and Herzegovina

OBJECTIVE: To evaluate the relationships between simple renal cysts and arterial hypertension and whether their evacuation decreases the blood pressure (BP). METHODS: In a cross-sectional design, we analyzed 184 study participants with cysts and compared hypertensive and nonhypertensive among them. Outcomes were the number, the size and the location of a cyst. In a cross-over design, we first evaluated the change in absolute value of SBP, DBP and mean BP in 62 hypertensive patients who underwent percutaneous evacuation of a cyst and then the decrease of BP as a categorical variable that comprised all study participants. RESULTS: There were 55% giant renal cysts among hypertensive and 24% among nonhypertensive patients (P = 0.0001). The prevalence rates of multiple

and peripheral cysts in hypertensive and nonhypertensive patients were similar to those of single and perihilar cysts, respectively. Significant differences in SBP, DBP and mean BP were found between pretreatment readings and 3 days, 1 month, 3 months and 6 months after cyst evacuation (P < 0001). The differences were significant in all hypertensive patients (P < 0.001). There were less hypertensive patients 3 days after treatment than before treatment (P < 0.0001). CONCLUSION: An apparent association between the size of a simple renal cyst and hypertension was found, and aspiration of cysts resulted in a reduction of BP. Location and number of cysts were not related to BP.

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68. Zerem E, Imamović G, Omerović S, Imširović B. Randomized controlled trial on sterile fluid collections management in acute pancreatitis: should they be removed? Surg Endosc. 2009;23(12):2770-2777.

The University Clinical Center Tuzla, Trnovac bb, Tuzla, Bosnia and Herzegovina

OBJECTIVE: To evaluate if percutaneous drainage of sterile fluid collections recurring after initial aspiration in acute pancreatitis yields better results than their conservative management. METHODS: Fifty-eight patients with fluid collections in acute pancreatitis were followed up prospectively. Forty of them with sterile fluid collections that recurred after initial aspiration were randomly assigned to two groups of 20 in each. One group was initially treated with conservative management and the other group with prolonged catheter drainage. Patients with unsuccessful initial treatment were converted to more aggressive procedure. Outcome measure was conversion rate to more aggressive procedure. RESULTS: Conversion to more aggressive procedure was done in 11 and 3 patients treated conservatively and with catheter drainage, respectively (p = 0.02). Four and 11 patients had bacterial colonization of their fluid collections in conservative management and drainage group, respectively (p = 0.048). Conservative treatment was successful in all six patients with sterile liquid collections < 30 ml. However, this treatment was unsuccessful in all seven patients with multiloculated and liquid collections >100 ml. CONCLUSIONS: Prolonged catheter drainage is more efficient for management of recurrent sterile fluid collections in acute pancreatitis than is conservative treatment. Conservative treatment is successful for patients with small fluid collections.

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69. Zerem E, Omerović S. Minimally invasive management of biliary complications after laparoscopic cholecystectomy. Eur J Intern Med. 2009;20(7):686-9.

The University Clinical Center, Tuzla, Bosnia and Herzegovina

BACKGROUND: We conducted this prospective study to evaluate the efficacy of percutaneous catheter drainage as a minimally invasive treatment in the management of symptomatic bile leak following biliary injuries associated with laparoscopic cholecystectomy. METHODS: Twenty two patients with symptomatic bile leak following laparoscopic cholecystectomy underwent percutaneous drainage of the bile collection under ultrasound control. In patients with jaundice and in those with persistent drainage, endoscopic retrograde cholecysto-pancreatography (ERCP) was performed immediately for diagnostic and for therapeutic intervention when appropriate. In other patients, ERCP was performed 4-6 weeks after the discharge from the hospital to document the healing of the leaking site. RESULTS: Five patients with jaundice were initially treated by a combination of endoscopic plus percutaneous drainage. One of them required surgical treatment following diagnosis of a major duct injury. The other 17 were treated by percutaneous drainage initially and for 14 of them it was definitive treatment. Three patients required sphincterotomy as additional treatment for stopping the leak. There were no complications related to the percutaneous drainage procedure. CONCLUSIONS: Most patients with bile leakage can be managed successfully by percutaneous drainage. If biliary output does not decrease, endoscopy is needed. In patients with jaundice endoscopic diagnostic and therapeutic procedures should be performed immediately.

By Nerma Tanović

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- **Case reports** and **letters** – up to 3 pages (maximum count 6000 characters with spaces),
- **Images in clinical medicine** – maximum 4 figures, the text of the legend up to 250 words (maximum count 1900 characters with spaces), up to 3 authors and no more than two references.

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Organization of the text. The text of original articles is usually divided into sections with the following headings: Introduction, Materials (Patients) and methods, Results, Discussion and Conclusion. This structure is not simply an arbitrary publication format, but rather a direct reflection of the process of scientific discovery. Long articles may need subheadings within some sections (especially the Results and Discussion sections) to clarify their content. Other types of articles, such as case reports, reviews, and editorials, are likely to need other more flexible structure of the text. If possible, use standard abbreviations. Non-standard abbreviations should be defined when first used in the text.

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8. Total number of pages, words and characters with spaces (Microsoft Word enables the simple acquisition of these data), number of figures and tables. A word count for the text only (excluding abstract, acknowledgments, figure legends, and references) allows editors and reviewers to assess whether the information contained in the paper warrants the amount of space devoted to it, and whether the submitted manuscript fits within the journal's word limits. A separate word count for the Abstract is also useful for the same reason.

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Abstract and Key Words are written on the second page. Because abstracts are the only substantive portion of the article indexed in many electronic databases, and the only portion many readers read, authors need to be careful that abstracts reflect the content of the article accurately.

An abstract (up to 250 words), without authors' names and institutional affiliations. Its structure should be similar to that of the text. For original articles, the abstract needs to have the structure with the following subtitles: Objective, Materials and methods, Results and Conclusion. Abstract for review articles and case reports also needs to have up to 250 words and does not have to be structured, but it has to have a Conclusion. Following the abstract, authors provide, and identify as such, 3 to 5 key words or short phrases that capture the main topics of the article. The key words should not repeat the title of the

manuscript. Terms from the Medical Subject Headings (MeSH) list of Index Medicus should be used; MeSH terms are available from: www.nlm.nih.gov/mesh/

Third page

Should carry the manuscript of article. Text should be under the following headings:

Introduction. Needs to be short and to specify to the reader, clearly and with arguments, reasons for the research presentation, and the novelties that the article brings. In Introduction maximum 3 to 4 pertinent and directly related works need to be cited. At the end of Introduction, an author needs to clearly specify the set aim of the research.

Methods. This part needs to provide the following information: selection and description of participants, precise technical information about all methods *(*describe the methods, apparatus, and procedures in sufficient detail to allow other workers to reproduce the results; give references to established methods, including statistical methods; identify precisely all drugs and chemicals used, including generic names, doses, and routes of administration and other specificities related to the presented research). Upon reporting about humane experiments, an author needs to indicate if the used procedures were in accordance with the Declaration of Helsinki from 1975 and its amendments from 1983. In addition, there needs to be stated if and which ethical committee gave consent for carrying out the research. A separate subtitle is *Statistical Analysis*. Authors need to indicate all statistical tests that were used. In addition, there needs to be stated the level of significance selected beforehand (P), that is which value P the authors considered to be statistically important (ex. 0.05 or 0.01, or some other). The results should be stated with pertaining confidence intervals (CI).

The editorship recommends to the authors to follow STARD instructions published in 2003 in the researches of diagnostic accuracy. At the end of the paragraph authors need to state which computer statistical program they have been using, as well as indicate the manufacturer and version of the program.

Results. Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Restrict tables and figures to those needed to explain the argument of the paper and to assess its support. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. The text must contain a clear designation as to where the tables and illustrations are to be placed relative to the text. Do not duplicate data by presenting it in both a table and a figure.

Discussion. Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results section. For experimental studies it is useful to begin the discussion by summarizing briefly the main findings, then explore possible mechanisms or explanations for these findings, compare and contrast the results with other relevant studies, state the limitations of the study, and explore the implications of the findings for future research and for clinical practice.

Conclusion. Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data. In particular, authors should avoid making statements on economic benefits and costs unless their manuscript includes the appropriate economic data and analyses. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledge. Anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. List the source(s) of funding for the study and for the manuscript preparation in the acknowledgements section.

References. Need to be on a separate page. Small numbers of references to key original papers will often serve as well as more exhaustive lists. Avoid using abstracts as references. References to papers accepted but not yet published should be designated as "in press" or "forthcoming"; authors should obtain

written permission to cite such papers as well as verification that they have been accepted for publication. If the paper has been published in electronic form on PubMed the confirmation of acceptance is not needed. Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source. Avoid citing a "personal communication" unless it provides essential information. For scientific articles, authors should obtain written permission and confirmation of accuracy from the source of a personal communication.

References should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by Arabic numerals in parentheses at the end of a sentence. Use the same number in the reference list. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure.

The titles of journals should be abbreviated according to the style used in Index Medicus. Consult the list of Journals Indexed for MEDLINE, published annually as a separate publication by the National Library of Medicine (available from: www.nlm.nih.gov/tsd/serials/lij.html). Examples of references please see on the following pages.

Tables. Need to be submitted separate from the main text. The preferred software for tables is Microsoft Excel (save each table in a file with single worksheet). Only tables made with table tools in Microsoft Word are acceptable. For the paper version, type or print each table on a separate sheet of paper. Number tables consecutively in the order of their first citation in the text. Use Arabic numerals. Each table needs to have an explanatory title. Place the title above the table. Give each column a short or abbreviated heading. Also, visibly indicate the position of each table in the text, using its assigned numeral at the end of the sentence which is relevant to the table(s). Tables should be positioned in the text where the author feels is appropriate but the Editor reserves the right to re-organize the layout to suit the printing process. Authors need to place explanatory matter in footnotes, not in the heading. Explain in footnotes of the table all nonstandard abbreviations. For footnotes use the following symbols, in sequence: *, †, ‡, §, ||, ¶, **, ††, ‡‡. Identify statistical measures of variations, such as standard deviation and standard error of the arithmetic mean. *Be sure that each table is cited in the text.* If you use data from another published or unpublished source, obtain permission and acknowledge them fully.

Figures (illustrations: diagram, photograph, photomicrograph, radiograph, drawing, sketch, picture, outline, design, plan, map, chart, etc.). Need to be submitted separate from the main text. They need to be submitted as photographic quality digital prints or, exceptionally, as professionally drawn and photographed original illustrations. Figures should be in a digital format that will produce high quality images. Formats recommended include: JPEG, GIF, TIFF, Microsoft Word, Excel. Sending original photographs and slides is permissible when they cannot be digitized without professional help. In this case, send an explanation in the cover letter. Using Arabic numerals, number figures consecutively in the order of their first citation in the text. Also, visibly indicate the position of each figure in the text, using its assigned numeral in parentheses. Figures should be positioned in the text where the author feels is appropriate but the Editor reserves the right to re-organize the layout to suit the printing process.

Supply a legend for each figure. Titles and detailed explanations belong in the legends, however, not on the figures themselves. Figures should be made as self-explanatory as possible. Letters, numbers, and symbols on figures should therefore be clear and even throughout, and of sufficient size that when reduced for publication each item will still be legible. Photomicrographs should have internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background. If photographs of people are used, either the subjects must not be identifiable or their pictures must be accompanied by written permission to use the photograph.

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Units of measurement

Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples. Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Abbreviation, Acronyms and Symbols

If possible for metric units use standard abbreviations. Non-standard abbreviations should be defined when first used in the text.

Sample references

Articles in journals

Standard journal article (*List the first six authors followed by et al.*):

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002;347(4):284-7.

More than six authors:

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. Brain Res. 2002;935(1-2):40-6.

Organization as author:

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. Hypertension. 2002;40(5):679-86.

No author given:

21st century heart solution may have a sting in the tail. BMJ. 2002;325(7357):184.

Volume with supplement:

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. Headache. 2002;42(Suppl 2):S93-9.

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Glauser TA. Integrating clinical trial data into clinical practice. Neurology. 2002;58(12 Suppl 7):S6-12.

Issue with no volume:

Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. Clin Orthop. 2002;(401):230-8.

Letters or abstracts:

Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. Eur Respir J. 2002;20(1):242. ; Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. Drug Alcohol Depend. 2002;66 Suppl 1:S105.

Article republished with corrections:

Mansharamani M, Chilton BS. The reproductive importance of P-type ATPases. Mol Cell Endocrinol. 2002;188(1-2):22-5. Corrected and republished from: Mol Cell Endocrinol. 2001;183(1-2):123-6.

Article with published erratum:

Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: a critical review. Clin Ther. 2000;22(10):1151-68; discussion 1149-50. Erratum in: Clin Ther 2001;23(2):309.

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Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. Blood. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Books and other monographs

Personal author(s):

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. Operative obstetrics. 2nd ed. New York: McGraw-Hill; 2002.

Organization(s) as author:

Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. Compendium of nursing research and practice development, 1999-2000. Adelaide (Australia): Adelaide University; 2001.

Chapter in a book:

Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

Conference paper:

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Dissertation:

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

Other published material

Newspaper article:

Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:

Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

Electronic material

CD-ROM:

Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Audiovisual material:

Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

Journal article on the Internet:

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Monograph on the Internet:

Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Homepage/Web site:

Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of a homepage/Web site:

American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html

Database on the Internet:

Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000 - [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.asp