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Bekir Misirlić (1931-1201) "A Small Piece of Garden", 1969, oil on canvas, 1150x1460 mm. Courtesy of Art Gallery of Bosnia and Herzegovina, Sarajevo.

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Academician Grujica Žarković (1915-2010)

Slobodan Loga
Husref Tahirović

Academician Grujica Žarković was the corresponding member of ANUBiH from 1967, and he received the status of academician in 1972, thus being, for a long time, a doyen-academician with the longest service in the current composition of the Academy. With his human, intellectual, scientific, pedagogical, research and other qualities, as well as with his world view, academician Žarković was a unique personality in our region. It is difficult to scrutinize comprehensively his forty-three years of work in the Department of Medical Sciences of the Academy of Sciences and Arts of Bosnia and Herzegovina (ANUBiH), since each year of that work brings a long list of activities in which academician Žarković participated.

Academician Žarković was born on the 12th of April 1915 in Bogotin (Glina municipality). He grew up and received education in the Croatian part of the former Military Frontier (Vojna Krajina). During his childhood, living in the rural environment, he truly sympathized with the severe life of peasants, and it encouraged him to search for solutions to their toilsome lives, and to advocate social changes which could have improved the position of that highly numerous part of the population at that time. The roots of his future orientation towards social aspects of medicine and left-wing political options should most probably be sought in his early cognitions of human inequality and injustice.

After graduation from the School of Medicinein Zagreb, he applied for the job of teaching assistant at the Department of Physiology of this School of Medicine, but the war did not allow him to learn the job application results, or engage in neuroscientific research. At the beginning of World War II his family was deported to a concentration camp, and afterwards expelled, whereas doctor G. Žarković, after a short engagement at a hospital in Nova Gradiška, joined partisans at Psunj in Slavonia.

Since 1941, during the National Liberation War, he served as Head of the Medical Corps of the Croatian Third Operative Zone, Head of the Medical Department in the headquarters of the Sixth Corps of the National Liberation Army and the Resistance Movement, Head of the Medical Corps of the principal headquarters of the National Liberation Army and the Resistance Movement in Croatia, member of the National Anti-Fascist Council of the People's Liberation of Croatia (ZAVNOH), and assistant Minister of Health on the National Committee of the Liberation of Yugoslavia.

After the war he played a number of responsible roles in the health care system, among others he was an assistant minister in the Ministry of Health of Yugoslavia, the Yugoslav consul in Australia, assistant Minister of Health of the Republic of Croatia and chief medical inspector in the Ministry of Health of Yugoslavia.

Many reforms, which academician Žarković recommended at that time, were accepted and have remained embedded till today within the health systems of the successor states of the former Yugoslavia, but not all, especially not the one, for which he was interested in the most: the right of citizens for equal health care. When they asked him if that idea was Utopia or reality, even in his 95th year of life, he was self-consistent and thought of it as the only way for the achievement of the community welfare.

In 1951 he was elected assistant professor for Hygiene at the School of Medicine in Sarajevo. Afterwards he left for postgraduate studies at Harvard School of Public Health in Boston where he gained his Master's degree in methodology of epistemological research. After obtaining a master's degree at Harvard, he visited several medical faculties in the USA and became acquainted with their attitudes towards doctors' education and specialization.

Upon his return from the USA, he modernized the classes of his subject, prepared and edited a course book in preventive medicine, collaborated with the Republic Institute for Health Protection and the Ministry of Health, and trained associates of the Institute for Preventive Medicine in synchronized performance of teaching practice, professional and scientific research tasks. He was farsighted. For some time he succeeded in his efforts to organize classes at the School of Medicine in Sarajevo on the basis that we know today as the principles of Bologna.

Twice he was the Dean of the School of Medicine in Sarajevo. He was the first at the School of Medicine in Sarajevo to perceive the importance of postgraduate studies in the development of medical science, and therefore, in 1962, organized postgraduate studies in Public Health and specialization in the methodology of the science research work.

As a delegate in the Social-Health Council of the Federal Assembly and a president of the Council of "the Federal Health Centre" in the nineteen-sixties, he opposed the preparation of legislation on decentralization of the health insurance funds and drafted his own counterproposal. Being feisty and courageous, he collected signatures of three thousand citizens, and requested from the Federal Assembly to take into consideration his "*Proposal for health service reform in the SFRY*" at the same time. His proposal was rejected for debate in the Assembly on a procedural basis.

During the next twenty years he had the impression that he was under a special regime, because he was consistently moved away from the politics of the health service organization in our region. However, besides this, he was honoured in expert and public circles. He was elected as president of the University of Sarajevo Association of Teachers, president of the Bosnia and Herzegovina Association of Medical Doctors, and president of the Union of Yugoslav Associations of Doctors. As a WHO consultant he visited India and Ceylon, participated at the congresses of medical doctors, led a couple of great research projects and performed regular teaching jobs.

He retired in 1975 when he was sixty years old, but he was very involved even after that. Whenever he had the chance, he spoke or wrote about the causes of the crisis of Yugoslav health policies, and about alternative strategies for the improvement of the citizens' health conditions. He published four monographs and series of articles about the population politics and advantages of the population planning for the poorer republics with high fertility.

In 1980 he published a course book entitled "*The Scientific Basis of the Health Care Organization*". The ideas which he then announced did not induce administrators in health care politics to change their course and style of work, which he criticized justly and uncompromisingly. According to the opinion of academician Žarković, there was amateurish management in our health care system which had a disastrous effect on the tremendous and socially significant health care system. Afterwards he organized postgraduate studies in management of health care systems and programs.

In 1992 he was a guest researcher at the Institute of Medical Informatics and System Researches in Munich where he studied and compared the characteristics of the former USSR health care systems and other socialist states of that time with health systems of other developed countries. On the basis of those comparisons, attitudes and suggestions of the international organizations, and study of the theory and practice of management in health care, he formulated alternative programs and strategies suitable for the

reform of health care systems in former socialist states. He published the results of his research in several articles and books, which were published in six languages.

He published one version of these books with colleague co-authors Hrabač and Nakaš in Bosnia and Herzegovina in 1999. Since this book did not produce the expected response, he published a monograph "Health Care Politics and Management of Health Care Systems" in 2004 as an ANUBiH publication.

Ten years ago the Department of Medical Sciences renewed and activated its former Committee for Health Care which was led by academician Žarković. The Committee gave three recommendations for reform of health care in Bosnia and Herzegovina, but there was no reaction from the responsible political factors in society.

Even in the tenth decade of his life he did not stop proposing new, contemporary and quality solutions for reforms of the health care system for the population. He was and remained a person with unquiet spirit, inquisitive, communicative, decisive and objective.

In the field of medicine he particularly dealt with the organization of the health care system, long-term planning in the health care system, health education and population problems.

In the area of the former Yugoslavia, in the field of epistemology, he was a pioneer in the research of mass chronic non-infectious disease problems, most significant being iodine deficiency disorder, rickets among children, traumatism, alcoholism, diabetes, heart diseases, neuroses, injuries of workers in industrial firms in Bosnia and Herzegovina, as well as the influence of social factors on the growth and development of children and children mortality. In the field of hygiene, he dealt with population food inspection, water supply problems, and especially radiation hygiene problems.

His publications, starting with the scientific and professional works, scientific reports, monographs and books, can be found nowadays in world index bases of medical journals, the libraries of the world health organizations, and other scientific and cultural institutions around the world. The study of academician Žarković's writing opus deserves to be approached scientifically and should become one of the future assignments of ANUBiH.

The greatness of academician Žarković's life work will be remembered for the numerous eminent scientists who developed under his mentorship and who collaborated with him over a long period of time.

Repeating the destiny of the people who were ahead of their time, he was often misunderstood by those who managed our health care system. However, his scientific and social opus in public health is priceless, if not at the time for his contemporaries, it will certainly be for future generations. With his inexhaustible, persistent work based on principles, deeply infused with humane intercession for health to become a value of all people, with his numerous scientific works and personnel which he directed into the modern flows of social medicine, academician Grujica Žarković held one of the most eminent positions in ANUBiH, at the University of Sarajevo, as well as at the region of former Yugoslavia and beyond.

Academician Žarković received several awards and medals (Commemorative Medal of the Partisans-1941, several war medals and 27th of July reward in 1963) but not those which were rightly his.

The departure of academician Žarković is an irreplaceable loss for ANUBiH. He was one of the most active and most appreciated members of ANUBiH, an unsurpassed reformer in our social service. He left an indelible mark on medical science and his profession. As a comfort we should hope that many young scientists and employees in public health service will find inspiration in his ideas for more active engagement in promoting public health.

The current state of knowledge of sudden infant death syndrome in Bosnia and Herzegovina and the outlook for the future

In this issue of the journal *Acta Medica Academia*, for the first time in a medical journal in Bosnia and Herzegovina, two papers are published on the subject of sudden infant death syndrome (SIDS). A detailed search in Pub Med, an index of citations of biomedical literature, using the key words SIDS and Bosnia and Herzegovina, we did not find a single publication on SIDS, which was in any way related to our country. Until recently, Bosnia and Herzegovina was part of the joint state of Yugoslavia, together with the newly created states of Slovenia, Croatia, Serbia, Montenegro and Macedonia; we were interested in how much was written about this problem in Yugoslavia, as well as in the newly created states. We discovered that Pub Med indexes about 20 general medical journals from the former Yugoslavia covering the years from 1948 to 2009. Conducting a search using the key words SIDS and the name of the state only two articles was located. They included an article by Kralik (1) in 2003 and comments by Zečević (2) on that paper published in 2004 in the Liječniki vijesnik (Doctor's Journal). This leads us to the conclusion that the problem of SIDS has not received significant attention in this region in the past 60 years.

Sudden infant death syndrome occurs throughout the world so it may be said with certainty that it also occurs in Bosnia and Herzegovina. Moreover, in personal communication with paediatricians of different generations from Bosnia and Herzegovina, the author of this article learned that paediatricians have seen and still witness this event, but they have not carried out any research on the subject, nor written about it.

However, on the basis of the fact that we have no written evidence of SIDS, the wider scholarly public could assume that doctors in Bosnia and Herzegovina do not recognize it, or it is such a common event that no one writes about it, or that there is no great interest in this issue in Bosnia and Herzegovina. It seems that this last assumption is the most acceptable, but regardless of which of them is correct, or if the reason is something completely other, the lack of documented, scholarly publications on demographic, sociological, epidemiological, clinical and forensic research into SIDS in Bosnia and Herzegovina impoverishes us in that aspect. On the other hand, it seems that we have forgotten the fact that these studies are important both for future generations of children and their doctors.

This issue of Acta Medica Academia contains two articles on SIDS. In the first article, Čajić et al. (3), presents a case of sudden infant death, illustrates the role of the primary health care institution, that if there is any suspicion surrounding the circum-

stances of the death that the medical institution insist that an thorough investigation be preformed. The goal of this investigation is to provide documentation and forensic evidence to support a SIDS diagnosis. Although this article does not contain anything new about cause or prevention, it provides published evidence that SIDS occurs in this region. Moreover, it opens the possibility for discussion of this problem among health workers and other experts in Bosnia and Herzegovina. In the second article, Professor Steven A. Koehler (4) states that sudden infant death, in the sense of discovery of its cause, is still today a medical and forensic mystery, that is, despite a great deal of research over many years, all attempts to establish the precise cause and the manner of death have failed. However, he still asserts that for a complete picture of SIDS, even basic information on this syndrome is needed from all countries of the world. Here he also mentions that developing countries or low and middle income countries as Bosnia and Herzegovina must understand their role and the importance of research into this problem in their setting and that data collected on the phenomenon should be shared with the entire world. In that sense the article presents general guidelines for research into SIDS in developing countries.

By publishing these two articles, we expect that their content will arouse the interest of medical community and other experts to devote more attention to this problem through retrospective or prospective research, to cover the epidemiological, sociological, clinical, forensic and other aspects of this phenomenon. The results obtained, as Steven A. Koehler (4) asserts, will certainly be of use to obtain a more complete picture of SIDS, and Bosnia and Herzegovina would no longer be a grey area in many scholarly or scientific reports in this field.

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Quantification of biodegradable PLGA nanoparticles for drug targeting

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Objective. The aim of this work was the development of appropriate analytical methods and assays for determining and monitoring composition and degradation of nanoparticles built from PLGA (poly D, L-lactid-co-glycolid), which can be reloaded with different drugs. A sensitive and precise method for monitoring of nanoparticle degradation in vitro was developed and optimized. Nanoparticles allow a selective enrichment of different drugs and knowledge of the nature and type of their degradation is essential for characterization and control of drug release and dosage. **Materials and methods.** The first method developed during this work to quantify the PLGA polymer matrix use advantage of the chemical reaction of aliphatic carboxylic acids with ferric chloride ($FeCl_3$) thus quantifying both degradation products of PLGA, lactic and glycol acids, at the same time. A second assay method of choice was to react to the polymer hydrolysate with lactate dehydrogenase, thus assaying selectively the lactic acid part. **Results.** During development of both of described methods was possible to determine dynamic range for PLGA matrix and nanoparticles, as well as to characterize impact of Pluronic F-68 and glycolic acid on lactate dehydrogenase activity. **Conclusion.** During our work we were able to develop two sensitive methods for monitoring of biodegradation of polymers which are consecutively used as a nanoparticle matrix in drug targeting.

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Key words: Drug targeting, Nanoparticles, Poly (lactid-co-glycolic) acid, Biodegradation.

Introduction

Recent developments in the field of medical nanotechnology and the possibility of chemical engineering of particles smaller in size than human cells allowed biochemical and pharmaceutical technology to realize drug targeting, which was already a vision since several decades. One possibility to gain increased enrichment of drugs in the respective tissue which has to be treated selectively is the reversible binding of drugs to nanostructures biocompatible carrier systems.

Such carriers are in the size of only a few nanometers thus being 100 to 200 fold

smaller than human cells. Examples for such carrier systems are spherical liposome and nanoparticles consisting of degradable, biocompatible polymers. Their small size combined with chemical engineering of their surface to bind selectively to the respective cells of choice, allow a directed enrichment of the drugs occluded in the degradable particles in diseases tissues and organs. Nanoparticles consisting of a (bio) degradable polymer matrix may carry the respective drugs either occluded or reversibly adsorbed. Various polymers have been used in drug delivery research that can effectively deliver the drug to a target site and increase the therapeutic effect, while minimizing any other side effect (1, 2).

One of the most important advantages of nanoparticles is their ability to cross physiological epithelial barriers because of their variable diameter in range from 10 nm to 1000 nm.

Colloidal carriers such as nanoparticles based on poly-lactic-co-glycolic acid (PLGA) have been extensively studied in medical and biotechnological sciences as drug delivery systems, which were suitable for most administration routes.

Poly (D, L-lactide-co-glycolide) (PLGA) was approved in the year 2000 by FDA (Food and Drug Administration) for application in drug targeting (3, 4).

Another unique properties of PLGA such as biocompatibility, biodegradability and controlled release, make this polyester one of the most promising material in medical and biotechnological research with high commercial interest.

It's also shown that PLGA has bioadhesive properties and binds to mucosa of gastrointestinal tract (5, 6, 7).

This effect can increase the residency time and may enhance the drug absorption time due to intimate contact with the epithelium cells. Also, biodegradable nanoparticles are of particular interest as they provide protection of fragile molecules against enzymatic and hydrolytic degradation in the gastrointestinal tracts.

In medicine PLGA is well known as a restorable suture material in surgery, which is randomly hydrolyzed in the organism to their biocompatible metabolites, lactic and glycolic acid.

Lactic acid is converted to pyruvate, which is degraded in the Krebs cycle via acetylating of coenzyme A, and carbon dioxide, which is mainly eliminated by respiration. Part of the glycolate is excreted directly via the urine; another part is oxidized to glyoxylate, which is converted to glycine, serine and pyruvate. (8, 9)

The rate of biodegradation depends on the lactic/glycolic acid ratio a 50/50 PLGA, as was used in this work, is degraded fully within one month.

The nanoparticles used were synthesized by double emulsifying techniques showing the advantage that hydrophobic as well as hydrophilic drug as e.g. therapeutic relevant peptides and proteins may be entrapped.

Up to now it quantification of nanoparticles from biodegradable and biocompatible PLGA was done by gravimetric methods after lyophilisation. Unfortunately this method allows only for quantification of the pure polymer, whereas in complex systems as in the aforementioned drug containing nanoparticles also the occluded drug and additives from the synthesis contribute gravimetrically and impair a correct result.

The object of the methods developed during this work to quantify the polymer matrix was to primarily hydrolyze the nanoparticles quantitatively and assay the monomers. One analytical technique was to take advantage of the chemical reaction of aliphatic carboxylic acids with ferric chloride ($FeCl_3$) thus quantifying both degradation products at the same time.

A second assay method of choice was to react to the polymer hydrolysate with lactate

dehydrogenase, thus assaying selectively the lactic acid part.

Materials and methods

Materials

Resomer 503 H (Poly (D, L-lactide-co-glycolic acid) (PLGA, lactide:glycolide = 50:50, inherent viscosity 0.32-0.44 dl/g, acid number > 3 mg KOH/g) was supplied by Boehringer Ingelheim (Ingelheim, Germany).

Glycylglycin hydrochloride, L-Glutamic acid, DL-lactic acid lithium salt, Glycolic acid, D-(-)-Lactic acid, L-(+)-Lactic acid, NAD: β-Nicotinamide adenine dinucleotide hydrate, from Yeast, L-lactic dehydrogenase, from Bovine Muscle, 686 Units/mg protein, D-Lactic Dehydrogenase from Lactobacillus leichmannii, 282 Units/mg protein, Glutamic-Pyruvic Transaminase, from Porcine Heart, 90 Units/mg protein, and Pluronic F-68 were purchased from Sigma- Aldrich (Vienna, Austria).

Methods

Preparation of PLGA nanoparticles

The nanoparticles were prepared by a water-in-oil-in water solvent evaporation technique which allows for entrapment of hydrophobic as well as hydrophilic drugs as e.g. therapeutic relevant peptides and purified via diafiltration using Vivaflow 50 to remove Pluronic F68, an additive essential for nanoparticle preparation and stabilization.

The amount of PLGA in nanoparticles after preparation procedure was quantified by lyophilisation. The nanoparticles were finally stored at -80°C until use.

Optical test for quantification of D, L-lactic acid

The optical enzymatic test for quantification of the amount of lactic acid in PLGA nanoparticles provides a sensitive method for continuous monitoring of lactate-dehydrogenase activity *in vitro* using a photometer. Lactate-dehydrogenase converts or hydrolyzes D, L- lactic acid, in presence of NAD, to pyruvate. In the final step of this enzyme-coupled assay, pyruvate will be converted to L-alanine in the presence of L-glutamate by the action of glutamate- pyruvate transaminase and also removed from reaction.

$$\text{Lactate} + NAD^+ \xrightarrow{\text{Lactate-dehydrogenase}} \text{Pyruvate} + NADH + H^+$$

$$\text{Pyruvate} + \text{L-Glutamate} \xrightarrow{\text{Glutamate-pyruvate transaminase}} \text{L- Alanine} + \text{2-Oxoglutarate}$$

Reaction with ferric (III) chloride

Chemical reaction of aliphatic carboxylic acids with ferric (III) chloride results in specific color change whose intensity is strongly dependent from pH value of solution.

In a strong acidic solution the color of $FeCl_3$ is pale, whereas in slightly alkaline solution $FeCl_3$ reacts with hydroxide ions to form a colloidal iron (III) hydroxide. Most suitable conditions for reactions with $FeCl_3$ are in solution at pH 7 where the formed complex is stable and can be identified spectrophotometrically by measuring the absorption at 360-370nm or at 450-460 nm (10).

As mentioned above PLGA (poly-lactic-co-glycolic acid) belongs to the family of aliphatic polyesters and contain two aliphatic carboxylic acids, lactic and glycolic acid, in equal amounts. Due to the advantage of the chemical reaction on aliphatic carboxylic acids with ferric chloride ($FeCl_3$), both products of PLGA degradation can be quantified at the same time.

Results

Impact of Glycolic acid on the activity of lactate- dehydogenase

For development of methods to quantify the PLGA polymer matrix it was very important to characterize the nature of interaction

between glycolic acid and lactate dehydrogenase. It's already known from literature that glyoxiclic acid in presence of cofactor NADH and glyoxiclic acid-reductase is converted to glycolic acid (11).

By this reaction a reduction of NADH-absorption 340 nm was measured.

$$\underset{\text{Glyoxiclic acid}}{CHO-COOH} + NADH + H^{+} \leftrightarrow \underset{\text{Glycolic acid}}{HO-CH2-COOH} + NAD^{+}$$

Experimental procedure

In order to characterize the type of interaction between glycolic acid and lactate dehydrogenase a stock of 5 mg (0.066 mmol) glycolic acid in 20 ml Glycylglycine buffer was prepared (end concentration of glycolic acid 250 µg/ml).

Standard-dilution of glycolic acid in Glycylglycine buffer in a range of 50 to 250 µg/ml were prepared and incubated in a water bath at 37 °C for 30 minutes.

Enzyme dilutions were diluted with Glycylglycine buffer and their protein amount was measured by Bradford just prior to use.

Test procedure

A master-mix containing 5 ml Glycylglycine buffer pH 10, 5 ml distilled water and 1 ml of 47 mM NAD was prepared. This Master-mix was incubated in a water bath at 37°C for 30 min. In each reaction tube, 2 ml of master-mix was mixed with 100 µl of glycolic acid standard dilutions. After incubation for 5 minutes in a water bath at 37°C the absorption of the samples was measured for 1 minute at 340 nm (3 ml SUPRASIL Quartz cuvettes were used). The obtained value was characterized as E1. As a blank we used 2 ml of Glycylglycine Buffer pH 10. Finally, the reaction was started by adding 1.12 U of L-lactate dehydrogenase or D- lactate dehydrogenase to a cuvette with glycolic acid and the absorption was measured every 2.5 minutes over a period of 25 minutes. This obtained value was characterized as E2.

For each measured concentration of glycolic acid a difference $\Delta E = E2 - E1$ was calculated and plotted versus time in [min] and the corresponding slope k was determined.

Finally, the concentration of glycolic acid was applied as a value of x-coordinate versus the corresponding slope k as ordinate values.

Figures 1 and 2 show that glycolic acid acts as a substrate for lactate dehydrogenase under our reaction conditions. But at the same time this molecule acts also as an inhibitor at the active site of this enzyme with respect to the substrate lactic acid.

Table 1 Measured value by reaction of glycolic acid with L-lactate dehydrogenase or D-lactate dehydrogenase

Concentration of Glycolic acid in µg/ml	Slope *k* after reaction with L-lactate dehydrogenase	Slope *k* after reaction with D-lactate dehydrogenase
50	0.0011	0.0009
100	0.0009	0.0012
150	0.0013	0.0018
200	0.0011	0.0020
250	0.0016	0.0013

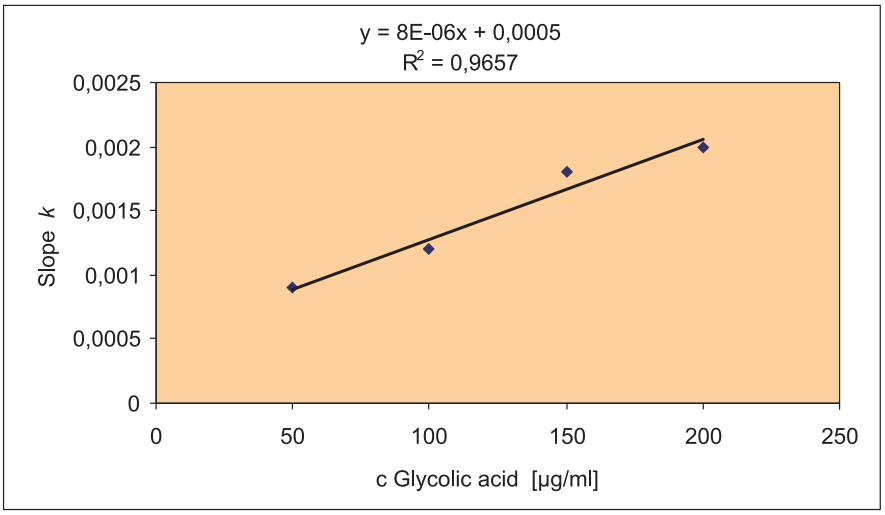

Figure 1 Reaction of Glycolic acid with L-Lactate dehydrogenase by measuring the absorbance at 340 nm

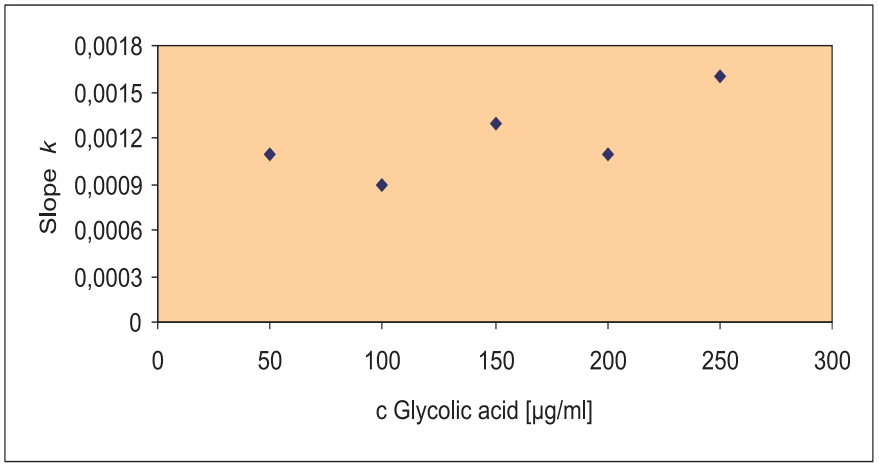

Figure 2 Reaction of Glycolic acid with D-Lactate dehydrogenase by measuring the absorbance at 340 nm

Determination of critical micelle concentration of anionic surfactant Pluronic F-68

In pharmacy surfactants are most frequently applied to improve solubility, stabilization and absorption of drug substances. In this work we used the anionic surfactant Pluronic F- 68 as an essential additive for stabilization of nanoparticle suspension in an end concentration of 1. 6% (w/v) (12).

The use of protective surfactants (i.e., polaxamers and poloxamines) could possibly prevent unwanted interactions between the drug and the PLGA as well as neutralize the acidity generated in the course of polymer degradation (13).

In order to characterize an influence of Pluronic F- 68 on the activity of Lactate dehydrogenase it was important to determine a critical micelle concentration of Pluronic F-68 in aqueous solution.

Experimental procedure

For determination of the critical micelle concentration of Pluronic F-68 a Sudan III

Table 2 Determination of critical micelle concentration of Pluronic F-68: obtained value

Concentration of Pluronic F-68 in % (w/v)	$\Delta E = E_{\text{with Pluronic F-68}} - E_{\text{without Pluronic F-68}}$ at 507 nm
0.10	0.073
0.16	0.092
0.25	0.093
0.50	0.119
1.00	0.263
1.50	0.389
2.00	0.577
2.50	0.746

(Sudan red, $C_{22}H_{16}N_4O$, MW 352.4 g/mol) was used as an indicator.

In 8 different reaction tubes, 2ml of the corresponding Pluronic concentration (0.1%; 0.16%; 0.25%; 0.5%; 1%; 1.5%; 2% and 2.5% (w/v)) was added and mixed with 200 µl NAD^+ and 100 µl L-lactic acid in concentration of 100 µg/ml. After 30 minutes of incubation in water bath at 37°C a small amount of Sudan III was added into each reaction vessel. Finally, after additional incubation for 15 minutes at 37°C, all samples were centrifuged (2 minutes by 14 000 rpm) and absorption of the samples was measured at 507 nm. As a blank we used the same preparation without Pluronic F-68.

Figure 3 shows that the absorption of Sudan III increases very abruptly at a concentration of 0.8% as a result of spontaneous micelle formation, and this point was defined as the critical micelle concentration of Pluronic F-68.

Optical test for quantification of D, L-lactic acid amount in PLGA and PLGA nanoparticles

In order to determine the amount of D, L-lactic acid in PLGA and PLGA nanoparticles, an enzyme-coupled assay of lactate dehydrogenase (LDH) was carried out. At pH 10, the activity of the lactate dehydrogenase that oxidizes lactic acid in presence of NAD^+ to pyruvate was examined.

Generation of a calibration line with L-lactic acid and glycolic acid

Resomer RG 503 H contains D, L-lactic acid and glycolic acid in equal amounts, an ideal ratio of both components for fast deg-

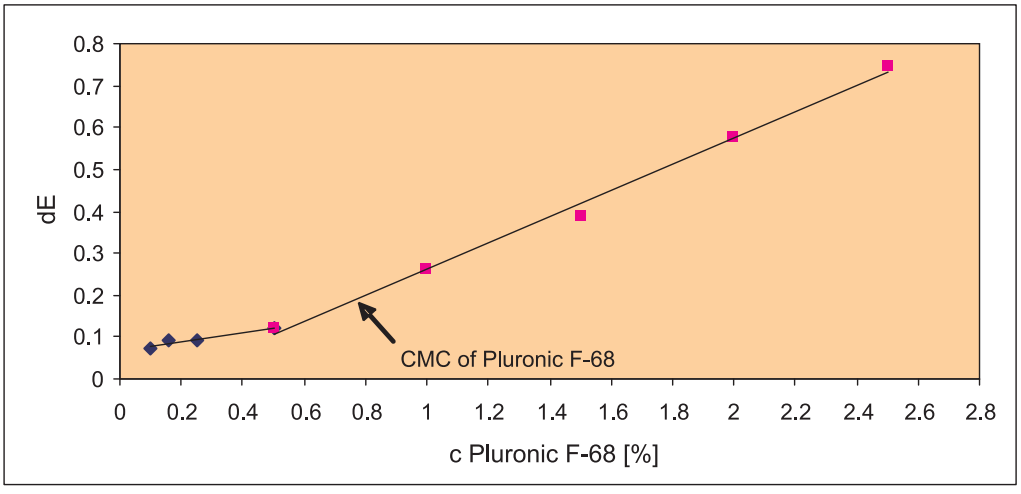

Figure 3 Critical micelle concentration CMC of Pluronic F-68

radation in human body. D, L-lactic acid is chiral and has two optical isomers, L-lactic acid and D-lactic acid. PLGA contains both optical isomers of lactic acid in the same amount. L-lactic acid is the biologically important isomer.

In previous experiment we could show that glycolic acid acts as a substrate and as an inhibitor for lactate dehydrogenase under our reaction conditions.

Therefore, for corrected generation of calibration lines a mixture of L-lactic aid and glycolic acid in a molar ratio 1:2, in the identical ratio as in PLGA was used.

A calibration line was generated by means of increasing L-lactic acid and glycolic acid amount. A stock containing 4 mg (0.044 mmol) L-lactic acid (end concentration 200 µg/ml) and 6.756 mg (0.088 mmol) glycolic acid (end concentration 337.7 µg/ml) in 0.01 M NaOH was prepared. Dilutions of a stock in the range of 50 to 200 µg/ml of L-lactic acid in 0.01 M NaOH were used for generation of a calibration curve. Finally, the pH-value of each sample was adjusted with 1 M HCl to 10 and incubated in a water bath at 37 °C for 30 minutes.

L-lactate dehydrogenase and Glutamate-pyruvate transaminase dilutions were diluted with Glycylglycine buffer pH 10 and their protein amount was measured by Bradford just prior to use.

Test procedure

A master-mix containing 7 ml Glycylglycine buffer pH 10, 7 ml distilled water and 1.4 ml of 47 mM NAD was prepared. This master-mix was incubated in awater bath at 37°C for 30 min. In each reaction tube, 2 ml of master-mix was mixed with 100 µl of stock solutions (50 to 200 µg/ml) and 0.126 U of glutamate-pyruvate transaminase. After incubation in water bath for 5 minutes at 37°C

Table 3 Measured value for L-lactic acid-calibration line

Concentration of L-lactic acid in [µg/ml]	Concentration of Glycolic acid in [µg/ml]	Slope *k*
50	84.425	0.0007
75	126.64	0.0009
100	168.85	0.0009
150	254.28	0.0013
175	296.49	0.0015
200	337.70	0.0017

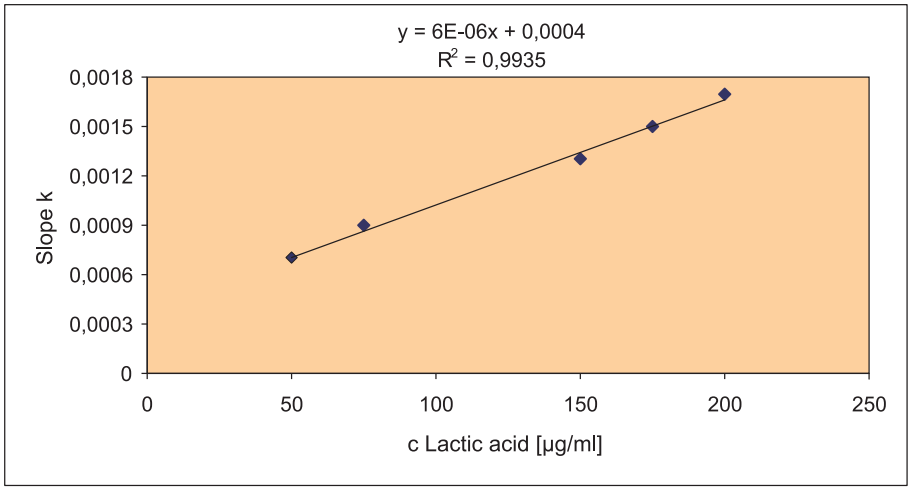

Figure 4 L-lactic acid-calibration line

the absorption of the samples was measured for 1 minute at 340 nm. The measured value was characterized as E1. As a blank 2 ml of Glycylglycine buffer pH 10 was used. Finally, the reaction was started by adding 1.12 U of L-lactate dehydrogenase to the cuvette with stock samples and the absorption was measured every 2.5 minutes over a period of 25 minutes. This measured value was characterized as E2.

For each measured concentration of stock dilutions a difference ΔE = E2 – E1 was calculated and plotted versus time in [min] and the corresponding slope k was determined.

Finally, the concentration of L-lactic acid was applied as a value of x-coordinate versus a corresponding slopes k as an ordinate values.

For generation of a calibration line a slope value for 100 µg/ml L-lactic acids was omitted, because of high deviation from other measured values as a result of influence of glycolic acid on enzymatic activity.

Quantification of L-lactic acid in PLGA polymer matrix

Hydrolyzation of PLGA

For this purpose 200mg of Resomer 503H was diluted in 5 ml of 1 M NaOH and incubated over night (~16 hours) in a water bath at 35°C to accelerate hydrolysis of the polyester into its monomers (end concentration of PLGA 40 mg/ml). After incubation the hydrolyzed PLGA stock solution was diluted with distilled water to form PLGA samples in concentration range between 200 µg/ml and 550 µg/ml. Finally, the pH-value of each sample was adjusted with HCl to 10.

Test procedure

In order to correlate a ratio of L-lactic acid in PLGA after hydrolyzation with the theoretical ratio in the used Resomer RG 503 L-LDH activity was determined, and using a calibration line an amount of L-lactic acid for the following samples was calculated.

Table 4 Determinated values by quantification of L-lactic acid in PLGA polymer matrix

Concentration of PLGA in [µg/ml]	Slope *k*	Calculated concentration of L-lactic acid in [µg/ml]
200	0.0009	83.33
250	0.0007	50
300	0.0009	83.33
400	0.0011	116.67
500	0.0015	183.33
550	0,0016	200

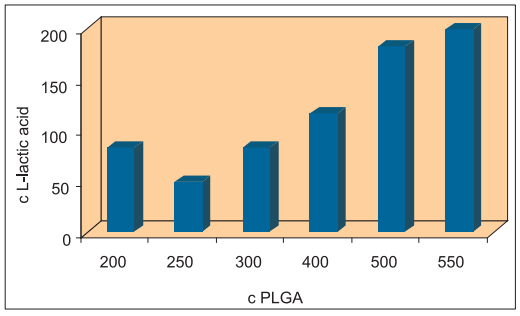

Figure 5 Correlation between concentration of PLGA and L-lactic acid

Figure 5 shows a linear correlation between the utilized PLGA concentration in the polymer matrix and the calculated concentration of L-lactic acid.

Quantification of L-lactic acid amount in purified PLGA nanoparticles

To correlate the amount of L-lactic acid in purified PLGA nanoparticles with theoretical amount of L-lactic acid in PLGA polymer matrix which is frequently used for nanoparticles preparation, L-LDH activity and corresponding amounts of L-lactic acid was also determined for the following samples:

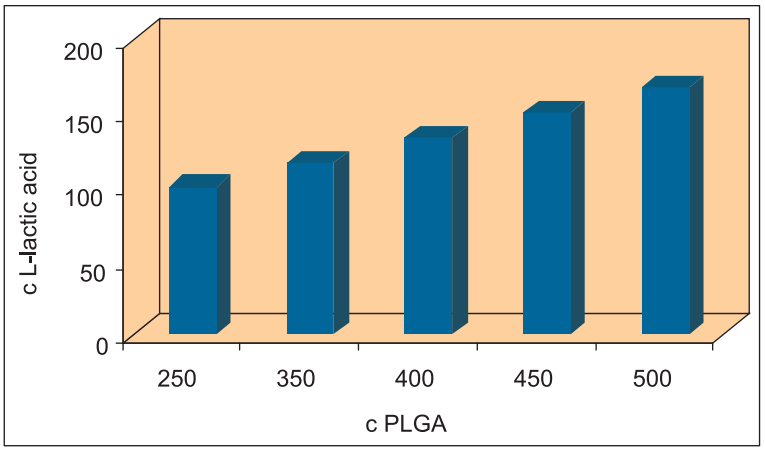

Figure 6 Correlation between concentration of PLGA and L-lactic acid in purified PLGA nanoparticles

Table 5 Determinated values by quantification of L-lactic acid in PLGA polymer matrix

Concentration of PLGA in [µg/ml]	Slope *k*	Calculated concentration of L-lactic acid in [µg/ml]
250	0.0010	100
350	0.0011	116.67
400	0.0012	133.33
450	0.0013	150
500	0.0014	166.67

Figure 6 shows that the calculated amount of L-lactic acid correlates with theoretical ratio of L-lactic acid in Resomer RG 503.

Reaction with ferric (III) chloride

Generation of a calibration line

For corrected generation of calibration line we used a mixture of racemic D, L-lactic acid and glycolic acid in equal molar ratio 1:1, as is used Resomer RG 503.

A calibration line was generated by means of increasing D, L-lactic acid and glycolic acid amount. A stock containing 12 mg (0.13 mmol) D, L-lactic acid lithium salt (end concentration 600 µg/ml) and 10.128 mg (0.13 mmol) glycolic acid (end concentration 506.4 µg/ml) in 20 ml 0.01 M NaOH was prepared. Six dilutions of a stock in the range of 50 to 360 µg/ml of D, L-lactic acid and accordingly 42.2 to 303.8 µg/ml in 0. 01 M NaOH were used for generation of the calibration curve.

Test procedure

As a sensitive reaction indicator 0.1 g $FeCl_3$ * 6 H_2O in 100 ml diluted HCl was used. In a first test step, 1 ml of stock dilutions (50-360 µg/ml D, L-lactic acid and 42.2 to 303.8 µg/ml glycolic acid) was reacted with 1 ml of $FeCl_3$ reagent. After incubation at room temperature (5 minutes) the absorption of each sample was measured for 1 minute at 360 nm and 370 nm. As a blank a mix of 1 ml $FeCl_3$ reagent and 1 ml 0.01 M NaOH was used.

Finally, the concentrations of D, L-lactic acid and glycolic acid were drawn as a value of x-coordinate versus a measured absorption as a ordinate values.

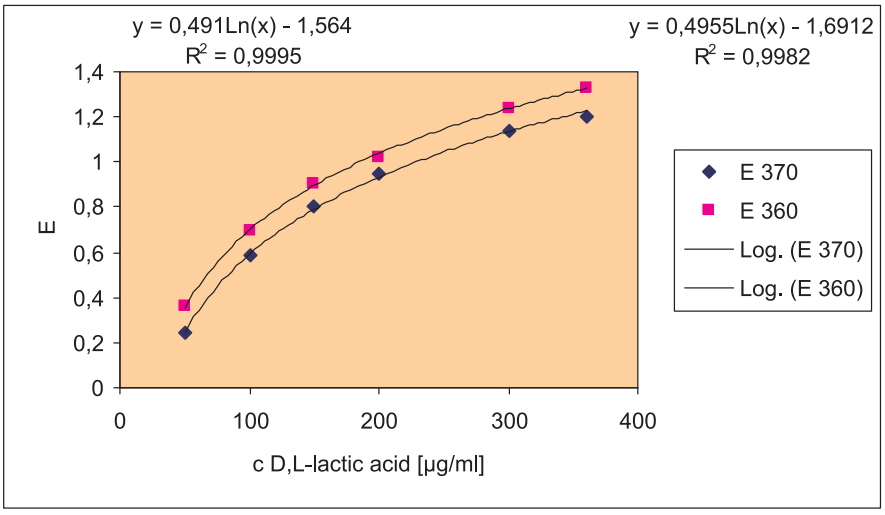

Figure 7 D, L-lactic acid-calibration line

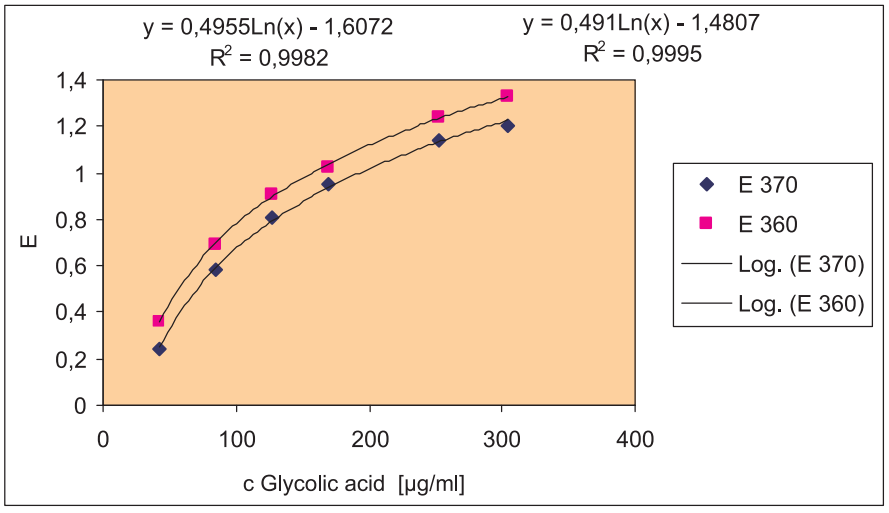

Figure 8 Glycolic acid-calibration line

Quantification of D, L-lactic acid and glycolic acid in a PLGA polymer matrix

The hydrolyzed PLGA stock solution was diluted with distilled water to form PLGA samples in a concentration range between 200 µg/ml and 600 µg/ml. 1 ml of PLGA dilutions (200-600 µg/ml) was reacted with 1 ml of $FeCl_3$ reagent. After incubation at room temperature (5 minutes) the absorption of each sample was measured for 1 minute at 360 nm and 370 nm.

A mix of 1 ml $FeCl_3$ reagent and 1 ml 0.01 M NaOH was used as a blank. Using a corresponding calibration line for D, L-lactic and glycolic acid the amount of both acids was calculated and compared to the concentrations of PLGA hydrolysates as given in the following tables.

Table 6 Results of $FeCl_3$ reaction for PLGA: measured values of samples at 360 nm and 370 nm: calculated concentration of D, L-lactic acid

Concentration of PLGA [µg/ml]	Absorbance at 370 nm	Absorbance at 360 nm	Calculated concentration of D, L-lactic acid at 370 nm in [µg/ml]	Calculated concentration of D, L-lactic acid at 360 nm in [µg/ml]
200	0.416	0.518	70.293	69.430
250	0.570	0.671	95.916	94.816
300	0.714	0.798	128.26	122.80
350	0.870	0.943	175.73	164.99
400	0.980	1.030	219.40	196.98
450	1.014	1.066	234.99	211.96
500	1.092	1.136	275.05	244.44
550	1.110	1.109	285.23	231.36
600	1.129	1.113	296.37	233.26

Table 7 Results of $FeCl_3$ reaction for PLGA: measured values of samples at 360 nm and 370 nm: calculated concentration of glycolic acid

Concentration of PLGA [µg/ml]	Absorbance at 370 nm	Absorbance at 360 nm	Calculated concentration of glycolic acid at 370 nm in [µg/ml]	Calculated concentration of glycolic acid at 360 nm in [µg/ml]
200	0.416	0.518	47.605	72.894
250	0.570	0.671	65.143	99.264
300	0.714	0.798	87.344	128.26
350	0.870	0.943	120.01	171.87
400	0.980	1.030	150.15	204.85
450	1.014	1.066	160.91	220.29
500	1.092	1.136	188.62	253.72
550	1.110	1.109	195.66	240.26
600	1.129	1.113	203.38	242.21

Quantification of D, L-lactic acid amount in purified PLGA nanoparticles

To compare the amount of D, L-lactic acid and glycolic acid in purified PLGA nanoparticles with theoretical amounts of both acids in PLGA polymer matrix which is frequently used for nanoparticles preparation, a test with $FeCl_3$ reagents was carried out and the corresponding amount of L-lactic acid and glycolic acid was also determined for the samples as shown in the following tables.

Table 8 Results of $FeCl_3$ reaction for PLGA nanoparticles: measured values of samples at 360 nm and 370 nm: calculated concentration of D, L-lactic acid

Concentration of PLGA [µg/ml]	Absorbance at 370 nm	Absorbance at 360 nm	Calculated concentration of D, L-lactic acid at 370 nm in [µg/ml]	Calculated concentration of D, L-lactic acid at 360 nm in [µg/ml]
200	0.251	0.333	50.384	47.634
250	0.436	0.516	73.188	69.148
300	0.640	0.716	110.47	103.92
350	0.688	0.775	121.71	117.18
400	0.818	0.893	158.22	149.02
450	1.004	1.063	230.29	210.67
500	1.103	1.162	281.22	257.74
550	1.152	1.191	310.46	273.42
600	1.186	1.212	332.51	285.36

Table 9 Results of $FeCl_3$ reaction for PLGA nanoparticles: measured values of samples at 360 nm and 370 nm: calculated concentration of glycolic acid

Concentration of PLGA [µg/ml]	Absorbance at 370 nm	Absorbance at 360 nm	Calculated concentration of glycolic acid at 370 nm in [µg/ml]	Calculated concentration of glycolic acid at 360 nm in [µg/ml]
200	0.251	0.333	34.018	50.181
250	0.436	0.516	49.584	72.600
300	0.640	0.716	75.124	108.70
350	0.688	0.775	82.839	122.45
400	0.818	0.893	107.95	155.37
450	1.004	1.063	157.67	218.96
500	1.103	1.162	192.89	267.39
550	1.152	1.191	213.13	283.50
600	1.186	1.212	228.41	295.78

Discussion

Determination of critical micelle concentration of anionic surfactant Pluronic F-68

Pluronic F-68 was used for preparation of nanoparticles in a final concentration of 1.6%. Through degradation of nanoparticles with NaOH a dilution step of 1:100 was carried out, which results in Pluronic F-68 concentration of 0.16% in measured samples. Comparison of the concentration of Pluronic F-68 in samples (0.16%) with maintained critical micelle concentration (0.8%) suggests that Pluronic F-68 has an insignificant influence on the activity of lactate dehydrogenase.

Optical test for quantification of L-lactic acid in PLGA polymer matrix and in purified PLGA nanoparticles

Figures 5 and 6 show a linear correlation between the utilized PLGA concentration and the calculated concentration of L-lactic acid. From the obtained results a detection limit for quantification of L-lactic acid in PLGA polymer matrix could be defined in the range of 250µg PLGA/ml and 550 µg PLGA/ml and 250 – 500 µg/ml in PLGA nanoparticles.

Reaction with ferric (III) chloride

Quantification of D, L-lactic acid and glycolic acid in a PLGA polymer matrix

According to the calculated values in Table 6, it can be deduced from the measured values at 370 nm, that the calculated concentration of D, L-lactic acid shows a higher correlation with the theoretical amount in Resomer RG 503. Dynamic range by this analytical method for D, L-lactic acid in polymer matrix is defined in a range between 300 µg/ml and 600 µg/ml. Due to the calculated concentration of glycolic acid as given in table 7, it is obvious that the measured values at 360 nm show a higher level of correlation with the theoretical amount of glycolic acid in Resomer RG 503. Dynamic range of quantification of glycolic acid in polymer matrix is defined in range of 250 µg/ml and 600 µg/ml. The light aberrances of measured results from the theory are caused by incomplete hydrolysis of PLGA.

Quantification of D, L-lactic acid and glycolic acid in purified PLGA nanoparticles

The results of ferric chloride reaction with the degradation products glycolic acid and D, L-lactic acid show a detection limit for lactic acid in PLGA nanoparticles in the range of 350 to 600 µg/ml. The detection limit for glycolic acid was 350 to 600 µg/ml in PLGA nanoparticles. The light aberrances of measured results from the theoretical values are a result of the purification process, during probably a small amount of polymer was washed out and the following incomplete hydrolysis of the nanoparticle-suspension.

Conclusion

The results of the assay technique employing the ferric chloride reaction with the degradation products glycolic acid and D, L-lactic acid show a detection limit for lactic acid in PLGA between 300 – 600 µg/ml and in PLGA nanoparticles between 350 – 600 µg/ml. The detection limit for glycolic acid was 200 – 600 µg/ml in PLGA and 350 – 600 µg/ml in PLGA nanoparticles.

Concerning the optical test for assaying L-lactic acid the detection limit was 250 – 550 µg/ml in PLGA and 250 – 500 µg/ml in PLGA nanoparticles.

Beyond that it was possible in our work to show that glycolic acid acts also as a substrate for lactate dehydrogenase under the applied reaction conditions. But at the same time this molecule acts also as an inhibitor at the active site of this enzyme with respect to the substrate lactic acid. This makes the development of the optical test more complicated, since an appropriate correction for the calibration curve had to be established.

Both methods were evaluated and compared to each other. It was shown that an enzymatic assay is more sensitive but the ferric chloride method is simpler if both glycolic acid and D, L-lactic acid should be quantified simultaneously. During our work we were able to develop two sensitive methods for monitoring of biodegradation of polymers which are consecutively used as a nanoparticle matrix in drug targeting.

The enzymatic assay turned out to be more sensitive and should be given preference to the ferric chloride method if both glycolic acid and D, L-lactic acid should be quantified simultaneously. The ferric chloride method is by far the simpler method but severely prone to mistakes.

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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Breastfeeding and the development of asthma and atopy during childhood: a birth cohort study

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Objective. Within the context of a population based-birth cohort, we investigated the association between breastfeeding and development of asthma and atopy in childhood. **Methods**. Children (n=1072) were followed from birth and reviewed at age one, three, five and eight years. Based on the onset and resolution of symptoms, we assigned children into the wheeze phenotypes (never, transient, intermittent, late-onset and persistent). Atopy was determined by skin testing and specific IgE measurement. According to the duration of breastfeeding, participants were assigned as not breastfed, breastfed ≤ four months and breastfed > four months. **Results**. In a multinomial regression model adjusted for gender, we found that breastfeeding > four months was protective of transient early wheeze (aOR: 0.61, 95% CI 0.41-0.90, p=0.01), with no significant association between breastfeeding and other wheeze phenotypes. In a multivariate model, we found a significant protective effect of breastfeeding >four months on doctor-diagnosed asthma by age eight (aOR 0.59, 95% CI 0.39-0.88, p=0.01). However, we observed a strong trend which failed to reach statistical significance for breastfeeding >four months to increase the risk of atopy at age one year (aOR 2.41, 95% CI 0.94-6.14, p=0.07). There was no significant association between breastfeeding and atopy at any other time point. **Conclusion**. Breastfeeding may prevent viral-infection induced wheezing illnesses in early childhood (transient early wheezing).

Key words: Breastfeeding, Asthma, Atopy, Childhood.

Introduction

Numerous studies have explored the association between breastfeeding in infancy and subsequent development of asthma and allergic disease during childhood. However, there has been considerable inconsistency in the direction of the reported associations, with different studies producing largely contradictory findings (1-4). For example, while some studies reported the protective effect of breastfeeding on the development of asthma and atopy (1), others have found that prolonged breastfeeding in infancy increases

the risk (2). In addition, the effect of breastfeeding may differ in children with different genetic predispositions, and on different phenotypes of childhood asthma. For example, most studies which reported that breastfeeding increases the risk of asthma development observed this association amongst children with a family history of allergic disease. Results from the Tucson Children's Respiratory Study have shown that while exclusive breastfeeding for ≥four months was unrelated to asthma in school age children amongst those with non-asthmatic mothers, it was associated with an increase in risk amongst children who had asthmatic mothers (3). In contrast, when recurrent wheeze during the first three years of life was used as an outcome, breastfeeding was shown to be protective, regardless of the presence or absence of maternal asthma (3). However, in a large population-based birth cohort study from New Zealand, children breastfed for at least four weeks in infancy had increased risk of having asthma and current wheeze at age 9 years, which remained positive after adjustment for parental history of allergic disease (4).

Special consideration needs to be given to the fact that the composition of breast milk varies between individuals, and this may have an impact on asthma and allergic diseases. A small study examining the level of TGF-β_1 and IL-10 in the colostrum and mature milk of allergic and non-allergic mothers found that TGF-β_1 level was significantly lower in the mature milk of allergic mothers (5).

So, why are there such inconsistencies in the reported association between breastfeeding in infancy and the development of asthma and allergies? A part of the answer is that in contrast to most other common complex diseases (e.g. diabetes and hypertension), asthma starts early in life and is an unstable phenotype, with symptoms progressing or remitting over time (6). One of the difficulties when studying this disease arises from the fact that asthma is clearly not a single disease, but a collection of diseases presenting as a syndrome or a collection of symptoms (7-10). This is particularly relevant during childhood, when wheezing may be a common symptom of a number of different diseases, with distinct aetiologies and different environmental risk factors (7, 9, 11). Therefore, the optimum study design to investigate early-life environmental associates of asthma is the population-based birth cohort (6). Using this study design to overcome problems of recall bias and carefully longitudinally phenotyping children, we investigated the association between breastfeeding and development of wheeze, asthma and atopy during childhood.

Methods

Study design, setting and participants

The Manchester Asthma and Allergy Study (MAAS) is an unselected, population-based birth cohort described in detail elsewhere (10, 12-22). Subjects were recruited prenatally and followed prospectively until age eight years. The study is registered as ISRCTN72673620 and approved by the Local Research Ethics Committee. All parents gave written informed consent and children gave their assent if appropriate.

Follow-up: Participants attended follow-up clinics at ages one, three, five and eight years (10, 13, 14, 16, 20-27). Validated questionnaires (28) were interviewer-administered at each time-point to collect information on parentally-reported symptoms, physician-diagnosed illnesses and treatments received.

Definition of exposures and outcomes

Breastfeeding: The information on breastfeeding and its duration was derived from

parental reports at first and third year reviews, based on the answers to questions "Did *you breastfeed your child?*" and "*How long did you breastfeed for (in months, weeks, days)*?". Breastfeeding was described as any breastfeeding during the specified time, including breastfeeding with supplementary formula milk. For the analysis of the association with the outcomes of interest (wheeze, asthma, atopy), we assigned children into three categories according to the duration of breastfeeding: (a) *not breastfed*, (b) *breastfed for ≤ four months* and (c) breastfed for *> four months*.

Wheeze: According to the parentally reported history of wheeze, we defined *current wheeze* at age one, three, five and eight years as a positive response to question "Has your child had wheezing or whistling in the chest in the last twelve months?". In addition, based on prospectively collected data, children were assigned to the following wheeze phenotypes (10, 21); *No wheezing* – no wheeze ever during the first eight years of life; *Transient early wheezing* – wheezing during the first five years, no wheezing after the age of five years; *Late-onset wheezing* – wheeze onset after the child's fifth birthday; *Persistent wheezing* – wheezing at all time points; *Intermittent wheezing* – wheezing at one time point during the first five years, wheezing at age eight years.

Asthma: Parentally-reported asthma was defined as a positive answer to the question *"Does your child have asthma now?"* and Doctor-diagnosed asthma ever as a positive answer to *"Has your doctor ever told you your child has asthma?" at the age of eight years.*

Atopic sensitisation: Atopic sensitisation was ascertained by skin prick testing (Bayer, Elkhart, IND, US) and/or measurement of specific serum IgE (ImmunoCAP; Phadia AB, Uppsala, Sweden) to common inhalant and food allergens (*Dermatophagoides pteronyssinus*, cat, dog, grasses, moulds, milk, egg and peanut); sensitisation was defined as a mean wheal diameter of 3 mm greater than negative control or allergen-specific IgE>0.35 kU_A/l to at least one allergen at age one, three, five and eight years.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 15.0 (SPSS Inc, Chicago Ill, USA). The association between breastfeeding and outcomes of interest (wheeze, asthma and atopy) was initially assessed using the Chi-squared test, with a significant result if p value was below 0.05. Odds ratio (OR) and 95% Confidence Intervals (CI) were determined using univariate logistic regression analysis. A multivariate analysis for wheeze/asthma was performed using a logistic regression model with adjustment for gender, maternal smoking and parental (maternal and paternal) asthma. For wheeze phenotypes, we carried out multinomial logistic regression analysis, before and after adjusting for gender. The association between atopic sensitisation and breastfeeding was tested in a multivariate logistic regression adjusted for gender and maternal atopy. The confounders used in these models were selected on the basis of previously published data, as well as their association with both breastfeeding and clinical outcomes in our study population.

Results

Participant flow and demographics

Of 1185 children whose parents gave consent to take part in the study, 1072 (90.5%) had data on breastfeeding available. Of these 1072 children, 358 (33.4%) were never breastfed, 357 (33.3%) were breastfed for four months or less and 357 (33.3%) were breastfed for longer than four months (Table 1). There was no significant difference in the duration of breastfeeding between boys

and girls. However, children who were born by caesarean section, who had parents who smoked, who were born into low income families and who had non-atopic fathers were less likely to be breastfed at birth (Table 1).

A total of 1028 children were reviewed at age one year, 987 at age three, 943 at age five and 905 at age eight; 343, 780, 758 and 736 participants underwent skin testing at ages one, three, five and eight years respectively. The prevalence of current wheeze was 35.4% (364/1028) in the first year of life, 22.8% (225/987) at age three, 22.0% (207/943) at age five and 17.7% (160/905) at age eight years. Of 905 children who had complete data on the presence of wheeze at each follow-up during the first eight years of life, 410 (45.3%) were classified as never wheezers, 225 (24.9%) had transient early wheeze, 100 (11.0%) intermittent wheeze, 51 (5.6%) late-onset wheeze and 119 (13.1%) persistent wheeze. Within the first eight years of life, 23.4% (204/870) children received doctor diagnosis of asthma; 10.7% (94/881) had parentally-reported current asthma at age eight years. As expected, the prevalence of atopy increased with age both when assessed by skin prick tests (SPT) and sIgE measurement (SPT; age one, 10.9% [50/459]; age three, 23.0% [205/893]; age five, 30.4% [262/861] and age eight 33.6% [281/837]; sIgE: age one, 16.6% [38/229], age three, 27.4% [62/226], age five, 33.5% [182/552] and age eight, 43.0% [230/535]).

Breastfeeding, wheeze and asthma

The association between breastfeeding and wheezing, doctor-diagnosed and parentally-reported asthma is shown in Table 2.

Current wheeze: There was a trend which failed to reach statistical significance for children who were breastfed >four months to wheeze less in the first year of life (OR 0.76, 95% CI: 0.56- 1.04, p=0.08). By the age of three years, children who were breastfed >four months were significantly less likely to have current wheeze (OR 0.66, 95% CI:

Table 1 Demographic characteristics of study population at the time of recruitment

Demographic characteristics	Never breastfed (n=358)	Breastfed ≤4 months (n=357)	Breastfed >4 months (n=357)	P value
Gender (n=1072) Male	192 (53.6%)	208 (58.3%)	182 (51.0%)	0.48
Vaginal delivery (n=852)	212 (72.9%)	237 (83.7%)	223 (80.2%)	**0.03**
Older sibling (n=913)	155 (51.7%)	144 (48.3%)	171 (54.3%)	0.50
Paternal annual income (n=973) < £10,000 £10,000- £20,000 £20,000- £30,000 >£30,000	 76 (23.5%) 139 (42.9%) 68 (21.0%) 41 (12.7%)	 46 (14.3%) 118 (36.8%) 92 (28.7%) 65 (20.2%)	 28 (8.5%) 113 (34.5%) 111 (33.8%) 76 (23.2%)	**<0.0001**
Maternal smoking (n=1066)	86 (24.1%)	47 (13.3%)	18 (5.1%)	**<0.0001**
Paternal smoking (n=1069)	122 (34.2%)	87 (24.4%)	65 (18.3%)	**<0.0001**
Maternal current asthma (n=1069)	55 (15.4%)	48 (13.5%)	48 (13.5%)	0.46
Paternal current asthma (n=1068)	20 (5.6%)	30 (8.5%)	33 (9.2%)	0.07
Maternal (SPT) atopy (n=1032)	199 (57.0%)	195 (57.4%)	210 (61.2%)	0.26
Paternal (SPT) atopy (n=1027)	202 (58.2%)	225(66.6%)	226 (61.1%)	**0.03**

Table 2 The association between breastfeeding and wheeze and asthma during the first 8 years of life (univariate analysis)

Wheeze	Outcome present						P value
	No		Yes				
	N	Percent	N	Percent	OR	95% CI	
Current wheeze at age 1 (N=1028)							
Never breastfed	213	61.2	135	38.8	1.00		
Breastfed ≤ 4 months	220	65.1	118	34.9	0.29	0.62, 1.16	0.29
Breastfed > 4 months	231	67.5	111	32.5	0.76	0.56, 1.04	0.08
Current wheeze at age 3 (N=987)							
Never breastfed	245	73.1	90	26.9	1.00		
Breastfed ≤ 4 months	251	78.0	71	22.0	0.77	0.54, 1.10	0.15
Breastfed > 4 months	266	80.6	64	19.4	0.66	0.46, 0.94	**0.02**
Current wheeze at age 5 (N=943)							
Never breastfed	239	76.8	72	23.2	1.00		
Breastfed ≤ 4 months	236	75.9	75	24.1	1.06	0.73, 1.53	0.78
Breastfed > 4 months	261	81.3	60	18.7	0.76	0.52, 1.12	0.17
Current wheeze at age 8 (N=905)							
Never breastfed	248	83.2	50	16.8	1.00		
Breastfed ≤ 4 months	242	81.8	54	18.2	1.11	0.73, 1.69	0.64
Breastfed > 4 months	255	82.0	56	18.0	1.09	0.72, 1.66	0.69
Parentally reported current asthma at age 8 (N=881)							
Never breastfed	263	90.7	27	9.3	1.00		
Breastfed ≤ 4 months	256	88.3	34	11.7	1.29	0.76, 2.21	0.34
Breastfed > 4 months	268	89.0	33	11.0	1.20	0.70, 2.05	0.51
Doctor diagnosed asthma ever by age 8 (N=870)							
Never breastfed	204	71.1	83	28.9	1.00		
Breastfed ≤ 4 months	221	77.3	65	22.7	0.72	0.49, 1.05	0.09
Breastfed > 4 months	241	81.1	56	18.9	0.57	0.39, 0.84	**0.005**

0.46-0.94, p=0.02). The association between breastfeeding and current wheeze was no longer significant at ages five and eight years (p=0.17 and p=0.69, respectively).

The results of multivariate models at ages one, three, five and eight years are shown in Table 3. In the multivariate logistic regression model adjusted for gender, maternal smoking and maternal and paternal current asthma, the association between breastfeeding and wheeze in the first year of life was not significant (aOR 0.88, 95 % CI 0.63-1.22, p=0.43, Table 3). In this model, significant associates of wheeze in the first year of life were male gender (aOR 1.50, 95% CI 1.15-1.96, p=0.003), maternal current asthma (aOR 1.98, 95% CI 1.38-2.83, p<0.0001) and maternal smoking (aOR 2.03, 95% CI 1.40-2.94, p<0.0001). However, the association between current wheeze at age three years and breastfeeding for longer than four months remained significant in a multivariate model (aOR 0.68, 95% CI 0.47-1.00, p=0.05). Another significant and independent associate of current wheeze at age three was maternal current asthma, with children of asthmatic mothers having 1.87 times higher odds of wheeze at the age three years (95% CI 1.26-2.77, p=0.002).

Wheeze phenotypes: In a multinomial regression model in which we explored the association between breastfeeding and phenotypes of wheezing during the first eight years of life (reference group: *no wheezing)*,

we found that breastfeeding >four months was protective of *transient early wheeze* (OR 0.60, 95% CI 0.40-0.89, p=0.01). There was no significant association between breastfeeding and other wheeze phenotypes. In a multivariate model adjusted for gender, the association between breastfeeding >four months and transient early wheeze remained significant (aOR: 0.61, 95% CI 0.41-0.90, p=0.01), with male gender being another significant independent risk factor (aOR 1.84, 95% CI 1.32-2.57, p<0.0001).

Asthma: Children who were breastfed >four months were less likely to have doctor-diagnosed asthma by age eight years (p=0.005, Table 2). However, there was no

Table 3 Multivariate analysis: breastfeeding and wheeze/asthma in the first 8 years of life

Wheeze	aOR	95% CI	p value
Current wheeze at age 1 (N=1023)			
Breastfed ≤ 4 months	0.91	0.66, 1.26	0.57
Breastfed > 4 months	0.88	0.63, 1.22	0.43
Male gender	1.50	1.15, 1.96	**0.003**
Maternal smoking	2.03	1.40, 2.94	**<0.0001**
Maternal asthma	1.98	1.38, 2.83	**<0.0001**
Paternal asthma	1.11	0.68, 1.80	0.69
Current wheeze at age 3 (N=983)			
Breastfed ≤ 4 months	0.77	0.54, 1.11	0.17
Breastfed > 4 months	0.68	0.47, 1.00	**0.05**
Male gender	1.20	0.89, 1.63	0.24
Maternal smoking	1.27	0.84, 1.94	0.26
Maternal asthma	1.87	1.26, 2.77	**0.002**
Paternal asthma	1.28	0.75, 2.22	0.37
Current wheeze at age 5 (N=938)			
Breastfed ≤ 4 months	1.09	0.75, 1.60	0.64
Breastfed > 4 months	0.87	0.58, 1.30	0.49
Male gender	1.49	1.08, 2.05	**0.02**
Maternal smoking	1.84	1.20, 2.83	**0.006**
Maternal asthma	1.83	1.22, 2.76	**0.004**
Paternal asthma	1.02	0.57, 1.81	0.95
Current wheeze at age 8 (N=901)			
Breastfed ≤ 4 months	1.09	0.71, 1.68	0.69
Breastfed > 4 months	1.09	0.70, 1.69	0.71
Male gender	1.27	0.89, 1.80	0.19
Maternal smoking	1.18	0.70, 1.98	0.53
Maternal asthma	1.47	0.93, 2.33	0.10
Paternal asthma	1.44	0.81, 2.57	0.22
Parentally reported asthma at age 8 (N=878)			
Breastfed ≤ 4 months	1.16	0.67, 1.99	0.60
Breastfed > 4 months	1.11	0.64, 1.92	0.72
Male gender	1.42	0.91, 2.22	0.12
Maternal smoking	0.63	0.29, 1.37	0.24
Maternal asthma	1.09	0.60, 1.97	0.79
Paternal asthma	2.01	1.05, 3.85	**0.04**
Ever doctor diagnosed asthma by age 8 (N=867)			
Breastfed ≤ 4 months	0.68	0.46, 1.00	**0.05**
Breastfed > 4 months	0.59	0.39, 0.88	**0.01**
Male gender	1.64	1.18, 2.29	**0.003**
Maternal smoking	1.21	0.76, 1.93	0.43
Maternal asthma	2.18	1.44, 3.31	**<0.0001**
Paternal asthma	1.87	1.10, 3.17	**0.02**

significant difference in the duration of breastfeeding between children who had parentally-reported current asthma at age eight and those who did not. In a multivariate model, we confirmed the significant protective effect of breastfeeding >four months on doctor-diagnosed asthma by age eight (aOR 0.59, 95% CI 0.39-0.88, p=0.01), with a strong trend for a similar association with breastfeeding ≤four months (aOR 0.68, 95% CI 0.46-1.00, p=0.05, reference group: never breastfed, Table 3). Male gender (OR 1.64, 95% CI 1.18-2.29, p=0.003), maternal current asthma (aOR 2.18, 95% CI 1.44-3.31, p<0.0001) and paternal current asthma (aOR 1.87, 95% CI 1.10-3.17, p=0.02) were other significant independent risk factors for doctor-diagnosed asthma by age eight years.

Breastfeeding and atopic sensitisation

The association between duration of breastfeeding and atopic sensitisation at ages one, three, five and eight years is shown in Table 4.

Table 4 The association between breastfeeding and atopy during the first 8 years of life (univariate analysis)

Atopy	Outcome present						
	No		Yes				
	N	Percent	N	Percent	OR	95% CI	p value
Atopy (SPT) at age 1 (n=343)							
Never breastfed	114	94.2	7	5.8	1.00		
Breastfed ≤ 4 months	92	88.5	12	11.5	2.12	0.80, 5.61	0.13
Breastfed > 4 months	102	86.4	16	13.6	2.56	1.01, 6.46	**0.05**
Atopy (sIgE) at age 1 (n=172)							
Never breastfed	47	88.7	6	11.3	1.00		
Breastfed ≤ 4 months	44	88.0	6	12.0	1.07	0.32, 3.56	0.91
Breastfed > 4 months	55	79.7	14	20.3	1.99	0.71, 5.60	0.19
Atopy (SPT) at age 3 (n=780)							
Never breastfed	203	79.6	52	20.4	1.00		
Breastfed ≤ 4 months	209	79.8	53	20.2	0.99	0.65, 1.52	1.00
Breastfed > 4 months	202	76.8	61	23.2	1.18	0.78, 1.79	0.44
Atopy (sIgE) at age 3 (n=164)							
Never breastfed	43	78.2	12	21.8	1.00		
Breastfed ≤ 4 months	40	76.9	12	23.1	1.08	0.43, 2.67	0.88
Breastfed > 4 months	42	73.7	15	26.3	1.28	0.54, 3.06	0.58
Atopy (SPT) at age 5 (n=758)							
Never breastfed	182	72.2	70	27.8	1.00		
Breastfed ≤ 4 months	179	72.2	69	27.8	1.00	0.68, 1.48	1.00
Breastfed > 4 months	182	70.5	76	29.5	1.09	0.74, 1.59	0.68
Atopy (sIgE) at age 5 (n=486)							
Never breastfed	118	72.8	44	27.2	1.00		
Breastfed ≤ 4 months	102	64.2	57	35.8	1.50	0.93, 2.41	0.10
Breastfed > 4 months	115	69.7	50	30.3	1.17	0.72, 1.88	0.53
Atopy (SPT) at age 8 (n=736)							
Never breastfed	175	73.2	64	26.8	1.00		
Breastfed ≤ 4 months	159	66.0	82	34.0	1.41	0.95, 2.09	0.09
Breastfed > 4 months	173	67.6	83	32.4	1.31	0.89, 1.93	0.17
Atopy (sIgE) at age 8 (n=471)							
Never breastfed	82	61.7	51	38.3	1.00		
Beastfed ≤ 4 months	92	56.8	70	43.2	1.22	0.77, 1.95	0.40
Breastfed > 4 months	105	59.7	71	40.3	1.09	0.69, 1.73	0.72

Table 5 Multivariate analysis: breastfeeding and atopy in the first 8 years of life

Atopy	aOR	95% CI	p value
Atopy (SPT) at age 1 (N=343)			
Breastfed ≤ 4 months	1.95	0.73, 5.19	0.18
Breastfed > 4 months	2.41	0.94, 6.14	0.07
Male gender	1.38	0.67, 2.85	0.38
Maternal atopy	2.36	0.94, 5.90	0.07
Atopy (SPT) at age 3 (N=770)			
Breastfed ≤ 4 months	0.94	0.60, 1.46	0.78
Breastfed > 4 months	1.20	0.78, 1.85	0.41
Male gender	2.05	1.41, 2.97	**<0.0001**
Maternal atopy	2.13	1.48, 3.08	**<0.0001**
Atopy (SPT) at age 5 (N=738)			
Breastfed ≤ 4 months	1.00	0.66, 1.50	1.00
Breastfed > 4 months	1.08	0.73, 1.62	0.69
Male gender	1.84	1.32, 2.58	**<0.0001**
Maternal atopy	1.45	1.04, 2.02	**0.03**
Current wheeze at age 8 (N=719)			
Breastfed ≤ 4 months	1.38	0.92, 2.07	0.12
Breastfed > 4 months	1.31	0.88, 1.95	0.19
Male gender	1.69	1.21, 2.35	**0.002**
Maternal atopy	1.47	1.06, 2.04	**0.02**
Atopy (sIgE) at age 1 (N=172)			
Breastfed ≤ 4 months	1.01	0.30, 3.41	1.00
Breastfed > 4 months	2.46	0.84, 7.15	0.10
Male gender	3.82	1.41, 10.40	**0.009**
Maternal atopy	1.17	0.45, 3.07	0.75
Atopy (sIgE) at age 3 (N=162)			
Breastfed ≤ 4 months	0.98	0.39, 2.50	0.97
Breastfed > 4 months	1.17	0.48, 2.87	0.73
Male gender	1.53	0.70, 3.35	0.29
Maternal atopy	2.44	0.98, 6.06	0.054
Atopy (sIgE) at age 5 (N=478)			
Breastfed ≤ 4 months	1.23	0.76, 1.97	0.40
Breastfed > 4 months	1.13	0.70, 1.80	0.62
Male gender	1.25	0.86, 1.83	0.24
Maternal atopy	1.30	0.89, 1.89	0.17
Atopy (sIgE) at age 8 (N=466)			
Breastfed ≤ 4 months	1.23	0.76, 1.97	0.40
Breastfed > 4 months	1.13	0.70, 1.80	0.62
Male gender	1.25	0.86, 1.83	0.24
Maternal atopy	1.30	0.89, 1.89	0.17

At age one year, we found that breastfeeding >four months increased the risk of atopy assessed by SPT (p=0.05, Table 4). Although the association between breastfeeding and atopy assessed by sIgE at the same time point was in the same direction, it did not reach statistical significance (most likely due to fewer children providing blood samples for measurement of specific IgE). We found no significant association between breastfeeding and atopic sensitisation at ages three, five and eight years, assessed by either SPT or sIgE measurement.

The results of multivariate models at ages one, three, five and eight years are shown in Table 5. In a multivariate logistic regres-

sion adjusted for gender and maternal atopy, we observed a strong trend which failed to reach statistical significance for breastfeeding >four months to increase the risk of atopy (SPT) at age one year (aOR 2.41, 95% CI 0.94-6.14, p=0.07). There was no significant association between breastfeeding and atopy at any other time (Table 5).

Discussion

Principal findings

Our findings indicate that children who were breastfed longer than four months were less likely to wheeze at age three years and were less likely to receive the doctor diagnosis of asthma during the first eight years of life. However, we found no association between breastfeeding and current wheezing at ages five and eight years. We confirmed the finding of the protective effect of prolonged breastfeeding on wheezing in early life in the analysis in which we used prospectively collected data to assign children to different wheeze phenotypes; children who were breastfed longer than four months were protected from transient early wheezing, with no association between breastfeeding and other wheeze phenotypes (intermittent, late-onset and persistent). We observed a strong trend for prolonged breastfeeding (more than four months) to increase the risk of atopy at age one year, but found no association between breast feeding and atopic sensitisation at ages three, five and eight years.

The strengths and limitations of the study

One of the strengths of this study is that data on breastfeeding was collected early in life, which reduced the chance of recall bias amongst parents of children who developed wheeze and asthma later in childhood. Also, data on wheezing and asthma were collected prospectively, increasing the accuracy of outcome measures and the validity of our findings. Moreover, due to the prospective nature of the study we had detailed information on multiple other factors known to increase the risk of wheeze and asthma during childhood (such as maternal smoking and parental asthma), which we used for adjustment in the multivariate analysis. However, one of the limitations is that at the time of clinical follow up at age one and three years, rather than assessing exclusive breastfeeding, we recorded it as any breastfeeding at a specified time. A further limitation is that some of the outcomes are based on parental report, which is unreliable as many parents have little understanding of what physicians mean by the term "wheeze"(24).

The meaning of the study

In this study we observed a significant protective effect of prolonged breastfeeding against wheezing during the first three years of life. However, this association was not confirmed in later childhood, and we found no relationship between breastfeeding and current wheeze at ages five and eight years. The finding that breastfeeding was protective against wheeze only among children who were classified as transient early wheezers may be interpreted as the non-specific effect of breastfeeding on anti-microbial immunity, with the consequent prevention of viral infections [given that a high proportion of early-life wheeze is caused by respiratory viruses (29)].

In addition to the effect of breastfeeding, we found that maternal smoking was a significant risk factor for wheezing during the first year of life. This is in agreement with a finding from another prospective study which found that children who were exposed to smoking in their household had 1.58 higher odds of wheeze in the first year of life (30), and with our previous findings,

which demonstrated that maternal smoking was an associate of early-life wheezing independently of lung function measured at age four weeks (31). Another significant predictor of childhood wheeze in our study, at both age one and three years was maternal asthma. Both passive smoking and maternal asthma are well described risk factors for early life wheezing and childhood asthma, respectively.

Our findings are in agreement with the Tucson Children's Respiratory Study, which reported that children who were exclusively breastfed for ≥four months were 2.2-times less likely to have recurrent wheeze in the first two years of life, independent of the presence of maternal asthma (3). However, similar to our findings, there was no association between exclusive breastfeeding and recurrent wheeze at age six years (3). Similar results were reported from the ALSPAC study (an unselected birth cohort of several thousand children), in which breastfeeding for longer than six months was protective against wheeze in the first three years of life, but not wheeze at age seven and eight years (32). These data indirectly support the concept that childhood wheezing illness is a conglomerate of diseases, with different environmental associates and probably different genetic backgrounds (6).

Although we found that breastfeeding for longer than four months tended to increase the risk of atopy at age one, this was not confirmed at later ages. The higher prevalence of atopic sensitisation at age one year among children who have been breastfed for longer than four months may be due to reverse causality (e.g. we demonstrated that these children were more often born to atopic fathers, who, in line with previous infant feeding recommendations may be more supportive and encouraging of their partners for prolonged breastfeeding). Similar observation of longer duration of breastfeeding among children of allergic parents compared to children of non-allergic parents was described in the Dutch PIAMA birth-cohort study (33).

In our study, the lack of association between breastfeeding and atopy at later ages is contrary to the findings from the PIAMA study, which reported a lower rate of atopic sensitisation to inhalant allergens (assessed by specific IgE measurement) at age eight years among children breastfed longer than four months (33). This difference may be explained by the fact that in our study we measured specific IgE to 10 common inhalant and food allergens, while the PIAMA study only looked at sensitisation to six inhalant allergens. Inevitably, we found a higher proportion of sensitised children within our study population at age eight years (40.8%) compared to children in the PIAMA study (27.5%).

Conclusions

Prolonged breastfeeding practice should be advocated as it prevents early life gastrointestinal and respiratory infections due the unique content of breast milk. Within the context of respiratory diseases during childhood, breastfeeding may prevent transient early wheezing which is predominantly induced by viral infection. However, we did not find any evidence that breastfeeding prevents asthma and atopic sensitisation at a later age, amongst school-age children.

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Relative dose intensity of systemic chemotherapy in an outpatient cancer center

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Objective. This study was undertaken to determine the average relative dose intensity (RDI) of chemotherapy administered to patients in a community-based outpatient cancer center. **Methods.** A retrospective review of medical records in an outpatient cancer center was conducted for patients initiating systemic chemotherapy in 2007 for a diagnosis of lymphoma, breast, lung, ovary, or colon cancer. Eighty-four records meeting the inclusion criteria were reviewed for demographic information, primary tumor type, chemotherapy regimen, staging at diagnosis, presence of disease progression, and mortality status. Regimen data included: chemotherapeutic agents used, dosages administered, dates of administration, treatment intent (adjuvant vs. metastatic), and granulocyte colony-stimulating factor (G-CSF) usage per cycle. Mean summary statistics were calculated and average RDI was analyzed. **Results**. The overall RDI at our institution was 83% (n=65). The RDI for those receiving adjuvant chemotherapy was 85% (n=51), whereas for those receiving chemotherapy for metastatic disease the RDI was 76% (n=14). Fifty-four percent (n=35) of the regimens met or exceeded the recommended minimum goal RDI of ≥ 85%. **Conclusions**. Overall the average RDI at our institution was 83%, slightly below the goal of ≥ 85%. Patients with potentially curable malignancies receiving adjuvant chemotherapy reached the threshold RDI; however, areas for quality improvement exist at our institution.

Key Words: Drug therapy, Chemotherapy, Dose intensity.

Introduction

Dose intensity (DI) is defined as the total amount of drug delivered to a patient over the total time course of treatment (1). Relative dose intensity (RDI) is the ratio of the dose intensity delivered to the reference standard dose intensity for a chemotherapy regimen (2). Retrospective analyses of randomized controlled clinical trials have suggested a strong association between RDI and disease-free and overall survival, especially for lymphoma and cancers of the breast, lung, ovary, and colon (2-13). In particular, data show increased survival for patients receiving greater than or equal to 85% RDI

and conversely, mortality curves similar to untreated populations when this threshold RDI was not administered (11-13). Reductions in chemotherapy dose intensity through dose reductions and/or delays may potentially compromise disease control and survival in patients with curable malignancies (2, 3). The primary objective of this study was to determine retrospectively the average RDI of chemotherapy administered to patients in an outpatient cancer center. In addition, the RDI was separately determined for patients receiving adjuvant chemotherapy and chemotherapy for metastatic disease.

Methods

Study design, setting, and population

A retrospective review was conducted of medical records in a community-based outpatient cancer center. Medical records were reviewed for patients initiating systemic chemotherapy in 2007 for a diagnosis of lymphoma or cancer of the breast, lung, ovary, or colon. Patients were excluded from the study if any of the following conditions were met: patient age less than 18 years, multiple primary tumor types, non-chemotherapeutic agent(s) administered as monotherapy, inpatient administration of chemotherapy, or unavailable chart.

Data collection

Eighty-four records meeting the above inclusion criteria were identified. These records were reviewed and data was extracted using a standardized data collection form that included demographic information, primary tumor type, chemotherapy regimen, staging at diagnosis, presence of disease progression, and mortality status. In determining the RDI, the following chemotherapy regimen data was collected: chemotherapeutic agents used, dosages administered, dates of administration, treatment intent (adjuvant vs. metastatic), and granulocyte colony-stimulating factor (G-CSF) usage per cycle.

After patient eligibility was determined and data collection was completed, the chemotherapy regimen data was entered into the NearSpace® RDI Calculator software program (14). For each chemotherapeutic agent, the software program calculated the RDI based on the total milligram dose of drug administered, the patient's body surface area (BSA) or target area under the curve (AUC), and the time course of treatment. The program expressed the RDI for each chemotherapy agent as the percentage of the dose intensity delivered relative to the reference standard dose intensity for that agent. For patients with multiple chemotherapy agents in their regimen, the RDI was determined separately for each agent and then the average RDI was calculated for the entire regimen.

Statistical analysis

All data was analyzed utilizing descriptive statistics. Summary statistics were calculated to convey the central tendency of RDI. These summary statistics were expressed as the sample mean.

Ethics

This study was conducted in accordance with the protections of human subjects as specified by our Institutional Research Review Committee.

Results

In 2007, We identified 84 patients who met the initial inclusion criteria. Nineteen patients were excluded: 5 patients received non-chemotherapeutic agent(s), 4 patients had inpatient administration of chemother-

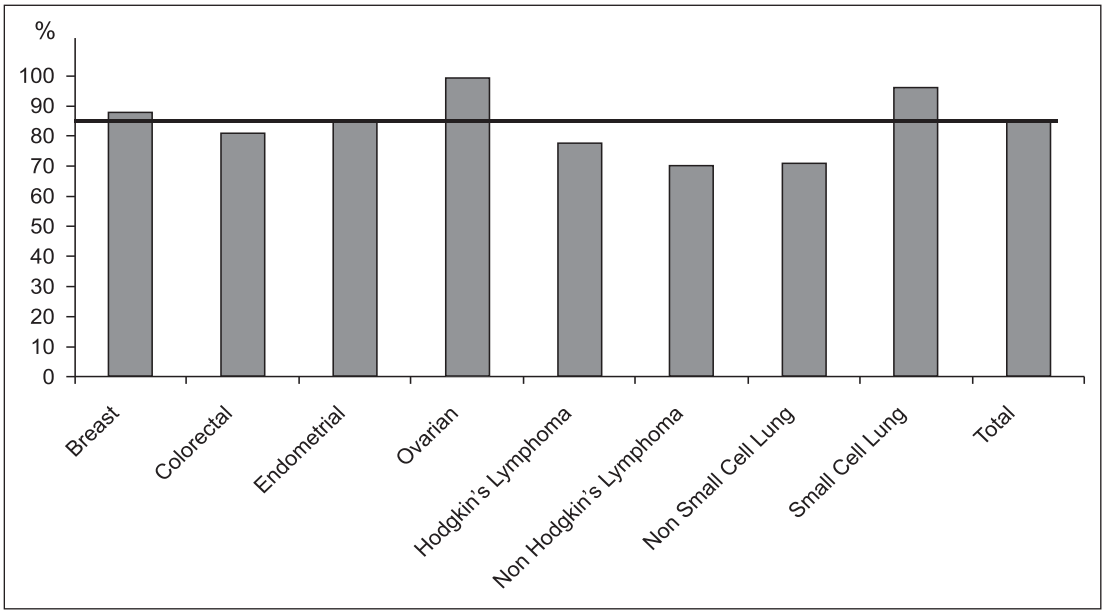

Figure 1 Average relative dose intensity by diagnosis. Black line denotes threshold RDI ≥ 85%

apy, 1 patient had more than one malignancy, and 9 charts were unavailable. Sixty-five patients were included in the final analysis. The overall RDI was determined to be 83%. The average RDI for patients receiving adjuvant chemotherapy (n=51) was 85% and for patients receiving chemotherapy for metastatic disease (n=14) the average RDI was 76%. The proportion of regimens that met or exceeded the 85% threshold RDI was 54%.

The average RDI was further analyzed by cancer diagnosis, disease progression, mortality status, and G-CSF use. RDI by cancer diagnosis is shown in Figure 1. The average RDI for patients diagnosed with breast, ovarian, and small cell lung cancer reached the desired goal of ≥ 85%, while the remaining cancer types did not.

Disease progression was documented in 22 patients. For these patients, the average RDI was 76%. Where disease progression was not documented (n=43), the average RDI was 86%. Analysis by mortality status revealed that the average RDI for expired patients was 73% (n=17), while patients who were alive averaged an RDI just above the desired range (86%, n=48). Use of G-CSF was documented in 69% (n=45) of patients; for 71% of cases, growth factors were administered as primary prophylaxis and for 28%, as secondary prophylaxis. The average RDI for patients who received G-CSF as supportive care during their chemotherapy was 82%.

Discussion

The overall RDI at our institution was slightly below the desired level of 85%; however, patients with potentially curable malignancies who received adjuvant chemotherapy just reached the threshold RDI. The results of this study provide valuable benchmarking data, allowing our institution to compare our practice patterns with that of other practices. In a nationwide survey by Lyman et al, 1,243 community oncology practices provided data for 20,799 patients with early-stage breast cancer (6). For patients receiving adjuvant chemotherapy, 55.5% received RDI < 85%. Another nationwide survey by

Lyman et al, focusing on 4,522 patients with aggressive non-Hodgkin's lymphoma, reported an RDI < 85% for 48 to 53% of patients receiving treatment at 567 oncology practices (7). Overall, our finding that 46% of our patients received RDI < 85% is consistent with results from these published studies. It is important to note that patients who experienced disease progression, or who expired, received chemotherapy at an RDI well below the threshold level. However, long term follow up (greater than one year) was not carried out in this analysis. More prospective studies are necessary to properly determine the impact of RDI on disease progression and survival, especially in the adjuvant setting, despite previous reports that reductions in chemotherapy dose intensity may compromise disease control and survival in patients with curable malignancies. Data shows increased survival for patients receiving greater than or equal to 85% RDI and conversely, mortality curves similar to untreated populations when this threshold RDI was not administered (12, 13). The use of referenced chemotherapy dose and timing are also important in order to achieve outcomes comparable to those achieved in clinical trials for that chemotherapy regimen (2).

Several limitations of this study should be considered. The RDI calculations included chemotherapeutic agents only. Targeted therapies, such as epidermal growth factor receptor inhibitors or vascular endothelial growth factor inhibitors, were not included in RDI calculations. To our knowledge, it has not been determined if these therapies confer a dose related impact on progression free survival or overall survival. Another factor to consider is that several patients expired prior to the completion of their planned treatment regimen (n=5). This group of patients had a low average RDI (40%) that negatively skewed the results of this study. Chart availability also limited the number of patients analyzed in this study. The majority of unavailable charts were cases in which patients were deceased.

The findings here indicate a need for quality improvement through the implementation of strategies for increasing the overall RDI administered to our patients. In order to improve the RDI administered at our institution, it is important to understand the barriers to delivery of full-dose, on-schedule chemotherapy. Reductions in dose and delays in therapy both hinder the delivery of full-dose, on-schedule chemotherapy and in turn, reduce the RDI achieved (15). Dose delays and reductions can be either treatment related or non-treatment related. Common treatment related causes include: empiric dose reductions, reductions due to myelosuppression (ie. neutropenia), and other drug specific toxicities (15, 16). Non-treatment related causes include: lack of patient and/or provider awareness of the importance of full-dose, on-schedule chemotherapy and visit cancellations (15). Patient-initiated visit cancellations often occur due to personal or family illness, social events, lack of transportation, or miscommunication. Typically, missed appointments are rescheduled for the following week, resulting in week-long chemotherapy delays. When chemotherapy dose delays and reductions are employed together to avoid excessive toxicity or to improve tolerability, negative outcomes of RDI reductions are magnified (16). Dose delays and reductions often result in only nominal decreases in toxicity, but considerable reductions in the capacity to attain a complete remission in patients with drug-responsive tumors (2, 17).

Many different strategies may be utilized in clinical practice to improve RDI. Practice-related interventions may include dose-dense or dense-intense treatment strategies, patient and family education, staff education, prospectively calculating and documenting regimen RDI, risk assessment for

febrile neutropenia, optimizing the use of supportive care agents (G-CSF), and utilization of a strict cancellation protocol (3, 15). Based on the findings from this study, our institution has undertaken a major quality improvement initiative to improve the RDI of systemic chemotherapy. Our main focus has been prevention of chemotherapy dose reductions and delays, specifically the evaluation of G-CSF use and visit cancellations. The next evaluation component is being conducted in four different phases: 1) a one-month pilot study to determine G-CSF utilization and systemic chemotherapy appointment cancellation frequency; 2) cancellation policy development, febrile neutropenia risk assessment tool implementation, and staff education; 3) cancellation policy and risk assessment tool implementation; and 4) post-intervention prospective determination of the RDI at our institution. This follow-up study will not only allow our institution to identify potential areas for improvement but to also to implement strategies that will enhance patient outcomes.

Conclusion

Overall the average RDI at our institution was 83%, slightly below the goal of ≥ 85%. Although patients with potentially curable malignancies receiving adjuvant chemotherapy reached the threshold of 85%, areas for quality improvement exist at our institution. Potential strategies for improvement include: staff education, optimized use of G-CSF, and a strict cancellation policy. Other institutions are encouraged to examine the RDI of systemic chemotherapy at their sites and to develop strategies with regard to improving and/or maintaining optimal RDI.

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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End-of-life care in the intensive care unit: the perceived barriers, supports, and changes needed

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Objective. To identify perceived barriers, supports and changes needed to improve end-of-life care (EOLC) in the intensive care unit (ICU) and to compare physicians' perceptions with those of nurses. **Methods.** We conducted a survey of critical care physicians and nurses in an academic medical center via a 3-item survey with open-ended statements regarding the strongest barriers, supports and changes needed to improve EOLC in ICU. **Results.** Thirty-four percent of all respondents identified physicians as the biggest barrier and thirty-three percent recognized nursing staff as the strongest support towards optimal EOLC. Improved communication was identified by 30% of respondents as the change most needed to improve EOLC. No significant differences between physicians and nurses were observed. **Conclusions.** Critical care physicians and nurses identified similar barriers, supports and the changes most needed to improve EOLC in the ICU. Recognition of physicians as the strongest barrier, and communication as the change most needed indicate areas for improvement. The finding of nurses as the strongest support for good EOLC provides the opportunity to strengthen their role in the care of the dying patient. Further study of these findings will help develop strategies to improve EOLC in the ICU.

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Key words: End-of-life care, Intensive care unit, Physicians, Nurses.

Introduction

In the United States, the majority of adult deaths occur in a hospital (1), and more than one in every five deaths occurs during or following admission to the intensive care unit (ICU) (2). Critical care physicians and nurses care for critically ill patients with the primary goals of saving lives and restoring function. Yet, despite advances in medical sciences and technology, the transition from primarily curative goals to palliative goals is frequent, which makes end-of-life care (EOLC) discussions regular occurrence in the ICU. Therefore, optimizing EOLC in the ICU is a critical component of the practice of ICU medicine.

With the advent of Hospice and Palliative Medicine, specialists in EOLC such as hospital based palliative care teams are playing

an increasingly important role in facilitating good EOLC in the ICU. While the number of hospitals with a palliative care team continues to increase, almost half of the hospitals with over 50 beds do not have a hospital based palliative care team (3), highlighting the need for critical care staff to be comfortable in their roles as providers of EOLC. However, even when consultative palliative care services are available, both the National Hospice and Palliative Care Organization and the American College of Critical Care Medicine emphasize the responsibility of intensivists in providing optimal EOLC (4, 5).

Cooperation and teamwork between ICU staff members is recognized as an integral component of optimal EOLC. Two essential parts of the ICU team are nurses and physicians. Both nurses and physicians identify EOLC as a frequent and challenging area of patient care (6). Previous studies comparing critical care physicians and nurses attitudes towards EOLC highlight discrepancies between their opinions (7, 8). Nurses experienced greater moral distress than their physician counterparts when dealing with EOLC (7). Although physicians felt that they included nurses and support staff in making decisions regarding EOLC; nurses perceived that their input into the plan of care was minimal (8).

The discrepancies in critical care staff's opinions regarding the delivery of EOLC suggest that there is need for improvement. Consequently, this study aimed to identify and compare the perceptions of critical care physicians and nurses in regards to barriers, supports and the changes most needed to improve EOLC in the ICU at our institution.

Methods

The study is a cross-sectional survey of critical care physicians and nurses working at the academic tertiary medical center in the Midwestern region of the USA. The study was approved by the Mayo Clinic Institutional Review Board. A survey packet was mailed to all critical care physicians, including fellows and nurses involved in direct patient care in adult ICUs at Mayo Clinic, Rochester, MN (total of 382 mailings). The packet contained the survey, a pre-addressed return envelope, and a cover letter explaining the survey. Participants were asked to complete next 3 open-ended questions relating to EOLC in the ICU; 1. The biggest barrier towards providing good End-of-Life Care in the ICU is… 2. The strongest support towards providing good End-of Life Care in the ICU is… 3. The one change that I would make to improve current End-of-Life Care is…

The survey was self-administered and anonymous, however participants were asked to identify themselves as a physician or nurse. All responses received with proper identification (MD or RN), were included in the analysis.

The investigators independently analyzed free text responses and categorized these in terms of content and themes. The responses that could not be categorized were grouped under "Other". Disagreement was resolved by consensus. The participants' responses were compared by dividing them by professional role as physician and nurse.

Statistical analysis

Chi square and Fisher's exact test for comparisons between two groups were used. A 2-sided *P* value of less than 0.05 was to be considered statistically significant. Statistical analysis was performed with JMP software (JMP, Version 5, SAS Institute inc., Cary, NC).

Results

Response rates

There were 263 individual responses to at least one question with proper identification

by professional role, out of 382 mailings for a total response rate of 69%. Sixty-nine percent, 65% and 54% of participants responded, respectively, to statements related to the strongest barriers, supports and changes needed to improve EOLC in the ICU (Table 1). Due to the open-ended nature of the survey, some participants recorded more than one response to each question, and therefore the total percentages for each question could exceed more than 100 percent (Tables 4, 5).

Table 1 Survey respondents and responses by each item

Surveyed	Total	Items					
		Barrier		Support		Change	
	N	N	%	N	%	N	%
Physicians	50	31	62	24	48	23	46
Nurses	332	232	70	224	67	184	55
Total	382	263	69	248	65	207	54

Strongest barriers to improvement of EOLC in the ICU

A total of eight distinct categories of barriers were identified. The most identified barrier by all participants was categorized as "physicians", as identified by 34% of respondents. Interestingly, both nurses and physicians identified physicians as the greatest barrier to optimal EOLC in the ICU with similar frequency (35% v. 29% respectively, p=0.52). Examples of responses that were categorized as "physicians" include "Drs don't like to give up even though families request comfort care" and "Physician unwillingness to "let patient go". Although not statistically significant, 23% of responses within the category of "physicians" specifically identified surgeons as hindering EOLC in the ICU. Other identified barriers along with their frequencies are listed in Table 2.

The second most commonly reported barrier, communication issues, was further broken down into three categories: communication between physicians and nurses, communication with families, and communication-in general, including communication between different services (Table 3). Overall, there were no statistically significant differences in the barriers identified by physicians and nurses.

Table 2 Barriers to end-of-life care in the intensive care unit

Barrier	Total		Nurses		Physicians		P
	N	%	N	%	N	%	
Physicians	90	34	81	35	9	29	0.52
Lack of Communication	62	24	53	23	9	29	0.45
Family Issues	33	12	26	11	7	23	0.07
Lack of Education	27	10	20	9	6	19	0.06
Over-Treatment	26	10	24	10	1	3	0.20
Inadequate Time	11	4	8	3	3	10	0.12
Pain	10	4	10	4	0	0	0.61
Advance Directives	9	4	6	3	3	10	0.07
Other	59	22	55	24	4	13%	0.25

Table 3 Lack of communication as a barrier

Communication	Total (63)	
	N	%
Between physicians and nurses	11	17
With Families	28	44
In general	24	38

Biggest support for good EOLC in the ICU

Thirty-three percent of respondents identified nursing staff as the strongest support for good EOLC in the ICU. There was no significant difference between responses from nurses and physicians (34% RNs and 29% MDs, p=0.63) identifying nurses as the biggest support. Some of the statements identifying nursing staff include "Nursing care given to patient and family", "Freedom to use nursing judgment to increase pain medication", "Good nursing support", "Time that we are able to spend with patients". Table 4 reviews additional categories of the responses. Examples of responses categorized as "Other" include: "Feeling comfortable with it yourself", and "It is the way I would like to be treated".

Table 4 Strongest supports for good end-of-life care in the intensive care unit

Support	Percentage*
Nursing Staff	33
Teamwork	17
Communication	14
Chaplain	11
Physicians	8
Comfort Care	8
Education	7
One on one nursing Care	6
Other	9

*Percentages add up to more than 100% because some respondents offered more than one answer.

The change most needed to improve EOLC in the ICU

Improved communication with patients, families, and among the ICU-team members, was identified by 30% of respondents as the change most needed to improve EOLC. As with the other categories, there was no significant difference between nurses and physicians (29% RNs and 30% MDs, p=0.81) identifying improved communication as the change most needed. Examples of statements categorized as communication include: "More communication between nurses/physicians and families", "Honest and compassionate communication between medical staff and patients/families early on in the patients stay here in the ICU", and "More openness to discussion". Other suggestions for the change most needed to improve EOLC in the ICU are listed in Table 5. Examples of responses categorized as "Other" include: "Quicker response from eye bank, life source, and funeral directors although the funeral home directors are usually better than donor personnel" and "Have a specialized team assists with the transition from 'fighting the illness' to providing a dignified death".

Table 5 The change most needed to provide good end-of-life care in the intensive care unit

Change Most Needed	Percentage*
Better Communication	30
More Education	17
Improved Comfort Care	11
Clear Goals/Advanced Directives	9
Change in Physician's Attitudes	6
Improved Environment	4
More Time Devoted to Dying Patients	4
More Family Support	2
Other	18

*Percentages add up to more than 100% because some respondents offered more than one answer.

Discussion

As the principal health care providers for critically ill patients, collaboration among critical care nurses and physicians is essential for providing good EOLC in the ICU. The lack of significant discrepancies between the critical care nurses' and physicians' perceptions of existing barriers, strengths, and ideas for improvement is very encouraging, but differs from previous studies. Although we are uncertain why our findings do not mirror previous literature, we hypothesize that increased focused education on EOLC at our institution (9) is likely the primary factor.

Interestingly, this study revealed that the perception of the physician as a barrier is maintained equally by both nurses and physicians. The perception by nurses of physicians being an impediment to EOLC has been demonstrated previously (6-8). However, self identification by physicians as creating a barrier to EOLC is novel. As we explore opportunities for improvement in EOLC in the ICU, it is critical to identify why physicians are perceived, by themselves and others, as the greatest barrier to the process. Previous studies on physicians' skills with EOLC have found that physicians are uncomfortable in providing EOLC (10, 11). At the time of this study, an online curriculum on EOLC (9) was a mandatory training requirement for fellows in critical care, and was available to all ICU staff. Although this study was not powered to detect differences between staff physicians and critical care fellows, we hypothesize that educational tools in EOLC will improve physician performance in this realm. In part, this finding may also represent the recognition by physicians of the disagreement that can often exist between various consultants.

Numerous physician-led consulting teams are typically involved in the care of an ICU patient. Conflicting assessments of prognosis and goals of therapy may contribute to physician impediment of optimal EOLC. A small qualitative nursing study subsequently validated by a larger investigation found that nurses felt physicians from different specialties not only had differing opinions about the patient's care and prognosis, but also gave conflicting information to family members (12, 13). Many of the respondents in our survey who categorized physicians as a barrier indicated that surgeons in particular impeded good EOLC. Examples of such responses include: "Physicians (particularly surgeons) seeing death of a patient as a direct reflection of their failure. "For example, a surgeon does a palliative surgery on a cancer patient and the pt dies from the cancer, surgeons don't want their patients to die because they just did surgery", "Death is often dragged out and prolonged", "Multisystem organ failure on 89 yr old equals death." This finding mirrored a trend found in another study done at our institution14.

Podnos and Wagman (15), highlight that surgical training has only recently begun to focus attention on palliative medicine principles. Previous work by Galante et al. (16) has identified deficiencies in education on EOLC for surgeons. An ethnographic study conducted at several ICUs by Cassell et al. (17) found that surgeons and intensivists viewed their roles and responsibilities towards patient care, and in particular EOLC, quite differently. Surgeons tended to view their role as preserving life at all costs, whereas medical critical care specialists more readily incorporated quality of life into their patient care values. Improved communication between healthcare teams and attention to the consistency of the messages to patients and their families is integral to improved EOLC. Closed ICU models of care, in which an intensivist oversees all care provided to a patient, will likely provide the best opportunity to improve the uniformity

of information regarding prognosis and recovery given to a patient and family.

In this study, physicians and nurses alike identified nurses as the strongest support for providing good EOLC in the ICU. Clearly ICU nurses spend considerably more time than physicians at the bedside with patients and their families. This additional time facilitates the building of relationships with patients and their families. These relationships between nurses and patients and families should be supported and encouraged to evolve in order to optimize EOLC in the ICU. One of the interventions described by Billings et al. (18) is a three-year project integrating the palliative care consultative service (PCCS) into the ICU setting included the Palliative Care Nurse Champions, which builds upon the existing support that critical care nurses provide for EOLC. This was a program whereby nurses who demonstrated interest in EOLC were selected to receive training on a variety of EOLC topics including communication skills, provided by a palliative care nurse-practitioner. These specially trained nurses served as advocates for good EOLC in the ICU. Subsequent training sessions filled up easily indicating the perceived benefit of the program. The final analysis of the project has not yet been reported.

Our findings are consistent with previous studies that indicate that providers of critical care consider communication as an important barrier, as well as the change most needed to providing good EOLC (7, 8, 19). Interestingly, in our survey, communication between nurses and physicians was the area within communication that was least identified as a barrier. This is in contrast to other studies which found larger discrepancies in the perceived lack of communication between nurses and physicians (8, 20). This finding may reflect the practice of multidisciplinary rounds, which are a daily occurrence at our institution. During these rounds, each ICU patient was discussed amongst physicians, nurses, and other critical care staff including case managers, pharmacists, and nutrition support, to develop a plan of care for that patient. This practice is supported by studies investigating interventions aimed at improving communication in the ICU, led to improved satisfaction and patient care (21, 22, 23). In this study, communication with families was the area within the communication category that was most frequently thought of as a barrier. Lilly et al. (24) studied the intervention of a weekly multidisciplinary meeting with patients and families, and found that ICU staff had higher rates of agreement on plans of care. Integration of ICU families into multidisciplinary rounds is one mechanism by which communication with patients and families may be enhanced.

At the time this study was conducted, the institution did have a hospital based palliative care team, although its role in the ICU was limited. The creation of a palliative care team was cited by respondents as a change that would improve EOLC. Palliative care teams are often interdisciplinary, and collaboration between the various disciplines is highly valued. In addition to providing and teaching the knowledge and skills associated with EOLC, the palliative care team can help foster a more supportive environment within the entire healthcare team as discussions are held regarding EOLC in the ICU. In their project integrating palliative care in the ICU, some of the interventions described by Billings et al. (18) include collaboration between the PCCS and ICU staff, palliative care nurse champions, and staff education on a variety of issues. Their findings thus far indicate that ICU staff has higher satisfaction and comfort with EOLC as a result of integrating the PCCS in the ICU.

Cooperation between critical care providers and palliative medicine services is integral to optimal EOLC in the ICU. The primary goals of critical care medicine is

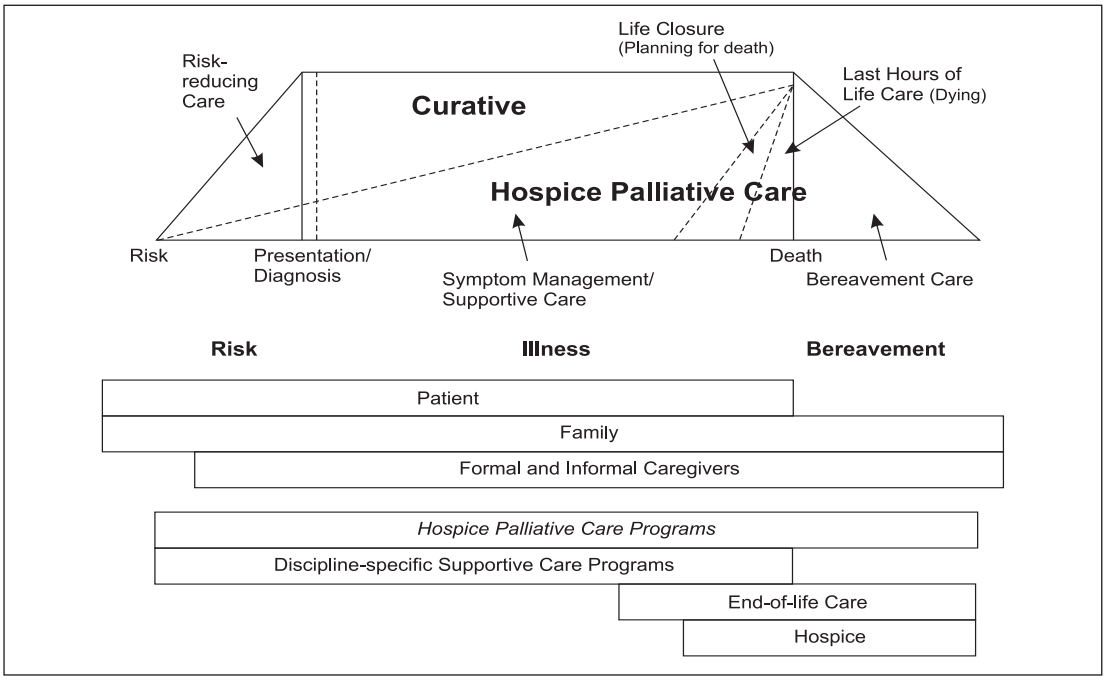

Figure 1 Continuum of curative modalities and palliative care
Palliative care within the experience of illness, bereavement, and risk. From Frank D. Ferris, MD, Medical Director, Palliative Care Standards/Outcomes, San Diego Hospice, 4311 Third Avenue, San Diego, CA, USA 92103–1407

saving or prolonging life, and that of palliative care is alleviating suffering and improving quality of life. In a model demonstrating the integration of these two disciplines, Byock (25) highlights that the primary goal of each discipline, is the secondary goal of the other. Ferris (26) further illustrates this model whereby curative modalities and palliative care coexist upon a continuum and should be addressed simultaneously upon presentation (Figure 1).

Limitations

The study was conducted at a single large academic institution in the Midwestern United States, and results may not be applicable to all critical care settings. The lack of significant differences in the views of physicians and nurses could potentially be due to the small sample size of physicians. While the open-ended nature of this survey did allow for a range of responses, the depth of responses was limited by the survey format of the study. Additionally, the perceptions of other critical care team members, such as case managers and hospital chaplains, the opinions of patients and family members, and the effects of an evolving hospital based palliative care team on EOLC in the ICU, are areas that were not examined and that deserve further study.

Conclusions

Both critical care nurses and physicians identified similar barriers, supports, and changes needed to improve EOLC in the ICU. Physicians were more than any other category considered to be the strongest barrier. Nurses were thought of as the strongest support by the most respondents. The

change most needed to improve EOLC in the ICU was found to be improved communication in general. Recognition and improved understanding of these factors is critical for designing strategies for improved EOLC in the ICU. This study illustrates the need to enhance physician education and the practice of EOLC, and improving teamwork between physicians of differing specialties. Additionally, increased attention to the development, and support of the role of nurses is integral to providing good EOLC. This study serves as a starting point for further focused quantitative and qualitative analysis, including in-depth interviews, of EOLC in the ICU.

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Assessment of anxiety and depression in adolescents with acne vulgaris related to the severity of clinical features and gender

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Objective. To determine anxiety and depression levels in adolescents with acne vulgaris in relation to clinical severity and gender. **Patients and methods.** Using a sectional study, the anxiety and depression level was analyzed of 90 adolescents of both sexes, aged 16 to 21 years, who were suffering from acne vulgaris. The study was conducted between February 1st 2008 and January 31st 2009 at the Dermatovenerology department of the Cantonal Hospital "Dr. Irfan Ljubijankić", Bihac. According to the type of efflorescence, its localization and severity of clinical features, respondents were classified in three groups: the first group consisted of adolescents with mild forms of acne, the second group had moderate forms of acne and the third had severe forms of acne. Evaluation of anxiety levels in patients with acne vulgaris was made using STAI questionnaire (Spielberger's state and trait Anxiety Inventory) that consists of 20 questions about the essential characteristics of anxiety as the condition, and the level of depression was determined using the BDS-scale (Back Depression Inventory) which consists of 18 items that were used by the respondents to perform self-evaluation of depression level. **Results.** In the study group there were 54 (60%) female respondents and 36 (40%) male, mean age 17.5 years (range 15.6-20.6). Of the total number of patients with acne vulgaris, nine of them or 10% (4 female and 5 male) had a mild form of acne (first group), 72 of them or 80% (46 female and 26 male) a moderate form of acne (second group) and 9 of them or 10 % (4 female and 5 male) a severe form of acne (third group) ($p > 0.95$). 1.1% of the respondents had an exceptionally low anxiety level, 13.3% had low level, moderate 76.8% and 8.8% had a high level. 37.8% had distinct signs of depression, the risk group consisted of 44.4%, and a normal value was found in 17.7%. The values of the STAI questionnaire expressed as median (range) in the first, second and third groups were 58.7 (39-70), 57.7 (20-70) and 60.2 (40-70) ($p = 0.36$), while the values of the BDS scale within the same groups were 13.4 (8-16), 15.1 (3-29) and 14.4 (7-24) ($p = 0.367$). **Conclusion.** The level of depression and anxiety in patients with acne vulgaris is not related to severity and patient sex.

Key words: Acne vulgaris, Adolescents, Anxiety and depression, STAI questionnaire.

Introduction

Acne vulgaris is a dermatosis which is most common during adolescence, when external appearance is very important, so that it can cause serious mental difficulties in young people (1). According to the clinical stage, acne vulgaris are classified into three clinical forms: mild, moderate and severe forms of acne (2).

The very beginning of adolescence is usually characterized by an increased interest in sexuality associated with ideas about how to adapt to new needs and new physical appearance. This is why young people are very sensitive in the beginning of the adolescent period to any changes that occur in the physical sphere. Changes that occur on the physical and physiological level during this period are extremely important for the further development of the person, they affect their emotional state, the formation of identity and achievement of social contacts (3). If the emotional atmosphere is not satisfactory, it creates an unstable or fragmented self-image. As a consequence an obsessive quality concern about skin integrity may arise, or impairment of the somatic perception, leading even to delusions (4). Their skin condition, in which the changes occur predominantly on the face, can have a significant impact on the quality of life (5), and the problems that often occur as a result of these changes are low self-esteem, anger, anxiety and depression.

A study involving 480 patients with various dermatoses found the highest incidence of depression and suicidal ideas in patients with severe forms of acne and severe psoriasis, while patients with mild and moderate forms of acne had the same level of depression and suicidal ideas as those suffering from atopic dermatitis, moderately severe forms of psoriasis and alopecia areata (6). Barankin and DeKoven (7) found that patients with acne have the same level of depression and anxiety as patients suffering from arthritis.

Unlike most other dermatologic diseases that may be limited to areas covered by clothing, acne vulgaris is often visible on the face. Individual and social perception in adolescents will lead to a sense of being tagged, which further may be followed with anxiety, fear and guilt. As direct consequences in young people depression and anxiety may be developed.

The aim of this study was to determine levels of anxiety and depression for adolescents with acne vulgaris in relation to the severity of the clinical features and their gender.

Patients and methods

The study was conducted at the Dermatovenerology Department of the "Dr. Irfan Ljubijankić" Cantonal Hospital, Bihac. Using a sectional study, the anxiety and depression level was analyzed of 90 adolescents of both sexes, aged 16 to 21 years, who visited the dermatological dispensary in the period from February 1st 2008 to January 31st 2009. Respondents used local therapy for acne. Adolescents were excluded from the study population who in addition to acne vulgaris had other chronic dermatoses and other diseases of organic systems that can lead to symptoms of depression and anxiety in patients. Dermatologic examination based on the clinical characteristics of the disease produced a diagnosis of acne vulgaris, while the patients were divided into three groups according to the type of efflorescence, their location and severity of the clinical features. The first group consisted of adolescents with a mild form of acne, the second group had a moderate form of acne and the third had the severe form of acne (Table 1).

After dermatological examination was completed, all respondents voluntarily completed the questionnaire "Spielberger's state

Table 1 Acne vulgaris clinical features in relation with analyzed groups

Group	Severity of clinical features, type of efflorescence, localization
First (n = 9)	Mild form of acne. The mildest form of the disease. Comedos prevail, infrequent small papulae and papulopustulae. Facially localized alterations.
Second (n = 72)	Moderate form of acne. Moderately severe form of disease. Comedos and red painful papulae prevail. As well as on the face, alterations can be observed on the back and sternal region also.
Third (n = 9)	Severe form of acne. The most severe form of disease. Prevalence of comedos, papulae, pustulae, crusts, stiffed and painful nodes of furunculous appearance, atrophic and hypertrophic scars. As well as on the face, also frequently affected are regions of torso and upper back, neck, upper arm and gluteus regions.

and trait Anxiety Inventory" (STAI) (8) to assess levels of anxiety, and to assess levels of depression using the Birleson scale of depression BDS-scale (Back Depression Inventory) (9).

The STAI-questionnaire for measuring anxiety as a state was composed of questions about the essential characteristics of anxiety, such as fidgeting, tension, nervousness and worrying. This questionnaire is used to evaluate how somebody feels at the moment of filling in the questionnaire. It consists of 20 questions which patients respond to by circling one of the answers on a four-level scale: not at all, slightly, moderately, and always so. Responses to each statement were scored from 1 to 4 points. The response that stated "not at all" was scored by 1 point, "slightly" by 2 points, "moderately" by 3 points, and "always so" by 4 points. All responses were not scored in the same way, so that in a certain number of responses a value of 4 points indicates the presence of high levels of anxiety, while in some other statements it points to the absence of anxiety. The total score is obtained by adding the score points for all 20 answers. The minimum score is 20, and the maximum is 80. After that, according to the appropriate tables taking age into account, we transformed the resulting score into standardized T values, and then we classified each patient into one of the following 6 related groups, based on the same tables and obtained results: 1st group (20-30 points) - extremely low level of anxiety, 2nd group - low level of anxiety (31-40 points), 3rd and 4th group – moderate level of anxiety (3rd group 41-50 points, and 4th group 51-60 points), 5th group – high level of anxiety (61-70 points), 6th group – extremely high level of anxiety (71-80 points) (8).

The BDS-Birleson Depression Scale was used to evaluate depressive symptoms in adolescents. It consists of 18 items that were used by the participants to perform self-evaluation of their depression level. The items consist of sentences that describe how the person felt during the last 7 days. Subjects self-evaluated their depression level by circling one of three options offered for each item: never, sometimes or often. Responses were scored by 0, 1 or 2 points. We obtained the total score by adding together the points for all 18 items. The minimum score is 0 and maximum is 36. Based on the total points obtained, the respondents were divided into three groups, with regard to the expression of depressive symptoms (the degree of depression). The first group comprises those with 0-10 points which is considered as the normal value, the second group are those with 11-16 points as a risk group, and the third group consists of respondents with 17 or more points who expressed signs of depression (9). The age of the subjects was calculated using a decimal calendar based on

the date of birth and date of examination, and it is expressed by decimal numbers (10).

Statistical analysis

The results are presented as median and range. To test the differences between the variables the Kruskal-Wallis test was used. The difference was considered significant if $p < 0.05$. For data processing we used the statistical program MedCalc software (version 8.1.0.0 for Windows, MedCalc).

Results

In the study group there were 54 (60%) female respondents and 36 (40%) male, mean age 17.5 years (range 15.6-20.6). Of the total number of patients with acne vulgaris, nine of them or 10% (4 female and 5 male) had a mild form of acne (first group), 72 of them or 80% (46 female and 26 male) had a moderate form of acne (second group) and 9 of them or 10 % (4 female and 5 male) had a severe form of acne (third group) ($p > 0.95$).

The mean age of subjects was expressed as median and range. In the tested sample it was 17.5 years (15.6 to 20.6 years), in the first group it was 19.3 years (15.7 to 20.3), in the second it was 17.5 years (from 15.6 to 20.6 years) in the third group it was 17.1 years (15.8 to 20.4 years). There was no statistically significant difference in age between the groups that were tested ($p = 0.717$).

Results obtained using the STAI-questionnaire showed that of the sample 1.1% of respondents (1 female and 0 male) had an extremely low level of anxiety, 13.3% (7 females and 5 males) a low level of anxiety, 76.8 % (39 female and 30 male) a moderate level of anxiety and 8.8% (7 females and 1 male) a high level of anxiety.

With analysis of levels of depression, the results obtained using the BSD-scale showed that 16 (17.7%) respondents (8 females and 8 males) had normal values, 40 (44.4%) respondents (26 female and 16 male) formed the risk group and 34 (37.8%) respondents (20 female and 14 male) had significantly expressed signs of depression.

Table 2 presents the values of the levels of anxiety measured by the STAI-questionnaire in relation to the analyzed group, and Table 3 shows the values of the level of depression measured by the BSD-scale in relation to the analyzed group.

Table 2 The values of anxiety levels measured by the STAI questionnaire in relation to the analyzed groups.

Grupa	Level of anxiety	
	Median	Range
First (n=9)	58.7	39-70
Second (n=72)	57.7	25-70
Third (n=9)	60.2	40-70

P= 0.36

Table 3 The values of depression levels measured by BDS-scale in relation to the analyzed groups.

BDS	Group			p
	First (n=9)	Second (n=72)	Third (n=9)	
Level of depression (Median, Range)	13.4 (8-16)	15.1 (3-29)	14.4 (7-24)	0.367

Discussion

What our study showed was that most patients had a moderate level of anxiety. The results show that there is approximately the same percentage of male and female respondents in the group with moderate and high levels of anxiety, i.e. there is no gender differences. According to Tadinac and associates (11) in patients with acne vulgaris there was no difference in the level of anxiety and depression by gender. Anxiety levels were higher in severe clinical forms of acne vul-

garis, but the difference was not statistically significant. This means that the severity does not play a significant role when it comes to predicting the level of anxiety in patients with acne vulgaris. Thus, adolescents with a mild clinical form of acne can be just as anxious as those with the severe form of acne vulgaris, that is to say we cannot use the clinical features as a basis to evaluate the psychological status of patients when it comes to anxiety. According to the literature, acne vulgaris is much more involved in psychiatric and psychological processes compared to other dermatological diseases. Around 80% of adolescents with acne vulgaris develop anxiety (12). According to Kellett and Gawkrodger (13) patients with acne showed a higher degree of anxiety and depression compared to other dermatological diseases, even in comparison to patients with cancer.

The results of our study showed that the largest number of respondents, when it comes to the level of depression, belonged to the risk group, and in patients who were in the risk group and the group with signs of depression, there was no gender difference. The level of depression was somewhat higher in the second group of respondents, compared to the first and third, but the difference was not statistically significant. According to studies, depression that occurs with acne is always an important indicator of disease. The general living and working capacity of these patients is reduced, similar to rheumatic, asthma and diabetes patients, and are often the most important problem in the treatment of acne, especially in mild to moderate forms of acne (11). Attitudes found in the literature differ. Buljan and Šitum (14) suggest that, in contrast to psoriasis, the severity of the clinical features of acne does not necessarily correlate with the severity of depression, as acne, regardless of its severity, causes depression, suicidal thoughts and suicide in 5.6% of patients. Suicide attempts are the central problem of depression. In fact, 50% of people who try to commit suicide are depressed, and 15% of depressed people do commit suicide. However, a study that included patients with various dermatoses showed the highest incidence of depression and suicidal ideas in patients with severe forms of acne and psoriasis, while patients with mild and moderate forms of acne had the same levels of depression and suicidal ideas as those suffering from atopic dermatitis, moderately severe forms of psoriasis and alopecia areata (6).

Conclusion

The level of depression and anxiety in patients with acne vulgaris is not related to severity and patient sex. When evaluation of the clinical status is performed and while the entire treatment is being planned, the patient's self-assessment of their depression and anxiety levels should be also considered in addition to the physician's evaluation, because for certain patients some relatively mild forms of skin disease can cause a violent emotional reaction, while others can face more serious illness with much greater ease.

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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Review article

The importance of a forensics investigation of sudden infant death syndrome: recommendations for developing, low and middle income countries

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Sudden infant deaths syndrome (SIDS), the sudden and unexpected death of a normal and healthy infant, has remained a medical and forensic mystery. Despite years of research all attempts to ascertain the exact cause and manner of death have failed. The information collected during the course of the comprehensive investigation by the various investigation agencies and analysis of the data has not been in vain. The epidemiological, demographic, and pathological data have identified distinctive features and risk factors associated with infants that died from SIDS. Epidemiological data has provided the unique characteristics of infants that died of SIDS that differentiates them from non-SIDS infants. Analysis of information from the death scene investigation has identified key risk factor behaviour associated with SIDS, namely the prone sleeping position. Pathological examination of the internal organs, specifically the brain, has shown some differences between SIDS and non-SIDS infants. However, to gain a complete picture of SIDS data, all countries around the world must provide information, even basic information, to understand this syndrome better. Developing countries must understand their role and importance in developing plans to investigate, collect, and disseminate SIDS data to the rest of the world. This paper provides general guidelines for the investigation of SIDS in developing countries.

Key words: Sudden infant deaths syndrome, SIDS, Death investigation, Forensics, Developing countries.

Introduction

One of the most challenging types of death investigation conducted by the Medical Examiners/Coroners (ME/C) Office and law enforcement agency involves the sudden and unexpected death of a previously normal and healthy infant. These deaths are generally classified as Sudden infant death syndrome (SIDS). Typically, a previously healthy infant is discovered dead by the parents during the morning hours. These cases are challenging, not only because of the high emotional cost to the family but also

the intense level of investigation required by both the forensic and the law enforcement agencies. The most distressing part of the investigations is the end-result, because it fails to provide to all parties involved a clear and identifiable cause and manner of death. The thoroughness of the death investigation and the accuracy of the information obtained are critical, not only for the local authorities conducting the investigation, but also for the medical, forensic, legal, and public health community at large. The information gathered from the various phases of the investigation includes anatomical data, past medical history (PMH) of the infant and mother, and the circumstances surrounding the death, which must be collected, analyzed and disseminated. This data must become available to the forensic, medical, and public health communities, as well as the general population. Information is critical to furthering our understanding of SIDS and possibly provide an explanation for these deaths. Our understanding of the SIDS phenomenon is directly related to the data collected during the death investigation conducted by the ME/C office and supplemented by reports by the medical response teams, hospital records and the police investigation. SIDS occurs in all countries around the world. Countries such as the U.S. have sophisticated forensic death investigation protocols, standardized data collection forms (1) and highly trained forensic epidemiologists that collect, analysis, summarise, and disseminate SIDS information. In developing countries, such as Bosnia and Herzegovina, issues relating to the economy, internal conflicts, manpower issues, and the availability of specially trained personnel and equipment can greatly impact the level of investigation and the type of information gathering relating to SIDS deaths. An examination of international data on SIDS has revealed that Bosnia and Herzegovina did not provide even basic data regarding SIDS. In Bosnia and Herzegovina the infant mortality rate is estimated at 21.05 deaths/1,000 live births (2) and according to the United Nations World Population Prospects report, for 2005-2010 (3), and the CIA World Fact book (4) this rate is similar to that of Libya (21.05/1000).

Developing countries should attempt to provide at least minimum, basic epidemiological information such as the total number of SIDS deaths and a breakdown by age, sex, and race. The estimated population of Bosnia and Herzegovina of 3.8 million is unique in one respect that its population is almost entirely white. This is important because it eliminates one of the major known risk factors for SIDS, which is being black (5).

The purpose of this paper is to underscore the importance of each aspect of the infant death investigation and provide general guidelines for developing countries in the investigation of SIDS deaths. This paper will also provide guidelines on the basic types of information that must be collected during the various phases of the death scene investigation, the requirements and importance of a complete post-mortem examination, how forensic epidemiologists have used the collected data to identify risk factors for SIDS, and some possible causes of the syndrome.

Definition

It is important to start with a clear definition of what constitutes an infant that died of SIDS. The initial definition of SIDS has gone thorough a great deal of fine-tuning since it was first used in 1969 (6). In 1989, the National Institute of Child Health and Human Development defined SIDS as: (1) the sudden and unexpected death of an infant under one year of age who was relatively healthy prior to death; (2) whose death remains unexplained even after the performance of a complete post mortem exami-

nation, including toxicological and genetic testing; (3) after the conduct of a thorough death scene investigation; and (4) a review of the infant's and mother medical history (7).

Listing the diagnosis of SIDS as the immediate cause of death on the death certificate (DC) can only be used if *all* the above conditions have been met. A diagnosis of SIDS is one of exclusion; therefore, only after all other possible causes have been investigated and eliminated should the medical examiner or coroner office conducting the investigation issue the cause of death as SIDS. There is some doubt regarding the appropriateness of using SIDS as the cause of death. The vast majority of SIDS deaths are classified with the immediate cause of death as *SIDS* and the Manner as *Natural*. However, others make an agreement for listing the cause of death as *Undetermined* and the manner of death as *Could Not be Determined* or *Undetermined* (8). Developing countries should create a national protocol detailing how all forensic pathologists should complete the DC of infants that died of SIDS. This will ensure consistently and provide a clear method to ascertain an accurate profile of the nation's SIDS mortality rates. SIDS deaths in older literature were referred to as "Crib Deaths" or "Cot Death" because the deaths typically occurred in the crib or cot (9).

Recently, the term Sudden Unexpected Death in Infants (SUDI) has emerged. It is a broad definition, which initially encompasses all infants that died suddenly and unexpectedly. Early in the investigation, the results of histology, microbiology, toxicology and the multi-professional reviews are incomplete, therefore the use of SUDI on the DC is appropriate. After all the various investigations are complete, the immediate cause of death should be changed to either the identified cause of death or, if all the findings are negative, the DC should be changed to SIDS (10).

First responders

The majority of SIDS infants are discovered either unresponsive or dead within the residence, resulting in a call for medical assistance. The first non-family individuals to interact with the infants are typically "first responders". Depending on the location, first responders consist of either Emergency Medical Technicians (EMT), paramedics, firefighters, or the police. The information they collect, practically their assessment of the infant's presentation and environment, can be critically important to the ensuing investigation.

In the case of an infant, the information provided by the parents or caregivers (babysitter) to the first responder, especially a detailed description of what position the infant was placed to sleep, the position and appearance upon discovery and the general sleeping environment, is important to the investigation. Statements given to the first responders are typically more accurate then those provided later to the police and to the death investigators from the ME/C office. For instance, a mother may have placed the infant on its stomach to sleep but knows that it is safer to place the infant on its back. Therefore, later when the parents are questioned by death investigators from the ME/C office they may provide statements that are inaccurate regarding the circumstances surrounding the events leading to the death. First responders must also note the general condition of the residence, with special attention given to where the infant was discovered and the position it was said to have been discovered in. Often the death scene has been altered by the time death investigators arrive at the residence, which can be several hours or even days after the death occurred.

The question, "What should EMTs or paramedics do if they encounter a clearly dead infant at a residence?" In the old days, EMT and paramedics were instructed to conduct a 'scoop and run' operation especially if it involved an unresponsive infant.

Today, with advances in training and equipment, more time is spent to assess, treat, and stabilize the patient at the scene prior to transport to the hospital (11). While each first responder station has its own individual protocol if the infant is clearly beyond medical intervention, all attempts should be made to place the infant back in the original position as discovered and prevent the parents from changing or altering the death scene. First responders must then immediately call the ME/C office and police to the scene. Once the police arrive, the security of the scene is transferred from the first responders to the police until investigators from the ME/C arrive and take jurisdiction of the scene and the investigation (11).

Developing countries must establish clear national guidelines when first responders encounter a deceased infant. As part of their training, they should be instructed on securing a death scene and informing the parents about the role and activities that will be conducted by the ME/C and police. In addition, standardized report forms for first responders should be developed to ensure that all relevant information is collected in a uniform manner which would result in easier analyses. The report should include the following information: the time the call for emergency medical assistance was made, the time of arrival at the scene, assessment of the infant, action taken by medical responders, and statements made as to the events leading to the emergency call.

The scene investigation

When an infant is declared dead, either by the EMT/paramedics at the residence or by a physician at the hospital, the death investigation begins. Two investigations are conducted simultaneously: one by the police and one by the medical examiners/coroners office. When a death occurs at a residence, the emergency call for medical assistance also results in a response by police. In the U.S. the ME/C office has jurisdiction of the death scene, however, this authority may vary country by country (11). A clear chain of command must be firmly established between law enforcement and the ME/C office as early as possible. At this phase of the investigation, the two investigation teams (law enforcement and ME/C) are both attempting to ascertain a history of the circumstances that occurred prior to the discovery of the deceased infant.

Police investigation

The police investigation involves questioning those that interacted with the infant prior to the incident, taking statements on the role and actions taken by each individual, and obtaining a detailed narrative of the circumstances immediately surrounding the discovery of the infant. The involvement of the police, especially at the beginning of the investigation, may alarm parents who have just suffered a loss (10). The police must make it clear, and with sensitivity, that they must conduct an investigation when the death is sudden and unexpected, and it is simply part of their overall duties and part of the standard protocol. While the death of an infant is initially suspicious the police should refrain from surrounding the home with police tape and handcuffing the parents. The axiom that it is a crime scene until proven otherwise should be adhered to in most cases, but some adjustment should be considered in the case involving the death of an infant. It is a death scene, therefore, the infant's room and the location where the infant was discovered should be secured until the forensic investigators arrive to take photographs and collect forensic evidence. Current police training in criminal investigations emphasizes the importance of 'the golden hour' – the first hour of evidence gathering that produces crucial evidence before it can be lost or contaminated. During this phase, the parents should be taken to

another part of the house and questioned by the police. If evidence such as the bedding (crib), mattresses, or sheets have to be removed the parents should be informed that they will be returned after the investigation. Police officers investigating these types of cases should be educated in the fact that the vast majority of babies' deaths are from natural causes. However, suspicion should arise only if there is material evidence of something irregular, such as medical evidence of injury or records of previous interaction with social services, Child protection units, or the police. The police will also question relatives and neighbours regarding the behaviour and activities of the family members (10).

In a death involving an infant, the police may be hesitant to conduct intensive questioning of the parents, as they would do if the victim were a middle aged male. The detectives may find it hard to accept that parents are capable of murdering or even harming their own children. In addition, they do not wish to conduct an aggressively interrogation of the parents as if they were murderers without any forensic evidence or visible trauma to the infant to warrant such an integration. Law enforcement offices conducting this type of investigation must however be aware that mothers are capable of harming their infants due to a condition called Munchausen's by Proxy and these infants deaths have been incorrectly classified as SIDS (12).

In developing countries, it may not be practical to educate and train all the detectives of the nation's police force in the proper methods to investigation a SIDS or possible SIDS deaths. Therefore, a small number of detectives in each region should receive special training on how to handle such cases and be assigned to these types of deaths when they occur.

Forensic investigation

The forensic medico-legal investigation is conducted by the ME/C office and is composed of three main sections: (1) Death Scene Investigation, (2) Autopsy and Toxicology, and (3) Review of the Past Medical History of the Infant and Mother. The ME/C investigation begins at the death scene by taking photographs of the overall residence then numerous images of the location and bedding where the infant was discovered. The investigators attempt to obtain an accurate representation of the events immediately prior to the death. Their examination focuses on three key factors: (1) the infant, (2) the sleeping location, and (3) the infant's environment. Critical information to be collected regarding the infant includes the time and method of the last feeding (breast or bottle), the time and position the infant was put down to sleep, and the time and position it was discovered. Anatomically correct dolls can be used to recreate the events leading up to the discovery (13). Next, the infant's sleeping location is examination in great detail. The crib is examined for general condition, the size and firmness of the mattress, and the number of covers and items within the crib are also noted. If the infant was sleeping in something other than a crib, that sleeping surface is photographed and described in detail. Information collected about the infant's environment should include the temperature of the infant's room and a search for possible sources of gas, carbon monoxide, carbon dioxide or other toxic gases. Air samples should be taken for future analysis. The parents' smoking habits, alcohol and drug use should be noted.

The level of training, experience, and equipment possessed by the ME/C death investigators affects the depth and level of detail of the information collected at the death scene. Death investigators in developing countries can gain additional training by enrolling in online classes offered by the FBI and CDC. Hands-on experience can be obtained by participating in exchange programs with countries like the U.S., Great

Britain, or Australia. In these programs, the foreign death investigators are exposed to the procedures and techniques used to investigate a SIDS death in the U.S. or another country. One of the most basic and important types of equipment that all death investigators should obtain is a digital camera. They have become extremely easy to operate, are inexpensive, and critically important if the case goes to trial.

Examination of the infant

After pronouncement of the death of the infant, it is then transported from the residence or hospital to the morgue. The infant will first be photographed as it arrived from the scene, then the clothing is removed and examined for trace evidence. The infant will then first undergo an external examination. This part of the examination looks for any signs of recent and remote injuries and/or trauma. The external examination may provide forensic evidence that will either support or call into question statements made by the parents. Changes after death such as rigor mortis and livor mortis may indicate clues to the infants' final position after death. This information should be carefully compared to the information provided by the parents. In addition, if evidence of recent or remote blunt forced trauma is identified, the parents must be questioned by the police and ME/C office about the possible etiology of these injuries. Complete radiographic images of the infant should also be taken and evaluated by a trained paediatric radiologist.

Next, the internal organs are examined using the standard "Y" shaped evisceration methodology followed by removal of the brain. The organs are examined in-situ first then removed, weighed and examined grossly and microscopically. A forensic pathologist should conduct the examination and the police officer or detective handling the investigation should be present during the forensic autopsy. Samples of each internal organ should be sent to the histology department, where microscopic slides are prepared. The ME/C office should save small samples of all the internal organs in formaldehyde for at least 5 years and the histological slides indefinitely. Where possible, these materials should be made available to research centres for study.

A complete forensic autopsy is critical because the diagnosis of SIDS requires that the post-mortem examination has been conducted and the results have failed to demonstrate a clear cause of death (8). The forensic examination typically does find some non-specific findings, such as upper respiratory infection and petechiae hemorrhage (14). Because the results of the SIDS autopsy are negative, a number of ME/C offices may choose not to perform one. Forensic pathologists that refuse to conduct an internal examination often offer the justification that they do not want to put the family through such an examination. This action in fact constitutes a disservice to the family, the medical community, and to those studying the syndrome. By refusing to perform a complete examination, diseases or conditions may be missed that can affect the mother's future children. Technically an infant that did not undergo an internal examination cannot be signed out on the DC as SIDS. The cause of death and manner of death must both be listed as *Undetermined*. This type of DC would cause additional stress and confusion for the family.

Toxicology/Genetic screening

A part of the complete internal examination includes the collection of various body fluids (blood, bile, urine, eye fluid, and CSF). Blood samples should be taken from a venous or arterial site (e.g. femoral vein). Cardiac puncture should be avoided as this may cause damage to intrathoracic structures

and make post-mortem findings difficult to interpret. Blood serum and urine should be sent to a forensic toxicologist for toxicology analysis for the presence of poisons and drugs. Blood cultures should be tested for microbiology cultures (aerobic and anaerobic). Cerebrospinal fluid (CSF) should undergo culture screening. Blood should be sent, ideally on a Guthrie card, for genetic screening (10). This screening should included markers for underlying metabolic diseases such as Medium chain acyl CoA dehydrogenase deficiency, or MCAD. MCAD is the most frequently metabolic disorder associated with SIDS. This screening is also important for the surviving siblings of SIDS, in order to prevent the tragedy of recurrent SIDS in some families (15).

A medico-legal system must be established in developing countries, either as a medical examiner or coroner system. In smaller countries, such as Bosnia and Herzegovina, a centralized system with one main ME/C office and a number of smaller satellite offices maybe the most economical. In order to gain experience and consistency, one or a small group of forensic pathologists should examine all sudden and unexpected infant deaths in the country. They should establish standard protocols so that all infants that die suddenly and unexpectedly undergo a through post-mortem examination. In developing countries, nurses and physicians should receive training regarding SIDS, and the importance and the need for a forensic investigation and autopsy. The establishment of a Child death review team, discussed later, will ensure that all infants receive a proper and correct investigation.

Past medical history of infant and mother

One of the functions of the ME/C office is to ascertain and review the past medical history of the mother and infant. Regarding the mother the following information should be abstracted from the medical records: age of each pregnancy, the interval between each pregnancy, number of pre-natal visits, and the use of drugs and tobacco during the pregnancy. Information collected about the delivery should include length of gestation, type of delivery, complications associated with the delivery, APGAR scores, and the birth weight and height of the infant. The medical history collected on the infant should also include growth history, number of post-natal visits, vaccination history, medications, and health immediately prior to death. The ME/C office has the authority to subpoena the mother's and infant's medical history from the birth hospital for review. The ME/C office should establish a good working relationship with the area hospitals to ensure that all relevant medical records are turned over to the ME/C office.

Child death review

Child death review teams (CDRT) can be organized at local, regional, or state level. They are designed to conduct retrospective reviews of all deaths under the age of 18 years that occur within that team's jurisdiction. The role of CDRT is to ensure that a thorough and complete investigation is conducted. The CDRT is a multidisciplinary team composed of the following individuals: first responders (ambulance and fire personnel), law enforcement officers, medical examiners/coroners officers, forensic epidemiologist, prosecutors, child protective service, paediatricians, emergency room personnel, and representatives from the school district. The objective of a CDRT is to ensure that all the available information from the different disciplines is presented and reviewed. In addition, it assists in creating an accurate portrait of the history of the child's death and ensures a consensus regarding the listed cause and manner of death. The end-result

of the CDRT is four-fold: first, the collection of epidemiological, demographic, and statistical data; second, the publication of monthly and yearly mortality reports; third, the suggestion of ways to improve the methodological investigation of infant/child deaths; and finally, to make recommendations to prevent future infant/child deaths (16).

CDRT have been operating in a number of states within the U.S. and a few developed countries. Developing countries should implement the creation of CDRT to provide a review of the thoroughness and completeness of these investigations. During the early phases of the development of a CDRT, there should be monthly meetings between the ME/C and law enforcement agencies to review methods and discuss ways to improve their joint investigation. In the early stages of the CDRT, they should initially concentrate on compiling basic epidemiological, statistical, and circumstantial data relating to SIDS deaths. Over time, other members should be added to the team.

End-results of SIDS investigations

All phases of the death investigation are important and provide, and will continue to provide, valuable information for our understanding of SIDS. The end result of years of collecting and examining large amounts of detailed epidemiological, anatomical data, and the features of the circumstances surrounding the deaths from around the world has resulted in a number of discoveries.

First, it clearly illustrated the unique epidemiological and demographic features of SIDS that differentiates it from other deaths during the post and perinatal period. Second, it identified risk factors associated with SIDS. Specific risks associated with SIDS include: being male, black, the prone sleeping position, excessive layers of clothing, and exposure to second hand smoke (11).

Based on the forensic epidemiological investigation of the data, the following features have been reported: (1) a unique death distribution with the majority of deaths occurring between 2-5 months of age, (2) a greater number of deaths during the winter months, (3) a higher death rate among black and male infants, (4) mothers are typically young, unmarried, of limited education, frequently use drugs and smoke, and of lower socioeconomic status (5). Environmental risk factors shown to be associated with SIDS include soft bedding, co-sleeping (bed sharing), and over-wrapping (17).

One of the most important discoveries was the risk associated with the prone sleeping position and SIDS. Based on epidemiological data analysis of the information from the death scene investigation, the majority of infants that died of SIDS where discovered in the prone sleeping position which led the National Institute of Child Health and Human Development in 1994 to initiate the "Back to Sleep" campaign in the United States. The campaign resulted in a dramatic decline in the incidence of SIDS in the U.S. (18) and other countries where the program was implemented.

Whereas the main function of the forensic autopsy is foremost the exclusion of identifiable diseases, identification of congenital conditions, and the discovery of trauma it has a second purpose for research. The examination of saved tissue samples and blood recovered from these infants will allow for detailed examination by researchers attempting to discover the possible cause of SIDS. The examination of brain tissue has shown some anatomical differences between SIDS and non-SIDS infants. Recent studies have shown that SIDS brains have fewer CO receptors (17). DNA samples can be used to search for genetic makers for SIDS (17). To date however, genetic analysis has not located any specific genotype differences that differentiate infants who die of SIDS that can be linked to specific clinically defined phenotypes (19). However, several differences

Table 1 Standard Protocol for Sudden infant death syndrome Investigation

Standard Protocol for Sudden Infant Death Syndrome Investigation	
Death Scene Investigation	Secure Death Scene Location Separate Infant and Parents Photo-document the Scene and Infant Conduct Witness Interviews (Parents/Care Givers) Re-enactment of the discovery of the infant (use dolls) Collect Atmospheric Air Samples Remove infant and other critical evidence Prepare Death Investigation Reports
Pre-Autopsy Review	Obtain and Review Infant's Past Medical Records Obtain and Review Mother's Past Medical Records Review Death Investigation Case Information Review First Responder Reports Review Police Reports
External Examination	1. Photograph Infant 2. Establish Infant Growth and Development Parameters 3. Document all signs of recent and remote trauma
Internal Examination	Gross Examination of Internal Organs Remove/Weight Internal Organs including the brain Microscopic Examination of Organs Collect Body Fluids: Blood, Bile, Urine, Eye Fluids Conduct Toxicological Analysis Conduct Microbiology/Genetic Screening Save representative samples of tissues
Post Examination Actives	Collection of Epidemiological Data Collection of Anatomical Data Collection of Pathological Data Dissemination of SIDS Information Publication of Case Reports Conduct Retrospective Studies

have been noted in gene polymorphisms among SIDS infants involving the sodium channel (SCN5A), potassium channel, and serotonin transport (5-HTT) genes (20, 21). Other polymorphisms have also identified genes that affect the autonomic nervous system development (PHOX2a, RET, ECE1, TLX3, EN1) (22) and the anti-inflammatory cytokines interleukin (IL-10) (23). The ME/C offices should freeze a small sample of blood from SIDS and non-SIDS cases for future comparison and research. This blood should be stored in a -70 freezer. Table 1 illustrates the standard protocol for the investigation of Sudden infant death syndrome. This table should be reproduced and provided to forensic death investigators and forensic pathologists and laminated copies should be posted in mortuaries around the county as an operating guide to the steps involved in a SIDS investigation.

Conclusions

The analysis of the information collected from various types of investigation of SIDS infants has resulted in the identification of a number of risk factors that have been associated with the phenomenon. The association of the sleeping position coupled with education campaigns has resulted in an overall decrease in the total number of SIDS deaths. However, the aetiology of the cause of these deaths has still not been ascertained. Those investigating these types of death, typically the ME/C, must go beyond their primary

function of determining the cause and manner of death and play a more active role in the collection of epidemiological, demographical, anatomical, and physiological information must be meticulously collected. In addition, biological specimens should be available for researcher centres. All countries, even developing ones must play an active role in the investigation of SIDS deaths. They must begin with a through investigation of the death scene and conduct a complete autopsy of the body. In addition, they must provide the results of their findings to the world's researchers. The ME/C office has a responsibility, not only to the infants' families to conduct this type of investigation, but also to future generations of infants. Developing countries have the advantage of starting with the established protocol, standards, and techniques developed by developed countries as their starting point.

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Review article

Microphthalmia-associated transcription factor (MITF) – from Waardenburg syndrome genetics to melanoma therapy

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Microphthalmia-associated transcription factor (MITF) was first discovered as protein coded by gene whose mutations are associated with Waardenburg syndrome. Later, MITF was shown to be key transcription factor regulating melanogenesis. Further studies have shown that in addition to regulating melanogenesis MITF also plays central role in regulation of melanocyte development and survival. MITF gene is amplified in a proportion of melanomas and ectopic MITF expression can transform melanocytes so MITF can function as melanoma "lineage survival" oncogene. Different studies have further revealed MITF's important but complex role in tumorigenesis and progression of melanoma. As expected from its important role in melanocytes and melanoma MITF is intricately regulated on all the levels from transcription to post-translational modifications. Although complex mechanisms of MITF functioning are still being revealed, MITF already has a valuable role in managing melanoma patients. Immunohistochemical analysis of MITF has shown both diagnostic and prognostic value in patients with melanoma. MITF is also a valuable specific marker for detection of circulating melanoma cells by reverse-transcription – polymerase chain reaction. MITF has recently been investigated as a potential target for melanoma therapy.

Key words: Microphthalmia-associated transcription factor, Melanoma, Melanocytes, Biological tumor markers, Waardenburg's syndrome.

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Introduction

Microphthalmia-associated transcription factor (MITF) was first discovered as a protein associated with Waardenburg syndrome. Further studies have revealed MITF as a master regulator of melanocyte function, development and survival. As expected from its role in normal melanocytes, MITF has also an important role in melanoma tumorigenesis and progression. The role of MITF in both normal melanocytes and melanoma is complex and depends on intricate system of regulation on different levels, from MITF transcription to post-translational modifications. That system, all components of which are still being discovered, enables MITF to adjust behaviour of melanocytes

and melanoma cells to various signals coming from within the cell and from the environment of the cell. Although all the components of MITF's functioning are still unknown, MITF already has a valuable role in managing patients with melanoma as diagnostic and prognostic marker. Also, several therapeutic approaches to melanoma targeting MITF are being explored. In addition to its role in melanocytes and melanoma, MITF also plays important role in several other cell types, like osteoclasts and mast cells, but the focus of this review will be on a complex role of MITF in melanocytes and melanoma, and on application of MITF in managing melanoma patients.

MITF and Waardenburg syndrome

MITF was discovered as a protein coded on a gene locus associated with Waardenburg syndrome in humans (1, 2). Waardenburg syndrome is a hereditary autosomal dominant disorder characterized by *heterochromia iridis*, patchy abnormal pigmentation of the hair and skin and sensorineural deafness (3). Clinically Waardenburg syndrome is classified in four types. Waardenburg syndrome type 1 and Waardenburg syndrome type 3 have been associated with mutations in *PAX3* gene (4). Waardenburg syndrome type 4 also known as Waardenburg-Shah syndrome has been associated with mutations in three genes, *SOX10*, gene for endothelin 3, and gene for endothelin receptor B (EDNRB) (5).

Waardenburg syndrome type 2 has been associated with mutations in one allele of MITF gene (6, 7). It has been shown that mutations in one allele of MITF gene do not influence the activity of protein coded on the other (non-mutated) copy of MITF gene (7). Therefore the dominant inheritance of Waardenburd syndrome type 2 has been explained as a result of haploinsufficiency, mechanism by which a MITF protein coded on a non-mutated allele of MITF gene can't reach the intracellular concentration necessary for its normal function (7). The symptoms of Waardenburg syndrome type 2 (white patches of the skin, altered iris pigmentation and loss of hearing associated with absence of melanocytes in *stria vascularis* of the cochlea) can be explained as a consequence of the melanocyte depletion (3). Mutations in MITF gene have also been associated with Tietz syndrome, which is a rare hereditary auditory-pigmentary disorder (8). The symptoms characteristic for Tietz syndrome are similar to the ones associated with Waardenburg syndrome type 2 but are present in a more severe form (8).

Mice with mutations in *microphthalmia* (*mi*) locus which is responsible for the synthesis of Mitf, a mouse homologue of MITF, have following disorders: loss of pigmentation, reduced eye size (microphthalmia), reduced number of mast cells, osteoporosis as a consequence of disturbed osteoclast function, and hearing impairment (9). These disorders implicate a role of Mitf in the development and function of melanocytes, mast cells, retinal pigment epithelial cells and osteoclasts.

MITF isoforms

Gene MITF is located on a third human chromosome in a region 3p12.3-3p14.1 and is responsible for the synthesis of nine so far described isoforms: MITF-M, MITF-A, MITF-H, MITF-B, MITF-C, MITF-D, MITF-Mc, MITF-E and MITF-J (2, 10-16) (Figure 1.). Protein MITF contains a basic domain required for DNA binding and helix-loop-helix and leucine zipper domains required for dimer formation (bHLH-LZ structure) (1-2). MITF is similar in amino acid sequence to other transcription factors that share the same bHLH-LZ structure: TFE3, TFEB and TFEC (17-19). MITF can bind DNA as a homodimer or as a heterodimer with transcription factors TFE3, TFEB and TFEC (17).

All the isoforms of MITF protein have a common C-terminal region containing a domain required for transcription activation and bHLH-LZ structure but each isoform has a distinct N-terminal region (20, 21) (Figure 1). The shortest isoform MITF-M consists of 419 amino acids and contains a unique N-terminal domain M (amino acid sequence: MLEMLEYNHY) and a six amino acids insert (ACIFPT) close to the basic region of the protein (18). It has been shown that also the MITF-M protein without this six amino acids insert is synthesized (18). Bismuth et al have shown that MITF-M isoform containing six amino acids insert can inhibit cell proliferation unlike the isoform without six amino acids insert (22). In accordance to this finding substantially increased proportion of isoform without six amino acids insert was found in metastatic melanoma in comparison to normal melanocytes (23). MITF-M is specifically expressed in melanocytes originating from neural crest and in melanoma cells (10, 11, 24, 25). The melanocyte-restricted promoter region from which MITF-M isoform is transcribed is responsible for such a specific expression of this isoform (26). A splice variant of MITF-M named MITF-Mdel containing two in frame deletions, 56 amino acids deletion in exon 2 (from V32 to E87) and 6 amino acids deletion in exon 6 (from A187 to T192), has been identified (27). Like MITF-M, MITF-Mdel is also specifically expressed in melanocytes and melanoma cells (27).

Isoforms MITF-A, MITF-H, MITF-B, MITF-C, MITF-Mc and MITF-J have a common B1b domain of 83 amino acid residues and each its unique N-terminal domain (A, H, B1a, C, Mc and J) (13-16, 18, 20) (Figure 1).

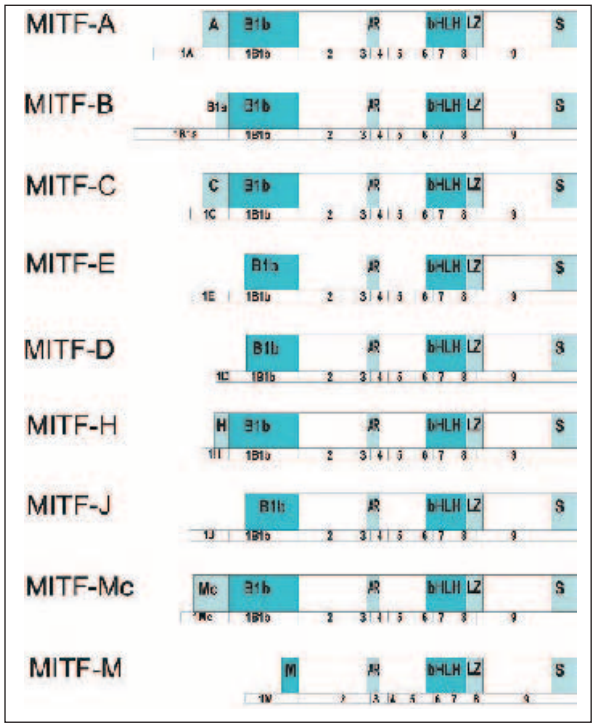

Figure 1 Structures of MITF isoforms. Schematic representations of all described isoforms of MITF protein (MITF-M, MITF-A, MITF-H, MITF-B, MITF-C, MITF-D; MITF-Mc, MITF-E and MITF-J) are shown. An activation region (AR), basic helix-loop-helix leucine-zipper region (bHLH-LZ), serine-rich region (S) and N-terminal regions encoded by isoform-specific exons (A, B1a, B1b, C, H, Mc and M) are indicated for each isoform. Corresponding exons of MITF gene (1A, 1B1a, 1B1b, 1C, 1D, 1E, 1H, 1J, 1Mc, 1M, 2, 3, 4, 5, 6, 7, 8 and 9) are indicated under the schematic representation of each isoform.

Domain A consists of 35 amino acid residues, domain H of 19, domain B1a of 10, and domain C of 34 amino acid residues (12, 20). Unlike the other isoforms, the translation of MITF-E and MITF-D, and probably also MITF-J isoform, does not start from the first exon, because it does not contain a code for methionine, but it starts from within B1b region (Figure 1.). Therefore the protein products for these three isoforms (MITF-E, MITF-D and MITF-J) are the same (13, 14, 16) (Figure 1).

Isoforms MITF-A and MITF-H are expressed in different cell types, including melanocytes and melanoma cells, with varying levels of expression depending on a cell type (10, 11, 24). Isoform MITF-C is expressed in different cell types, but not in melanocytes and melanoma cells (11). Of all the isoforms isoform MITF-A is the most abundantly expressed in retinal pigment epithelial cells (10). The expression of MITF-D isoform has been found in retinal pigment epithelial cells, macrophages, osteoclasts and mast cells, which are all cells affected by mutations in mouse MITF gene (13). In other cell types analyzed, including melanocytes and melanoma cells, no expression of MITF-D isoform has been found (13). Isoforms MITF-E and MITF-Mc are specifically expressed in mast cells (14, 15).

MITF gene

Gene MITF consists of nine first exons (1M, 1A, 1H, 1B, 1C, 1D, 1E, 1Mc and 1J) encoding the synthesis of N-terminal domain specific for each isoform and eight exons shared by all isoforms (12, 16) (Figure 1). 3' part of exon B (B1b) is also a second exon in isoforms MITF-A, MITF-H, MITF-C, MITF-D, MITF-E, MITF-Mc and MITF-J (12, 16) (Figure 1). So the isoforms MITF-M and MITF-B are encoded by nine exons, while the isoforms MITF-A, MITF-H, MITF-C, MITF-D, MITF-E, MITF-Mc and MITF-J are encoded by ten exons. Each isoform is transcribed from its own unique promoter, suggesting the functional diversity of these isoforms in different tissues (12). The amino acid sequences of homologues of human MITF protein have been determined in mice (1), rats (28), chicken (29), hamsters (30), quails (31) and zebrafish (*Danio rerio*) (32). All of these sequences are highly homologous with the one of human MITF protein. Analysis of publicly available genomic sequence data indicates the existence of genes homologous to human *MITF* gene also in other vertebrate as well as invertebrate species (33).

Function of MITF

MITF is a transcription factor that activates the transcription of genes for tyrosinase, tyrosinase-related protein 1 (TYRP1), and dopachrome tautomerase (DCT), enzymes specifically expressed in melanocytes that have a key role in synthesis of pigment melanin (34-36). MITF activates the transcription of these and other target genes by binding in their promoter regions to a restricted subset of E-box motives containing canonical CATGTG sequence flanked by a 5' thymidine (37). More recent analysis of more than 40 MITF target genes came out with T-C-A-T/C-G-T-G-A as a MITF-binding consensus sequence (38). The regulation of DCT promoter is more complex than for tyrosinase and TYRP1, as some other proteins like CREB and SOX10 cooperate with MITF in activating DCT expression, while PAX3 has an antagonistic effect on activation of DCT expression by MITF (39-41). In addition to activating the transcription of genes involved in melanin synthesis, MITF also activates the transcription of genes involved in melanosome structure (*PMEL17/SILV/GP100*, *MLANA/MELAN-A/MART-1*), melanosome biogenesis (ocular albinism type 1 gene (*OA1*)), and melanosome transport

(*RAB27A*), which makes MITF a central regulator of melanogenesis on a transcription level (38, 42-45). MITF also activates the transcription of gene for melanocortin 1 receptor (MC1R), a receptor on plasma membrane of melanocytes for α-melanocyte stimulating hormone (α-MSH) (38, 45, 46). Binding of α-MSH to MC1R is first step in the mechanism of the hormonal regulation of pigmentation, the mechanism that also involves activation of MITF as an important downstream step (45). Therefore, activation of *MC1R* transcription by MITF represents a positive feedback mechanism in the hormonal regulation of pigmentation.

Association of mutations in human *MITF* gene with Waardenburg syndrome and study of mice with mutations in *microphthalmia* locus coding for mouse homologue of MITF imply important role for MITF in melanocyte development and survival. Several experiments have confirmed that MITF-M has an important role in differentiation and normal function of melanocytes. Induced expression of gene for MITF-M in NIH/3T3 fibroblasts in which it is normally not expressed converted these cells into cells expressing melanocyte-specific genes for tyrosinase and TRP-1 (47). In one experiment zebrafish (*Danio rerio*) embryos lacking melanophores due to mutations in *nacre*, a functional homologue of *MITF* gene, were transfected with wild-type *nacre* gene, which was sufficient to restore the development of melanophores (32). In another experiment embryonic stem-like cells form medaka (*Orysias latipes*) were transfected with the melanocyte-specific isoform of Xiphophorus *mitf* gene and cells with all the characteristics of differentiated, functional pigment cells were observed (48). Unlike for melanocytes, differentiation of retinal pigment epithelium cells and melanogenesis in these cells is not regulated by melanocyte specific MITF-M isoform but by other MITF isoforms like MITF-D, MITF-H and MITF-A (10, 49).

Several targets of MITF, including genes that play important role in the control of apoptosis and cell cycle, have been identified elucidating the important role of MITF in melanocyte development and survival. MITF controls the transcription of *BCL2*, gene for Bcl-2, an important inhibitor of apoptosis (50). The importance of this interaction for melanocyte survival was shown in experiment in which overexpression of *BCL2* rescued melanocytes from apoptosis induced by dominant-negative MITF mutation (50). Regulation of *BCL2* expression by MITF could explain reduced number of melanocytes in persons with Waardenburg syndrome type 2. Mutated MITF in persons with Waardenburg syndrome type 2 is less efficient in inducing the expression of *BCL2*, and reduced expression of *BCL2* has a consequence of more melanocytes dying by apoptosis. MITF induces the transcription of another inhibitor of apoptosis, BIRC7 (also known as melanoma inhibitor of apoptosis (ML-IAP) or LIVIN) (51). MITF also regulates the expression of MET, receptor for hepatocyte growth factor (HGF), the activation of which protects melanocytes from apoptosis (52-53). MITF promotes proliferation of melanocytes by regulating the transcription of several genes involved in the cell-cycle regulation. In melanocytes and melanoma cells MITF binds to a sequence upstream of the transcription start of cyclin-dependent kinase 2 (*CDK2*) gene and induces transcription of this important cell-cycle regulator that induces cell-cycle progression (54). MITF induces the expression of the gene for transcription factor TBX2 that prevents senescence and cell-cycle arrest through repression of cyclin-dependent kinase inhibitor 1A (p21) expression (55-57). Another mechanism by which MITF promotes proliferation of melanocytes and melanoma cells is through activation of the

expression of *DIAPH1*, a gene for Dia1 protein that controls actin polymerization (58). Activity of Dia1 results in increased degradation of cyclin-dependent kinase inhibitor 1B (p27), which leads to an increased cellular proliferation (58). MITF trough activation of *DIAPH1* expression also reduces invasiveness of melanoma cells (58). In contrast to described pro-proliferative effects, MITF also has anti-proliferative effects. MITF activates transcription of genes for two proteins that induce cell cycle arrest, cyclin-dependent kinase inhibitor 1A (CDKN1A/p21) and cyclin-dependent kinase inhibitor 2A (CDKN2A/p16) (59, 60). The effect of MITF on cell cycle arrest indicates the important role of MITF in melanocyte differentiation. It is possible that level of expression of MITF determines whether it will have pro-proliferative or anti-proliferative effect. It was shown that both MITF depletion and MITF forced expression inhibit proliferation of melanoma cells while normal level of MITF expression favours cell proliferation (61). Some other genes that play a role in promoting melanocyte survival and preventing apoptosis have been identified as MITF targets, like gene for a DNA repair enzyme apurinic / apyrimidinic endonuclease1 (APEX1), gene for a transcription factor hypoxia inducible factor 1 a (HIF1A), and gene for endothelin receptor B (ENDRB) (38, 62-64). Also some other MITF target genes that are not directly related to melanocyte survival and development have been identified, like *TRPMN1*/*melastatin*, glycoprotein-nmb (*GNMB*), and *SNAI2*/*SLUG*, a gene that plays important role in epithelial-mesenchymal transition (38, 65-67).

In addition to its' central role in melanocyte development and biology, MITF is also important for osteoclasts and mast cells development and function (68). In osteoclasts MITF has been shown to activate the transcription of several genes for proteins important for osteoclast function like tartarate-resistant alkaline phosphatase (TRAP), cathepsin K, OSCAR, E-cadherin, OSTM1 and Clcn7 (69, 70). In mast cells MITF activates the expression of several genes important for mast cell differentiation and function like genes for mast cell proteases 2, 4, 5, 6, and 9, granzyme B, tryptophan hydroxylase, Kit, and some others (71, 72).

Regulation of MITF

MITF is regulated on different levels, form transcription to post-translational modifications. It was shown that Wnt signalling pathway induces the transcription of MITF (73). Wnt are secreted cysteine rich glycoproteins that play an important role in embryonic development and differentiation. Wnt proteins are especially important for differentiation of melanocytes and other neural crest derived cells (74, 75). Binding of Wnt molecules to specific cell-surface receptors of the Frizzled family activates these receptors and initiates the sequence of signals that leads to increased stability and accumulation of cytoplasmic β-catenin which then enters the nucleus and interacts there with lymphoid enhancer factor 1/ T cell factor (LEF1/TCF) transcription factor inducing the transcription of LEF1/TCF target genes (75) (Figure 2.). In a promoter region of MITF-M a functional binding site for LEF1/LCF was discovered which explains the mechanism of activation of MITF expression by Wnt signaling pathway (73). MITF also interacts directly with LEF1 to activate expression of some MITF target genes, as well as expression of MITF itself (76, 77). It was shown that dickkopf 1 (DKK1), an inhibitor of Wnt pathway, has a suppressing effect on the expression of MITF (78).

MITF expression can also be activated by α melanocyte-stimulating hormone (αMSH) signalling pathway (36, 79). αMSH is synthesized and secreted in epidermal keratinocytes and binds to the melanocor-

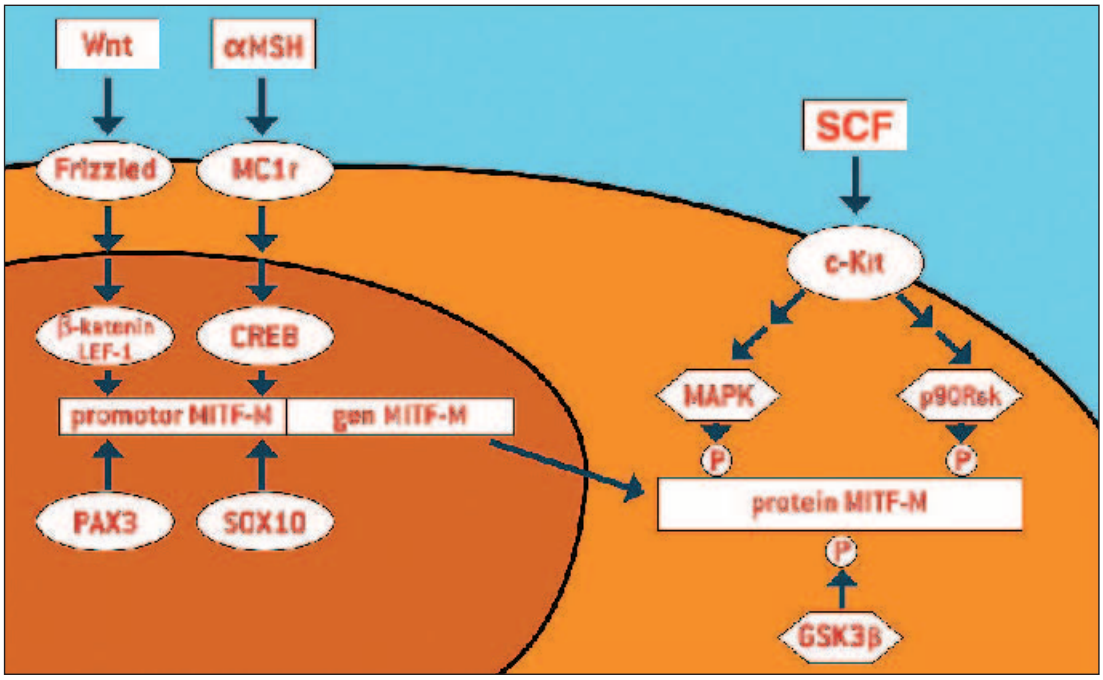

Figure 2 Schematic representation of regulation of MITF gene expression and MITF protein activity. MAPK (mitogen activated protein kinases), p90Rsk, and GSK3b are protein kinases that phosphorylate MITF protein on Ser 73, Ser 409, and Ser 298, respectively and thus regulate its activity. CREB (cAMP response element binding protein), PAX10, SOX10 i LEF1 are transcription factors that induce transcription of MITF gene. Wnt, SCF (stem cell factor), and αMSH (a melanocyte stimulating hormone) are growth factors that activate cellular signaling pathways leading to activation of named protein kinases and transcription factors. c-Kit is receptor for SCF (stem cell factor), Frizzled is receptor for Wnt, and MC1r is receptor for αMSH.

tin 1 receptor (MC1R) on cell surface of melanocytes activating a signalling cascade that involves activation of adenylate cyclase that synthesizes cyclic adenosine monophosphate (cAMP). One of the effects of increased level of intracellular cAMP is activation of cAMP response element-binding (CREB) transcription factor by phosphorylation. Activated CREB can bind to a target sequence in the promoter region of MITF-M and activate its transcription (36, 79).

In addition to LEF1/TCF and CREB, SOX10 and PAX3 transcription factors also bind to promoter region of MITF-M and activate its expression (80-84). Mutations in *PAX3* gene are associated with Waardenburg syndrome type 1 and 3 while mutations in *SOX10* gene are associated with Waardenburg syndrome type 4 (85, 86). Role of PAX3 and SOX10 in regulation of MITF expression can explain some of the symptoms associated with Waardenburg syndrome type 1, 3, and 4. It was shown that cooperation between SOX10 and CREB is required to activate MITF expression, which can explain how ubiquitous CREB can regulate cell-type specific expression of MITF (87). Waardenburg syndrome type 4 can also be caused by mutations in gene for endothelin (EDN) or gene for its receptor EDNRB. Binding of EDN to EDNRB on melanocytes starts a signal cascade that leads to increased expression of MITF and phosphorylation of MITF at Ser 73 (64). MITF transcription can be activated also by transcription factors Onecut-2 and peroxisome proliferator-activated receptor γ (PPARγ) (88, 89). Recently it was shown that interleukin-1 can significantly down-regu-

late the expression of MITF-M in melanoma cells expressing interleukin 1 receptor (90).

It was shown that oncogenic BRAF with V600E mutation found in ~60 % melanomas also regulates MITF by two different mechanisms (91-93). First mechanism involves $BRAF^{V600E}$ activation of extracellular-signal regulated kinase 2 (ERK2) that then phosphorylates MITF thus inducing its degradation (92). The other mechanism involves $BRAF^{V600E}$ mediated induction of the expression of transcription factor BRN2 that is not normally expressed in melanocytes and that can bind to MITF promoter and induce the transcription of MITF (93). These apparently contradictory effects of $BRAF^{V600E}$ on MITF can be reconciled in a previously described model according to which normal level of MITF activity promotes cell proliferation while both its downregulation and upregulation suppress this pro-proliferative action of MITF.

MITF is also regulated by post-translational modifications. Binding of stem cell factor (SCF) to its cell surface receptor c-Kit starts a signalling cascade that leads to phosphorylation of MITF on Ser 73 by a mitogen activated protein kinase (MAPK) extracellular-signal regulated kinase 2 (ERK2) and on Ser 409 by serine-threonine kinase p90 ribosomal s6 kinase (RSK) (94, 95). It was shown that Ser 73 phosphorylation of MITF enables binding of transcription coactivator p300/CREB-binding protein (CBP) to MITF increasing the activity of MITF as transcription factor (96). Both Ser 73 and Ser 409 phosphorylations also downregulate MITF by enhancing its ubiquitination followed by proteosome degradation (95). MITF can also be phosphorylated at Ser 298 by glycogen synthase kinase 3β (GSK3β) which activates MITF by enhancing its binding to its target DNA sequences (97). The importance of this phosphorylation is confirmed by finding of point mutation in a subset of Waardenburg syndrome 2 patients that results in substitution of Ser 298 with a proline in MITF (97). It was shown in osteoclasts that MITF is phosphorylated on Ser 307 by p38 MAPK as a part of NF-κB signalling pathway, which increases MITF activity in inducing the transcription of its target genes (98). The activity of MITF can also be modulated by sumoylation at Lys 182 and Lys 316 mediated by protein inhibitor of activated STAT3 (PIAS3) (99). It was shown that sumoylated MITF has decreased transcriptional activity on target genes with more than one MITF binding site (99). It was shown that in melanocytes and in melanoma cells MITF can be cleaved by caspases after Asp 345 producing C-terminal fragment that has pro-apoptotic activity (100).

The expression of *MITF* can also be regulated by microRNAs (miRNA). It was shown that expression of miRNA-137 suppresses MITF expression (101, 102). Also miRNA-182, that is frequently upregulated in melanoma, suppresses the expression of MITF (103). MITF expression is also regulated by miRNA-148 (102).

It was shown that activity of MITF as transcription factor depends on its interaction with several other proteins. MITF binds a known transcriptional co-activators p300/CBP, which enhances MITF's activity as a transcription factor (96, 104). It was shown that interaction with p300/CBP turns MITF from repressor to activator of *DCT* gene (105). MITF was shown to interact with Rb to activate *CDKN1A* gene expression (59). Direct interaction of MITF with β-catenin increases activation of expression of MITF target genes (106). It was shown that activity of chromatin-remodelling enzymes SWI/SNF is required for MITF activation of some MITF target genes (*TRP1* and tyrosinase gene) but not for other MITF target genes (*MC1R*) (107).

MITF and melanoma

Cronin et al analyzed somatic mutations in MITF and *SOX10*, upstream regulator

of MITF, and found them in more than 14 % primary melanomas and 22 % metastatic melanoma (108). Garraway et al. have shown that MITF gene was amplified in 10.5% of primary melanomas and 15.2% of metastatic melanomas, but no amplification was detected in melanocytic nevi, which are considered a pre-malignant lesions associated with melanoma (109). Also, the amplification of MITF gene was associated with decreased 5-year survival in the same study (109). In another study strong MITF gene amplification in metastatic melanoma patients was also associated with reduced disease-specific survival (110). Furthermore, it was shown that ectopic expression of MITF together with V600E mutated BRAF can transform immortalized melanocytes genetically engineered to have inactivated CDKN2A/CDK4/RB and p53 pathways and to express human telomerase reverse transcriptase (hTERT) (109). Also it was shown that MITF is at least partly responsible for melanoma chemoresistance (109). Based on these results it was proposed that MITF might play a role as "lineage specific" oncogene in melanoma. "Lineage specific" (also called "lineage survival" and "lineage addiction") oncogenes are genes that play important role in normal proliferation and survival of particular cell lineage during development and deregulated expression of which in a subset of cancers of the same cell lineage is important for cancer survival and progression (111). It is possible that MITF amplification is one way to compensate for MITF downregulation trough $BRAF^{V600E}$-ERK, supporting a role for MITF as "lineage specific" oncogene in melanoma. The role of MITF as a "lineage specific" melanoma oncogene is in apparent contradiction with its role in melanocyte differentiation and cell cycle arrest exhibited trough activation of *CDKN2A* and *CDKN1A* gene transcription (59, 60). These contradicting roles of MITF could be reconciled if we hypothesize that MITF plays a role as "lineage specific" melanoma oncogene only in subset of melanoma cells in which CDKN2A/CDK4/RB pathway is inactivated, for example by mutations in *CDKN2A* gene which are well documented in melanoma (112). This explanation is supported by finding that all cell lines with MITF gene amplification in previously described study by Garraway et al also had CDKN2A pathway inactivation (109). In the same study MITF (in cooperation with $BRAF^{V600E}$) could transform melanocytes that had inactivated CDKN2A pathway (109). Also, it was shown that inactivation of *CDKN2A* can enable melanocytes to escape MITF induced growth inhibition while maintaining MITF expression (60). Studies that have shown that MITF is important activator of expression of several genes that play important role in melanoma cell survival, growth and proliferation, like *BCL2*, *CDK2*, *HIF1A*, *TBX2*, *BIRC7/ML-IAP* support the role of MITF as "lineage specific" oncogene in melanoma (50, 51, 54, 55, 57, 63).

In a different genetic context when CDKN2A/CDK4/RB pathway is not inactivated it is most probable that MITF activity should be kept at a certain level to promote melanoma cell survival and proliferation because too high expression of MITF would lead to cell cycle arrest and too low or no expression would lead to apoptosis. In line with this model is two-fold regulation of MITF by V600E mutated BRAF in melanoma. As previously described, $BRAF^{V600E}$ found in ~60 % melanomas, downregulates MITF trough ERK2 mediated phosphorylation and upregulates MITF transcription trough BRN2 transcription factor (92, 93). It is probable that in that way oncogenic $BRAF^{V600E}$ keeps MITF at a level needed to maintain melanoma cells proliferation and survival. Several studies have shown results corroborating that model. It was shown that upregulation of MITF expression in mela-

noma cells inhibits their proliferation (92). Also, MITF reexpression in melanoma cells that do not express MITF reduced their tumorigenecity in vivo (25). Transfection of aggressive UISO-Mel-6 melanoma cells with *MITF-M* decreased their proliferation and metastatic potential leading to a less aggressive phenotype (113). Expression of MITF was studied by immunohistochemistry and was shown to decrease with melanoma progression (114). Another study has shown that MITF expression analyzed immunohistochemicaly is associated with longer overall survival and disease-free survival and fewer lymph node metastases (115). On the other hand, abolished MITF expression in melanoma cells resulted in profound apoptosis that could be rescued by *BCL2* or *BIRC7/ML-IAP* overexpression (50, 51). In other study downregulation of MITF suppressed colony formation by melanoma cells that could be rescued by overexpression of *CDK2*, a cell cycle regulator which was shown to be controlled by MITF and indispensable for growth and cell cycle progression only in melanoma cells (54). Kido et al have shown that both depletion and forced expression of MITF significantly inhibited melanoma cell proliferation (61). Although many of the previously mentioned studies indicate important role for MITF in melanoma, MITF was not expressed in a proportion of melanoma samples analyzed, indicating that there are different subsets of melanomas which differ regarding the role and importance of MITF for their progression and survival (115-117). Furthermore, it is possible that importance and role for MITF in melanoma can change during melanoma progression (118).

Several studies have shown that MITF is involved in other aspects of melanoma behaviour in addition to regulating melanoma cell survival and proliferation. MITF can regulate melanoma angiogenesis by activating the expression of *HIF1A*, which in turn activates the expression of vascular endothelial growth factor (*VEGF*) (63). Through regulating the expression of *DIAPH1*, gene for Dia1 protein, MITF in addition to increasing proliferation also reduces invasiveness of melanoma cells (58). MITF induced by HGF signalling upregulates the expression of MET receptor and so plays a central role in HGF-MET regulated invasion of melanocytes and melanoma cells (52). MITF also activates the expression of TRPM1/melastatin, a prognostic factor in melanoma patients the expression of which is inversely correlated with melanoma metastatic potential and prognosis so it may play a role as melanoma metastasis suppressor (65). MITF can also be implicated in regulation of melanoma invasion and metastases trough regulating the expression of *SNAI2*/*SLUG*, a gene that plays important role in epithelial-mesenchymal transition (67). Hoek et al analyzed expression profiles for 86 melanomas and separated them based on expression profile in two cohorts, one with high proliferative and low metastatic potential characterized by high MITF expression and other with low proliferative and high metastatic potential characterized by low MITF expression (119). In a further study it was shown that during tumour progression melanoma cells could switch from one to other of these two expression profiles (118). MITF was shown also to play important role in regulating cellular response to reactive oxygen species through regulating the expression of apurinic/apyrimidinic endonuclease 1(APE-1/Ref-1) (62). The expression of MITF makes melanoma cells more resistant to H_2O_2-induced cell death implicating another role for MITF in melanoma carcinogenesis (62).

Studies showing that signalling pathways deregulation of which is implicated in melanoma tumorigenesis and progression regulate MITF expression and activity further imply an important role for MITF in mela-

noma. MITF is an important downstream target of Wnt/β-catenin signalling pathway that is deregulated with aberrant nuclear accumulation of β-catenin in significant proportion of melanomas (73, 120). Widlund et al. have shown that β-catenin is important for growth and survival of melanoma in a manner dependent on downstream activation of MITF (121). As previously elaborated, MITF is downstream target of c-Kit signalling pathway (95). Somatic oncogenic mutations in *KIT*, gene coding for c-Kit, have been implicated in melanomas arising on acral, mucosal and chronically sun-damaged cutaneous surfaces and therapy targeting c-Kit has shown promising results in these melanoma patients(122, 123). Also, MITF expression is induced by TYRO3, which is overexpressed in melanoma and plays important role in melanoma tumorigenesis and progression (124). Furthermore, miRNA-182, which is upregulated in melanoma and plays a role in melanoma progression regulates MITF transcription further emphasizing role of MITF in melanoma (103).

In addition to melanoma, MITF has also been implicated in the development of clear cell sarcoma and TFE3 and TFEB, transcription factors closely related to MITF, have been implicated in the development of some other cancers (125).

MITF as immunohistochemical marker for melanoma

The most common routinely used markers for immunohistochemical diagnosis of melanoma, S100 and gp100 (detected with HMB45 antibody) show either relatively low specificity (S100) or relatively low sensitivity (gp100/HMB45) (126). Therefore, a particular interest was shown for the results of the study in which all the tissue samples of primary melanoma and melanoma metastases, including the ones that were negative for S100 and gp100, stained positively with a nuclear staining pattern when D5 antibody specific for human MITF was used (127). In that study samples of healthy skin and benign melanocyte lesions were also positive for MITF. However, none of the samples of tumours of non-melanocyte origin was positive for MITF.

Such highly specific and sensitive immunohistochemical staining for MITF in melanocyte lesions was confirmed in other studies (128, 129). In one study MITF was analyzed as immunocytochemical marker for melanoma, showing specificity of 97% and sensitivity of 100% superior to S100 and gp100 in the same study (130). However, some other studies have shown a lower sensitivity (88% and 81%) of MITF protein as a marker for immunohistochemical diagnosis of melanoma (116, 117). In one study only 64% of S100 positive, HMB45 negative, epitheloid melanomas stained with MITF (131). Also staining for MITF not specific for melanocyte lesions has been shown in some studies. In one study 1 out of 8 breast carcinomas, 2 out of 17 renal carcinomas, and 2 out of 5 leiomyosarcomas were MITF positive (117). In other study immunoreactivity for MITF was seen also in macrophages, lymphocytes, fibroblasts, Schwann cells, and smooth muscle cells at various sites, and in tumours derived from these cells (132).

MITF was specially investigated as a marker for immunohistochemical diagnostics of desmoplastic melanoma, a rare histological type of melanoma that is often difficult to distinguish from some other tumours or benign lesions, but did not show value for that purpose. In one study only one out of 30 investigated histological samples of desmoplastic melanoma was positive for MITF (116). Another study has shown that MITF is neither specific nor sensitive marker for immunohistochemical diagnostics of desmoplastic and spindle-cell melanomas (133). MITF has also shown a value as immunohistochemical marker for the

detection of melanoma metastases in sentinel lymph nodes (134). Using the specific antibodies the expression of MITF protein was confirmed in cell lines and tissue specimens of other tumours of melanocytic origin: uveal melanomas, central nervous system melanocytomas and clear cell sarcomas (134-136).

In one study the expression of MITF protein was investigated as a prognostic factor in patients with intermediate-thickness (1-4 mm) cutaneous melanoma (115). The expression of MITF evaluated semiquantitatively by immunohistochemistry in that study was associated with statistically significantly longer overall survival and disease-free survival. In another study MITF gene amplification was analyzed by quantitative real-time PCR in tumour tissue samples from metastatic melanoma patients (110). In that study strong MITF gene amplification was associated with a reduced disease-specific survival but no correlation was found between MITF copy number and chemotherapy response. These results indicate that MITF gene copy number could be a valuable prognostic marker but not a predictive marker for chemotherapy response in patients with metastatic melanoma.

MITF as a marker for the detection of circulating melanoma cells

The detection of circulating melanoma cells by reverse-transcription – polymerase chain reaction (RT-PCR) has been investigated as a potential prognostic and predictive marker in melanoma patients. The most widely used melanoma-specific marker for RT-PCR detection of circulating melanoma cells is tyrosinase. Tyrosinase has shown high specificity, low threshold for the detection of circulating melanoma cells and association with overall and progression-free survival in many studies (137). Still, the clinical value of tyrosinase is limited due to significant proportion of patients with confirmed distant metastases being tyrosinase negative (137, 138). Several studies have shown that analysis of additional markers together with tyrosinase can improve the detection of circulating melanoma cells (137, 139).

We were first to confirm that MITF-M can be analyzed as a specific marker with a low threshold for the detection of circulating melanoma cells by RT-PCR (140). In that study, we have shown that analysis of MITF-M as an additional marker to tyrosinase improves the detection of circulating melanoma cells in melanoma patients (140). Koyanagi et al. have subsequently shown that MITF detection in blood by real-time quantitative RT-PCR is a significant independent prognostic factor for relapse-free and overall survival and can indicate subclinical metastatic disease and predict treatment outcome in melanoma patients (141). In a recent study we investigated MITF as a marker for the detection of circulating melanoma cells by RT-PCR on 201 melanoma patients in all stages of the disease (142). In this study positive value of MITF was associated with significantly shorter overall survival and progression-free survival.

A recently identified splice variant of MITF-M, MITF-Mdel is widely expressed in melanocytes, melanoma cell lines and tissues, but almost undetectable in non-melanoma cell lines or peripheral blood mononuclear cells from healthy donors (27). Therefore, MITF-Mdel is also a promising marker for the detection of circulating melanoma cells by RT-PCR.

MITF as target for melanoma therapy

The role of MITF in normal melanocyte development and in melanoma progression and survival makes it a potential target for melanoma therapy. Electroporation mediated transfer of short interfering RNA specific for MITF gene was studied in mice with

melanoma. That treatment induced apoptotic death of tumour cells leading to significant growth retardation of the tumour (143). Another approach using histone deacetylase (HDAC) inhibitors has been studied showing that HDAC inhibitors suppress the expression of MITF-M in melanoma cells and systemic HDAC inhibitors treatment significantly suppressed the growth of melanoma in a human melanoma xenograft model (144).

Conclusion

Results of many different studies described in this review have established MITF as a master regulator of melanocyte and melanoma function, development and survival. MITF is already routinely applied as melanoma marker and has shown promising results as a target for melanoma therapy. Still, regulation of MITF in melanocytes and melanoma cells is immensely intricate and consequently its role and importance in melanoma is complex and depending on different factors. As described previously, just one example of complex role of MITF in melanocytes and melanoma is finding that MITF has both proproliferative and antiproliferative effects. Also, as described, MITF gene is amplified in a proportion of melanomas where MITF plays a role of a "lineage specific" oncogene, while in another proportion of melanomas MITF is not expressed at all. Therefore, further elucidation of complex regulation and effects of MITF in different genetic, intracellular and extracellular contexts could enable further and clinically more relevant applications of MITF, primarily in the management of melanoma patients.

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Pyogenic discitis in adolescence: a case report and review of the literature

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Back pain in adolescents can have many underlying reasons. Even though discitis is an uncommon condition, health care professionals should suspect discitis in children and adolescents with back pain with or without a history of trauma. With early diagnosis and treatment, favorable results can be achieved in these patients. Our report concerns a successfully treated discitis with osteomyelitis in a 16 year old patient with subsequent three year follow up.

Key words: Pyogenic discitis, Adolescence.

Introduction

Common causes of low back pain in children, such as muscle strain, herniated disk, spondylolysis, spondylolisthesis and Scheuermann's kyphosis, are frequently diagnosed and given proper treatment. Due to the low incidence, a delay in diagnosis of less common causes of low back pain such as systemic disease (storage disease), neoplastic disease or infection (discitis) may result in long term disability (restriction of mobility and local kyphosis) (1) of the involved spine. In this case report, we describe the outcome and long term follow up of a 16 year old boy who presented with low back pain, was diagnosed with discitis and treated successfully.

Case report

A 16-year-old male presented to his pediatrician's office with complaints of intermittent low back pain for the previous four weeks. He recalled falling backwards while playing volleyball 1-2 months before presentation. He had no recollection of fever, chills or other physical ailments. His pain was isolated to the lumbar region without any radicular symptoms or weakness. Physical

exam demonstrated tenderness to palpation at the lumbar spine and surrounding paraspinal musculature. He did not have any signs of myelopathy or spinal cord impingement such as muscle weakness, altered reflexes or sensation. Radiographs (Figure 1) demonstrated a slight, if any, decrease in the disk space height of L2-3 but no other significant findings that would correlate with low back pain in a young adult. After completing a course of physical therapy without any pain alleviation, MRI of the lumbar spine was obtained (Figure 2). The results demonstrated mild superior endplate compression of L3 with mild diffuse bone marrow edema of the L3 body and pedicle consistent with spondylodiscitis.

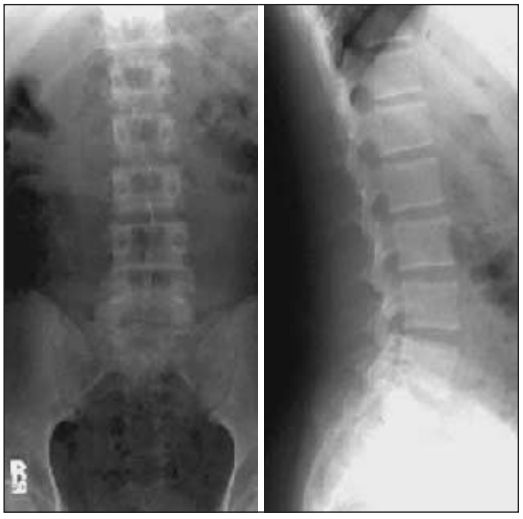

Figure 1 LS spine radiographs at initial presentation

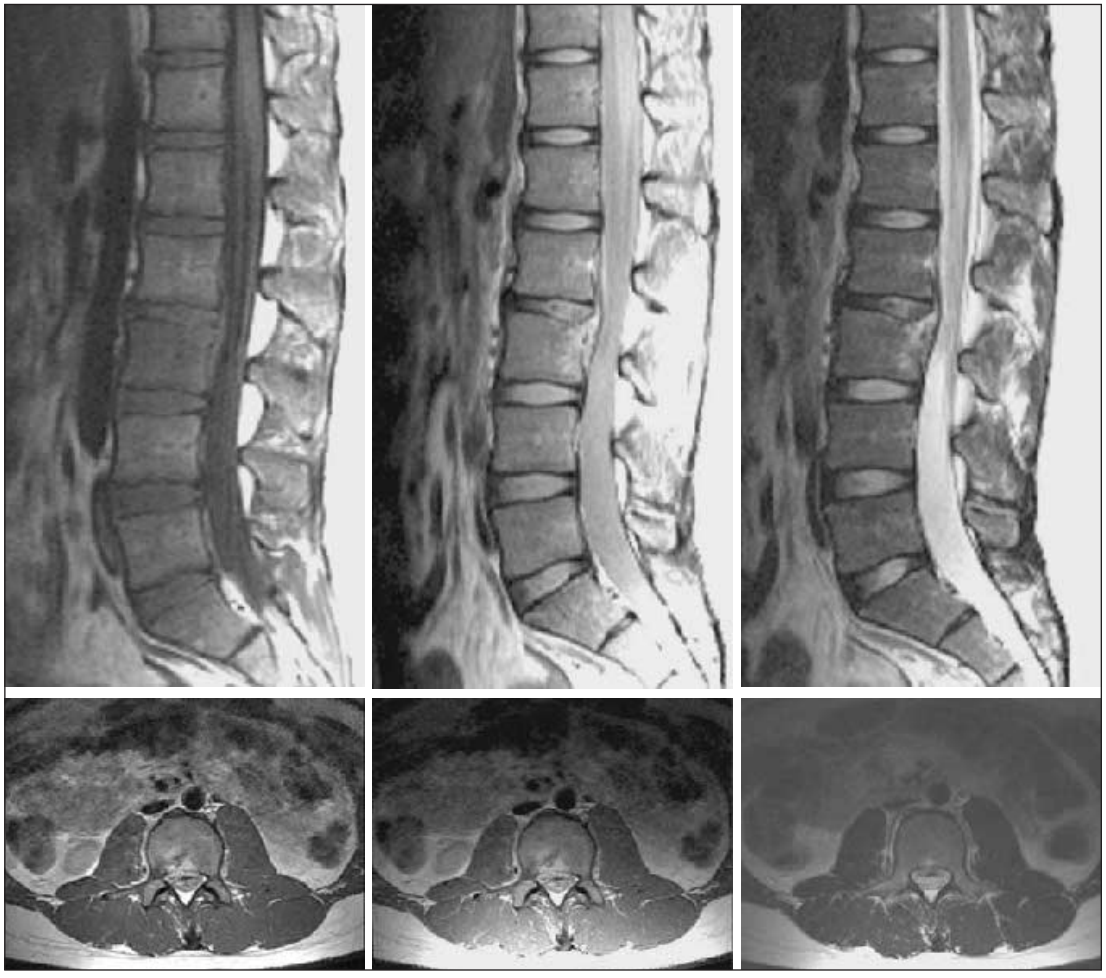

Figure 2 MRI of the LS spine with and without gadolinium showing the inflammatory changes in the intervertebral disc at the onset of treatment. No signs of the spinal canal compromise are seen

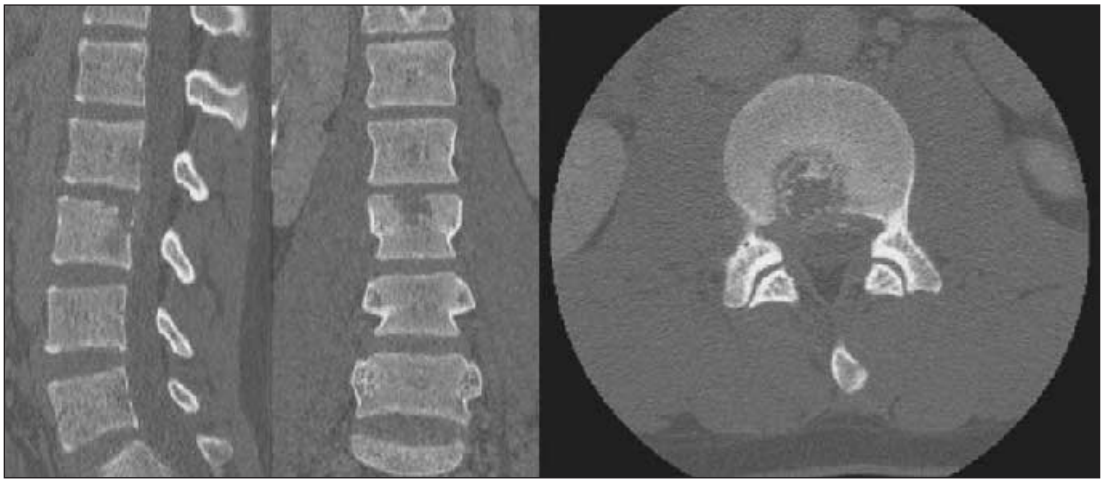

Figure 3 CT scan show extensive bone resorption of the involved level

This patient was admitted to hospital for further work up. Admission lab testing revealed an erythrocyte sedimentation rate (ESR) of 80 and C-reactive protein of 2.8 (0-0.8 mg/dl) but a normal white blood cell of 10.8 without a left shift. Due to the unusual findings within the L 3 vertebral body and lumbar disc, a CT guided biopsy (Figure 3) of both the L3 body and L2-3 disc was obtained.

We obtained peripheral blood cultures which were subsequently negative. Initially the patient was treated empirically with intravenous Vancomycin until the cultures were finalized. Vancomycin was discontinue promptly because the patient developed a "Red Man Syndrome".

The cultures of the biopsy specimens demonstrated Methacillin sensitive Staphylococcus aureus within both the L3 body and L2-3 disc which were consistent with the blood culture results and confirmed acute and chronic inflammation consistent with osteomyelitis. The treatment intravenous Nafcillin was started at that time. At the end, the patient was treated with an eight-week course of intravenous Nafcillin, a low profile bed rest for 4 weeks and thoracolumbar orthosis for a total of three months.

Follow up radiographs (Figure 4) and MRI (Figure 5) demonstrated resolution of

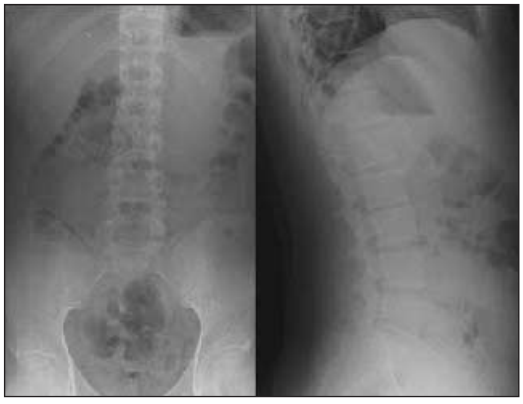

Figure 4 Follow up radiograph 4 months after initial treatment showed disc space narrowing

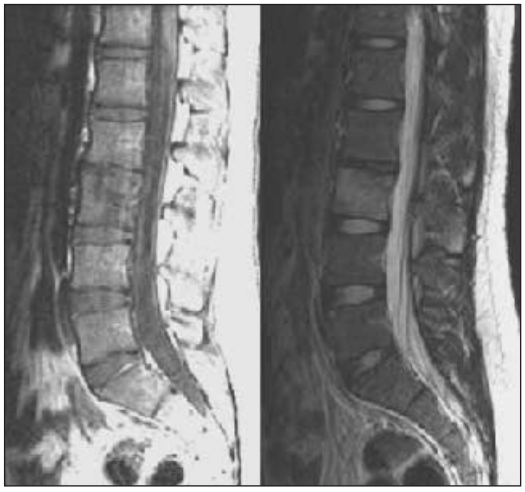

Figure 5 Follow up MRI 1 month after treatment showed slow revolution of disc and bone edema

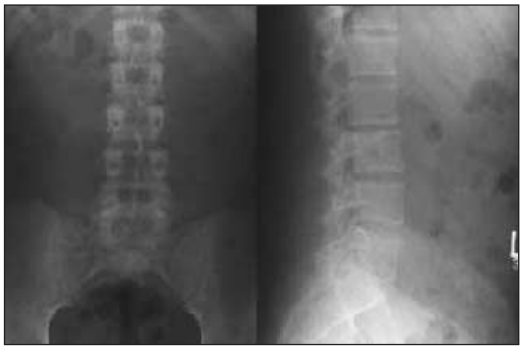

Figure 6 L-S spine radiographs after 1year and 6 months follow up still showed L 2-3 disc space narrowing

the bone edema with the only residual finding being a decreased disc space between L2 and L3. After 8 weeks from the initiation of treatment the ESR and white blood cell count were normal. Symptomatically, the patient's back pain resolved.

At the one year and two year follow up visits, radiograph show only mildly decreased disc space height (Figure 6). The patient was asymptomatic at those visits.

Discussion

Discitis in childhood and adolescence is a relatively rare condition. Average age of diagnosis is between 5-7.5 years old and it most frequently occurs in the age groups of 0-2, 10-12, 12-14 years respectively; L2-3 and L3-4 discs are the most common involved vertebral levels (2, 3). Adolescent discitis can be explained by seeding of microorganisms in the existing vascular channels that penetrate from the vascular loop into the disc proper. These channels usually persist until the third decade of life (4). However, the vascular theory can not be the only explanation of the entire phenomenon since benign bacteremia exists daily and is mostly transient and totally asymptomatic.

Morrissy and Haynes postulated that local trauma and hematoma were predisposing factors which can lead to bone infection (5). Infarction of a bone segment must occur in the vertebral body before osteomyelitis develops. Bacteria penetration in the vertebral body by lodging in the area with poor blood supply, is followed by its subsequent multiplication leading into symptoms of infection. Scoles and Quinn (6) believes that discitis is probably a common clinical manifestation of trauma, viral inflammation or bacterial infection. In our case, the history of trauma while playing volleyball may be a precipitating cause of discitis.

Plain spinal x-rays are an initial part of back pain investigation. The typical finding on the radiographs is narrowing of the intervertebral disc space which occurs 2-3 weeks after the onset of the symptom. This is followed by erosion of the subchondral bone plate and osseous eburnation (3, 7). MRI is a useful diagnostic tool in adolescents. Ring et al. (9) showed that the appearance on MRI was similar to that in an adult patient. A typical finding in the vertebral body on the T1-weighted image is a low intensity signal which may be less obvious than in the adult since the bone marrow is predominately hematopoietic in nature. A T2-weighted image of the infected disc usually shows increased signal intensity but these changes may not be observed in children (8, 10, 11).

The mainstream treatment of discitis is antibiotics (12), although there are reports of its self-limited course in some patients with prolonged recumbency (7, 10). Crawford used antibiotics only in 41% of his patients and reported similar outcomes (4). Since the most common causative organism is *Staphylococcus aureus*, some authors recommended starting antibiotics empirically even if the biopsy was negative (3, 4, 8, 13, 14). The route of administration should initially be intravenous. Intravenous antibiotics lead to a predictable and rapid resolution of the symptoms (8). The acute symptoms usually resolve in 2-3 days. After 4-6

weeks of treatment, intravenous antibiotics can be changed to an oral form only if acute back symptoms resolve (6). Other forms of non-operative treatment consist of bed rest, bracing and traction; however, these methods should not be used as the sole treatment because of the high complication rate (8).

Blood cultures should be obtained prior to antibiotic administration. A needle biopsy may be necessary in cases when systemic symptoms persist for 3-4 days in spite of broad-spectrum antibiotics. A needle biopsy under a CT-scan guidance or fluoroscopy is the method of choice. An open biopsy may be performed only if the close method fails. Needle biopsy yields positive results in only 60% of cases (4, 6, 12, 14, 15).

The indication for a surgical treatment is limited to cases where failed antibiotic treatment. Debridement, decompression or correction and stabilization of a deformity are necessary due to abscess formation, spinal cord compression and/or development of a spinal deformity (14). On follow up radiographs, most disc spaces had persistent endplate sclerosis with narrowing of the intervertebral disc space. In cases where disc height loss was more than 50%, there was usually progression to bony fusion of the adjacent levels.

Conclusion

Healthcare professional should have a high suspicion for discitis in children with back pain since early diagnosis has very good prognoses for recovery. If symptoms are not responding to NSAIDs, pain medication or physical therapy, discitis should be included in the differential diagnosis. Our diagnostic method of choice after plain x-rays is MRI with contrast medium. Laboratory diagnostic tests such as blood cultures, ESR, complete blood count and tissue culture from needle guided biopsy are obtained routinely. The treatment choices include intravenous antibiotics, bed rest and bracing. Surgical intervention is rarely indicated.

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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Primary jugular foramen meningioma with unusual extensive bone destruction: case report and review of literature

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Jugular foramen meningiomas are exceedingly rare tumors of the fossa jugularis. These meningiomas are characterized by an invasive growth pattern with extensive skull base infiltration in all directions and by the mixed permeative-sclerotic appearance of the bone margins of the jugular foramen. We report an unusual case of a primary jugular foramen meningioma in a 30-year-old woman. The unenhanced high-resolution CT of the temporal bones revealed extensive bone destruction around the left jugular foramen as well as bone destruction of the basilar part of the left occipital bone without sclerosis. These findings are unusual for meningiomas and correspond more to glomus jugulare tumors. In the literature, we did not find a case similar to ours. Conclusion. MRI and CT imaging provide accurate distinction between meningioma and glomus tumors or schwannomas in most cases. From high-resolution CT scans, in the case where a permeative-destructive pattern is dominant, and with the absence of hyperostosis and bone thickening around the jugular foramen, the differential diagnosis between jugular foramen meningiomas and other tumors, especially glomus jugulare tumors, is difficult. In that case the correct diagnosis should be based on the MRI findings.

Key words: Jugular foramen, Meningioma, Glomus jugulare tumor, Bone destruction.

Introduction

The most common tumor that develops in the jugular foramen (JF) is a glomus jugulare tumor. Neural sheath tumors follow, such as schwannomas and neurofibromas, which share a common site of development in the JF, while meningiomas are the most rare tumors (1). Jugular foramen meningiomas (JFM) can be classified as primary if the tumor originates from the JF, although these are exceedingly rare, or secondary, when the tumor is centered in the posterior fossa, most commonly in the cerebellopontine angle or petroclival region, with extension into the JF (1, 2). Primary JFM appear

to behave differently from meningiomas that involve JF secondarily (3, 4, 5). These meningiomas are characterized by an invasive growth pattern with extensive skull base infiltration in all directions (3, 6, 7), and by the mixed permeative-sclerotic appearance of the bone margins of the JF (2, 8). In this article, we describe a very unusual case of primary JFM with permeative erosion of JF margins and extensive destruction of affected bones without sclerosis, which is not typical for JFM. We also review the literature on this rare entity.

Case report

Three years ago, a 30-year-old woman with a one year history of progressive left hearing loss, pain in the left half of the face, headache and dizziness, came to the Department of Radiology, and computed tomography (CT) of the temporal bones was performed. CT detected a widened left JF with erosions of its margins without sclerosis and soft tissue mass into the mesotympanum and hypotympanum. The ossicles were intact. The ostheolitic changes were seen on the posterior and inferior wall of the petrous portion of the temporal bone. The radiologist suspected a glomus jugular tumor. Digital subtraction angiography (DSA) revealed the vascular tumor with dual feeding arterial supplies, from the left external carotid artery and the left vertebral artery (posterior inferior cerebellar artery), without intensive tumor blush. Without preoperative magnetic resonance imaging (MRI) the patient underwent surgery at another hospital. Left suboccipital craniectomy via retrosigmoid approach was performed and subtotal tumor removal was achieved. Histopathological examination revealed meningothelial meningioma. In the next few months, she underwent gamma knife radiosurgery twice at another hospital. After surgery, the previous symptoms persisted, with swallowing

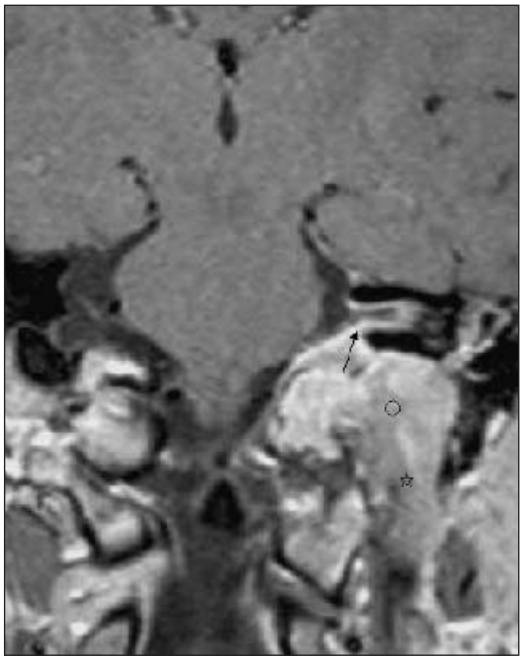

Figure 1 The coronal contrast-enhanced T1-weighted image shows intense enhancement in the mass centered in the jugular foramen (circle) with extensive infiltration of surrounding skull base. The mass spreads into the posterior fossa, the internal auditory canal (arrow) and inferiorly into the nasopharyngeal carotid space (star). High-velocity flow voids in the mass are absence. Also, the MR image shows different signal intensities between the intra- and extracranial components of JFM

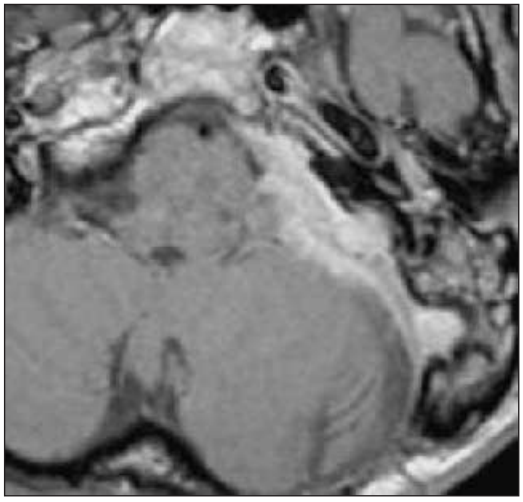

Figure 2 The axial contrast-enhanced T1-weighted image shows en plaque involvement of the posterior fossa with prominent dural tails and medial spread in the skull base to the mid clivus

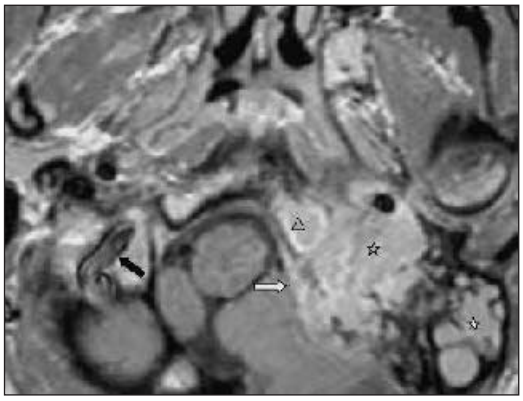

Figure 3 The axial contrast-enhanced T1-weighted image shows the mass in the left fossa jugularis and the carotid space (star) with medial spread into skull base to the mid clivus (triangle), and en plaque involvement of the posterior fossa (white arrow). The left internal jugular vein is incorporated into the mass with the absence of its normal flow void. Note normal flow voids on the right (black arrow). The mastoid air cells are filled with fluid (white star)

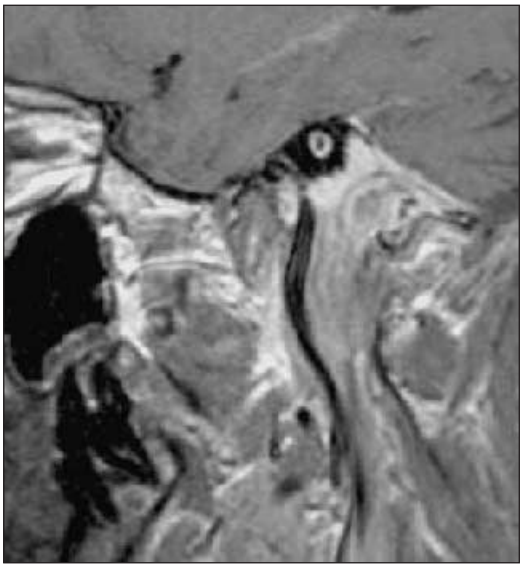

Figure 4 The sagittal contrast-enhanced T1-weighted image shows the primary jugular foramen meningioma with inferior involvement of the nasopharyngeal carotid space and anterior displacement of the carotid artery

dysfunction and dysphonia in addition. Ten months after surgery, postoperative MRI of the brain was performed. The MRI findings showed an extra-axial mass isointense to hypointense on T1-weighted sequences and intermediate on T2-weighted sequences, with strong enhancement after contrast administration. The mass was centered in the left JF with extensive en plaque involvement of the posterior fossa and prominent dural tails, which spread into the internal auditory canal (Figure 1, 2). The mass medially involved the clivus, while inferiorly it involved the carotid space (Figure 2, 3, 4). The MR signal intensity of the intracranial component for JFM was higher than of the extracranial component (Figure 1, 4).

One year after MRI, a control CT showed widening of the left JF with permeative erosion of its margins without sclerosis and extensive bone destruction around the left JF, as well as bone destruction of the basilar part of the left occipital bone, including the left occipital condil, the clivus and the lateral wall of the foramen magnum (Figure 5, 6). The walls of the carotide canal, the vestibular aqueduct, as well as the internal auditory canal were eroded (Figure 7). At this time the tympanic cavity was completely opacified while the ossicles were intact as they were on the first CT scans (Figure 5).

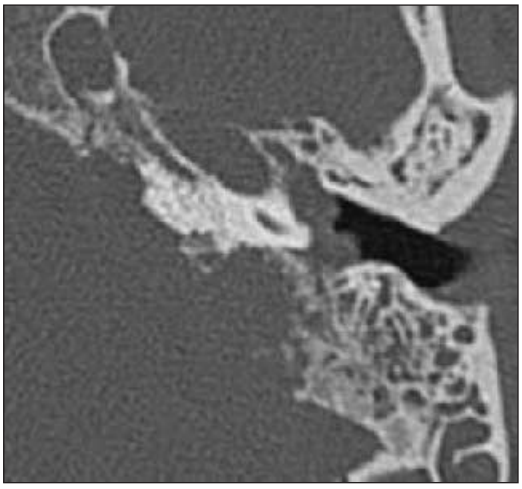

Figure 5 The bone window image from the axial CT scan shows widening of the left jugular foramen with permeative erosion of its margins without sclerosis. The image shows a soft tissue mass in the cavum tympani, as well as fluid in the mastoid air cells on the left

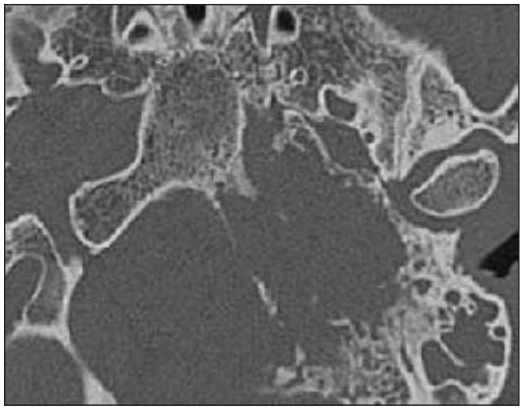

Figure 6 The bone window image from the axial CT scan shows extensive bone destruction of the basilar part of the left occipital bone

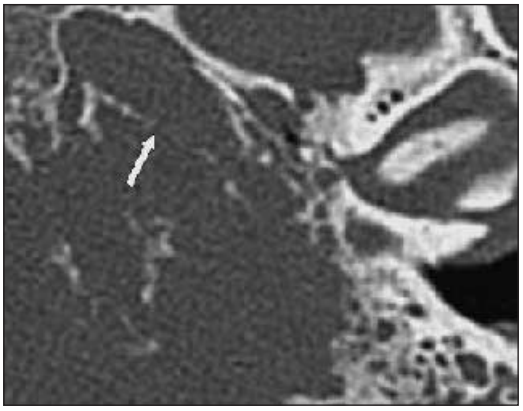

Figure 7 The bone window image from the axial CT scan shows the eroded wall of the left carotid canal (arrow)

Discussion

The jugular fossa is an anatomically complex region which consists of major vessels, the jugular bulb and vein, and multiple cranial nerves, including the cranial nerve IX, X, and XI (1). Due to this fact, patients with jugular fossa tumors (JFT) may have multiple cranial nerve dysfunctions. JFT are rare in clinical practice. JFM are most often considered in the differential diagnosis of glomus jugulare tumors (GJT), which account for 90% of JFT. They are followed by schwannomas of the lower cranial nerves, and then JFM, which account for about 0.7–9.3% of posterior fossa meningiomas (9, 10). Approximately, 112 cases of JFM have been reported in literature (8) and 40 of them are primary JFM (3). Samii and Ammirati in their series of 420 skull base meningiomas found only three primary JFM (0.7%) (8). In many of the reported cases, JFM mimicked GJT and lower cranial nerve neuromas (11). This is very important because different JFT have different surgical risks, and preoperative differential diagnosis is important for surgical planning and evaluation of postoperative morbidity (12). The identity of most lesions can be determined by a combination of spiral CT and MRI (2, 13). CT is useful for analysis of the JF bone margins as well as of adjacent skull base foramina. MRI with gadolinium shows the characteristics of a tumor, its vascularization, extension and its relationship to neighboring structures (1). Primary JFM often present very invasive features, infiltrating the surrounding skull base in all directions (3, 5, 6, 7, 8). This pattern of spread can be referred to as "centrifugal" and usually involves the temporal bone, including the middle ear cavity laterally and invading the skull base, including the jugular tubercle, hypoglossal canal, occipital condyle, and clivus medially. Inferior extracranial spread occurs into the nasopharyngeal carotid space of the deep suprahyoid neck. Further superior intracranial spread is seen along the intracranial dural reflections. This spread along the dura is termed "en plaque" and is characteristic of primary JFM. A globose appearance is seen less commonly in tumors with intracranial extension (2, 8). Theoretically, the pattern of spread allows differentiation between primary meningiomas, and the other fossa jugularis tumors (8). GJT typically involve the hypotympanum superolaterally, with limited involvement of the carotid space inferiorly. Infrequently, they extend medially into the jugular tubercle, hypoglossal canal,

and clivus (2). Unlike paragangliomas, JF schwannomas follow the course of the IX, X, and XI cranial nerves from the brainstem, with variable inferior spread. However, the pattern of spread is not totally reliable and can not form a basis on which to make a distinction between these entities (8). A helpful differentiating feature of JFM is the absence of the flow voids which are characteristic of GJT (2, 9). Macdonald et al. reported five cases of primary JFM. All of them were characterised by centrifugal infiltration of the surrounding skull base. Posterior fossa involvement had an "en plaque" appearance in four cases. All cases showed prominent dural tails. Flow voids were absent in all cases (2). We found all these features in our case.

On DSA, paragangliomas unlike meningiomas have typical angiographic appearance- a hypervascular mass, with enlarged feeding arteries, intense tumor blush, and early draining veins (13). In our case DSA revealed a vascular tumor without intense tumor blush what excluded GJT from the differential diagnosis of JFTs.

A recent study by Shimono et al. demonstrated differences in MR signal intensity and contrast enhancement between the intra- and extracranial components of JF meningiomas. The signal intensities of the intracranial component of JFM were significantly higher than those of the extracranial component on T1-, T2-, and postcontrast T1-weighted images (14). We also noted these different signals that are the best visualised on the postcontrast T1-weighted images (Figure 1). Primary JFM cause irregular enlargement of the JF. On CT scans, the JF margins have a mixed permeative-sclerotic appearance. On the other hand, GJT causes a permeative-destructive pattern, with erosion of the JF margins and infiltrated bone, without preservation of the underlying architecture or bone density. Neuroma gradually enlarges the JF by pressure erosion and gives an expanded and scalloped, but well-defined corticated margin to the JF (2, 9). In our case primary JFM caused permeative erosion of the JF margins and extensive bone destruction of affected bones (the petrous part of the left temporal bone and the basilar part of the left occipital bone) without any sclerosis. These findings are unusual for meningiomas and correspond more to GJT. Chen et al. reported a case of an angiomatous type of JFM with bony destruction around the JF without sclerotic change, which may be related to the histologic type of this tumor (12). In our case the etiology is unknown. It is not related to the histologic type of the tumor because it was a meningothelial meningioma, which is the most common tumor of the fossa jugularis (6).

Conclusion

Correct preoperative differential diagnosis of JF tumors is important for surgical planning and helps to avoid surgical pitfalls. MR and CT imaging provide an accurate distinction between meningioma and glomus tumor or schwannoma in most cases. High-resolution bone window CT is helpful for diagnosis, but in the case of the absence of hyperostosis and bone thickening around the jugular foramen, and when a permeative-destructive pattern is dominant, differential diagnosis between JFM and other tumors, especially GJT, is difficult by high-resolution CT. In that case the correct diagnosis should be based on MRI finding.

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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Clinical and therapeutic data of a child with ecthyma gangrenosum

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The presence of the *Pseudomonas aeruginosa* infection in healthy children is very uncommon. Infants may occasionally present with community-acquired sepsis due to *Pseudomonas aeruginosa*, preceded by prolonged contact with contaminated bath water. Ecthyma gangrenosum is a characteristic dermatological manifestation caused most commonly by *Pseudomonas aeruginosa*. We describe the clinical data and therapeutic management of a 6 month-old infant with ecthyma gangrenousum caused by *Pseudomonas aeruginosa*, complicated with septicaemia and meningitis. The infant was immunodeficient as indicated by hypogammaglobulinemia, neutropenia, and a low level of C3 complement component.

Key words: Pseudomonas aeruginosa, Ecthyma gangrenosum, Immunodeficiency, Neutropeni, Infant.

Introduction

Ecthyma gangrenosum (EG) is a characteristic dermatological infection caused most commonly by *Pseudomonas Aeruginosa* that penetrates deep, involving the dermis (1, 2, 4). The lesions begin as pink macules and progress to purple nodules, which become hemorrhagic eventually turning to ulcers with ecchymotic and gangrenous centres, with an eschar formation surrounded by an intense red areola (1, 2). These lesions are characterised by metastatic dissemination, bacterial invasion of blood vessels and deep abscesses (1). Ecthyma gangrenousum is caused by direct inoculation or it could be secondary to septicaemia. The most important predisposing factor that can lead to ecthyma gangrenousum is the presence of any kind of immunodeficiency associated with severe neutropenia (3, 10, 12). Other predisposing factors are low-birthweight, malnutrition, cystic fibrosis and immunosuppressive therapy.

Case report

A six-month-old child from Patos (South Albania) was admitted to the PICU of the University Hospital Center in Tirana, after 10 days of fever, vomiting and skin lesions. The initial lesion was a pink macula, which progressed to a purple nodule that became hemorrhagic and finally necrotic with scar

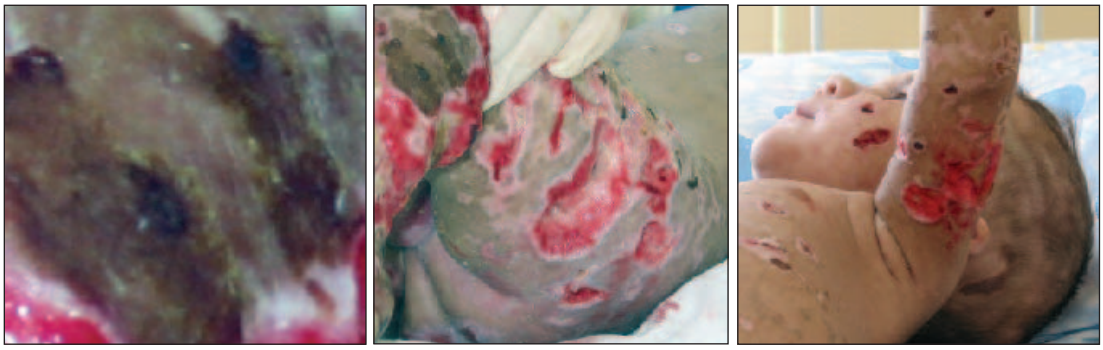

Figure 1 Distribution of ulcers and necrotic lesions in our patient.

formation, surrounded by an intense red areola.

Subsequent lesions with similar progression developed on the lower and upper extremities, despite outpatient treatment with oral antibiotics (amoxicillin and clavulanic). The child presented with a high fever (temperature 39-40°C) and vomiting. On physical examination the child was toxic febrile, drowsy, with oedema, tachypnoe, tachycardia, bulging fontanel and characteristic skin lesions: pink macules, purple nodules, hemorrhagic nodules progressing to ulcers and necrotic lesions (Figure 1).

Laboratory data: Red Blood Cells: 4130000/µl; White Blood Cells: 2400/µl; absolute neutrophil count: 480 cells/mm^3; Platelets: 97000/µl; erythrocyte sedimentation rate: 42 mm/hour; C-Reactive Protein-190mg/dl; Fibrinogen-550 mg/dl. The cerebrospinal fluid (CSF) was turbid, with leucocytosis greater than 1000/mm^3 and neutrophilic predominance (85%). Bacterial cultures were obtained from skin, blood, stool and urine, and antibiotic treatment with ceftriaxone + ampicillin was started. Dexamethason and mannitol were added for the management of meningitis). Despite the treatment new lesions appeared on both trunk and extremities, associated with deep abscesses. CSF and blood culture grew *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was also isolated from the samples obtained from the necrotic skin lesions. Stool and urine cultures were negative. These result confirmed the diagnosis: Ecthyma gangrenosum caused by *Pseudomonas aeruginosa*, complicated with sepsis and meningitis. Having completed the analysis of clinical data with etiological diagnosis, the decision was taken to treat the patient with combination therapy according to literature data (10, 14): ceftazidime + gentamicin, intravenous immunoglobulin for three consecutive days, followed by ceftazidime + ciprofloxacin. Immunologic function evaluation revealed low levels of C3 complement (0.368 g/l) and IgM (0.28 g/l). Elisa HIV was negative.

Three weeks after the beginning of therapy the clinical improvement was evident. The fever decreased, the cerebrospinal fluid was sterile and the erythrocyte sedimentation rate returned to normal. The culture obtained from the skin lesions produced no growth of pathogens. Although child improved clinically, and while awaiting surgical correction of the deep and large necrotic lesions, due to the immunologic status of the patient, local debridement was done, associated with antibiotic coverage to prevent a bacterial superposition. Forty days after admission, the patient was transferred for plastic surgical correction. After the reconstructive surgery, the child's situation appears satisfactory.

Discussion

The presence of *Pseudomonas aeruginosa aeruginosa* infection in previously healthy and imunocompetent children is very uncommon. Infants may occasionally present with community-acquired sepsis due to *Pseudomonas aeruginosa aeruginosa*, but this is very rare and is preceded by prolonged contact with contaminated bath water (1). The clinical appearance is very characteristic. The main site of EG lesions is the gluteal or perineal region (57%), although these lesions can spread to other body sites, as in our patient, in whom metastatic lesions appeared on both the trunk and lower extremities. Once considered unusual, ecthyma gangrenousum has received special attention in medical literature in recent years (4, 5, 11, 12, 13, 14). This disease has been related to life-threatening septicaemic infections and high mortality (8, 9, 11, 13, 14). Mortality rates of Pseudomonas sepsis in immunocompromised persons range from 38 to 96 %, whereas the mortality rate in non-bacteremic patients is 15.4%. (13, 14) Delayed presentation for medical evaluation and treatment in the case reported here was a complicating factor probably contributing to the severity of the condition

One major clinical feature in this patient was the presence of neutropenia. We found a low level of C3 complement component and hypogamaglobulinemia, that indicate an inefficient phagocytosis (1, 14) and this may explain why this patient's case was complicated with sepsis and meningitis. Appropriate antibiotic coverage for *Pseudomonas aeruginosa* and surgical debridement of the necrotic areas are the key to successful treatment. In default of other antipseudomona antibiotics in our country, ciprofloxacin was selected in this case despite the young age of the patient, due to the lack. Intravenous immunoglobulin was administered as well, and may have contributed to the favourable outcome. This successful empirical intervention has been previously reported in children with sepsis secondary to *Pseudomonas aeruginosa* (11).

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Sudden infant death syndrome: a case report in Bosnia and Herzegovina

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Sudden infant death syndrome (SIDS) is an idiopathic condition that affects seemingly healthy infants under one year of age, whose death remains unexplained after the performance of a complete postmortem examination, toxicological analyses, genetic testing, death scene investigation, and a review of the mother's and infant's medical history. Detailed epidemiological, pathological, and forensic information has been reported regarding SIDS from the developed countries. However, SIDS information from developing countries is either widely scattered or non-existent. This is the first published case report of SIDS from the country of Bosnia and Herzegovina. A previously healthy 3 months old, white male infant was found dead after being placed to sleep in the prone position. The features of this case report closely parallel the classical features of SIDS cited in the world literature. The infant was healthy, male, between the ages of 2-12 months, discovered in the prone position and had a negative postmortem investigation. It is important for developing countries such as Bosnia and Herzegovina to conduct detailed forensic investigations of deaths from SIDS and provide epidemiological, pathological, and circumstances of that information to the world's collective knowledge.

Key words: Sudden infant death syndrome, Bosnia and Herzegovina.

Introduction

Sudden infant death syndrome (SIDS) is defined as the sudden death of an infant whose cause remains unexplained even after a detailed review of the infant's and mother's medical history and medical records, a complete forensic autopsy and toxolgical examination, and a thorough investigation of the circumstances under which the death occurred (1, 2, 3). Although SIDS is defined as a syndrome and therefore the result of more than one disease, many researchers still believe SIDS is a unique entity due to its quality, which includes a greater frequency between the ages of 2-4 months, more frequent incidence among black male infants and the presence of intrathoracic petechiae (4). In developed countries, SIDS represents one of the most common causes of death after the

neonatal period (5). In Japan, its incidence is the lowest (0.09 per 1000 infants), and New Zealand, the largest (0.80 per 1000), while in the United States (U.S.) the mortality rate is 0.57 per 1000 infants (6). The incidence rate varies from as low as 0.046/1,000 in Hong Kong (7) to as high as 6.7/1,000 among the Native American Indians (8). The incidence in the developing countries such as Bosnia and Herzegovina is mostly unknown.

The etiology of SIDS is still rather vague (9). There are various theories, but none has been proven. The current model to explain SIDS is the "Triple Risk Model". The model suggests the interaction of three factors simultaneously that affects the infant: (a) an underlying vulnerability in the infant, (b) a critical developmental period, and (c) an exogenous stressor (10). The critical period for SIDS is the first year of life with the greatest risk occurring between the ages of 2-6 months. A major exogenous stressor associated with SIDS is the prone sleeping position. A number of studies in the world literature have rejected the association between the use of medicines and vaccinations and SIDS (11, 12, 13).

Risk factors for SIDS include modifiable risk factors such as the prone sleeping position, soft bedding, overheating due to over wrapping, and maternal smoking and drug abuse. Other modifiable risk factors associated with SIDS are poor prenatal care, teenager pregnancy and lack of prenatal care. Intrinsic risk factors include prematurity, male gender, and black race. A previous history of SIDS in the family, artificial feeding, difficulty sucking and swallowing, sleep apnea, periodic and episodic occurrence of "disappearance" of the child have also been reported to be linked with SIDS (14).

By presenting this case we are emphasizing the reality that SIDS could occur in all countries of the world and that pediatricians and community health services should educate parents about the recommended measures to eliminate risk factors for SIDS, and reduce infant mortality in cooperation with parents. In addition, it is necessary to emphasize to the medical and legal community the importance of conducting a proper death scene investigation and forensic examination of all infants that die suddenly and unexpectedly.

Case report

The circumstances of the death

In August 2007 a 3 month-old white male infant was brought to the Pediatric Services of Sokolac Health Centre in full cardiac arrest and was pronunced dead on arrival. The infant lived with his parents and the maternal grandfather. According to the mother the infant was healthy prior to death. At 11:30 AM the infant was bottle-fed cow's milk and placed in his crib by his mother, face down and covered with blankets.Approxaintly 1-2 hours later the mother reentered the room and discovered that the infant was dead. He was in the prone position, partially covered by blankets, with his hand on the pillow. There was no foam or vomit noted around the infant's mouth, according to the mother.

The infant was born by a spontaneous vaginal delivery in the hospital maternity ward, to a 22-year-old, healthy mother, who received prenatal care and had an uncomplicated pregnancy. His birth weight was 3150 grams and length 53 centimeters. This was the mother's second pregnancy. Her first was at age 20 which resulted in the birth of a healthy infant from a previous marriage.

Regular check-ups at one, two and three months of life revealed that the infant's growth and development were within normal limits. The infant was vaccinated according to the standard protocol, receiving BCG vaccine, and two doses of Hepatitis B vaccine. Five days before his death he received one dose of combined diphtheria, tet-

anus and pertussis vaccine and inactivated polio vaccine. According to the mother, no post-vaccination reaction, such as fever, was observed .

The family history revealed that the maternal uncle had died at the age of 2 months and the maternal great-uncle had died at the age of 3 months, both as previously healthy infants. At the time of this infant's death the mother was unemployed and externally emotionally unstable.

The father of this infant is a 36 year-old physical laborer. The father was born with a cleft lip and with limited mental function. The father's sister was treated for epilepsy in early childhood. The social status of the family was on the verge of poverty.

Death scene investigation

The death was reported to the local police and they conducted a detailed death scene investigation. The infant's room was noted as humid. Note the summer season, beginning in August. The infant's sleeping environment consisted of a crib. The crib contained several blankets and a large soft mattress (Figure 1).

Figure 1 The appearance of the crib immediately after the death

Forensic investigation of the infant

A forensic autopsy was performed on the white male infant, of the stated age of 3 months at the Institute for Forensic Medicine in Sarajevo. The infant was 62 cm long and weighted 5850 grams (50th percentile). The external examination revealed that the extremities displayed rigor mortis. There were no signs of external trauma or violence. Mild perioral cyanosis, with red spots were noted on the back and neck region (Figure 2). Examination of the internal organs showed general edema and cyanosis. However, these morphological changes were minor and nonspecific to a specific cause of death.

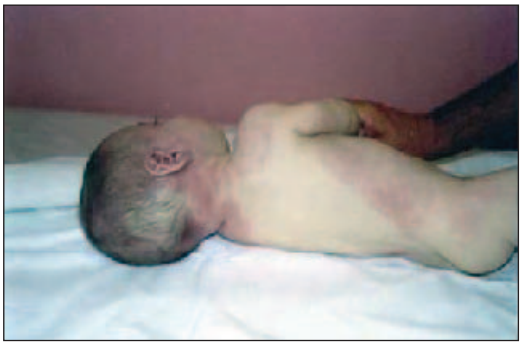

Figure 2 The appearance of the child in the outpatient clinic during the external inspection

The conclusions of the forensic autopsy examination and the death scene investigation ruled the death of this infant as natural death. The morphological changes seen in the external and internal organs, specially the edema of the pulmonary tissue and brain, were non-specific findings. Such morphological changes are typically seen in infants that die from sudden infant death syndrome.

Discussion

Sudden infant death syndrome (SIDS) is an idiopathic condition that typically affects infants during their first year of life and is characterized by a negative post mortem examination, death investigation, and medical history. The syndrome has been present from antiquity - there are even references

in the Old Testament of the Bible (15). The overall number of SIDS in the U.S. and other developed countries has been dramatically decreasing since 1992 corresponding with the "Back to Sleep" campaign (15). While the cause of SIDS still remains elusive, epidemiological studies have identified modifiable risk factors associated with SIDS, which include infants sleeping in the prone position, infants sleeping on a nonstandard sleep surface, and bed sharing.

A great amount of information is known about the features of SIDS in the developed countries. However, in developing countries such as Bosnia and Herzegovina even basic epidemiological data on infants who have died of SIDS is lacking. It is critical that pediatricians educate future mothers of the risk of SIDS especially the modifiable behaviors associated with SIDS. The legal and forensic community must also play a part in conducting thorough and complete investigations of all deaths involving infants. In addition, forensic epidemiologists must work with these developing countries and the forensic establishment to create SIDS databases and to analysis and interpret the data. The collection of information is important for two reasons: first, to establish the epidemiological patterns of SIDS deaths in Bosnia and Herzegovina and allow for comparisons to patterns cited in other countries; second, to allow for the identification of the many unique risk factors specific to the region of Bosnia and Herzegovina.

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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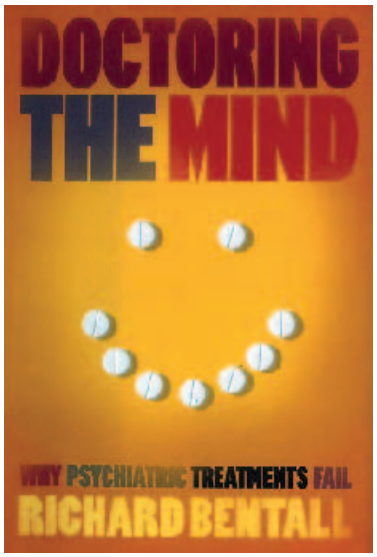
15. Mitchell EA. SIDS: Past, Present and Future. Acta Paediatrica. 2009;98:1712-19.

Richard P. Bentall: "Doctoring the Mind. Why Psychiatric Treatments Fail".

London, Allen Lane, 2009, ISBN 978-0-713-99889-4.



R. P. Bentall is Professor of Clinical Psychology at the University of Bangor in Wales (UK). He has published extensively in the field of the causes and treatment of severe mental illnesses. His previous book, "Madness Explained: Psychosis and Human Nature", won the British Psychological Society Book Award for 2004.

In terms of his view of psychiatry Bentall is a member of a loosely tied group of psychiatrists and psychologists, mostly of British origin, who have, over the last fifteen years or so, fiercely criticized the foundations of contemporary psychiatry. Patrick Bracken and Philip Thomas, from Bradford, in the UK, are the most prominent psychiatrists in the aforesaid group. They appear to be more philosophically minded than Bentall. Bracken and Thomas maintain that in postmodern times psychiatry should also be postmodern. They dub such postmodern psychiatry *postpsychiatry*. Bentall, on his part, has named his approach *rational antipsychiatry*. (Interestingly enough, Bentall makes no reference to Bracken and Thomas in his book.)

The biomedical psychiatric model that has by and large prevailed in psychiatry for the last three to four decades, is the target of an all-out attack by postpsychiatrists and rational antipsychiatrists alike. They especially critically examine the corollaries of the biomedical model: belief that psychiatric disorders are genetic diseases ("the fundamental error of psychiatry" (p. 113); that antipsychotic drugs are successful "at helping some of the most distressed and vulnerable people in our society"; that diagnoses are of much help in providing services to the mentally ill; that symptom reduction should be the major goal of psychiatric treatment; that there is a clear boundary between severe mental illnesses and normality; and that psychological and social treatment is second-rate treatment of people with severe mental illness.

Indeed, there are a great many imperfections, contradictions and dilemmas in contemporary psychiatry, and all of them should be thoroughly scrutinized for the sake of patients, in the first place. Also, there is no doubt that the dominance of the biomedical model has had, to put it mildly, a negative impact on the overall care of people with mental illness. Furthermore, the congruity between modern day positivism and the financial interests of pharmaceutical companies in preserving the biomedical as the only valid psychiatric model, is not difficult to detect. However, it seems that recent critics of psychiatry have pushed the pendulum too far in the opposite direction. They have been too eager to challenge virtually all aspects of contemporary psychiatry, quite often failing to substantiate their allegations. For example, it is hard to lend credence to Bentall's assertion that "severe mental illness is an understandable reaction to the tribulations of life" (p. 269). Here is another example of Bentall's flawed way of looking at some basic aspects of today's psychiatry. When he says "most doctors are valued members of psychiatric teams, but they have few unique skills (for example, other mental health professionals can learn to prescribe drugs)" (p. 269), he overlooks the fact that only those who are knowledgeable in neuro- and psychopharmacology, and neurophysiology and biochemistry as well, are able to prescribe drugs in a proper way. "Prescribing drugs" is not a matter of simply "learning to prescribe drugs".

In conclusion, I would say that this book confirms the impression gained by the reader of his previous book: Bentall, along with the British postpsychiatrists, has come out from under Laing's overcoat. This refers primarily to Bentall's claim that each psychosis becomes understandable when one looks closely at psychotic experience, that in the treatment of psychotic people psychological methods should take precedence over biological ones, and that psychiatric diagnoses are no more than labels.

Dušan Kecmanović

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International publications of authors from Bosnia and Herzegovina in Current Contents indexed publications in the first half of 2010

1. Babić D, Martinac M, Bjelanović V, Babić R, Sutović A, Sinanović O. Aggression in war veterans suffering from posttraumatic stress disorder with co-morbid alcoholism. Coll Antropol. 2010 Mar;34 Suppl 1:23-8.

Department of Psychiatry, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

For thousands of years it has been known that aggression as a symptom appears in numerous psychiatric disorders and diseases. During the last decade the appearance of the aggressive behavior related to the posttraumatic stress disorder (PTSD) has been frequently investigated, often associated with war trauma. The goal of this study is to analyze the impact of alcoholism on a way war veterans suffering from chronic PTSD express and control aggression. The sample included 240 war veterans with chronic PTSD. The subjects were divided in two groups. PTSD group (n=147) and controlled group composed of those suffering from alcoholism in addition to PTSD (n=93). In this study, the following psychological instruments were used: The Harvard trauma questionnaire for PTSD diagnosis (HTQ); the questionnaire for self-evaluation of aggression (STAXI); The Profile Index Emotion (PIE); questionnaire for auto-diagnosis of alcoholism (CAGE). The obtained results indicate that subjects who have PTSD with co-morbid alcoholism are more deprived, aggressive ($p < 0.001$) and oppositional ($p < 0.05$) in comparison to subjects whose PTSD is not combined with alcoholism (PIE). The aggression is statistically more expressed in subjects with PTSD who have also been diagnosed with alcoholism on all subscales in comparison to subjects with PTDS who have not been diagnosed with alcoholism: the current state of aggression, the general state of aggression, aggression towards an unfair treatment, aggression directed inwards and outwards ($p < 0.001$); aggression towards nonspecific provocation and a general way of expressing aggression ($p < 0.05$) (STAXI). Subjects that had PTSD combined with alcoholism show a higher degree of aggression in comparison to subjects with PTDS who are not diagnosed with alcoholism.

2. Bašić B, Beganović A, Samek D, Skopljak-Beganović A, Gazdić-Šantić M. Ten years of monitoring the occupational radiation exposure in Bosnia and Herzegovina. Radiat Prot Dosimetry. 2010 Apr-May;139(1-3):400-2. Epub 2010 Feb 11.

Radiation Protection Centre of the Institute of Public Health of Federation of Bosnia and Herzegovina, M. Tita 9, Sarajevo, Bosnia and Herzegovina.

Monitoring of occupationally exposed persons in Bosnia and Herzegovina started in 1960s and it was interrupted in 1992. Dosimetry service resumed in 1999 when the International Atomic Energy Agency provided Harshaw 4500 TLD-reader and the first set of TLDs for the Radiation Protection Centre (RPC) of the Institute of Public Health of the Federation of Bosnia and Herzegovina. In January 2009, the RPC covered 1279 professionals with personal dosimetry, which is more than 70 % of all radiation workers in the country. Most of the TLD users work in medical institutions. In period 1999-2003 RPC provided 984 workers with dosemeters. In the next 5 y period (2004-2008), the number of persons covered by do-

simetry increased by an average of 51 %. The mean and collective effective dose in the period 1999-2003 were 1.55 mSv and 1.54 personSv, respectively. In the period 2004-2008, the mean doses changed by 1 % on average, but the collective effective dose increased by 53 % for all practices. Mean and collective effective dose were 1.57 mSv and 2.34 personSv, respectively. The highest personal doses are associated with industrial radiography, than exposures in nuclear medicine. Radiology plays a significant role in collective dose only, whereas other exposures are low. Results correspond to results found in the literature. New practices in industry and medicine emphasise the need for more personal dosemeters, as well as specialised dosemeters for extremities monitoring, etc.

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3. Beganović A, Kulić M, Spužić M, Gazdić-Šantić M, Skopljak-Beganović A, Drljević A, Džanić S, Bašić B, Lincender L. Patient doses in interventional cardiology in Bosnia and Herzegovina: first results. Radiat Prot Dosimetry. 2010 Apr-May;139(1-3):254-7. Epub 2010 Mar 11.

Department of Medical Physics and Radiation Safety, Clinical Centre of Sarajevo University, Bolnicka 25, 71000 Sarajevo, Bosnia and Herzegovina.

Cardiologists at the Cardiac Centre of the Clinical Centre of Sarajevo University performed invasive cardiology procedures in one room equipped with a Siemens Coroskop (Siemens Healthcare, Erlangen, Germany) unit with the possibility of digital cine imaging. The number of procedures performed with this unit is 1126 per year. The number of adults performing only diagnostic procedures is 816, therapeutic procedures 62 and both diagnostic and therapeutic 228. Twenty diagnostic examinations but no therapeutic procedure are performed on children per year. The workload is increasing year by year, with an average increase of 26 % per year. The X-ray system does not have a kerma area product (KAP) meter installed; therefore an external KAP meter was mounted on the X-ray tube. Gafchromic dosimetry films (International Specialty Products, Wayne, USA) were placed under the patient to record the skin dose distribution. The peak skin dose (PSD) was calculated from the maximum optical density of the dosimetry films. Dose measurements were performed on 51 patients undergoing therapeutic procedures (percutaneous transluminal coronary angioplasty and stent placement). Two patients received doses (KAP) larger than 100 Gycm(2). The PSD was higher than 1 Gy in 3 out of 16 evaluations, and one of these patients received a skin dose >2 Gy. No deterministic skin effects were recorded. The dosimetry results are similar to results reported in other countries. Invasive cardiac procedures deliver high doses to the skin that could cause deterministic effects (erythema). Physicians performing these procedures should be aware of these risks. More efforts should be put into the training of cardiologists in radiation protection.

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4. Curić I, Curić S, Nikolić J, Vasilj I. Public health services in Herzegovina region during 1992-1995 war. Coll Antropol. 2010 Mar;34 Suppl 1:321-4.

Clinic for Infectious Diseases, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The aim of this study was to describe the situation and the development of Croatian Defense Council medical corps during the 1992-1995 war in Bosnia and Herzegovina. The paper provides an overview and describes the main events that lead to development of the medical care in the wartime conditions, with special emphasis on the public health system in Herzegovina region. This included the creation of three distinctive public health system settings: initial, integral and post-war period, all marked by certain specificities in organization and delivery of the public health and overall health care to both military and civilians. The knowledge and skills gathered during this period can be useful in situations that involve the need for fast public health actions, such as various natural disasters and disease outbreaks, and could be used for establishing highly mobile response public health teams. Furthermore, the experiences gathered during these periods may be useful during the planning phases of the health care reforms, all aiming to deliver the best possible health care to the entire population.

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5. Čarapina M, Jurić M, Bubalo P, Leventić M. Myoepithelial carcinoma of the parotid gland. Coll Antropol. 2010 Mar;34 Suppl 1:283-6.

Clinical Institute of Pathology, Cytology and Forensic Medicine, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

Rare malignant tumor of the salivary gland, a myoepithelial carcinoma, arose de novo in the right parotid gland. The initial tumor was composed predominantly of myoepithelial cells. Subsequently the tumor recurred three times, with infiltration of the bones of the cranial base. Histological examination showed sarcomatoid neoplasm composed of malignant spindle cells with high mitotic rate and perineural invasion. There was no involvement of cervical lymph nodes. Immunohistochemistry demonstrated myoepithelial differentiation: tumor cells were positively stained with vimentin, alpha smooth muscle actin and S-100 protein antibodies, and focal positively was noticed with cytokeratin (AE1/AE3) antibody. Large number of tumor cells nuclei was reactive with the monoclonal anti-p63

antibody, clone 4A4. Myoepithelial carcinomas exhibit a wide spectrum of morphological heterogeneity and for that reason could be confused with many tumors. Cytoarchitectural patterns and immunohistochemical profile are crucial for identification. These tumors are malignant neoplasms with diverse clinical outcomes, sometimes very aggressive.

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6. Ćavar M, Sekulić D, Čuljak Z. Complex Interaction of Religiousness with other Factors in Relation to Substance Use and Misuse Among Female Athletes. J Relig Health. 2010 May 6. [Epub ahead of print]

Faculty of Science, Mathematics and Education, University of Mostar, Mostar, 88000, Bosnia and Herzegovina.

Strength of religious faith (SRF) is rarely studied as a protective factor against substance use and misuse in sports. Herein, we studied the potential buffering effect of the complex socio-educational, sports, and religiousness factors in the protection against substance use and misuse, including cigarettes, analgesics, appetite suppressants, potential doping behavior, and binge drinking. The sample of subjects included 40 high-class female athletes (22-26 years of age). Using a strictly anonymous questionnaire, we investigated different social, educational, and sports factors (including SRF measured by the Santa Clara Strength of Religious Faith Questionnaire) in relation to substance use and misuse. Following the calculation of simple correlations, multiple regression analysis revealed that in combination with low sports experience, SRF has a significant buffering effect against binge alcohol drinking and consumption of appetite suppressants. The data are discussed in comparison with previous findings and theoretical background. Future studies should study the topic while observing samples of recreational and competitive athletes of both genders.

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7. Đođibegovic J, Marjanović A, Šober M, Škrbo A, Sinanović K, Larssen T, Grung M, Fjeld E, Rognerud S. Levels of persistent organic pollutants in the Neretva River (Bosnia and Herzegovina) determined by deployment of semipermeable membrane devices (SPMD). J Environ Sci Health B. 2010 Feb;45(2):128-36.

University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

The main objective of this study was to determine levels of certain persistent organic pollutants (POPs) in Neretva River, Bosnia and Herzegovina (BiH), which is currently facing implementation of the Stockholm Convention on persistent organic pollutants (POPs) and environmental protection strategies. This is the very first report on the deployment of semipermeable membrane devices (SPMDs) in BiH. SPMDs were used for continuous 3-weeks sampling of POPs at three locations, covering 220 km long stream of the Neretva River. Water concentrations of polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) and polybrominated diphenylethers (PBDEs) were calculated using performance reference compounds (PRCs). The total OCP concentrations ranged from 40 to 140 pg L(-1) and most of compounds were detected only in lower course of the river. Total PAH ranged from 160 to 4000 pg L(-1) and show a clear spatial variation. Dominant PAHs were phenanthrene, fluoranthene, fluorene and acenaphthene. Total PCB ranged from undetectable to 120 pg L(-1). From the group of 15 PBDE congeners investigated, only PBDE-47 and PBDE-99 were detected. Since the concentrations of broad spectrum of POPs found in the Neretva River are quite low, future actions should be focused on preservation rather than on sanitation measures. Regular monitoring should anyhow be established.

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8. Fajkić A, Lepara O, Voráček M, Kapusta ND, Niederkrotenthaler T, Amiri L, Sonneck G, Dervić K. Child and adolescent suicides in Bosnia and Herzegovina before and after the war (1992-1995). Crisis. 2010;31(3):160-4.

Institute of Pathophysiology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

BACKGROUND: Evidence on youth suicides from Southeastern Europe is scarce. We are not aware of previous reports from Bosnia and Herzegovina, which experienced war from 1992 to 1995. Durkheim's theory of suicide predicts decreased suicide rates in wartime and increased rates afterward. AIMS: To compare child and adolescent suicides in Bosnia and Herzegovina before and after the war. METHODS: Data on youth suicide for prewar (1986-90) and postwar (2002-06) periods were analyzed with respect to prevalence, sex and age differences, and suicide methods. Suicide data from 1991 through 2001 were not available. RESULTS: Overall youth suicide rates were one-third lower in the postwar than in the prewar period. This effect was most pronounced for girls, whose postwar suicide rates almost halved, and for 15-19-year-old boys, whose rates decreased by about a one-fourth. Suicides increased among boys aged 14 or younger. Firearm suicides almost doubled proportionally and were the predominant postwar method, while the most common prewar method had been hanging. CONCLUSIONS: The findings from this study indicate the need for public education in Bosnia and Herzegovina on the role of firearm accessibility

in youth suicide and for instructions on safe storage in households. Moreover, raising societal awareness about suicide risk factors and suicide prevention is needed.

9. Gazibegović-Busuladžić A, Milošević DB, Becker W, Bergues B, Hultgren H, Kiyan IY. Electron rescattering in above-threshold photodetachment of negative ions. Phys Rev Lett. 2010 Mar 12;104(10):103004. Epub 2010 Mar 12.

Faculty of Science, University of Sarajevo, Zmaja od Bosne 35, 71000 Sarajevo, Bosnia and Herzegovina.

We present experimental and theoretical results on photodetachment of Br(-) and F(-) in a strong infrared laser field. The observed photoelectron spectra of Br(-) exhibit a high-energy plateau along the laser polarization direction, which is identified as being due to the rescattering effect. The shape and the extension of the plateau is found to be influenced by the depletion of negative ions during the interaction with the laser pulse. Our findings represent the first observation of electron rescattering in above-threshold photodetachment of an atomic system with a short-range potential.

10. Goletić T, Gagić A, Rešidbegović E, Kustura A, Kavazović A, Savić V, Harder T, Starick E, Prašović S. Highly pathogenic avian influenza virus subtype H5N1 in mute swans (Cygnus olor) in Central Bosnia. Avian Dis. 2010 Mar;54(1 Suppl):496-501.

Sarajevo Veterinary Faculty, National Reference Laboratory for Avian Influenza and Newcastle Disease, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina.

In order to determine the actual prevalence of avian influenza viruses (AIVs) in wild birds in Bosnia and Herzegovina, extensive surveillance was carried out between October 2005 and April 2006. A total of 394 samples representing 41 bird species were examined for the presence of influenza A virus using virus isolation in embryonated chicken eggs, PCR, and nucleotide sequencing. AIV subtype H5N1 was detected in two mute swans (Cygnus olor). The isolates were determined to be highly pathogenic avian influenza (HPAI) virus and the hemagglutinin sequence was closely similar to A/Cygnus olor/Astrakhan/ Ast05-2-10/2005 (H5N1). This is the first report of HPAI subtype H5N1 in Bosnia and Herzegovina.

11. Hadžović E, Betz G, Hadžidedić Š, El-Arini SK, Leuenberger H. Roller compaction of different pseudopolymorphic forms of Theophylline: Effect on compressibility and tablet properties. Int J Pharm. 2010 Jun 19. [Epub ahead of print]

Industrial Pharmacy Lab, Department of Pharmaceutical Sciences, University of Basel, Klingelbergstr. 50, 4056 Basel, Switzerland; Bosnalijek d.d., Development Department, Jukićeva 53, 71000 Sarajevo, Bosnia and Herzegovina.

The effect of roller compaction on disintegration time, dissolution rate and compressibility of tablets prepared from Theophylline anhydrate powder, Theophylline anhydrate fine powder and Theophylline monohydrate was studied. In addition, the influence of adding microcrystalline cellulose, a commonly used excipient, in mixtures with these materials was investigated. Theophylline anhydrate powder was used as a model drug to investigate the influence of different compaction pressures on the tablet properties. Tablets with same porosity were prepared by direct compaction and by roller compaction/re-compaction. Compressibility was characterized by Heckel and modified Heckel equations. Due to the property of polymorphic materials to change their form during milling and compression, X-ray diffraction analysis of Theophylline anhydrate powder, Theophylline anhydrate fine powder and Theophylline monohydrate powders and granules was carried out. After roller compaction the disintegration time and the dissolution rate of the tablets were significantly improved. Compressibility of Theophylline anhydrate powder and Theophylline anhydrate fine powder was decreased, while Theophylline monohydrate showed higher compressibility after roller compaction. Microcrystalline cellulose affected compressibility of Theophylline anhydrate powder, Theophylline anhydrate fine powder and Theophylline monohydrate whereby the binary mixtures showed higher compressibility than the individual materials. X-ray diffraction analyses confirmed that there were no polymorphic/pseudopolymorphic changes after roller compaction.

12. Hudić I, Fatušić Z, Kamerić L, Mišić M, Šerak I, Latifagić A. Vaginal delivery after Misgav-Ladach cesarean section - Is the risk of uterine rupture acceptable? J Matern Fetal Neonatal Med. 2010 Jan 19. [Epub ahead of print]

Clinic of Gynecology and Obstetrics, University Clinical Center Tuzla, Tuzla, Bosnia and Herzegovina.

Objective. To evaluate whether the single-layer closure as is a routine by the Misgav-Ladach method compared to the double-layer closure as used by the Dörfler cesarean method is associated with an increased risk of uterine rupture in the subsequent pregnancy and delivery. 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. All patients with one previous cesarean section who at-

tempted vaginal birth following cesarean section were managed from 1 January 2002 to 31 December 2008. Exclusion criteria included multiple gestation, greater than one previous cesarean section, previous incision other than low transverse, gestational age at delivery less than 37 weeks and induction of delivery. We identified 448 patients who met inclusion criteria. Results. We found that 303 patients had a single-layer closure (Misgav-Ladach) and 145 had a double-layer closure (Dörffler) of the previous uterine incision. There were 35 cases of uterine rupture. Of those patients with previous single-layer closure, 5.28% (16/303) had a uterine rupture compared to 13.11% (19/145) in the double-layer closure group ($p < 0.05$). Conclusion. We have not found that a Misgav-Ladach cesarean section method (single-layer uterine closure) might be more likely to result in uterine rupture in women who attempted a vaginal birth after a previous cesarean delivery. This cesarean section method should find its confirmation in everyday clinical practice.

13. Ibralić I, Sinanović O, Memišević H. Age at menarche and premenstrual syndrome in adolescent girls with intellectual disability in Bosnia and Herzegovina. Res Dev Disabil. 2010 May-Jun;31(3):800-3. Epub 2010 Mar 6.

Center Vladimir Nazor, Sarajevo, Bosnia and Herzegovina.

The issues involving menstruation are the topic of many scientific inquiries in the fields of medicine, psychology, sociology and anthropology. The aim of this study was to determine the age at menarche and the most common symptoms of premenstrual syndrome (PMS) in adolescent girls with intellectual disability. The main method of data collection was through the use of a survey and an interview with the girls. The sample consisted of 31 adolescent girls with intellectual disability and 31 adolescent girls without intellectual disability serving as a control group. Both groups were between the ages of 14 and 18 years. The results of this study revealed higher variability of age at menarche in girls with intellectual disability compared to girls without intellectual disability. The symptoms of PMS were almost equally distributed in both groups of girls. Many girls with intellectual disability do not have enough knowledge about menstruation. More attention needs to be given to treating the symptoms of PMS and educating the girls in a school setting.

14. Ivanković A, Ravlija J, Škobić H, Vasilj I, Ivanković Z, Pejanović-Škobić N, Pavleković G. Health status of population in Federation of Bosnia and Herzegovina in 15 years of transitional period. Coll Antropol. 2010 Mar;34 Suppl 1:325-33.

Medical Faculty, University of Mostar, Mostar, Bosnia and Herzegovina.

War in Bosnia and Herzegovina lasted from 1991 to 1995 and resulted in profound consequences marked by the large number of victims, increase in the diseases and disorders prevalence, that were not common before it occurred. The effects it had on health status of the entire population was reflected through many negative demographic trends, increasing prevalence of chronic diseases and the spread of a number of unhealthy behavioral patterns and a lot of migrations. All this presents a problem for institutions of health system which are attempting to control these negative influences especially during the transition period, marked by the direct adverse consequences of the 1991-1995 war. The present paper presents a summation of various sources which are attempting to provide a synthetic overview and provide basic information in relation to the health status of the population, and also to provide a baseline evaluation for deployment of public health interventions.

15. Jurić M, Čarapina M, Gilja A, Šimić G. Knowledge, attitudes and behaviors of young people related to drinking and driving in Mostar region, Bosnia and Herzegovina. Coll Antropol. 2010 Mar;34 Suppl 1:39-44.

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

The traffic accidents became the leading cause of morbidity and mortality among young population groups during the late 60s and early 70s. Among several European countries that are in transition, Bosnia and Herzegovina takes the leading place in fatal traffic accidents. In this study we have investigated knowledge, attitudes and behavior of young people related to alcohol impaired driving. Our aim was to investigate the patterns and behavior among young people that could be useful for public health intervention among them. This is of special interest as there is a lack of such information from Bosnia and Herzegovina. The study was performed in the city of Mostar, Bosnia and Herzegovina. The study included 189 examinees of both genders, aged between 18 and 24 years, who have been divided in two groups: students of the University of Mostar and those who did not go for further education after high school (nonstudents). Sampling was performed in July 2006 in Mostar and surrounding area. The Gallup organization questionnaire was used. Descriptive statistic test and chi-square were used in statistical analysis. Result of this study could be helpful in taking preventive measures for lowering number of traffic accidents among young people or they could be base in some protective programs for increasing traffic safety.

16. Jurić M, Novaković J, Čarapina M, Knežević E. Treatment cost of patients with maxillofacial fractures at the University Hospital in Mostar 2002-2006. Coll Antropol. 2010 Mar;34 Suppl 1:199-203.

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

The aim of this study was to establish the costs structure of medical treatment for the patients with maxillofacial fractures, to perform a treatment cost evaluation, describe the factors which considerably influence the costs and discover the ways of achieving financial savings in treated patients. The study group consisted of patients with maxillofacial fractures who were admitted and treated at the Department of Maxillofacial Surgery of the University Hospital Mostar in the period from January 2002 until December 2006. Data for the study were collected from the patients' databases, case histories and data obtained on the basis of individual payments for the treatment that was collected by Finance Department of the University Hospital of Mostar Most patients in this study were men (83%), of average age 34 +/- 19 years. Zygomatic bone fracture was the commonest injury. Open surgical procedure was performed in 84.7% of treated cases. The costs for the open procedure were considerably higher than conservative treatment. Medication cost made up a total of 37.9% and cost of hospital accommodation 27.3% out of total hospital charge. Cost reduction in treated patients with maxillofacial fractures should be achieved through protocols of urgent treatment of maxillofacial trauma patients immediately after sustaining an injury and with earlier discharge of the patients when postoperative complications are not expected.

17. Klarić M, Frančišković T, Pernar M, Nemčić Moro I, Milićević R, Černi Obrdalj E, Salčin Satriano A. Caregiver burden and burnout in partners of war veterans with post-traumatic stress disorder. Coll Antropol. 2010 Mar;34 Suppl 1:15-21.

Department of Psychiatry, Faculty of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina.

War veterans diagnosed with chronic post-traumatic stress disorder (PTSD) experience serious difficulties in social, professional and family life. Consequently, their wives often become indirect victims of their husbands' dysfunction. The purpose of this study was to assess the caregiver burden and burnout level in partners of veterans suffering from PTSD, especially in cases where partners suffer from their own PTSD symptoms. The experimental group consisted of 154 wives or partners of war veterans treated for PTSD caused by the war trauma in University Hospital Mostar. The control group was made of 77 wives or partners of war veterans without PTSD. The study was based on the General Demographic Questionnaire, the Harvard Trauma Questionnaire, Bosnia-Herzegovina version, Caregiving and the Experience of Subjective and Objective Burden and the Maslach Burnout Inventory. The wives of PTSD affected veterans scored significantly higher in all subscales of the Caregiver Burden Questionnaire and the Burnout Inventory. The results indicated that subjective demand burden, subjective stress and burnout were significantly higher in relationships in which both partners suffer from PTSD compared to couples in which only the veteran suffers from PTSD and couples in which none of the partners has PTSD. Living with a veteran diagnosed with PTSD places a heavy burden on the wife and poses a serious risk of burnout, which has to be taken into account in treatment planning.

18. Klupka-Sarić I, Galić M. Epidemiology of multiple sclerosis in western Herzegovina and Herzegovina--Neretva Canton, Bosnia and Herzegovina. Coll Antropol. 2010 Mar;34 Suppl 1:189-93.

Department of Neurology, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The aim of this study was to investigate the selected indicators of multiple sclerosis (MS) in Herzegovina (Western Herzegovina Canton and Herzegovina-Neretva Canton). By using all available health and medical sources in the studied area and using McDonald's criteria, a total of 96 patients were identified in the period from 1996 to 2006. Results of the study show that the crude prevalence of MS was 30.99/100,000 (95% confidence interval [CIC 24.8-37.2), the highest one in the municipality of Posusje (49.6/100,000) and the lowest one in the municipalities of Neum and Ravno (no recorded cases); the female/male ratio was 1.5; the mean age of the patients on the prevalence day was 41.4 +/- 10.2 years and the mean age at the disease onset was 30.7 +/- 6.4 years; the most often clinical course of the disease was relapsing-remitting (58%), secondary progressive course was present in 28% patients, primary progressive in 9% and progressive relapsing in 1% of patients; the most frequent initial signs of the disease were motor (33%) and sensory ones (24%). According to the results of the study, the south-western part of Bosnia and Herzegovina is an area on the crossing from moderate risk to high risk zone for MS. The distribution of MS is heterogeneous. MS was more prevalent in the municipalities with colder climate and more winter precipitation and it is not present in the coastal region with warmer climate and almost without winter precipitation.

19. Kordić M, Babić D, Petrov B, Kordić J, Jelavić B, Pivić G. The meteorological factors associated with suicide. Coll Antropol. 2010 Mar;34 Suppl 1:151-5.

Health Centre Široki Brijeg, Široki Brijeg, Bosnia and Herzegovina.

Looking through the history, people have always been associating suicide with weather conditions, trying to understand and identify the relationship between meteorological factors and suicide. The aim of this study was to determine and analyze the meteorological conditions in the time of attempted or committed suicides, and examine the possible link between the changes of meteorological factors and the frequency of suicidal behavior. Retrospective study of pairs covered the period from January 2003 to January 2006. Examinees included in the study were persons who committed or attempted suicide in the region of Mostar. Meteorological factors included the days of attempts or committing of suicide, with meteorological factors of the day immediately prior to the days of attempts or committing of suicide, as well as with average monthly values of meteorological factors. Meteorological factors of the days with suicidal behavior were similar to the day prior to the days with suicidal behavior, but there was significant difference between meteorological factors of the days with suicidal behavior and average monthly values of meteorological factors: maximum pressure was significantly lower in the days with suicidal risk, as well as pressure gradient. Regarding the seasonal periods, examinees most frequently attempted to commit suicide in April. Results indicate that meteorological factors do not act as an acute stress factor for suicide behavior, but its change over time may be the trigger for a suicide attempt.

20. Kozomara D, Galić G, Brekalo Z, Šutalo N, Kvesić A, Šoljić M. A randomised two-way comparison of mastectomy performed using harmonic scalpel or monopolar diathermy. Coll Antropol. 2010 Mar;34 Suppl 1:105-12.

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

The purpose of this study was to perform an overall evaluation and comparison of the success rate of modified radical mastectomy by harmonic scalpel and monopolar electrocauter The prospective study included all of the patients that were planned for and mastectiomized because of breast carcinoma during July 1st 2008 until December 21st 2008 at the Department of Surgery and Urology, University Hospital Mostar. Duration of the surgical procedure, intraoperative blood loss and operational drain secretion was measured and registered. Leukocyte number (Le), interleukin 6 (IL-6), C-reactive protein (CRP) and erythrocyte sedimentation rate was tested and registered out of peripheral venous blood before the operation, 4 hrs after it, as well as on the first, second and third day after the operation. Every patient was tested for postoperative pain intensity, amount of administered analgesics during hospital stay, number and types of postoperative complications; also the time needed for return to everyday activities was registered. 61 patients were included in the study. 31 patients were operated with the harmonic scalpel, and 30 of them with the monopolar electrocauter. There is no statistically significant difference between the operation time in the two groups: 78.50 +/- 17.50 minutes by harmonic scalpel and 82.50 +/- 18.50 minutes by electrocauter ($p = 0.796$). The smaller amount of intraoperative blood loss is statistically significant in the group of patients mastectomized by harmonic scalpel 78 +/- 31 ml compared to 256 +/- 112 ml in the group mastectomized by electrocauter ($p < 0.001$); as is the total operational drain secretion: patients mastectomized by harmonic scalpel 540 +/- 390 mL compared to 960 +/- 710 mL in patients mastectomized by electrocauter ($p < 0.001$). There is no statistical difference in the number of leukocytes in blood after modified radical mastectomy using the harmonic scalpel or electrocauter ($p = 0.957$), or in erythrocyte sedimentation rate ($p = 0.114$), CRP ($p = 0.071$) and IL-6 ($p = 0.082$). The duration of postoperative hospital stay does not differ statistically between the two groups, nor does the postoperative pain intensity, amount of administered analgesics, number or types of postoperative complications, as well as the time needed for return to everyday activities. Therefore using the ultrasound harmonic scalpel in comparison to monopolar electrocauter brings certain advantages, which however do not contribute significantly to the total success rate of the operation.

21. Kvesić A, Šoljić M, Galić G, Lončar Z, Brekalo Z, Martinovć Z, Šutalo N, Tomić M, Lekić A. The relation between the serum calcium level and the complication incidence in haemodialysis uremic patients. Coll Antropol. 2010 Mar;34 Suppl 1:173-80.

University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The mineral metabolism disorder is the most influential factor of the morbidity and mortality incidence of haemodialysis uremic patients. The second most influential factor is the infection, which is the most frequent complication with an undesirable outcome. In recent times, the relation of the increased serum calcium and phosphorus level on the one hand, and the morbidity and mortality of that population in case on the other, has been observed. However, insufficient professional and scientific thought has been given to the relation of the lower serum levels of the aforemen-

tioned minerals and the morbidity and mortality incidence. We have researched the relation between lower serum calcium level (hypocalcaemia) and the complication incidence, especially infection. Throughout the time period of 18 months, 120 haemodialysis uremic patients were observed and 76 (63.3%) of them had serum calcium level below the lower threshold of referent values (9.0-9.5 mg/dL). In the patients with a lower serum calcium level (hypocalcaemia) a significant infection incidence (chi2 = 3.99; p = 0.0468), a significant sepses incidence (chi2 = 8.016; p = 0.04), a significant total complication incidence ($p < 0.05$) were determined, as well as a higher vascular access local infection incidence, but without statistically significant research results of this relation (chi2 = 0.098; p = 0.7598). We are of the belief that the incidence of the vascular access local infection should be examined on a greater number of patients; therefore, the significance of the examined relation in such an instance would be expected. The total infection incidence in all 120 observed patients is 3.8 for 100 months. It is to be concluded that the research findings indicate the association regarding the appearance of low serum calcium concentration (hypocalcaemia) and an increased complication incidence, especially the inflammation that leads to the requirement of further research in order to decrease morbidity, and consequently also the mortality of the observed population of patients by means of programmed therapy approach.

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22. Lakičević G, Splavski B, Brekalo Z. The value of stereotactic biopsy in improving survival and quality of life for malignant brain glioma patients. Coll Antropol. 2010 Mar;34 Suppl 1:93-7.

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

The purpose of the study was to investigate possible differences in the survival and outcome of malignant brain glioma patients when treated by two different methods of surgery. During a 3-year period, 32 glioma patients underwent surgery and oncological protocol afterwards. The patients were divided into two groups according to the surgical method applied. The case group comprised 11 patients in whom a stereotactic biopsy was performed, while the control group consisted of21 patients who were operated on by radical surgery (craniotomy and maximal reduction of the tumor mass). All survived patients were clinically examined at follow-ups (one year and 2 years following the surgery). The monitored variables for both groups were the tumor pathohistology (the tumor type), the survival rate (time between surgery and follow-up), and the outcome assessed by The Extended Glasgow Outcome Scale. Data statistical analysis was done to compare various investigated variables in two different groups of patients. The majority of patients treated by a stereotactic biopsy survived for more than 2 years following the procedure. The great part of patients treated by radical surgery died or was severely disabled at follow-up examination. The survival and outcome for the patients in whom a stereotactic biopsy was performed were notably better comparing to the patients who were treated by radical surgery. Consequently, it appears that a stereotactic biopsy is surgical option for primary treatment of selected patients with malignant brain glioma when the survival and quality of life are concerned.

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23. Marković-Peković V, Stoisavljević-Šatara S, Škrbić R. Outpatient utilization of drugs acting on nervous system: a study from the Republic of Srpska, Bosnia & Herzegovina. Eur J Clin Pharmacol. 2010 Feb;66(2):177-86. Epub 2009 Oct 9.

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

PURPOSE: The aim of this study was to analyse the utilization patterns of drugs acting on the nervous system in the Republic of Srpska, Bosnia & Herzegovina between 2002 and 2008. METHODS: This was a retrospective study aimed at analysing outpatient utilization of drugs reimbursed by the Health Insurance Fund, with a focus on the utilization of drugs acting on the nervous system. Anatomical therapeutic chemical/defined daily dose methodology was used to monitor drug utilization, and the drug utilization 90% (DU90%) method was used to assess drug prescribing. RESULTS: The most highly used drug subgroups were psycholeptics and antiepileptics followed by the psychoanaleptics. Anxyolitics comprised the most prescribed pharmacological subgroup over the whole study period, but a decrease was observed in 2007 and 2008. Following updating of the list with selective serotonin re-uptake inhibitor drugs, particularly sertraline, antidepressant use increased fivefold in 2008 compared to 2006. Tramadol was the predominant opioid analgesics in terms of utilization, while the use of oral morphine was low. Diazepam was the most highly prescribed drug, followed by phenobarbital and carbamazepine. The list update with the new generation drugs was immediately reflected in the DU90% profile. CONCLUSIONS: The observed tendency toward increased total drug utilization observed in our study is comparable to worldwide trends. Implementation of new clinical guidelines for nervous diseases and updating of the list of reimbursable drugs with the addition of new ones contributed to the observed improvement in prescribing patterns in primary healthcare during the study period. The DU90% is shown to be a simple rough method for assessing prescribing quality. More stratified analyses should be performed on a routine basis to ensure a rational use of medicines and a cost-efficient use of limited healthcare resources.

24. Milardović R, Corssmit EP, Stokkel M. Value of 123I-MIBG Scintigraphy in Paraganglioma. Neuroendocrinology. 2010;91(1):94-100. Epub 2009 Sep 25.

Institute of Nuclear Medicine, Clinical Center of the Sarajevo University, Sarajevo, Bosnia and Herzegovina.

AIM: The aim of the study was to evaluate the current role of (123)I-MIBG scintigraphy in the detection and follow-up of patients with paragangliomas. MATERIALS AND METHODS: 117 patients were referred for diagnostic (123)I-MIBG scintigraphy based on a strong clinical suspicion, positive familial history and genetic testing, or for follow-up of paragangliomas. (123)I-MIBG images were analyzed and correlated with (111)In-octreotide scintigraphy, CT or MRI results. Accuracy of the imaging method was calculated per patient and per tumor per site. RESULTS: A total of 117 patients were referred for (123)I-MIBG diagnostic imaging; 80 patients were diagnosed with paraganglioma; 66 patients had a single neuroendocrine tumor and 14 patients multiple tumors. The total number of all lesions in these patients was 172. (123) I-MIBG scintigraphy demonstrated 65 lesions in 56 patients (overall sensitivity: 56.3%, specificity: 84%). Lesion-per-site analysis revealed that sensitivity and specificity significantly varied per tumor site (lowest sensitivity for the head and neck: 17.5% and lowest specificity for the abdomen: 87.5%). Hormones were elevated in 85 patients: 55 (123)I-MIBG tumors were positive and 35 tumors were negative. In 16 patients (13.7%) with a genetic burden and a single neuroendocrine tumor, (123)I-MIBG whole-body imaging was successful at detecting a second tumor. In 2 patients (1.7%) with paragangliomas, (123)I-MIBG unexpectedly detected metastases, so the restaging was properly done. CONCLUSION:(123)I-MIBG scintigraphy remains important in pheochromocytoma and functioning neuroendocrine tumors. The value of (123)I-MIBG scintigraphy is high in familial syndromes with multiple neuroendocrine tumors at different sites, multifocal tumors, and relapsing and metastatic disease. Copyright 2009 S. Karger AG, Basel.

25. Miljko M, Čarapina M, Prlić J, Arapović D. Chronic synovitis after synovial sarcoma resection: a case report. Coll Antropol. 2010 Mar;34 Suppl 1:291-4.

Institute of Radiology, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

We present a diagnostic course and treatment of synovial sarcoma in a young 16-year-old male patient. The clinical course with special emphasis on the imaging techniques is presented here, providing an overview of this subject and offering a useful educative material. The course of the follow-up is also described, largely relying on MRI in diagnosis course. Changes in the synovial tissue of the knee were recorded, later to be classified as the sarcoma. Post-operative course suggested the existence of chronic synovitis. Further studies are needed to fully understand the changes that may affect knee mechanics after surgery and/or inflammation factors secreted by tumor cells.

26. Mimica M. Implementation of new educational methods: how to overcome obstacles? Coll Antropol. 2010 Mar;34 Suppl 1:11-4.

Clinic for Internal Medicine, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

A presentation of new educational methods was organized for students and teachers of the Faculty of Medicine in Mostar in 2006. Afterwards, the teachers and the students were given a questionnaire to fill on their attitude towards actual, traditional versus new educational methods. According to the results of the questionnaire, a lot of students and the majority of teachers prefer status quo, and, moreover, only 1/3 of teachers support implementation of the new educational methods. Due to the results of this survey, implementation of new educational methods was postponed for the following two years. Since the management of the Faculty of Medicine in Mostar was well aware of the fact that new educational methods help in teaching students about fundamental principles of critical thinking, life-long learning and constructive intellectual conflicts, it was concluded that implementation of new methods should be a necessity. In this academic year (2008/2009), after discussion at the Faculty Council, a new course "Modern educational methods" has been introduced into the curriculum. As the first phase, a new transitional method named "Contradiction with Evidence Based Solution" has been introduced in the workshop session. The proposed new method is a combination of the old, traditional presentation done by a teacher, and new methods which are student-centred. Realistic expectation is that implementation of other new educational methods would be easier after introduction of this transitional method. This implementation trial should be seen as a pilot project that could be introduced to other faculties, at first to those of the University of Mostar, and afterwards to other universities in the region.

27. Novaković J, Mardešić-Brakus S, Vukojević K, Saraga-Babić M. Developmental patterns of Ki-67, bcl-2 and caspase-3 proteins expression in the human upper jaw. Acta Histochem. 2010 Jun 30. [Epub ahead of print]

Department of Anatomy, Histology and Embryology, School of Medicine, University of Mostar, Bijeli brijeg bb, 88000 Mostar, Bosnia and Herzegovina.

The distribution of the Ki-67, bcl-2 and caspase-3 proteins was immunohistochemically analyzed in the developing human upper jaw (5th-10th gestational weeks). During this period, proliferative activity gradually decreased from higher levels at the earliest stages (50-52%) to lower levels, both in the jaw ectomesenchyme and in the epithelium. The highest expression of bcl-2 protein was found in the epithelium and ectomesenchyme of areas displaying lower rates of cell proliferation. High levels of caspase-3 protein were detected during the earliest stages of jaw development, indicating an important role for apoptosis in morphogenesis of early derivatives of the maxillary prominences. The number of Ki-67, bcl-2 and caspase-3 positive cells changed in a temporally and spatially restricted manner, coincidently with upper jaw differentiation. While apoptosis might control cell number, bcl-2 could act in suppression of apoptosis and enhancement of cell differentiation. A fine balance between cell proliferation (Ki-67), death (caspase-3) and cell survival (bcl-2) characterized early human upper jaw development. A rise in the number of apoptotic cells always temporally coincided with the decrease in number of surviving bcl-2 positive cells within the palatal region. Therefore, the upper jaw development seems to be controlled by the precisely defined expression of genes for proliferation, apoptosis and cell survival.

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28. Ostojić L, Damjanović V, Vasilj I, Jančić E, Maslov B. Biomedical scientific productivity of the Mostar University Faculty of Medicine and University Hospital Mostar in 1999-2008. Coll Antropol. 2010 Mar;34 Suppl 1:7-10.

Faculty of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina.

The aim of this study was to investigate the scientific productivity of the Mostar University Faculty of Medicine and University Hospital Mostar. All articles that were indexed by PubMed with the keyword Mostar were included in the analysis. During 1999-2008, a total of 76 articles were published, with a total of 366 authorships contributed by a total of 228 unique authors, whereas a total of 161 of these authors (70.6%) coauthored a single article only. The average number of co-authors was 4.6 per article. There was a strong increasing linear trend in the total number of published articles. The most published articles were related to clinical research, whereas the least were recorded in the basic biomedical sciences, suggesting the need to increase the research capacity in basic biomedical sciences. The large percent of single-authorship authors that were recorded suggest almost a sporadic rather than systematic publication output. Likely improvements to this situation include the creation of the newly formed doctoral (PhD) course due to start next year and several other ways in which scientific research in biomedicine can be increased in basic, clinical and public health sciences.

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29. Ostojić Z, Grle M, Moro G, Zubak Z, Ostojić M. Bone fractures in a 53-year-old patient with parathyroid adenoma--a case report. Coll Antropol. 2010 Mar;34 Suppl 1:295-8.

Department of Orthopedics, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The study reports a case of primary hyperparathyroidism in a middle-aged patient who was first admitted for persistent ankle pain and local swelling. The subsequent clinical procedures suggested cystic changes in several leg bones, which were later shown to be caused by the parathyroid adenoma. Clinical presentation of the primary hyperparathyroidism can be highly misleading, sometimes causing various clinical procedures before it is certainly diagnosed.

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30. Ostojić Z, Moro G, Kvesić A, Roth S, Bekavac J, Manojlović S. Treatment of peritrochanteric fractures by the use of gamma nail. Coll Antropol. 2010 Mar;34 Suppl 1:243-6.

Department of Orthopedics, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The Gamma nail was designed to treat unstable intertrochanteric and subtrochanteric fractures. In this study we analysed a total of 60 patients (44 men and 16 women), who were surgically treated for the peritrochanteric fracture in period 2006-2007 at the University Hospital Mostar. After the surgical treatment good bone healing was achieved in 50 patients (83.3%). A total of five patients had delayed healing or protrusion of the cervical screw, and in two patients nails were not appropriately distally locked. During the follow-up period a total of 7 patients died. The average operation time was 40 minutes, and the average blood loss was 400 mL, which is a comparable result with the previously published studies. In conclusion, although most of the peritrochanteric fractures treated at the University Hospital Mostar were fixated by gamma nail, the final decision regarding the operational technique should be left to surgeon's judgment, since the efficacy of the treatment plan is highly dependent on experience of the operational team and surgeon's operational technique.

31. Ostojić Z, Prlić J, Juka K, Ljubić B, Roth S, Bekavac J. Results of treatment of displaced supracondylar humeral fractures in children by K-wiring. Coll Antropol. 2010 Mar;34 Suppl 1:239-42.

Department of Orthopedics, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The supracondylar fracture of the humerus in children remains the most challenging injury for the orthopedic surgeon. It is important to consider the options of treatment very carefully and tailor the treatment according to the characteristics of each fracture. In this study we observed outcomes of surgical procedures using the Kirschner-wire for the dislocated (displaced) supracondylar fractures in 135 children (mean age 6.7 years). In 96 patients closed reposition (reduction) and fixation with crossed K-wire was done. A total of 41 children were operated by the means of open reposition and crossed K-wire fixation. Another 41 underwent similar (1-mm) K-wire application. In classifying the fractures Gartland classification of the supracondilar fractures of the humerus was used. Postoperatively, cubitus varus was found in seven, and cubitus valgus in three children (5% and 2% respectively). Stiffness of the elbow was recorded in 18 patients, while the paresis of the ulnar nerve was recorded in three cases (13 and 2% respectively). In conclusion, we can suggest crossed fixation while applying the K-wire throughout two cortexes, since such technique ensures the most superior fixation and stable osteosynthesis.

32. Perić B, Perko Z, Pogorelić Z, Kraljević J. Laparoscopic cholecystectomy in Cantonal Hospital Livno, Bosnia and Herzegovina and University Hospital Center Split, Croatia. Coll Antropol. 2010 Mar;34 Suppl 1:125-8.

Dr. Fra Mihovil Sučić Cantonal Hospital, Livno, Bosnia and Herzegovina.

Cholecystectomy is the most frequently performed operation in abdominal surgery. The aim of this study was to compare the operative procedure and outcomes of the laparoscopic cholecystectomy in two hospitals, the University Hospital Center Split and the Regional Hospital in Livno. A total of 97 patients who underwent laparoscopic cholecystectomy for cholelithiasis at University Hospital Center Split and 86 patients from Regional Hospital in Livno, both groups sampled in 2005 were included in this study. Differences in patients' age, gender, operation time, total hospital stay, number of trocarsi ports, antibiotic and parenteral therapy, and complications were analyzed. There were significantly fewer men than women who underwent laparoscopic cholecystectomy in both hospitals. The mean age of the patients undergoing laparoscopic cholecystectomy at University Hospital Center Split was higher than that of the patients at Regional Hospital in Livno. The operation time was shorter at the University Hospital Center Split than that at Regional Hospital in Livno. There was a significant difference, in favor of the University Hospital Center Split, in the number of patients who received postoperative antibiotics and parenteral therapy, with fewer patients who received postoperative therapy in Split. At the Regional Hospital in Livno fewer trocars were used for laparoscopic cholecystectomy. The average hospital stay of patients undergoing laparoscopic procedures at University Hospital Center Split was shorter than that of patients at Regional Hospital in Livno. Two complications occurred in postoperative period at the University Hospital Center Split and one complication was noticed in hospital in Livno. In conclusion, there were no major complications in postoperative period. It is also encouraging to find that there was significant improvement of surgical approach and technique at the hospital in Livno during the period of time analyzed in this study.

33. Planinić D, Bodina I, Perić B. Prevalence of odontogenic keratocysts associated with impacted third molars. Coll Antropol. 2010 Mar;34 Suppl 1:221-4.

Dental practitioner, Međugorje, Bosnia and Herzegovina.

In this study we analyzed the prevalence of the odontogenic keratocyst (OKC) associated with impacted third molars and evaluated OKC reactivity with the antibodies against cytokeratins (CK), particulary for CK10. Tissue samples were obtained from the proximity of the impacted molar. Differences between genders, age groups and localization of cysts were assessed using the chi2-test and relative risk (RR), and associated confidence interval. Cysts were found in 75 cases, and the radicular ones prevailed (63%), followed by follicular and OKC (13% and 12% respectively). The RR for the upper jaw cysts was almost twice greater than for the mandible. For the OKC exclusively, the upper-lower jaw RR was 1:2. The RR for all cysts increases with age, while decreases for the OKCs. The risk for the OKC occurrence decreased with age, with no significant differences between age groups. Finally, based on the results from this study we cannot suggest that the CK10 staining should be considered an useful marker in differential diagnosis of the OKCs.

34. Resić H, Mašnić F, Kukavica N, Spasovski G. Unusual clinical presentation of brown tumor in hemodialysis patients: two case reports. Int Urol Nephrol. 2010 Apr 28. [Epub ahead of print]

Clinic for Hemodialysis, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Brown tumor or osteoclastoma is a lytic bone tumor, which is common in secondary hyperparathyroidism (1.5-13%) in chronic dialysis patients, mainly in those with untreated renal osteodystrophy. Brown tumor appears as a result from excess osteoclast activity and consists of collections of osteoclasts intermixed with fibrous tissue and poorly mineralized woven bone. It can be manifested as a single or multiple bone lesions. Although invasive, it has no malignant potential and should be distinguished from giant cell tumors of the bone. Two unusual cases of brown tumor in dialysis patients are reported. We present a first patient with five subtotal parathyroidectomies between 2002 and 2009 and a tendency toward recurrence of secondary hyperparathyroidism (sHPTH). The double MRI check up could not reveal any ectopic parathyroid gland. Although the patient had permanently high PTH values, serum calcium level was never above the normal range. However, the brown tumor in the cervical spine was destructing the cervical vertebrae and required surgical intervention. Despite the conservative treatment with calcium and non-calcium-based binders and various forms of vitamin D, the patient's clinical and biochemical condition improved only after the use of cinacalcet. The second patient, a 58-years-old female on chronic hemodialysis since 1998, was found with high PTH serum levels in 2009. The development of sHPTH was scintigraphically confirmed and surgically treated. During the late 2008, she started feeling pain, numbness and swelling of the 3rd right hand finger, prior to the full clinical manifestation of the tumor. The CT scan of the right hand showed osteolytic changes and soft tissue destruction of the middle phalanx of the 3rd right hand finger. This formation corresponded to an unusual presentation of brown tumor associated with sHPTH. As expected, after the parathyroidectomy, there was no marked change in the destructed bone of the 3rd right hand finger middle phalanx, but only a gradual improvement in the subjective clinical condition of the patient. Based on these two reports, we would recommend that in cases of severe or recurrent sHPTH either total parathyroidectomy or early administration of calcimimetics should be considered. Furthermore, the implementation of regular checkup and treatment according to the KDIGO guidelines should be advised and clinical appearance of any bone tumor immediately checked for an association with sHPTH, which is a rather common entity in dialysis patients.

35. Šarac Z, Perić B, Filipović-Zore I, Ćabov T, Biočić J. Follicular jaw cysts. Coll Antropol. 2010 Mar;34 Suppl 1:215-9.

Department of Oral and Maxilllofacial Surgery, Faculty of Medicine, University of Mostar, Mostar, Bosnia and Herzegovina.

The aim of this study was to examine the occurrence, localization, size, ways of diagnosing and treatment of a foIlicular jaw cyst. Assessment of the patients' motives and their earlier health status was recorded, as well as their postoperative clinical course. Most of the patients were admitted because of pain, swelling, trismus, or other difficulties associated with cyst formation. Follicular cysts with persisting primary predecessor had an asymptomatic development, and were discovered after orthodontic examination or by chance. In most cases pathohistological finding and description of the formation have coincided with each other ($p < 0.05$). Cysts of different sizes were treated by different surgical approaches, most commonly alveolotomy and cystectomy in small cysts, while alveolotomy and cystectomy with suction or iodine tampon in large cysts. Cooperation of a dentist, an oral surgeon, a pathologist, and other specialists can lead to early diagnose and prevention of further growth of a follicular jaw cyst, thus preventing substantial bone damage.

36. Šimić D, Prohić A, Šitum M, Zeljko Penavić J. Risk factors associated with the occurrence of basal cell carcinoma. Coll Antropol. 2010 Mar;34 Suppl 1:147-50.

Clinic for Dermatology and Venerology, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

Basal cell carcinoma (BCC) is the most frequent malignant skin tumor, which is associated with both genetic factors and environmental influences. The objective of this study was to investigate the risk factors associated with the occurrence of BCC in the inhabitants of the Western Herzegovina area. The study took place during 1997-2003. We examined the risk factors which are presumably associated with the occurrence of BCC: skin type, exposure to UV rays and family occurrence of BCC, supplemented by the examination of the skin type, UV rays and existence of malignant tumors amongst the family members. We recorded a high correlation between the type of skin and the risk of occurrence of BCC. Long term and frequent skin exposure to UV rays were also associated with BCC. We also recorded increased risk for BCC in persons whose family members suffered from malignant skin tumors. Avoiding exposure to the sun as well as protection from UV rays may decrease the risk of BCC.

37. Šimunović N, Rončević Z. Innocent murmurs: parental viewpoint, what are the most common misconceptions and how to avoid them. Coll Antropol. 2010 Mar;34 Suppl 1:89-92.

Clinic for Pediatrics, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The aim of this study was to investigate the parental reaction after they have been informed that their child has a heart murmur. This study also explored whether their reaction was influenced by the fact that the heart murmur is innocent, which actually means that the child is healthy. One hundred parents participated in this cross-sectional study. According to the statistical results, minor parent concern was notable after cardiologist's examination and consulting. Whereas before the cardiologist's examination 98% of parents were concerned about their child's health, later, less than half of them, or to be exact, only 38% of them, were still concerned. Before the questionnaire was filled, according to gender distribution, males were less concerned than females. Regarding the number of children, parents having three or more children were less worried before the medical examination. Before the examination, only 17% of parents were completely confident that their child had no heart complaint, and after a month 60% of parents had the same opinion. After cardiologist's examination and educational consulting, parental concern dropped significantly, which points to an obligatory need to thoroughly familiarize parents with their child's health condition.

38. Škobić H, Sinanović O, Škobić Bovan N, Ivanković A, Pejanović Škobić N. Prevalence of alcohol abuse and alcoholism in general population of Mostar region, Bosnia and Herzegovina. Coll Antropol. 2010 Mar;34 Suppl 1:29-31.

Department of Neurology, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The aim of this study was to determine the prevalence of alcohol abuse and alcoholism in the general population of Mostar region, Bosnia and Herzegovina. This study was conducted on a stratified sample of 704 participants. The prevalence of alcohol abuse was determined using standardized questionnaire on alcohol consumption--Michigan Alcoholism Screening Test. Prevalence of alcohol abuse with high risk for alcoholism was 9.9% and prevalence of alcohol addiction was 2.1%. In student population, there were 3.9% of alcohol addicts and 11.1% of persons with high risk of alcoholism. In high school population, there were 1.7% of alcohol addicts and 14.4% of persons with high risk of alcoholism. In Mostar region there was a high prevalence of alcoholism and problematic drinking, especially in high school and student population. There is a need for extensive preventive measures that have to include education, early diagnosis and intervention.

39. Šutalo N, Maričić A, Kozomara D, Kvesić A, Štalekar H, Trninić Z, Kuzman Z. Comparison of results of surgical treatments of primary inguinal hernia with flat polypropylene mesh and three-dimensional prolene (Phs) mesh--one year follow up. Coll Antropol. 2010 Mar;34 Suppl 1:129-33.

Department of General Surgery, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

The aim of this study was to compare the results of the surgery of inguinal hernias using flat polypropylene mesh and three-dimensional prolene (PHS) mesh. The study included two groups of 40 male patients, aged 18-50 years, with the diagnosis of inguinal hernia. One group was operated with a flat polypropylene mesh, while the second group was operated with three-dimensional prolene (PHS) mesh. The study has shown that the operation with three-dimensional prolene mesh lasted 15 minutes longer and that the patients had stronger inflammatory response. Statistically, there was no significant difference in post-operative pain intensity, post-operative use of analgesics, length of hospitalization, return to daily activities, early and late post-operative complications. No recurrence was registered in any of the groups. The analysis of results indicates that there is no difference in treatment of inguinal hernia with flat polypropylene and three-dimensional prolene (PHS) mesh.

40. Tahirović H, Toromanović A. Glycemic control in diabetic children: role of mother's knowledge and socioeconomic status. Eur J Pediatr. 2010 Aug;169(8):961-4. Epub 2010 Feb 19.

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

The aim of this study is to investigate the role of mother's knowledge and socioeconomic status (SES) of the family on glycemic control in diabetic children. Our sample was taken from successive admissions to the outpatient's diabetes clinics in Tuzla, Bosnia and Herzegovina. Diabetes knowledge was assessed using the Michigan Diabetes Research and Training Center Diabetes Knowledge Test. Glycemic control was assessed by glycosylated hemoglobin (HbA(1C)). The mother's demographics were obtained by self-report. To categorize families' SES, parents' level of education, and current employment were recorded and analyzed using the Hollingshed two-factor index of social position. As expected, higher mother's knowledge was significantly associated with lower HbA(1C) ($r = -0.2861705$, $p = 0.0442$). Also, a significant correlation was found between the families' SES and HbA(1C) levels ($r = 0.4401921$; $p = 0.0015$). Mothers with more knowledge have children with better metabolic con-

trol, and low SES is significantly associated with higher levels of HbA1c. Improvement of mothers' knowledge and family SES may improve glycemic control and ultimately decrease acute and chronic complications of diabetes in children.

41. Tulumović D, Imamović G, Mešić E, Hukić M, Tulumović A, Imamović A, Zerem E. Comparison of the effects of Puumala and Dobrava viruses on early and long-term renal outcomes in patients with haemorrhagic fever with renal syndrome. Nephrology (Carlton). 2010 Apr;15(3):340-3.

University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina.

AIM: The clinical course and outcome of patients with haemorrhagic fever with renal syndrome (HFRS) caused by Puumala (PUUV) and Dobrava viruses (DOBV) were analyzed and whether it left long-term consequences on kidney function after 10 years was evaluated. METHODS: Cross-sectional studies were conducted to test the kidney function and blood pressure of HFRS-affected patients and to follow them up 10 years after. Eighty-two PUUV- and 53 DOBV-induced HFRS patients and 14 and 31 participants 10 years after having contracted PUUV- and DOBV-related diseases, respectively were evaluated. RESULTS: Serum creatinine concentrations were 279.5 and 410 mcmol/L in PUUV and DOBV groups, respectively (P = 0.005). There were six and 13 anuric (P < 0.05), none and seven dialysis-dependant (P < 0.05), and nine and 18 hypotensive patients (P < 0.05) in PUUV and DOBV groups, respectively. After 10 years, glomerular filtration rates were 122.1 + or - 11.1 and 104.7 + or - 20.2 mL/min (P < 0.05) in PUUV and DOBV groups, respectively. CONCLUSION: During the acute phase, DOBV causes more severe renal impairment than PUUV infection. After 10 years follow up, renal function was found within normal limits, although after DOBV infection glomerular filtration rate (GFR) was significantly lower than after PUUV infection.

42. Vulić D, Lee BT, Dede J, Lopez VA, Wong ND. Extent of control of cardiovascular risk factors and adherence to recommended therapies in US multiethnic adults with coronary heart disease: from a 2005-2006 national survey. Am J Cardiovasc Drugs. 2010;10(2):109-14.

Department of Internal Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina.

Guidelines for cardiovascular risk factor control in people with coronary heart disease (CHD) focus on compliance with beta-adrenoceptor antagonists (beta-blockers), angiotensin receptor blockade (ACE inhibitors/angiotensin II receptor antagonists [angiotensin receptor blockers; ARBs]) [ACE/ARBs], and lipid-lowering agents, with goals for BP of <140/90 mmHg and low-density lipoprotein cholesterol (LDL-C) levels of <2.6 mmol/L (100 mg/dL). Most data derive from registries of hospitalized patients or are from clinical trials. Little data exist on goal attainment and adherence with therapy among CHD survivors of major US ethnic groups in the real-world setting. We assessed levels of cardiovascular risk factor control and adherence with recommended therapies among US CHD survivors. We identified 364 US adults (representing 12.8 million in the US with CHD) aged 18 years and over in the National Health and Nutrition Examination Survey 2005-6 with known CHD. We calculated proportions of patients who were receiving recommended treatments, and who achieved goal targets for BP, LDL-C levels, glycosylated hemoglobin (HbA(1c)), and nonsmoking status, and differences between actual and goal levels ('distance to goal'), stratified by sex and ethnicity. Overall, 58%, 38%, and 60% of CHD survivors were receiving beta-adrenoceptor antagonists, ACE/ARBs, and lipid-lowering medications, respectively (22% received all three). However, treatment rates for beta-adrenoceptor antagonists and lipid-lowering agents were lower ($p < 0.05$ to $p < 0.01$) in Hispanics (36% and 27%, respectively) and non-Hispanic Blacks (47% and 42%, respectively) than in non-Hispanic Whites. Moreover, lipid-lowering treatment rates were lower in females (50%) than in males (67%) [$p < 0.01$]. Overall, 78% were nonsmokers while 68% achieved goal levels for BP, 57% for LDL-C levels, and, if diabetic, 67% for HbA(1c). Only 12% met all four goals. Non-Hispanic Whites had the lowest SBP and DBP as well as HbA(1c) ($p < 0.05$ to $p < 0.01$ across ethnicity). In those who did not achieve goal levels, distance to goal averaged 1.0 mmol/L (37.0 mg/dL) for LDL-C levels, 15.6 mmHg for SBP, and 1.3% for HbA(1c). Despite clear treatment guidelines, we show that many US adults with CHD, especially Hispanics and non-Hispanic Blacks, are neither receiving recommended treatments nor adequately treated in terms of BP, LDL-C levels, and HbA(1c). Greater efforts by healthcare systems to disseminate and implement guidelines are needed.

43. Zeljko-Penavić J, Šitum M, Šimić D, Vurnek-Živković M. Quality of life in psoriatic patients and the relationship between type I and type II psoriasis. Coll Antropol. 2010 Mar;34 Suppl 1:195-8.

Department of Dermatology and Venerology, University Hospital Mostar, Mostar, Bosnia and Herzegovina.

Psoriatic patients, along with skin changes, frequently show various psychological changes such as depres-

sion, anxiety and have overall lower quality of life. The aim of this study was to evaluate the quality of life in patients with psoriasis compared to other dermatological patients, as well as to investigate the differences between the two subgroups--type I and type II psoriasis. A total of 94 dermatological patients were included. The patients were divided into two groups, the first group made of psoriatic patients which was further divided into two subgroups, and the second, control group made of patients with other skin diseases. DSQL quality of life questionnaire was used. The study showed that among psoriatic patients there was no significant difference in the quality of life, but there was a significant difference between the psoriasis type I and the control group, which could be explained by the strong influence of the disease on the quality of life in psoriatic patients.

44. Zerem E, Imamović G. Comments on the article about the treatment of peripancreatic infection. World J Gastroenterol. 2010 May 14;16(18):2321-2.

University Clinical Centre Tuzla, Tuzla, Bosnia and Herzegovina.

We read with great interest the article by Tang et al published in issue 4 of World Journal of Gastroenterology 2010. The results of their study indicate that percutaneous catheter drainage in combination with choledochoscope-guided debridement is a simple, safe and reliable treatment procedure for peripancreatic infections secondary to severe acute pancreatitis. However, there are some points that need to be addressed, including data about the patients in the study and their clinical characteristics, data about infection and superinfection during the treatment and type of treatment of patients with acute necrotizing pancreatitis.

by Nerma Tanović

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Sample references

Articles in journals

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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.

Issue with supplement:

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

Issue with no volume:

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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.

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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.

Article published electronically ahead of the print version:

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:

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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/.

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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

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