



SCRIPTA MEDICA

ASSOCIATION OF MEDICAL DOCTORS OF THE REPUBLIC OF SRPSKA,
FACULTY OF MEDICINE, UNIVERSITY OF BANJA LUKA

2019

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EDITORIAL

Science Metrics Systems in Biomedical Sciences: Current Trends

ORIGINAL ARTICLES

The Effects of Gasotransmitters Inhibition on Homocysteine Acutely Induced Changes in Oxidative Stress Markers in Rat Plasma

The Prevalence of Smoking and its Impact on Disability in Multiple Sclerosis

Does Gender Influence Quality of Life in Children with Atopic Dermatitis?

Students' Attitudes About the Ethics of Using Animals for Experimental Purposes

REVIEW ARTICLE

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CASE REPORT

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BANJA LUKA, 2019



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Science Metrics Systems in Biomedical Sciences: Current Trends

Enver Zerem^{1,2}

ABSTRACT

The publication of scientific research is principally information published in scientific journals and they are cornerstone of knowledge dissemination, as well as an essential criterion for academic and scientific evaluation, recruiting funds and career progression. However, it is very difficult to apply right measures and scientific criteria which can objectively assess scientific research. For many years there has been a great interest in scientific ranking and evaluation of scientific journals, but also of scientific contribution of scientists. It is generally accepted that the IF (WoS) and the total number of citations of articles published in the journal, are the most relevant parameters of the journal's significance. However, the scientific significance of a scientist is much more complicated to evaluate and the value of their scientific production cannot be directly reflected by the importance of the journals in which their articles are published.

The majority of existing science metric systems, which evaluate the achievement of scientists are focused solely on the number of citations of their articles. Based on a long term of academic experience, the author describes and evaluates the most known scientific databases which are used in biomedical sciences. Also, the author proposes Z-score as a new science metric system which takes into account the current IF (WoS) and total number of citations of the journal in which the article is published, as well as author's contribution to the scientific article. In that way, proposed criteria greatly remedy major discrepancies in evaluating scientific production of individual authors and institutions.

Keywords: science metrics systems, scientific impact factor, Z-score, number of citations, author contribution.

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Society invests significant resources in biomedical research, seeking for ways to improve human health. However, the efficiency of society's investments in biomedical research, in terms of improved health outcomes, has not been fully confirmed. Social and scientific importance and the quality of scientific research highly depend on the usefulness of the research results. The scarcity of scientific research funds and the tendency to direct the funds towards high-quality research accentuates the importance of measuring and assessing the quality of research and knowledge valorization. However, it is very difficult to apply the right measures and scientific criteria which can objectively assess scientific research¹⁻³.

The product of scientific research is principally information published in scientific journals and they are cornerstone of knowledge dissemination, as well as an essential criterion for academic and scientific evaluation, recruiting funds and career progression^{4,5}. It is true that, beside evaluation of scientific publications, there is a wide range of other scientific activities which also reflect scientific credibility such as⁵:

- number and quality of extramural grants,
- leadership in national or international academic societies,
- service on editorial boards of respected journals,
- service on government sponsored national peer review committees,

- the number of PhD students delivered,
- the amount of coverage of one's scientific output in the lay press, etc.

Although, those activities are important and give certain significance to the scientific credibility of a scientist, the relevant science metrics systems only cover publications, and omit other criteria of scientific relevance, which are typically used in judging promotions and tenure of scientists. The reason for this is the fact that these activities, regardless of their importance, are very heterogeneous since each of them has specific characteristics and requires different parameters for evaluation. Hence, for these parameters of scientific relevance there are no universal evaluation criteria and their value is mainly assessed individually depending on the purpose of the assessment ^{5,6}.

1. The ranking of scientists and scientific journals

For many years there has been a great interest in scientific ranking and evaluation of scientific journals, but also of scientific contribution of scientists. The most known scientific databases which are used in biomedical sciences are:

Current Contents (CC)

- Web of Science (WoS)
- Journal Citation Reports (JCR)
- Index Medicus, Medline, PubMed
- Excerpta Medica (EMBASE)
- Scopus,
- Scholar,
- H-index

Most of those scientific bases (except for Scopus, Scholar, H-index and WoS which rank scientists) present and rank scientific journals only.

1.1. Current Contents (CC)

Current Contents is a platform for Clarivate Analytics (formerly Thomson Reuters). It is the most appreciated database that is usually available through the Web of Science. The reasons for its popularity are the relatively high journal selection criteria, its coverage of all areas of science, the update frequency, the author's summary, the author's address, the names and addresses of the publisher, and its ability to review the content of a particular issue of the journal. Current Contents is published in seven following sections (each containing more than 1000 sources):

- Current Contents / Agriculture, Biology & Environmental Sciences

- Current Contents / Arts & Humanities
- Current Contents / Clinical Medicine
- Current Contents / Engineering, Computing & Technology
- Current Contents / Life Sciences
- Current Contents / Physical, Chemical & Earth Sciences
- Current Contents / Social & Behavioral Sciences

Through above noted seven sections Current Contents covers all areas of science. Most biomedical journals are included in the Current Contents/Clinical Medicine section, but some biomedical journals are classified under the sections of Current Contents/Life Sciences and Current Contents/Social & Behavioral Sciences ^{5,6}.

1.2. Web of Science (WoS)

The Web of Science is a platform for Clarivate Analytics, which provides access to quoted databases covering all areas of science. The platform contains more than 33,000 indexed journals and nearly one billion records of quoted references, and includes articles, conference proceedings, reports, patents, and more. The following databases are available through the Web of Science interface ^{6,7}:

- Science Citation Index Expanded
- Social Sciences Citation Index
- Arts & Humanities Citation Index
- Emerging Sources Citation Index
- Book Citation Index - Science
- Book Citation Index - Social Sciences & Humanities
- Conference Proceedings Citation Index- Science
- Current Chemical Reactions
- ESCI Backfiles
- Index Chemicus

1.3. Journal Citation Reports (JCR)

On the basis of the data obtained from the citation databases (Science Citation Index and Social Science Citation Index) Eugene Garfield created a special statistical database and named it Journal Citation Reports (JCR). At the end of June each year, Clarivate Analytics publishes the JCR list for the previous year. Impact factor (IF) is a quantitative aid for ranking, evaluating, categorizing and comparison of the journals. IF is calculated as an quotient when dividing the number of citations received during the year that refer to articles from the previous two years. It is gen-

erally accepted that IF and the total number of citations of all articles published in the journal represent a relevant measure of their value and scientific influence. For some domains, it is much more relevant to have an IF for a 5-year period rather than for a standard 2-year period ^{8,9}.

1.4. Index Medicus, Medline, PubMed

Index Medicus is the print version of the bibliographic citation database of the National Library of Medicine, and MEDLINE is its online counterpart. PubMed is an online database that provides access to citations in MEDLINE as well as those from additional life sciences journals. MEDLINE and PubMed are among the most popular and widely used literature databases for health care professionals. Nearly 4500 journals are indexed in MEDLINE, and even more are indexed in PubMed ¹⁰.

1.5 Excerpta Medica (EMBASE)

Embase is a highly versatile, multipurpose and up-to-date biomedical research database. Produced by Elsevier, it covers the most important international biomedical literature containing over 32 million records from over 8,500 currently published journals from 1947 to the present. Embase's international coverage expands across biomedical journals from 95 countries and is available through a number of database vendors ⁹.

1.6. Scopus

Scopus is a quotation database that indexes sources from all over the world, and includes more than 69 million records for 21,950 reviewed journals, 280 trade journals from almost all fields of science, more than 560 series of books, 8 million conference proceedings and more than 39 million patents (Wikipedia).

1.7. H-index

Almost all relevant scintimetric indexes which evaluate the achievement of scientists are focused on the number of citations of their articles. The best-known scintimetric system which assesses the individual scientific contribution of scientists is the so-called H-index which is calculated as the lowest ranked article which number of citations matches its ranking number (for example, a scientist whose H-index is 10 must have at least 10 articles which have 10 or more citations).

However, H-index has considerable shortcomings because the system is based solely on the evaluation of the number of individual article ci-

tations. Therefore, H-index obviously favors older articles which are available for quotation for a longer time, and negatively impacts on assessment of scientific value of new articles and scientific production of perspective scientists. Besides, H-index does not take into account the individual contribution of each author in an evaluated article, since according to H-index all authors of an article are treated as equal. Hence, H-index does not tackle the ever-present problem of expanding author lists with authors whose contribution may be minute or none ^{11,12}.

1.8. Other scientific database

There are several other databases such as: Google Scholar, PageRank index; Altmetrics; g-index; e-index; i-index; total publications; total number of citations etc ^{9,13,14}.

2. Does Zerem-score bring improvements?

The existence of such a large number of science metric systems shows that there is no perfect scientific metric index that accurately measures the scientific contribution of scientists and scientific journals. It is generally accepted that the IF (WoS) and the total number of citations of articles published in the journal, are the most relevant parameters of the journal's significance. The scientific significance of a scientist is much more complicated to evaluate than that of a scientific journal, since the scientific production value cannot be directly reflected by the importance of the journal in which the article is published ⁵.

As a director for scientific research in the institution where I used to work every year I was in charge of annual assessment of the employees' scientific contribution. At the beginning, it seemed like a simple job. The scientific contribution was ranked on the basis of IF of the journal in which the article was published. However, some authors had published more articles throughout the year. There were different types of articles which were published in journals indexed in different scientific bases. Also, those articles were new, therefore they had not been cited by other authors. It is known that, the number of citations of a particular article, as the relevant measure of the value of that article, has its limitations since it requires excessive time lag and gives advantage to older articles of similar quality. Furthermore, the contribution of all the authors in a scientific article is usually not the same. Therefore, it is very difficult to apply the right measures and scientific criteria which



can objectively assess a new scientific research and provide precise qualitative and quantitative data on which new articles could be evaluated. Based on the long term experience, I was proposing the new criteria (named Z-score) which can objectively estimate the scientific effect of scientists and institutions. The Z-score criteria have been published in the *Journal of Biomedical Informatics* ^{5, 12, 15}.

According to the Z-score criteria the overall scientific score of an author is calculated as the sum of two scores (author contribution score-ACoS and author citation contribution score-ACCS). ACoS is calculated as the scientific value

CONCLUSIONS

Whether scientists like it or not, the societal impact of their research is an increasingly important factor in their academic and scientific evaluation, recruiting public funds for scientific research and career progression [5]. This has always been the case, but current trends in scientific and academic community increasingly emphasize the need to improve criteria and establish measures that can objectively assess the societal impact of research and would provide better qualitative and quantitative data which will enable the societal and scientific community to objectively assess the value of scientists and scientific research. This is especially important in developing countries where a complex interrelation between politics and the academic community significantly impacts on the process of acquisition of scientific and academic titles ^{5, 16, 17}.

However, it is very difficult to apply right measures and scientific criteria which can objectively assess scientific research, providing precise qualitative and quantitative data on which funding agencies could base their decisions. The existence of a large number of science metric systems shows that there is no perfect scientific metric index that can accurately measure the scientific

of the journal in which an article is published and the authors' specific contribution in this article. ACCS is calculated as the scientific values of the particular article expressed through the number of quotations that this article has received and the authors' specific contribution in the article. In order to successfully apply and calculate Z-score, we have created the adequate computer software - Z-score calculator, which encompasses all the parameters described in the proposed criteria. Also, Z-score calculator is designed to be compatible with all browsers and it is capable of automatic collection of data once linked to a browser ^{5, 15}.

contribution of scientists and scientific journals. It is true that the current science metric systems have multiple shortcomings and are not ideal for an objective assessment of scientific research and the scientists' significance. However, without the introduction and application of internationally recognized scientific criteria in the evaluation of scientific research, and the coordination of academic progress in accordance with these criteria, there is room left for the decision makers within the academic community to lower the criteria margin to the level which they subjectively consider relevant, without complying with the internationally recognized criteria. Therefore, the application of internationally recognized scientific criteria in the evaluation of scientific research is necessary and these criteria should be constantly improved. I consider Z-score criteria sustainable and capable of objective estimation of the scientific effect of scientists and institutions. I hope this article contributes to the discussion about science metric systems, raising questions and motivating the expression of different viewpoints with the intention to improve science metric systems and make them more objective and competent in the complex process of evaluating scientific production in biomedical research.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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The Effects of Gasotransmitters Inhibition on Homocysteine Acutely Induced Changes in Oxidative Stress Markers in Rat Plasma

Marko Djuric¹, Sanja Kostic², Dragana Loncar-Stojiljkovic³, Slavica Mutavdzin⁴, Mirjana B. Colovic⁵, Danijela Krstic⁶, Predrag Stevanovic¹ and Dragan M. Djuric⁴

ABSTRACT

Background: The importance of homocysteine (Hcy) is increasingly recognized in last few decades as an independent risk factor for atherosclerosis and thrombosis, but there is lack of data referring to influence of Hcy on plasma oxidative stress parameters as well as the role of gasotransmitters in these effects. Therefore, this study aim was to assess the role of gasotransmitter inhibitors in Hcy-induced effects on plasma oxidative stress in rats.

Material and Methods: Study involved 96 male *Wistar albino* rats divided into 8 groups: 1) Control group – saline (1ml 0.9 % NaCl i.p.); 2) DL-Hcy (8 mmol/kg i.p. DL homocysteine (DL-Hcy); 3) L-NAME (10 mg/kg i.p. N ω -Nitro-L-arginine methyl ester (L-NAME), inhibitor of NO production); 4) ZnPPR IX (30 μ mol/kg i.p. protoporphyrin IX zinc (ZnPPR IX), inhibitor of CO production); 5) DL-PAG (50 mg//kg i.p. DL-propargylglycine (DL-PAG), inhibitor of H₂S production); 6) DL-Hcy+L-NAME (8 mmol/kgi.p. DL-Hcy + 10 mg/kg i.p. L-NAME); 7) DL-Hcy+ZnPPR IX (8 mmol/kgi.p. DL-Hcy + 30 μ mol/kg i.p. Zn PPR IX), and 8) DL-Hcy+DL-PAG (8 mmol/kg i.p. DL-Hcy + 50 mg//kg i.p. DL-PAG). In all experimental groups, tested substances were administered in a single dose, intraperitoneally, 60 minutes before animals' euthanasia. In the collected blood samples malondialdehyde concentration, catalase, glutathione peroxidase and superoxide dismutase activity were measured.

Results: Applied substances induced rapid and strong increase of plasma antioxidant enzymatic activity probably as a compensatory response to its pro-oxidant influence.

Conclusion: The effects of Hcy on the activity of plasma antioxidant enzymes are in part mediated via interaction with gasotransmitters.

Keywords: gasotransmitters, homocysteine, oxidative stress markers, rat plasma.

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INTRODUCTION

Homocysteine (Hcy) is a S-containing amino acid and its plasma concentrations can be raised by various constitutive, genetic and lifestyle factors. Hyperhomocysteinemia is a modest independent predictor (risk factor or marker) of cardiovascular disease, stroke, neurodegeneration

and cancer.^{1,2} Some recent studies have shown that Hcy toxicity is mainly caused by oxidative stress via increasing inflammatory response³ and augmenting reactive oxygen species (ROS).⁴ Hyperhomocysteinemia is related with increased ROS formation, including the superox-

ide anion radical (O₂⁻) and hydrogen peroxide (H₂O₂),⁵ and in that way it leads to oxidative stress and cellular damage.⁶ Oxidative stress may influence the endogenous antioxidant system that includes glutathione-dependent enzymes (glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST)), superoxide dismutase (SOD), and catalase (CAT) antioxidant enzymes.^{6,7} There is a evidence that hyperhomocysteinemia may impair the glutathione-related antioxidant defense system,^{8,9} however data about its effect on SOD activity are still controversial. Wilcken et al. have revealed a positive correlation between SOD activity and Hcy levels in patients with homocystinuria,¹⁰ while other authors showed that hyperhomocysteinemia decreased erythrocyte SOD activity in patients with cardiovascular disease.^{9,11}

On the other hand, there are increasing number of assumptions that signaling gaseous molecules as nitric oxide (NO), carbon monoxide (CO) and hydrogen sulfide (H₂S)¹² may have an important role in effects of Hcy-thiolactone on the myocardial function and coronary circulation.¹³ This is very plausible having in mind that gasotransmitters have an important role in the regulation of inflammation, oxidative stress, modulation of mitochondria respiration and activation of antioxidant enzymes.¹⁴ With participation of NO, S-nitroso-Hcy inhibits hydrogen peroxide.¹⁵ H₂S also decreases level of Hcy in plasma¹⁶ and it is strong antioxidant.¹⁷ CO is the most stable gasotransmitter, and it is capable of exerting its effects during long time and distances.¹⁸

However, Hcy-thiolactone and gasotransmitters connection is still not fully understood. Thus, the aim of this research was to examine the effects of acute administration of DL-Hcy alone or in combination with specific inhibitors of different gasotransmitters, such as N ω -nitro-L-arginine methyl ester (L-NAME), Zinc protoporphyrin IX (ZnPPR IX) and DL-Propargyl Glycine (DL-PAG) on oxidative stress markers in the rat plasma – catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) activities and malondialdehyde (MDA) concentration.

METHODS

Physiological Assay and Experimental Protocol

Male Wistar albino rats (n = 96, 12 in each experimental group, 10 weeks old, body weight (250 \pm 30 g) have been used for investigation. Animals were housed in strictly controlled conditions (air temperature of 22 \pm 1°C, relative humidity of 50%, a cycle of brightness: darkness 12:12 hours, starting bright period at 8 AM), with free access to water and standard food. In all experimental groups, tested substances were administered in a single dose, intraperitoneally (i.p.), 60 minutes before euthanasia of animals. All animals were divided into 8 groups, and received: 1) Control group – saline (1 ml 0.9% NaCl i.p., pH 7.4); 2) DL-Hcy group (8 mmol/kg i.p. DL homocysteine); 3) L-NAME group (10 mg/kg i.p. L-NAME as inhibitor of NO production via inhibition of nitric oxide synthase); 4) ZnPPR IX group (30 μ mol/kg i.p. ZnPPR IX as inhibitor of CO production via inhibition of heme oxygenase-1); 5) DL-PAG group (50 mg//kg i.p. DL-PAG as inhibitor of H₂S production via inhibition of cystathionine gamma lyase); 6) DL-Hcy+L-NAME group (8 mmol/kg i.p. DL-Hcy + 10 mg/kg i.p. L-NAME); 7) DL-Hcy+ZnPPR IX group (8 mmol/kg i.p. DL-Hcy + 30 μ mol/kg i.p. ZnPPR IX), and DL-Hcy+DL-PAG group (8 mmol/kg i.p. DL-Hcy + 50 mg//kg i.p. DL-PAG).

All experimental procedures were done in accordance with prescribed legislation (EU Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 86/609/EES) and the principles of ethics.

Biochemical analyses

Sixty minutes after administration of tested substances, the rats were euthanized by decapitation. Blood was collected through a glass funnel and placed in test tubes coated in heparin. The blood samples were left at the room temperature for 15 min and afterwards centrifuged (15 min \times 3000 rpm). The obtained plasma was used for the analyses.

The evaluated parameters were determined in the control condition, and then in acute series of experiments.

Determination of Hcy

The plasma samples were analyzed using the electrochemiluminescence method (ECL- electrochemiluminescence immunoassay system,

ADVIA Centaur XP System, Siemens Healthcare GmbH, Erlangen, Germany); the range of reference values was $\text{Hcy} < 15 \mu\text{mol/l}$.

Determination of lipid peroxidation products (MDA)

MDA content in plasma was determined by using thiobarbituric assay.¹⁴ 500 μl of 25% HCl and 500 μl of 1% thiobarbituric acid (in 50 mM NaOH) was added in 500 μl of sample. The mixture was heated for 10 minutes on boiling water bath, and cooled to room temperature. Then 3 mL of n-butanol was added and shaken on a Vortex for 30 seconds. In order to successfully separate phases, samples are centrifugated (10 minutes at $2000 \times g$). Content of malondialdehyde was determined spectrophotometrically by measuring the absorbance of the organic phase (upper layer) at 532 nm. The blanks contain 50 mM NaOH instead of thiobarbituric acid, and they are prepared for each sample separately. The values of MDA content (nmol of MDA/ml plasma) was determined on the basis of absorbance values and molar absorption coefficient of the malondialdehyde-thiobarbituric acid complex.

Determination of CAT activity

CAT activity was measured by an essay that accompanies the degradation of H_2O_2 according to Beutler.¹⁹ 50 μl of a suspension of plasma was added to the quartz glass tubes at room temperature, consisting of 2.975 ml of 50 mM phosphate buffer solution in 0.4 mM EDTA. The enzyme reaction is initiated by adding 30 μl of 3% H_2O_2 . Reduction in absorbance due to enzymatic degradation of H_2O_2 (at 240 nm for 3-5 minutes) was monitored. CAT activity was expressed as U/ml of plasma. One unit (U) of enzyme activity was defined as 1 micromol of spent H_2O_2 /min.

Determination of SOD activity

The activity of total SOD was measured according to the method of Misra and Fridovich.²⁰ 10-30 μl of plasma was added in 3 ml of a 0.5 M EDTA-sodium carbonate buffer (pH 10.2). The enzymatic reaction started by adding 100 μl of epinephrine (30 mM in 0.1 M HCl). SOD activity was measured at absorbance of 480 nm during 4 minutes. One unit (U) of SOD was defined as the amount of enzyme that inhibits the rate of the epinephrine oxidation for 50%. The enzyme activity was expressed as U/ml of plasma.

Determination of GPx activity

Procedure for measuring of GPx activity begins with preparation of reaction cocktail which con-

sists of 8.9 ml of phosphate buffer, 50 μl of 200 mM reduced glutathione (GSH), 1 mg of β -NADPH, and 100 ml of 100 units/ml GSH-reductase from baker's yeast (*Saccharomyces cerevisiae*). pH value of reaction cocktail was adjusted to 7 (50 mM NaH_2PO_4 + 0.40 mM EDTA). 3 ml of a reaction cocktail and 0.3 ml of plasma sample was added in a quartz glass cuvette (room temperature). Cuvette was placed in a spectrophotometer, and 50 μl of 0.042% H_2O_2 was added in order to start enzymatic reaction ($A_{240} = 0.52$ to 0.56). The decline of absorbance ($\lambda = 340 \text{ nm}$) in intervals of 15 seconds during the 4-5 minutes was monitored. GPx activity was expressed as $\Delta A/\text{min}/\text{ml}$ of plasma.²¹

Chemicals used

All chemicals were of p.a. grade quality and were purchased from Sigma Aldrich (Germany).

Statistical analyses

The parameters of descriptive statistics were used. For testing statistical significance after testing normality of distribution, one-way analysis of variance (ANOVA), followed by Tukey's Post Hoc Test was used. Statistical calculation was done using SPSS computer program (SPSS Inc. Chicago, SAD). Values are presented as mean \pm SEM. $P < 0.05$ was considered statistically significant.

RESULTS

Determination of Hcy

In all plasma samples levels of measured Hcy were more than $65 \mu\text{mol/l}$, indicating moderate hyperhomocysteinemia (30-100 $\mu\text{mol/l}$), except in the Control group in which it was $10.4 \pm 0.6 \mu\text{mol/l}$.

Plasma MDA values

In all experimental groups: DL-Hcy ($6.09 \pm 0.85 \text{ nmol/ml}$ of plasma, Figure 1. a), L-NAME ($5.63 \pm 0.85 \text{ nmol/ml}$ of plasma, Figure 1. a), DL-PAG ($5.28 \pm 1.37 \text{ nmol/ml}$ of plasma, Figure 1. a), DL-Hcy+L-NAME ($4.10 \pm 0.74 \text{ nmol/ml}$ of plasma, Figure 1. b), DL-Hcy+ZnPPR IX ($4.28 \pm 0.59 \text{ nmol/ml}$ of plasma, Figure 1. b), DL-Hcy+DL-PAG ($9.90 \pm 0.94 \text{ nmol/ml}$ of plasma, Figure 1. b), except ZnPPR IX alone ($17.30 \pm 4.74 \text{ nmol/ml}$ of plasma, Figure 1. a), the administration of tested substances induced significant decrease in MDA values compared with control condition ($15.37 \pm 1.41 \text{ nmol/ml}$ of plasma) (Figure 1 a, b).

Plasma CAT activity

In relation to control group (16.40±2.11 U/ml of plasma, Figure 2. a, b), the administration of all tested substances induced significant increase in CAT activity: L-NAME (50.59±3.58 U/ml of plasma, Figure 2. a), ZnPPR IX (67.54±2.08 U/ml of plasma, Figure 2. a), DL- PAG (118.69±12.13 U/ml of plasma, Figure 2. a), DL-Hcy+L-NAME (127.87±19.17 U/ml of plasma, Figure 2. b), DL-Hcy+ZnPPR IX (181.19±16.64 U/ml of plasma, Figure 2. b), DL-Hcy+DL-PAG (109.58± 22.43 U/ml of plasma, Figure 2. b), however only in DL-Hcy group (26.49±4.22 U/ml of plasma, Figure 2. a) there was no significant change in this parameter.

Plasma GPx activity

Levels of GPx were significantly increased by all of the applied substances: L-NAME (3.37±0.23 U/ml of plasma, Figure 3. a), ZnPPR IX (4.50±0.13 U/ml of plasma, Figure 3. a), DL-PAG (7.91±0.80 U/ml of plasma, Figure 3. a),

DL-Hcy+L-NAME (7.60±0.77 U/ml of plasma, Figure 3. b), DL-Hcy+ZnPPR IX (12.59±0.69 U/ml of plasma, Figure 3. b), DL-Hcy+DL-PAG (6.37±0.72 U/ml of plasma, Figure 3. b), except DL-Hcy alone (1.76±0.28 U/ml of plasma, Figure 3. a), compared with control condition (1.09±0.14 U/ml of plasma, Figure 3. a, b).

Plasma SOD activity

Administration of all tested substances: DL-Hcy (30.41±0.71 U/ml of plasma, Figure 4. a), L-NAME (31.07±0.40U/ml of plasma, Figure 4. a), DL-PAG (30.25±0.54 U/ml of plasma, Figure 4. a), DL-Hcy+L-NAME (32.98±0.94 U/ml of plasma, Figure 4. b), DL-Hcy+ZnPPR IX (33.36±0.43 U/ml of plasma, Figure 4. b), DL-Hcy+DL-PAG (32.66±1.20 U/ml of plasma, Figure 4. b) caused significant increase in SOD level compared with control condition (25.31±0.96 U/ml of plasma, Figure 4. a, b), except ZnPPR IX alone (26.46±2.65 U/ml of plasma, Figure 4. a).

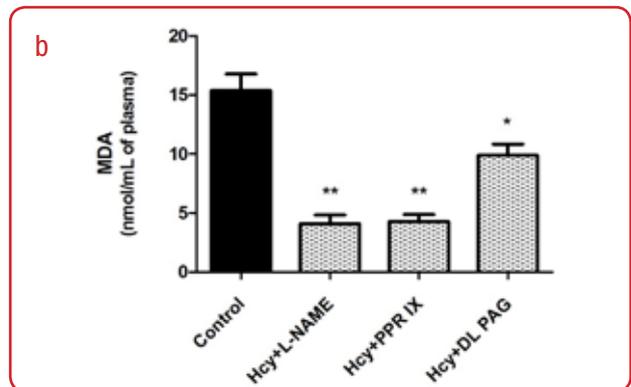
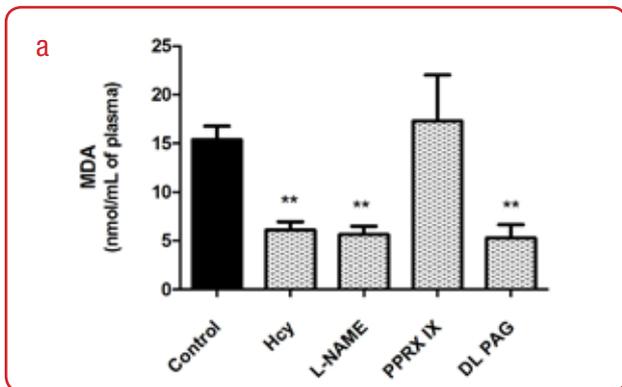


Figure 1. The effects of Hcy and gasotransmitters inhibition separately (a) and its combination (b) on MDA concentration in rat plasma. Values are presented as mean ± SEM. *P < 0.05; **P < 0.01 compared to control group.

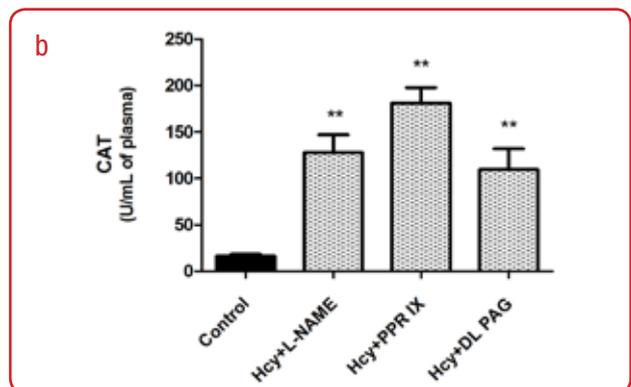
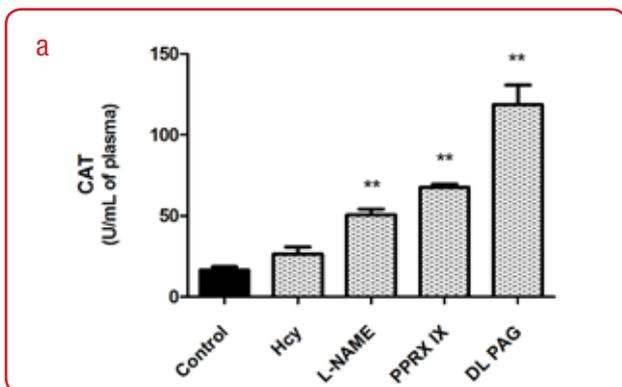


Figure 2. The effects of Hcy and gasotransmitters inhibition separately (a) and its combination (b) on CAT activity in rat plasma. Values are presented as mean ± SEM. **P < 0.01 compared to control group.



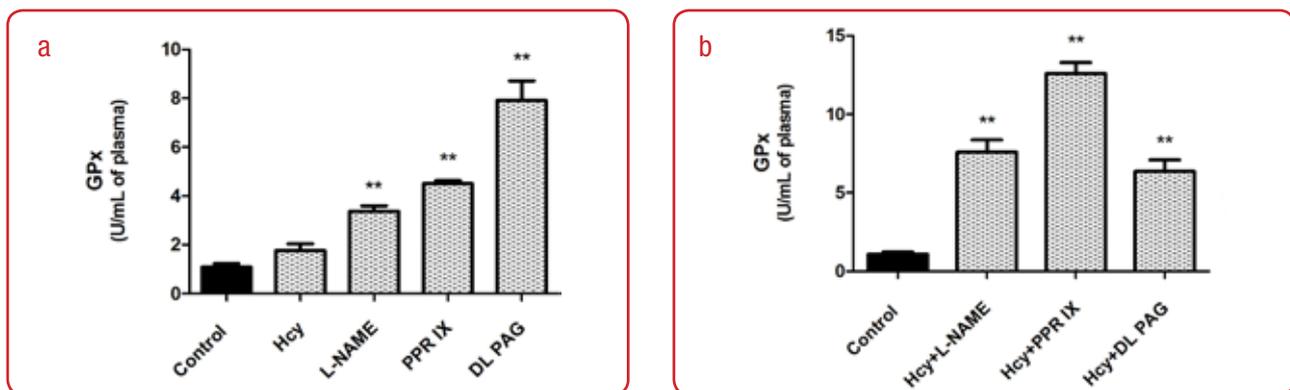


Figure 3. The effects of Hcy and gasotransmitters inhibition separately (a) and its combination (b) on GPx activity in rat plasma. Values are presented as mean \pm SEM. ** $P < 0.01$ compared to control group.

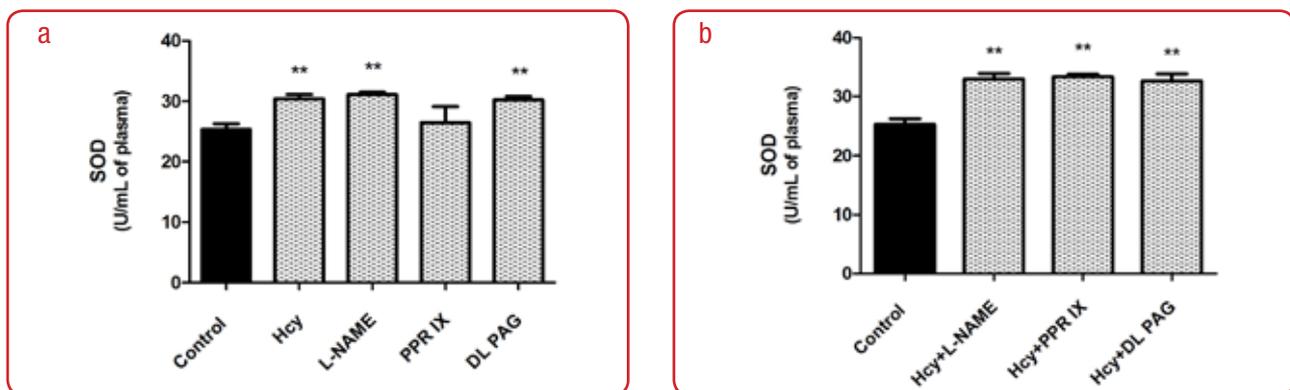


Figure 4. The effects of Hcy and gasotransmitters inhibition separately (a) and its combination (b) on SOD activity in rat plasma. Values are presented as mean \pm SEM. ** $P < 0.01$ compared to control group.

DISCUSSION

In present investigation DL-Hcy caused decrease of MDA level in the plasma, indicating that acutely applied Hcy does not increase lipid peroxidation in plasma of rats. Similar results were observed in case of NO or H₂S inhibition, or during synergistic application of each inhibitor with Hcy. Therefore, we can assume that (except CO) gaseous molecules could induce plasma lipid peroxidation, while in presence of Hcy positive effect of their blockage seems to be even more obvious. Decreased level of MDA in all groups may be consequence of increased activities of antioxidant enzymes in same groups during the same time. We previously noted that thiolactone form of Hcy alone or in combination with gasotransmitter inhibitors non-significantly decreased level of lipid peroxidation measured in coronary venous effluent.¹³

In the second part of our research, we focused on the effects of Hcy and different gasotransmitters production inhibition and on antioxidative

enzyme system such as GPx, CAT, SOD. The obtained results have shown that acute intraperitoneal administration of DL-Hcy induced acute increase of antioxidative enzymatic activity, which correlates with decreased values of MDA. These findings probably mean that DL-Hcy induce strong pro-oxidant effects taking into consideration rapid and significant increased activities of antioxidant enzymes (CAT, GPx, SOD) in plasma following intraperitoneal administration of this substance in rat, and probably as an adaptive response to its pro-oxidant effects. This is in accordance with other study that also showed increase of CAT and SOD activity after acute application.¹¹ However, decrease of plasma MDA level during the same period lead to the conclusion that there was an inverse response to the raise of antioxidant enzymes.

On the other hand, chronic application of Hcy induce drop in antioxidant enzymatic activity,²² probably due to increased time of exposure. In-

terestingly, in our investigation we have noticed reduced levels of MDA in almost all groups, and significantly increased levels of GPx in the same groups. It seems logic, considering that GPx by reducing H₂O₂ level, inhibit lipid peroxidation directly or indirectly by mediation of lipid peroxides.²³

During inhibition of gasotransmitter production, it has been observed increased activity of plasma antioxidant enzymes, indicating that NO, CO or H₂S could react with these enzymes in plasma. However, during inhibition of gaseous signaling molecules production, Hcy con-

tinues to potentiate raise in activity of SOD, CAT and GPx. These results showed no changes in dynamics of measured plasma antioxidants in presence or in absence of gasotransmitter synthesis alone, suggesting that the influences of Hcy are not quite clear. At the end, it is important to emphasize that determination of each gasotransmitter effect was assessed indirectly by their inhibition of production, which could be limitation of this study also. Addition of any data on mRNA and also cellular data would provide more evidence to the notion that enzyme involved in oxidative stress are induced or suppressed.

CONCLUSION

Gasotransmitters inhibitors increased significantly antioxidant enzymes activities and that increase was higher when they were administered in combination with Hcy. This indirectly indicates that gasotransmitters have an important role in oxidative stress protection.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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AUTHORSHIP STATEMENT

Marko Đurić: performing of experimental procedures, statistical analysis, interpretation of data, preparation of the manuscript; Sanja Kostić: statistical analysis, interpretation of data, preparation of the manuscript; Dragana Lončar-Stojiljković: interpretation of data, analysis of results, preparation of the manuscript; Mirjana Čolović: biochemical analysis; Danijela Krstić: biochemical analysis, interpretation of data; Predrag Stevanović: interpretation of data, analysis of results; Dragan Djuric: study design, performing of experimental procedures, statistical analysis, interpretation of data, analysis of results, preparation of the manuscript.

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The Prevalence of Smoking and its Impact on Disability in Multiple Sclerosis

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ABSTRACT

Introduction: Etiology of multiple sclerosis (MS) involves multifactorial interactions among genetic and environmental factors. Cigarette smoking is one of the most important environmental factors that increase the disease risk by about 50%. The aim of the research is to assess the prevalence of smoking in MS patients compared to the general population, and to assess the association between smoking and physical disability in MS patients.

Materials and Methods: A cross-sectional study included 100 MS patients and 50 healthy people. For estimation of the degree of physical disability, the Expanded Disability Status Scale (EDSS) was used, and for clinical and demographic data we used a general questionnaire. In order to collect data on the smoking, the questionnaire for examining risk factors for vascular disease among patients with MS was used.

Results: Analysis of smoking incidence showed that, between MS and control group, smoking had been a dependent variable ($\chi^2 = 6.258$, $p = 0.04$). In smokers, seen in both groups, there was no statistically significant difference in the number of cigarettes, nor in the duration of smoking. There was no significant correlation between smoking in MS group and the index of disease progression ($r=0.216$, $p=0.133$). Also, the relationship between EDSS and smoking had no statistical significance, as well as between the disease course and smoking ($\chi^2 = 1.531$, $p = 0.216$).

Conclusion: In view of the relevance of smoking in MS, it is important to modify this environmental factor and thus prevent its effect on the onset and progression of MS.

Keywords: multiple sclerosis, smoking

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INTRODUCTION

Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory, demyelinating disease of the central nervous system and one of the most common causes of disability in the category of younger adults.¹

The disease has a very heterogeneous clinical course and symptoms. Since the average duration of the disease is longer than twenty years, the accumulation of clinical disorders may significantly affect the daily living of patients.² The etiology of MS involves multifactorial interac-

tions among genetic and environmental factors. Genetic predisposition is only a part of the risk for developing MS, while lifestyle and environmental factors are key participants in the development of the disease. The influence of these factors on the pathogenetic mechanisms is very important and some of them can be modified. Proven risk factors for developing the disease are female gender, smoking, low levels of vitamin D, dietary habits, Epstein Barr virus infections and obesity in childhood and adolescence.³ Cigarette smoking is, however, one of the most

important environmental factors that increase the risk of disease by about 50%. These are data from previously performed studies that indicate that the precise effects of smoking on the onset and development of MS vary in different contexts and different populations.⁴

Consequently, in this study we especially emphasized this issue in our community with all the specifics that characterize it, looking at cigarette smoking as one of the leading risk factors for both cerebrovascular disease (CVD), respiratory diseases, neoplastic diseases, and for the onset, progression, and impact on the therapy of MS. This applies both to active smokers and people exposed to secondhand smoke.

Since the '60s of the last century, smoking has been identified as a significant risk factor for the occurrence and activity of MS. In the study of the Israeli researchers, it has been shown that premorbidly there was a greater percentage of smokers among patients suffering from MS than in the general population (44: 36%).⁵ Most subsequent researches in this period have been related to the data from the questionnaire, and also confirmed the above results.^{6,7}

Recently, considerable research has been done using the control of the nicotine metabolite (cotinine) in the blood, and it was shown that nicotine alone was less important for the onset and progression of the disease than the non-nicotine substances produced by the combustion of tobacco. Most of these studies are related to patients tested after the diagnosis of MS.⁸⁻¹⁰ A prospective study by Salzer et al. confirmed that smoking was as a risk factor for MS, which increased the risk for MS by 50% in patients with elevated levels of cotinine in the blood.¹¹

According to the study of Hedstrom et al, patients who are smokers and use interferon beta 1b therapy have an increased risk of the development of neutralizing antibodies to the drug in comparison to the non-smokers (OR 1.9, $p = 0.002$). Such a correlation was not observed in former smokers.¹²

Olsson et al. in their study showed that smoking had a significant impact on the occurrence of neutralizing antibodies to natalizumab, which reduces or disables the therapeutic effect of this drug.

It was also shown that smoking is associated with a greater number of relapses in natalizumab-treated MS smokers compared to those who did not smoke.¹³

The effect of smoking on the presence of antibodies against John Chunningam (JC) virus in the population of MS patients was also studied, and it was established that smoking is not associated with an increased prevalence of the presence of this virus.¹⁴

Since the eighties of the last century to date, there were numerous studies related to this issue in the world and several in the region; however this is the only study which included patients from the Republic of Srpska.⁴

AIM OF THE STUDY

The aim of our research was to assess the frequency of cigarette smoking in a population of MS patients, compared to the general population matched by gender and age, as well as to estimate the connection of smoking and physical disability measured by The Expanded Disability Status Scale (EDSS) in patients suffering from MS.¹⁵

METHODS

This cross-sectional study was conducted as part of the examination of patients affected by MS in ambulances or during hospitalization at the Department of Neurology, University Clinical Center of the Republic of Srpska, Banja Luka. The sample consisted of 100 MS patients and 50 healthy people. The inclusion criteria were: MS diagnosed according to the McDonald criteria, age 18-69 years, and consent to participate.³ The exclusion criteria were: An exacerbation of the last month, and the use of corticosteroids of the last month.

The study was conducted for twelve months. Participants, both MS patients and healthy people, were introduced with the basic elements of research and voluntarily signed the consent for participation in the study, with the prior approval of the Ethics Committee of University

Clinical Center of the Republic of Srpska, Banja Luka to conduct research in this institution. For the study, it was used a general questionnaire, consisted of questions related to the clinical characteristics of patients: age at onset of MS, duration of illness, the course of MS and value of EDSS score at the time of testing. In order to determine the degree of physical disability, it was used the Expanded Disability status scale (EDSS).¹⁵ In order to collect data on risk factors, it was used the questionnaire for risk factors for vascular diseases in patients with MS. The questionnaire was based on the literature data for the purpose of scientific research at the Institute of Epidemiology, School of Medicine, University of Belgrade. The questionnaire consisted of four parts. Regarding smoking, data were related to the status of participants in relation to smoking (yes/no, ex-smoker), the number of cigarettes smoked per day and the duration of smoking habit (from- to).

Statistical analyses included methods of descriptive statistics (mean, standard deviation, mode, median, skewness/kurtosis), χ^2 test, Student t-test, variance analysis (ANOVA), correlations (Pearson's and Spearman's tests) and regression analysis.

RESULTS

Regarding clinical data relevant to this study, it is important that when it comes to the course of MS, the majority of our patients (72%) had a relapsing-remitting form of MS, 27% had a secondary progressive MS, and 1% had primary progressive MS. The average value of EDSS score in our group of patients was 3.7 ± 2.1 . The mean age of study subjects at enrolment was 41.9 ± 10.1 (range 20-64) in experimental group, and 42.1 ± 12.3 (range 18-63) in control group. Out of the 100 subjects in experimental group, 25 (25%) were male and 75 (75%) female, and out of 50 subjects in control group 14 (28%) were male and 36 (72%) were female.

A detailed analysis of the frequency of smoking in studied groups showed that, in comparison to affiliation to MS or control group, smoking has been a dependent variable ($\chi^2 = 6.258$, $p = 0.04$). Figure 1 shows that the structure of the response varied between the experimental and control group.

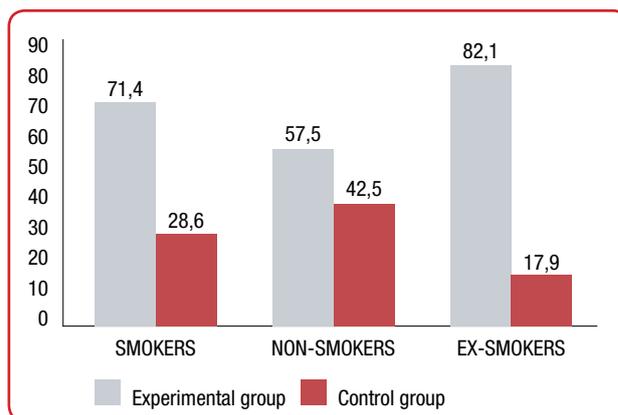


Figure 1: Representation of smoking in the control and experimental group (%)

Among smokers, analyzed in both groups, there was no statistically significant difference in the number of smoked cigarettes nor the duration of smoking (table 1).

Table 1: Representation of smoking in the control and experimental group (%)

Group	Number	Number of cigarettes		Duration of smoking	
		Average value	Standard deviation	Average value	Standard deviation
Experimental	58	15.17	8.10	18.47	10.54
Control	19	16.12	6.77	23.16	10.18

In accordance with the objectives of the research, in terms of finding a connection between smoking in the experimental group and index of disease progression, we used the Pearson correlation coefficient. However, in relation to the analyzed variable, the correlation coefficient had a low absolute value, which means that there was no statistically significant correlation with an index of disease progression ($r=0.167$, $p=0.251$).

Then it was used the Spearman correlation coefficient, but for our variable mentioned coefficient could not be interpreted (had no statistical significance). It was shown that the relationship between EDSS and smoking had no statistical significance.

We have also analyzed the relationship between the disease and smoking. In order to establish a possible relationship between these parameters we used chi-square test, but we did not find any significance ($\chi^2 = 1.531$, $p = 0.216$).



DISCUSSION

A significant number of studies showed that smoking had increased, even up to 50%, the risk of developing of MS.^{5, 6, 8, 16, 17, 18, 19}

Our analysis showed that smoking, in relation to the affiliation to the experimental or the control group, was a dependent variable ($p = 0.04$). Data from our survey also show that the proportion of smokers in examined groups was statistically significantly different, as confirmed by the chi-square test ($\chi^2 = 6.258$). There were 71.4% of smokers in the experimental group and 28.6% in the control group. The complete structure is shown in Figure 1. Specifically, while in the experimental group share of smokers and ex-smokers dominates, the same share does not apply to the control group.

Pekmezović et al. conducted a survey in the Belgrade area and showed that smoking is significantly more frequent in the experimental group than in the control group. They also found that smoking was a significant and independent risk factor for MS.²⁰

This is also shown in two studies by Hedstrom et al, where smoking is identified as a risk factor for the development of MS.^{21,22}

Salzer et al. found that the riskiest group were young adults up to 26.4 years of age.¹¹ Therefore, the same researchers conducted a study on the risk of passive smoking on developing of MS and concluded that the risk was significantly higher in patients who were exposed to inhalation of tobacco smoke than in those who were not. This risk increased with longer exposure to the agent.²³ Salzer et al. analyzed blood samples in women who smoked during the gestation period and did not observe a higher risk of MS after childbirth, nor after 26.4 years of age of children whose mothers smoked during pregnancy.²³ A weak link between smoking during pregnancy and the postpartal onset of MS was also shown by Montgomery and al.²⁴ In a study published in 2007 it has been shown that children whose parents smoke have a greater risk of developing MS, and this risk increased with higher exposure to secondhand smoke.²⁵ Research of Tardiua et al. has shown that there is a significantly higher risk for the development of MS in children who had a clinically isolated syndrome, and who were exposed to second-

hand smoke compared to those who were not.²⁶ Similar results about young adulthood and exposure to risk factors (elevated BMI, cytomegalovirus infection, the level of vitamin D), and smoking as the most important risk factor for the appearance of the MS in that age were provided by other researchers.^{27, 28, 29}

Based on our results we found that in smokers, seen in both groups, there was no statistically significant difference in the number of cigarettes, nor in the duration of smoking.

However, Iranian researchers in a study published in 2013 in a population of patients with MS in their own country, established that the risk of MS was significantly higher in male smokers and in those who have been taking of large amounts of cigarettes.³⁰

The same year, the results of another study were published, which showed the similar results as the above-mentioned study, with addition that this group of researchers found a statistically significant positive correlation between the number of cigarettes (more than ten) during the day, and the development of secondary progressive MS ($p = 0.007$).³¹

Recently, studies that assess the effect of smoking on the progression of the disease, i.e. transition from relapsing-remitting (RR) form into a progressive form can be found. In our study, we did not find results seen in many previous studies, in terms of an interaction between the disease and smoking ($\chi^2 = 1.531$, $p = 0.216$), nor the impact of smoking on the index of the disease progression ($r = 0.167$, $p = 0.251$). This can be explained by a certain specificity of our participants who were under regular medical control and without any significant influence of other comorbidity conditions, as well as the data presented in Figure 1 which show that the high percentage of participants of the experimental group (82.10%) are former smokers, in contrast to the control group where the percentage was 17.90%.

The study of Hernan et al. showed that smokers with the RR form of MS are three times more likely to move to secondary progression than

non-smokers, as only 20 of the 179 patients who had never smoked or were former smokers had a progressive course of the disease.⁷

In the study of Sundstrom et al, out of 122 patients with newly diagnosed MS, 72% of them were ex-smokers, and those who started smoking before the age of 15 developed the secondary form of the disease after an average of six years of follow up. Forty percent of ex-smokers who started smoking after the age of 15 years developed a secondary progression, and it happened with only 26% of non-smokers.⁸

Di Pauli et al followed 129 patients with the clinically isolated syndrome (CIS) who were at high risk for the MS according to the MRI and cerebrospinal fluid analyses. After three years, 75% of smokers developed MS, compared to 51% of non-smokers.³²

In contrast to these results, Seine et al. examined 205 women with clinically definite MS from Portugal and noted that smoking had a protective effect on women with ApoE4 allele. Smokers had lower scores on the EDSS ($p= 0.023$) and MSSS scale ($p= 0.033$).³³

Only one study, by Pittas et al, took into account the potential confounding effect of other habits of people with disease.³⁴

In several studies, cigarette smoking is shown to be a factor associated with an increased level of gadolinium discolored lesions, and the volume of T2 hyperintense lesions on MRI, and brain atrophy. However, none of these studies had a control group of people without MS, which would give more informative results.^{35, 36}

Several other studies have shown a positive correlation between cigarette smoking and disease progression.³⁷

Manouchehrinia et al. analyzed data from 895 patients, of whom 49% were smokers at the time of onset of the disease and achieving the diagnosis. Their results showed that smoking was associated with increased severity of the disease and more rapid progression. In fact, the cessation of

smoking before or after the diagnosis of the disease was associated with a slower progression.³⁸ Results from a study done in 2012 showed that cigarette smoking increased the risk of reaching the value of six on the EDSS, in relation to other factors that have been analyzed (coffee and alcohol drinking, consumption of fish).³⁹

Despite the above data that unambiguously show the impact of smoking on the onset and progression of MS, it has not yet been determined exactly which is the true mechanism responsible for these results. This refers to the biochemical processes related to smoking, genetic mechanisms and influence on the development of comorbidities, which then have an impact on MS.^{10,38,40} Probably there are more associated mechanisms which can not be completely separated, as well as with other CVD risk factors which affect MS.

CONCLUSION

In view of the above facts, obtained in this and other studies on the effects of smoking on the onset and progression of MS, the impact of the therapy, the mortality and the number of hospital days, it would be very important, in each community individually including our own, to eliminate this environmental modifiable factor. This way it is possible to avoid an adverse impact on the health of the entire population, and the impact on the overall quality of life of patients with MS. And, finally, it is especially important that individuals with a history of MS in the family, should be advised about the impact of smoking in MS, and the importance of preventing their children's exposure to secondhand smoke.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Does Gender Influence Quality of Life in Children with Atopic Dermatitis?

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ABSTRACT

Introduction: Atopic dermatitis (AD) is a chronic skin disease that has a significant impact on quality of life (QoL). The aim of this paper was to evaluate if gender affects some aspects of QoL in children with AD.

Materials and methods: The cross-sectional study was conducted at the Clinic of Dermatology and Venereology, Clinical Center of Montenegro, between August 2017 and July 2018 and included 200 children aged 5–16 years with AD diagnosis. The severity of disease was measured by the Three Item Severity (TIS) score, while QoL was assessed with the Children's Dermatology Life Quality Index (CDLQI). Socio-demographic data on children with AD were collected by a short questionnaire.

Results: The difference in overall CDLQI between boys and girls was not statistically significant. The domains that were most affected by AD were school/holidays (mean score 2.27 ± 0.65), and symptoms (mean score 2.22 ± 0.70). Swimming/sports activities, school/holidays, teasing/bullying and sleep disturbance were more affected in girls. Overall CDLQI score and CDLQI subscale scores (except school/holidays and sleep in boys) significantly correlated with TIS. According to multivariate logistic regression analyses statistically significant differences between two genders were not found for age, AD severity, concomitant atopic disease, and family history of atopic disease.

Conclusion: Although we did not find differences between the two genders in the overall health related QoL, this study confirmed the tendency for AD to have a more severe impact on girls' lives. These results may influence treatment and counselling of children affected with AD.

Key words: atopic dermatitis, children, gender, quality of life, CDLQI, TIS, dermatology, epidemiology

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INTRODUCTION

Atopic dermatitis (AD) is the commonest chronic inflammatory skin disease in children with a significant burden on healthcare resources.^{1,2,3}

Although AD may occur at any age, it most often begins in infancy and childhood. The prevalence estimates of AD vary worldwide, with highest prevalence in developed, high-income countries, where AD affects over 20% of children.^{4,5} Over the last few decades the number of AD patients has doubled in most parts of the world, especially in low-income countries.³ AD has a great im-

act on quality of life (QoL) of the affected children.⁶⁻⁹ In order to effectively use QoL results, it is important to know about existing gender differences in QoL assessment. However, previously reported results concerning gender differences in QoL of young (0-4 age) and older children (5-16 ages) with AD were contradictory.¹⁰⁻¹⁶

The purpose of this study was to analyze gender differences in health-related QoL in older children (5-16 years of age) suffering from AD.



METHODS

Study design and participants

A cross-sectional study was carried out at the Clinic of Dermatology and Venereology, Clinical Center of Montenegro (CCM) in Podgorica between August 2017 and July 2018. The study included 200 children (78 boys and 122 girls) aged 5 to 16 years with confirmed AD diagnosis made using Hanifin and Rajka criteria.¹⁷ The study was approved by the Ethics Committee of the CCM, Podgorica. Written informed consent was obtained from parents of all 200 children with AD.

Data collection

Socio-demographic data on children with AD (child's gender, age, concomitant atopic disease and family history of atopic disease) were collected by a short questionnaire.

Disease severity was assessed using the Three Item Severity (TIS) score, which corresponds well with the more detailed objective SCORAD (SCORing Atopic Dermatitis) index.^{18,19} It is a simple scoring system which use three of the intensity items of the SCORAD index: erythema (0–3), edema (0–3), and excoriations (0–3) in one or several different representative areas with the maximum score of 9. Based on the TIS, the severity of AD was classified into mild (<3), moderate (3–5) and severe (≥6).

The health related QoL of children was measured using the Children's Dermatology Life Quality Index (CDLQI). The CDLQI is a specific QoL instrument for measurement of impact of skin diseases on QoL of children aged between 5 and 16 years.²⁰ It is 10 item structured questionnaire that assess symptoms and feelings (2 items), leisure (3 items), school or holidays (1 item), personal relationships (2 items), sleeping (1 item) and treatment (1 item). All questions are related to the week preceding its application. The item related to assessment of impact of AD on school activities has an alternative option to assess the effect of AD on holiday activities, in case the child is evaluated during school holidays. If both options are rated by a child, higher of the two scores is included in the total CDLQI score. Answers are scored on a 4-point scale from 0 (not at all) to 3 (very much). The CDLQI total score is calculated by summing the score of

each question (0–3) resulting in a minimum of 0 and a maximum of 30. The higher the score, the more QoL is impaired. Serbian version of CDLQI has been validated previously.²¹ The severity banding of total CDLQI score is suggested as follows: 0 to 1 – no effect; 2 to 6 – small effect; 7 to 12 – moderate effect; 13 to 18 – very large effect and 19 to 30 – extremely large effect of AD on child's life.²²

Statistical analysis

Descriptive statistics were used to describe the cohort of children. Continuous variables were presented as mean ± SD, and categorical values as frequencies and percentages. To assess differences between variables in boys and girls, Hi square test and Student's t-test were used where appropriate. Correlation between CDLQI scores and AD severity (TIS score) was assessed using Pearson's correlation coefficients. Multivariate logistic regression analyses were performed with gender as dependent variable and age, concomitant atopic disease, diseases severity (TIS score), CDLQI overall score and family history of disease as independent variables. Cronbach's alpha was applied to assess the reliability of CDLQI. A two-tailed probability value of 0.05 or less was considered significant. All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS), version 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Socio-demographic and clinical characteristics of the study sample (n = 200) according to gender are presented in Table 1. There were no significant differences between boys (78) and girls (122) in mean age, concomitant atopic disease, family history of atopic diseases, and diseases severity.

The overall CDLQI score and scores for all 10 items of CDLQI according to gender are shown in Table 2. The mean CDLQI score of the total sample was 17.11 ± 5.89. The difference in overall CDLQI between boys and girls was not statistically significant (P = 0.217). Overall, the domains that were most affected by AD were school/holidays (mean score 2.27 ± 0.65), symptoms (mean score 2.22 ± 0.70), followed by friendships (1.86 ± 0.85) and leisure/hob-

Table 1: Characteristics of the study sample according to gender

Characteristic	All	Boys	Girls	<i>P</i>
Total sample	200 (100)	78 (39.0)	122 (61.0)	
Age				
Range	5–16	5–16	6–16	
Mean ± SD	11.68 ± 2.73	11.74±2.54	11.64±2.86	0.793*
Atopy				
AD alone	78 (39.0)	34 (43.6)	44 (36.1)	0.287**
AD and atopic disease	122 (61.0)	44 (56.4)	78 (63.9)	
Family history of atopic disease				
Yes	155 (77.5)	56 (71.8)	99 (81.1)	0.122**
NO	45 (22.5)	22 (28.2)	23 (18.9)	
Disease severity, n (%)				
TIS (mean ± SD)	5.10 ± 0.90	5.00±0.88	5.16±0.91	0.211*
Mild (TIS = 0–2)	/	/	/	
Mild (TIS = 0–2)	164 (82.0)	66 (84.6)	98 (80.3)	0.441**
Severe (TIS ≥ 6)	36 (18.0)	12 (15.4)	24 (19.7)	

* t-test; ** χ^2 test

AD – atopic dermatitis; Atopic disease – asthma, allergic rhinitis, and/or allergic conjunctivitis; SD – Standard deviation; TIS – Three-Item Severity score.

Table 2: The Children's Dermatology Life Quality Index (CDLQI) according to gender

CDLQI (mean ± SD)	All (n = 200)	Boys (n = 78)	Girls (n = 122)	<i>P</i> *
Total score	17.11 ± 5.89	16.20 ± 5.94	17.70 ± 5.80	0.217
1. Symptoms	2.22 ± 0.70	2.18 ± 0.72	2.25 ± 0.70	0.516
2. Feelings	1.61 ± 1.11	1.54 ± 1.09	1.65 ± 1.13	0.492
3. Friendship	1.86 ± 0.85	1.90 ± 0.81	1.84 ± 0.87	0.620
4. Clothes/shoes	1.59 ± 0.89	1.67 ± 0.92	1.54 ± 0.86	0.329
5. Leisure/hobbies	1.86 ± 0.80	1.72 ± 0.85	1.95 ± 0.76	0.051
6. Swimming/sports	1.56 ± 1.04	1.36 ± 1.13	1.69 ± 0.97	0.035
7. School/holidays	2.27 ± 0.65	2.13 ± 0.69	2.36 ± 0.60	0.013
8. Teasing/bullying	1.68 ± 0.80	1.44 ± 0.85	1.84 ± 0.73	0.001
9. Sleep	1.20 ± 0.85	1.03 ± 0.90	1.31 ± 0.80	0.020
10. Treatment	1.28 ± 0.72	1.26 ± 0.75	1.29 ± 0.71	0.714

* t-test **Bold** values stand for statistical significance.**Table 3:** Distribution of the overall CDLQI scores of boys and girls with AD according to the CDLQI banding

CDLQI score	Total N (%)	Boys N (%)	Girls N (%)	<i>p</i>
No effect (0–1)	/	/	/	
Small effect (2–6)	4 (2.0)	4 (5.1)	/	
Moderate effect (7–12)	44 (22.2)	22 (28.2)	22 (18.3)	0.009
Very large effect (13–18)	64 (32.3)	18 (23.1)	46 (38.3)	
Extremely large effect (19–30)	86 (43.4)	34 (43.6)	52 (43.3)	

bies (1.86 ± 0.80). Swimming/sports activities, school/holidays, teasing/bullying and sleep disturbances were more affected in girls (Table 2). Statistically significant difference in distribution of the overall CDLQI scores of boys and girls

with AD according to the CDLQI banding is presented in Table 3. The effect of AD was more likely to be moderate in boys and very large in girls ($p = 0.009$).

Table 4: Correlation between CDLQI scores with AD severity (TIS score)

CDLQI score	Boys Coefficient *	<i>p</i>	Girls Coefficient *	<i>p</i>
Overall CDLQI	0.39	<0.001	0.62	<0.001
Symptoms and feelings (questions 1 and 2)	0.37	0.001	0.53	<0.001
Leisure (questions 4, 5 and 6)	0.27	0.018	0.49	<0.001
School/holidays (question 7)	0.08	0.458	0.37	<0.001
Personal Relationships (questions 3 and 8)	0.46	<0.001	0.47	<0.001
Sleep (question 9)	0.20	0.084	0.49	<0.001
Treatment (question 10)	0.35	0.001	0.53	<0.001

CDLQI – Children's Dermatology Life Quality Index

*Pearson's correlation coefficient. TIS – Three-item severity score; AD –Atopic dermatitis.

Bold values stand for statistical significance.

Overall CDLQI score and CDLQI subscale scores (except school/holidays and sleep in boys) significantly correlated with TIS (Table 4). In comparison with boys, stronger correlation was seen in girls for overall CDLQI score ($r = 0.62$ for girls and $r = 0.39$ for boys) and CDLQI subscale scores. However, CDLQI overall score did not correlated significantly with the age of boys and girls ($r = -0.09$, $p = 0.411$ for boys and $r = -0.10$, $p = 0.271$ for girls).

DISCUSSION

In this study the difference in overall CDLQI score between boys and girls was not significant ($P = 0.217$) that is in agreement with the results of previous studies.^{15,16} The international multi-centre study on self-assessed QoL in 167 AD non-matched children, 5–16 years old, from Ukraine, Czech Republic, Singapore, and Italy did not find any significant gender differences between boys and girls in CDLQI results.¹⁵ In the matched analysis of the same international study in which each child in the group of boys ($N = 36$) was matched to a corresponding child in the group of girls ($N = 36$) from the same country whose age and SCORAD value were almost identical, Chernyshov et al. did not find differences in overall CDLQI.¹⁶ On the contrary, Kiebert et al. found a significant gender difference in QoL of older children with AD with significantly higher CDLQI scores in girls.¹⁰

In the present study girls with AD in comparison with boys, assessed CDLQI on swimming/sports, school/holidays, teasing/bullying and

According to multivariate logistic regression analyses statistically significant differences between two genders were not found for age, AD severity measured by TIS, concomitant atopic disease, and family history of atopic disease (data not shown).

The good internal consistency of the CDLQI was demonstrated with a Cronbach's alpha coefficient of 0.88.

sleep significantly higher. However, we did not find statistically significant differences between the two genders in the CDLQI subscale symptoms and feeling that is in contrast with the study conducted by Balci et al.²³ In the matched study by Chernyshov et al. the CDLQI subscale on symptoms and feeling was assessed significantly higher by girls with AD. A gender difference was found for the item feelings while there was no gender difference in the assessment of the item symptoms. Girls were more embarrassed, self-conscious, upset and sad because of AD.¹⁶ Like Chernyshov et al. we did not find gender differences in problems with issues of clothes and shoes (changing and wearing different and special clothes or shoes because of child's skin).¹⁶ In contrast, Hon et al. reported that girls had more problems with clothes and shoes than did boys.¹²

In our study overall CDLQI score correlated well with disease severity that is in accordance with previous studies.^{24-26,15, 9,16} Besides the overall CDLQI score, the CDLQI subscale scores

on symptoms and feelings, leisure, personal relationships, and treatment correlated well with disease severity in both genders, while significant correlation on school/holidays and sleep was found only in girls. In comparison with boys, stronger correlation was seen in girls for overall CDLQI score ($r = 0.62$ vs. $r = 0.39$) and all CDLQI subscale scores. In the matched study conducted by Chernyshov et al.¹⁶ two separate items in boys (symptoms and feelings) and five items in girls (symptoms, feelings, friendship, playing and doing hobbies, and swimming) significantly correlated with AD severity. In our study the CDLQI item on problems with swimming and other sports was the sixth highly scored item in girls. In the study by Ang et al.²⁷ this item was the second highly scored item, and one of the lowest scored items in the study by Chernyshov et al.¹⁶

To the best of our knowledge this is one of a few studies on gender differences of QoL in children with AD in Western Balkan with relatively high number of patients. However, some limitations of the present study should be mentioned. First, only children with moderate to severe forms of AD were enrolled in the study. The reason is that the most severe forms of AD are treated at the CCM, a tertiary health care center. Because of

that it is not possible to generalize the results of our study to other patients affected by AD in the population of Montenegro. Second, like most authors, we did not apply matching in the analysis, although it has been recommended that gender differences can be adequately studied only if the children in the girls' and boys' groups were individually matched across groups for age and severity of AD.¹³

Although we did not find differences between the two genders in the overall health-related QoL, we confirmed the tendency for AD to have a more severe impact on girls' lives. For example, swimming/sports activities, school/holidays activities, teasing/bullying and sleep disturbance were more affected by girls. Symptoms, such as itching, scratching and pain and feeling such as embarrassment or self-conscious, upset or sad, were increased with AD disease severity in both genders, especially in girls who in general need more attention to their AD-related psychological problems. These results may influence treatment and counselling of children affected with AD. Doctors should focus on the prevention of avoidance behaviour in girls who have more severe AD, because such avoidance behaviour may affect other domains of girls' lives, like friendships, hobbies, sports, and even school work.

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permission to translate and use the CDLQI in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Students' Attitudes About the Ethics of Using Animals for Experimental Purposes

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ABSTRACT

This study aims at discovering the ethics level of the students who are attending the Faculty of Medical Sciences, the Faculty of Engineering Sciences and postgraduate students with experience of use laboratory animals in experimental purposes at University of Kragujevac. Focus of this study is on ethical segment of use of laboratory animals for experimental purposes and the correlation between the manifested level of ethics and various other factors like gender, high-school education, GPA (grade point average), educational level of parents and desired affiliation after the graduation.

The cross sectional study was conducted with 175 participants. In data collection, a questionnaire was used consisting of questions concerning sociodemographical characteristics and two scales – Gallup and Beckstead's scale and a scale specially designed for the purposes of this research. The data were processed by using the methods of descriptive statistics, t-test for independent samples, single factor analysis of variance and Pearson's correlation coefficient.

The results have shown that the correlation between the manifested level of ethics of using animals in experimental purposes and gender, high-school education, grade point average, field of study or desired affiliation after the graduation is statistically significant. The differences in the results between students of pharmacy and the students of engineering sciences are also statistically significant. Cronbach's alpha were estimated at 0.786, which implies that the internal consistency of scales shows significantly high level of reliability. The students from both fields, pharmacy and mechanical engineering, have shown a considerable level of ethics concerning the use of animals for laboratory purposes.

Key words: ethics, laboratory animal, attitude

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INTRODUCTION

Even though scientific experiments have been constantly contributing to the development of modern science, experiments on live animals have always been a matter of controversy since the ethics of such actions has been often questioned. The principles of modern ethics, oriented towards biocentrism, require that experiments conducted on animals should be reduced in number as much as possible and that they should be performed taking into consideration the welfare of animals so that the animals' pain, suffer, fear and stress are minimalized.¹

The ethical experiments involving the procedures of manipulation with experimental animals aim at the acquisition of new knowledge in the field of biomedicine and pharmacology, and thus they contribute to a general development of medicine and pharmacology.² These procedures include the deliberate sacrifice of experimental animals in order to get isolated organs or to sacrifice an animal at the end of the experiment. The death of animals must occur in an instant (the procedures which meet this criterion include the triple dose of anesthetics, inhalation of



CO₂ or other gases in special chambers, cervical dislocation, decapitation, etc.) and must not cause suffering or pain to an animal.²

According to the data obtained by World Society for the Protection of Animals (WSPA) by the end of 2006, only 65 countries out of 120 had national legislation dealing with legal aspects of animal protection.³ The first law concerning the animal welfare was adopted in Republic of Serbia in 2009. The Animal Welfare Act regulates the number of important issues concerning the preservation and the promotion of animal welfare in various situations, including the use of animals in experimental or other scientific purposes which may cause injury, pain, suffering, fear and stress to an animal, impair its health and cause permanent or temporary disruption of physical, psychological, or genetic integrity and finally result in its death.⁴

The attitudes of individuals concerning this matter, and ethics in general, mainly arise from their attitudes towards the consequences of such actions, normative beliefs about the expected behaviour and controlling beliefs regarding the feasibility of conduct.⁵ Attitudes towards animals are influenced by many psychological factors. An insight into the attitudes can be obtained by using various different data collection methods such as a questionnaire.^{6,7}

A review of national academic and scientific literature leads to a conclusion that there is a relatively small number of studies dealing with this aspect of ethics in medical workers and students. The idea for this research arose from this particular deficiency.

Assuming that this topic did not get the attention it deserves and that there is no a 'sufficient' level of awareness among the general population, the study was conducted on two groups of university students. The students of the first group study medical sciences whereas the students of the second group study technical sciences.

The objective of this paper is to determine the level of ethics among the students attending the Faculty of Engineering Sciences (students do not have any laboratory experience), the Faculty of Medical Sciences (which students have had contact with laboratory during their studies and who have learnt about experiments on animals)

and postgraduate students (who have almost everyday experience with laboratory animals) and the correlation between that level and various other factors like gender, high-school education, GPA (grade point average), educational level of parents and desired affiliation after the graduation.

METHODS

The research is conducted on participants who attend the Faculty of Medical Sciences and the Faculty of Engineering Sciences at University of Kragujevac. It includes 175 participants, among them 62 males and 113 females. This study has three groups: 73 students who attend the Faculty of Engineering Sciences, 78 students who attend the Faculty of Medical Sciences, and 24 postgraduate students.

This study is observational and its methodology corresponds to a study of prevalence where the participants are filling in a questionnaire at the same time. The participants involved in this study participated voluntarily and anonymously.

For data collection, a questionnaire is used consisting of 3 parts and general part referring to some basic information about the study itself (Appendix 1). The first part consists of questions about the sociodemographic characteristics of the participants (gender, age, high-school education, GPA, desired affiliation after graduation, the educational level of their parents and whether they have a pet). The second segment is Gallup and Beckstead scale (from 1988),⁸ which was reused in Metzger's study (2014),⁹ while the third part is dedicated to a scale designed for the purposes of this research.

Respondents were asked to indicate (in the second, third and the fourth part of the questionnaire) the degree of agreement with each statement on the Likert's scale, from 1 (completely disagree) to 5 (totally agree). The final score was calculated by adding points for separate statements thus obtaining the total score. Then, as in original studies, the mean value was determined. Higher means values indicate a higher level of ethics. Similarly, the statements considered as negative attitudes towards the issue were

scored inversely in respect to those which speak positively. This procedure is often used in order to avoid the tendency of respondents to consistently agree or disagree with statements. In Gallup and Beckstead's scale, such statements were under the numbers 3, 4, 6, 8, 10, 11, 12, 14, and in the scale designed for this particular research under the numbers 2 and 5. In the fourth part of the questionnaire, each claim was scored individually and then the mean value was obtained. For the second part of the questionnaire, the total number of points that a respondent could have achieved on the scale ranged from 14 to 70 with higher scores implying a higher level of ethics, while in the third part, the score ranged from 9 to 45.

This study has investigated whether there is an impact of gender, type of high-school education, the field of study at university and prior knowledge on the subject, grade point average, desired

affiliation after graduation, owning a pet and the educational levels of parents (which in this case are independent variables) on the level of ethics concerning the use of experimental animals for laboratory purposes in students (dependent variable, outcome).

The statistical analysis of the data was conducted by using the software package IBM SPSS Statistics v20. The results were obtained through the methods of descriptive statistics (perceptual distribution, mean and standard deviation). In order to determine the influence of individual factors on the ethics a t-test for independent samples and single factor analysis of variance (ANOVA) were used. The results were considered statistically significant if the probability of the null hypothesis (p) was < 0.05 . Pearson's correlation coefficient was used to determine the relationship between quantitative variables.

RESULTS

During the winter semester of the school year 2014/2015, 180 respondents (the students of integrated academic studies of pharmacy and postgraduate students from the Faculty of Medical Sciences and the students from the Faculty of Engineering Sciences) underwent the interviewing. 175 questionnaires out of 180 were filled in and returned (the percentage of answers

97.22%). The average age of the participants amounts 21.5 ± 1.489 .

Results for the Gallup and Beckstead scale and the scale designed for this research are presented in Tables 1, 2 and 3 in the form of mean values and standard deviations. The frequency distribution histograms are shown in Figures 1 and 2.

Table 1: Results for all participants (n=175)

	Gallup and Beckstead scale	The scale designed for this research
Mean value and standard deviation	47.09 \pm 7.42	29.15 \pm 6.48
Minimal values	19	9
Maximum values	62	45
Percentiles	25.	42.00
	50.	48.00
	75.	52.00

The differences in ethics in respect to gender

In order to find an answer to the question of whether there is a difference in the level of ethics among students of different gender a t-test for independent samples is used. Females achieved slightly higher average scores on the Gallup and Beckstead scale (48.04 ± 6.247) in respect to males (45.34 ± 8.977), t-test indicated that this

difference is statistically significant ($t = -2.109$; $p = 0.038$). Regarding the scale designed for the purpose of this research, female participants achieved a slightly higher average score (30.04 ± 5.755) in comparison to males (27.55 ± 7.410), and this difference was statistically significant too ($t = -2.291$; $p = 0.024$).

The differences in ethics in respect to high-school education

A t-test for independent samples was used for determining the potential links between the ethics level of the students and their high-school education. The students who attended medical high school (50.37 ± 5.254) had higher average scores for the Gallup and Beckstead scale in respect to students who have completed other high-schools (44.85 ± 7.859). However, the statistical tests show that the difference is statistically significant $t(175) = 5.568$; $p = 0.00$. In case of the scale designed for this research, students who completed medical schools (32.23 ± 4.079) have also shown higher average score

in respect to students who finished other high schools (27.06 ± 6.978). The t-test proves that there is a statistically significant difference between the two groups of students $t(175) = 6.165$; $p = 0.00$.

The differences in ethics in respect to the field of study

To determine whether there is a difference in ethics level among the students in respect to the faculty they attend, an ANOVA was used. The objective of this paper was to determine the level of ethics among the students attending the Faculty of Engineering Sciences (students who do not have any laboratory experience), the stu-

Table 2: Results for the scales in respect to participant characteristics

	Variable	Number of participants
Gender	Male	62 (35.4 %)
	Female	113 (64.6 %)
High-school education	Medical high-school	71 (40.6 %)
	Other schools	104 (59.4 %)
A University student attends	Faculty of Medical Sciences	78 (44.6 %)
	Faculty of Engineering Sciences	73 (41.7 %)
	Postgraduate student	24 (13.7 %)
GPA	<7.0	0 (0%)
	7.01-7.5	11 (6.3 %)
	7.51-8.0	37 (21.1 %)
	8.01-8.5	37 (21.1 %)
	8.51-9.0	52 (29.7 %)
	9.01-9.5	31 (17.7 %)
Desired affiliation after the graduation	>9.51	7 (4.0 %)
	Working in a pharmacy, clinical pharmacist	43 (24.6 %)
	Working as pharmaceutical company representative	13 (7.4 %)
	Working in academic and scientific research sector	37 (21.1 %)
	Working in education sector	24 (13.7 %)
	Working in the production of medicines sector	8 (4.6 %)
	Working in the medicines' quality control sector	1 (0.6 %)
Other	49 (28.0 %)	

dents from Faculty of Medical Sciences (students have seen laboratory during their studies and have learnt about experiments on animals) and postgraduate students (postgraduate students and students who volunteer in laboratory - who have almost everyday experience with laboratory animals). In both cases, Gallup and Beckstead scale and the scale designed for this research, statistically significant relation was found ($F = 35.853$; $p = 0.000$ and $F = 42.183$; $p = 0.000$, retrospectively). Results for both scales are presented in Figure 3.

The difference in ethics in respect to desired affiliation after graduation

ANOVA was used in order to determine whether there is a statistically significant difference between the level of ethics and desired affiliation of students. In Gallup and Beckstead scale and in the scale designed for this research, statistically significant relation was found ($F = 3.473$; $p = 0.003$ and $F = 7.283$; $p = 0.000$, retrospectively). The results for both scales are presented in Figure 4.

Students who want to work with patients directly have higher average scores on the Gallup and Beckstead scale (50.67 ± 5.437) compared to students who wish to engage in any other job positions (45.92 ± 7.619) and this difference is statistically significant ($t(175) = 4.481$; $p = 0.000$). On the scale designed for this research, students who want to work with patients have also higher average scores on the scale (32.81 ± 4.553) than those who do not (27.96 ± 6.581). The difference is statistically significant ($t(175) = 5.391$; $p = 0.000$).

The differences in ethics in respect to GPA

In order to determine the relationship between the average point grades and scores on the scales designed to evaluate the level of ethics, Pearson's correlation coefficient was used. The results have shown that there is statistically significant correlation between GPAs and scores on Gallup and Beckstead scale ($r = 0.235$; $p = 0.002$) – correlation is significant at the 0.01 level (2-tailed). In case of the scale designed for this research, the relation is also statistically significant ($r = 0.208$; $p = 0.006$) - correlation is significant at the 0.01 level (2-tailed). Finally, a conclusion can be drawn that there is significant correlation between the level of ethics and GPA.

The differences in ethics and parents GPA Single factor analysis of variance (ANOVA) was used to determine the correlation between the ethics level and the education of parents for both parents separately. The results show that in both cases, on Gallup and Beckstead and the scale designed for this research, the correlation be-

tween the education of fathers and the ethics is not statistically significant ($F = 0.610$, $p = 0.692$ and $F = 0.525$, $p = 0.757$, respectively). Statistically significant difference in ethics depending on the educational level of mothers+ is shown for scale designed for this research ($F = 3.374$, $p = 0.006$) and there is no statistically significant difference for Gallup and Beckstead scale in correlation between the education of mothers and the ethics ($F = 1.912$, $p = 0.095$).

The differences in ethics in respect to owning a pet

A t-test for independent samples was used to determine if there is a correlation between the ethics of using animals in experimental purposes of a person and the fact whether a person owns a pet. Students who own a pet had slightly higher average scores on Gallup and Beckstead scale (47.27 ± 6.243) in comparison to those who do not (46.88 ± 8.573). On the scale designed for this research, the students who own a pet had a slightly higher average scores (29.74 ± 5.902 versus 28.51 ± 7.046). In both cases, the results of the t-test have shown that there is no statistically significant correlation ($t(175) = -0.348$; $p = 0.728$ and $t(175) = -1.259$; $p = 0.210$, respectively).

The results of this study obtained through fourteen one-sample t-tests

The results of this study, obtained through fourteen one-sample t-tests, for each individual point on the scale, are presented in Table 4. The results have a confidence interval of 95% which indicates that the level of significance is 0.05.

Table 3: Results on the scales in respect to education of parents and owing a pet

	Variable	The number of participants
Mother's educational level	Elementary school not completed	1 (0.6 %)
	Elementary school	8 (4.6 %)
	High school	98 (56.0 %)
	College	19 (10.9 %)
	University	46 (26.3 %)
	Other, unknown	3 (1.7 %)
Father's educational level	Elementary school not completed	1 (0.6 %)
	Elementary school	10 (5.7 %)
	High school	100 (57.1 %)
	College	26 (14.9 %)
	University	37 (21.1 %)
	Other, unknown	1 (0.6 %)
The owner of a pet	No	83 (47.4 %)
	Yes	92 (52.6 %)

Table 4: Results of the study

Question	
1. Research on animals has little or no bearing on problems confronting people.	2.73
2. An intrinsic interest in the animal for its own sake is sample justification for doing animal research.	3.10
3. I am very concerned about pain and suffering in animals.	3.56
4. I would rather see humans die or suffer from disease than to see animals used in research.	1.72
5. Since many important questions cannot be answered by doing experiments on people, we are left with no alternative but to do animal research.	3.71
6. I have seriously considered becoming a vegetarian in an effort to save animal lives.	2.13
7. New surgical procedures and experimental drugs should be tested on animals before they are used on people.	3.86
8. There are plenty of viable alternatives to the use of animals in biomedical and behavioral research.	3.63
9. Many important biomedical breakthroughs are a consequence of animal research.	3.94
10. Animals should be granted the same rights as humans.	2.98
11. Most psychological research done on animals is unnecessary and invalid.	2.88
12. We need more regulations governing the use of animal research.	3.82
13. Most laboratory animals are better housed, fed, cared for, and protected from pain and suffering than many humans.	3.28
14. Animal research cannot be justified and should be stopped.	2.20

The evaluation of competitive validity and reliability of the questionnaire scale designed for this research

In order to determine the competing validity between Gallup and Beckstead scale and the scale designed for this research we used Pearson's correlation coefficient. The results show that there is a statistically significant and strong positive correlation between the results on Gallup and Beckstead scale and the scale of this research ($r = 0.208$; $n = 175$; $p = 0.006$). This can be taken as an evidence that there is a competitive validity. The significance interval of 0.01 implies that the confidence interval is 99%. The diagram showing the distribution of the correlation between these two variables, with the regression line and the equation, is presented in Figure 5.

DISCUSSION

The cultural differences, in attitudes towards animals, should be understood and respected in order to promote tolerance in multicultural education. It is shown that European students and U.S. students (though to somewhat lower extent) do not approve cruelty towards animals to the same extent as students from some Asian countries.¹⁰ The students from Europe have manifested a greater concern about the suffer of animals than Asian students, but not in term of respect towards them.^{11, 12}

It is generally assumed that female students

The reliability of the measuring instrument is a metric characteristic which indicates the extent to which the same results can be expected when the same measuring device is reused in future studies. The most commonly used method for evaluating the confidence interval is to calculate it by using the reliability of internal consistency, i.e. by calculating the Cronbach's coefficient alpha, where the coefficient value ≥ 0.7 is considered to be a good indicator of reliability ($0.7 \leq \alpha \leq 0.9$). Cronbach's alpha were estimated at 0.786, which implies that the internal consistency of scales is significantly high.

have greater concern for the suffering of animals and that they express greater care towards their welfare than male students do. This study confirms such hypothesis and our results are consistent with those reported in literature. This hypothesis can also be projected on general population. The female subjects in almost all situations exhibit greater care and a higher degree of empathy towards animals in comparison to male subjects.¹³⁻¹⁷ It is believed that women and men are born with equal potential for the development of empathy and ethics, but that difference exist later in life, as a result of environ-

mental influences, motivation and education.^{18,19} The results of this study confirm the previously mentioned statements since the results have shown that the differences between two genders are statistically significant. This thesis cannot be applied to veterinary students whose attitudes are more closely connected to their possession of pets in an early age than to gender differences.^{20, 21}

This study has shown the statistically significant correlation between the ethics level and whether the students want to engage in direct contact with patients or not at their desired job position. Due to working in a pharmacy or working as a clinical pharmacist, the individual is in direct contact with patients in relation to other affiliations offered. Therefore, it was assumed that the level of ethics and empathy could be related to desired affiliation or, to be more precise, with a fact whether an individual desires to work with patients.²²⁻²⁴ which has been shown in this study according to the results because there is statistically significant difference between the ethics in respect to the high-school education students completed. The interest in certain field of study and motivation to choose certain professional profile is under the influence of various different factors such as a talent and skills for certain activities, the expected salary, social trends, parental influence, labor market conditions, etc.^{25, 26} However, it is generally accepted that both working in pharmacy store and as a sales representative require exceptional social skills. This can be one of the reasons why statistically significant correlation between the ethics and the desired affiliation was found.

Family environment in an early age has an influence on later interpersonal relationships. Moreover, it provides a solid basis for the development of empathy and also shapes the sense of ethics.^{27, 28} If the level of education is taken as an indicator of the awareness of the issue, then it is expected that sense of ethics towards animals is passed to the offspring and their attitudes can have a strong influence on the attitudes a child would have. This study has shown that there is no statistically significant difference between the levels of empathy students have towards animals and parental education, except statistically significant correlation between the results on scale designed for the purpose of this research and education of mothers.

Gallup and Beckstead conducted a research including 263 students from different departments of the State University in New York in 1988. One of the conclusions of this study is that there is some degree of concern for the welfare of animals used in scientific experiments but also that there is a need to evaluate the real necessity of conducting such research experiments.⁸ Metzger replicated the previously mentioned research to determine whether there was a change in students' attitudes towards experiments which use animals due to a constant declining public support for their realization.⁹

The assessment of the points given in the questionnaire should enable the evaluation of whether the decline in value of the point is a result of a growing concern for animals and/or a declining assumed values of using animals in research.[9] The data from the Gallup and Beckstead study were published as a frequency of response, and then transferred to average value of every point with one-sample t-tests. Fourteen one-sample t-tests were also carried out in the study conducted by Metzger. In Metzger's study, there is a statistically significant difference (for all points, except for 2 and 13) with the results of Gallup and Beckstead study determined by using the Bonferroni correction, which is $\alpha = 0.0035$.⁹

The increasing values in different time periods in which the three studies are conducted are noticeable in terms of points given under the numbers 8, 10 and 13. The results obtained in this study are in the range of the previous two studies in terms of points 1, 3, 4, 6, 11 and 14. The other values of the points do not deviate from the results obtained through the previous studies. The results on the points 4, 6, 11 and 14 represent the concern for the animal welfare while the points 5, 7 and 9 express the evaluation of research in which animals are used.

The questionnaire should be used on a greater number of participants, so that psychometric characteristics and the validity of the scale are tested in detail. This study is conducted on a small sample of students attending two faculties, which may be a potential reason why the statistically significant correlation was not found in all parameters. Additionally, all the data was collected through self-expression so the reliability of the results relies completely on the honesty and truthfulness of the students.

CONCLUDING REMARKS

The population of students who participated in this research, from both the Faculty of Medical Sciences and the Faculty of Engineering Sciences, manifested a satisfactory level of ethics. For pharmacists, it is extremely important that in addition to theoretical knowledge they acquire through their studies, they also possess a well developed ethics and the sense of empathy towards the animals they use in their experiment. The empathy for experimental animals helps them judge the rationality of their experimental undertakings and prevents them from victimizing animals without a real necessity. Moreover, the empathy for animals is just an integral part of the empathy towards living beings in general, including the other people. Even for those phar-

macy students who will not work in academic and scientific field in the future, and thus probably will not be in position to work with laboratory animals the empathy towards animals is extremely important. Empathy, in general, will help them understand their patients better and take a better care of them. In such supportive atmosphere it would be much easier to gain a patient's trust and thus all the relevant information needed in making the decisions about the most rational treatment. The students who participated in this study, at least judging by their empathy towards animals, have a huge potential to become excellent medical workers who would properly treat their patients.

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Fetal Syndromes - Diagnosis and Management using 3D-4D Sonography

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INTRODUCTION

Three-dimensional ultrasound (3D US) has become a very powerful and progressively popular ultrasound technique in the last three decades (1989-2019).¹ Today, it is used in regular prenatal assessment in everyday clinical practice as additional method to two-dimensional ultrasound (2D US).

Over the time, 3D US equipment and technology dramatically enhanced the quality of images, shortened the time of acquisition, and at the same time improved our ability to assess and visualize the normal and to detect abnormal development of a embryo and fetus in utero.¹⁻¹³

Acquired volumes can be stored, re-loaded and re-evaluated at any time. Different imaging modalities can be applied for more detailed survey. Particular region of interest such as small defects can be displayed in the ideal plane that sometimes cannot be achieved by conventional 2D US technique.^{3,4,9,10} This increases the accuracy of the detection and diagnosis of various malformations and fetal syndromes.²

Diagnosing fetal syndromes is a major challenge both in the prenatal and postnatal period.² The syndrome is like a big puzzle whose parts need to be carefully assembled to get a whole picture.² Good multidisciplinary approach, proper communication and collaboration with parents and all physician's involved are required in the diagnostic process. All available resources and tools are needed to increase diagnostic precision in the prenatal diagnosis of fetal syndromes.⁴

The introduction of highly specialized software systems for three-dimensional and four dimensional ultrasound (3D/ 4D US) enable detailed assessment of the fetal anatomy and the evaluation of the dynamics of structural and functional development of the fetus in real-time.^{2,4} Owing to this, clinical practice has also gained new functional tests such as the Kurjak's antenatal neurodevelopmental test (KANET); named in honor of its author; used for the evaluation of fetal brain function.^{2-4,24-31} Antenatal detection and diagnosis of fetal anomalies and syndromes is shifted from the 2nd to the 1st trimester of preg-

nancy^{2,4,10-12} partly due to technological advances and benefits of imaging quality with 3D/4D US technology, as reported by many authors.

When to suspect a syndrome? Defining a syndrome

Common terminology used to describe fetal syndromes can sometimes be confusing. A wide variety of terms and synonyms are used. Sometimes, there is a lack of good definitions of how many major and minor criteria should be present to diagnose each syndrome. The difference in prenatal detection rates for each region or country can be partly explained by differences in screening policies and follow-up practices, as well as the possible variations in practitioners' skills and available equipment.⁴

Detecting one anomaly should always raise doubts about the presence of other anomalies and should therefore serve as a trigger that will encourage us to further investigate and raise awareness of the possible existence of syndromes.^{2,4-9}

Other triggers can be: positive personal or family history of syndrome, or a child born with a syndrome, consanguinity, exposure to teratogens (drugs, radiation) and other harmful agents (e.g. infections, TORCH, Zika). Genetic syndromes in children are most commonly diagnosed on the basis of craniofacial dysmorphic features.^{5,8,34,35}

Clinical dysmorphology is a branch of clinical genetics devoted to the study of abnormal human development, with emphasis on syndromes that reflect as changes in morphology of the body.³²⁻³⁵ There are several pathophysiological mechanisms of poor fetal development (fetal maldevelopment) and these include: malformation, deformation, disruption or dysplasia.

Malformations: due to abnormal embryonic development, commonly defined as single, localized poor formation of tissue, which has genetic etiology. This anomaly then raises a number of other defects (e.g. anencephaly). The recurrence risk for malformations generally range from 1% to 5%.⁷

Deformation is a result of extrinsic mechanical forces on otherwise normal tissue, deforming it (e.g. abnormal faces, pulmonary hypoplasia and limb contractures that result from pro-

longed oligohydramnios or primary renal agenesis in Potter syndrome.

Disruption results from an extrinsic insult that destroys normal tissue, altering the formation of affected structure (e.g. amniotic band syndrome).

Dysplasia: when the primary defect is absence of normal organization of cells into tissue, we speak of dysplasia (e.g. achondroplasia).

Any of the mechanisms of fetal maldevelopment can result in altered morphology of fetal organs and systems, which can result in the formation of a fetal syndrome if many organs are involved. The term "**Syndrome**" originates from ancient Greek meaning "running together"³⁹, representing a specific pattern of associated signs, symptoms, dysmorphic features and/or behaviors occurring together in the same person.^{2,6,40}

We are currently aware of thousands of syndromes and their variants 4-8 and this number is rising every day. It is particularly important to learn how to identify and find the right diagnosis in severe cases. In fact, it is estimated for the human genome to have about 80,000 genes, it should probably be discovered as many rare syndromes. Over 300 syndromes are associated with some type of facial anomaly.^{2,4,8,9} The incidence of fetal syndrome varies.⁸ It is estimated that the real occurrence of most syndromes is likely to be much greater, but due to natural selection there is no further development.

Terminology used to describe fetal syndrome can sometimes be very confusing. Namely, different terms and their synonyms are used at the same time. In some cases, it is not well defined how important the main criteria would be, and how many of the subordinate criteria should appear for setting up a particular diagnosis. It is important to point out the difference and to distinguish between the terms: syndrome, sequences and associations.

Syndrome: the occurrence of anomalies, multiple malformations and / or sequences, together in a recognizable sample. They are mainly the result of one, usually genetic abnormality and have the same etiology; as for example in trisomy 21 (Down Syndrome).

Sequence: The sequence occurs when one developmental disadvantage results in a cascade of secondary deficiencies that cause tertiary and so on, such as: Pierre-Robin sequence: primary defect is mandibular hypoplasia, retrognathia that causes glossophtosis, secondary defect, which further disturbs normal, physiological closure of the soft palate and causes a tertiary defect: the palathoshisis of soft palate. (retrognathia-> glossophtosis-> palathoshisis). In 25% of cases, the fetus will have Stikler syndrome.⁴¹ The sequence may be an isolated finding, associated with some other defect or a part of a syndrome.

Association: Connecting random combinations of inherited anomalies that may be the result of numerous pathogenic genetic factors (unlike syndrome). Example: VATER / VACTERL association (acronym for) (anomalies of the vertebrae - V; anal atresia- A; cardiac defects- C; trachea - oesophageal fistula - TE, kidney anomalies - R, anomalies of the limbs - L). This association is typically defined by the presence of at least three of the above-mentioned congenital anomalies.⁵⁻⁸

Some fetal syndromes can be detected prenatally while others cannot; some are expressed prenatally while others are not.⁴ In many cases definitive diagnosis can be made postnatally, many years later.² It is estimated that about 1 out of 10 people, or a total of 30 million, live with rare diseases in the United States. Globally, it is about 350 million people who have one of over 7,000 known rare diseases. As already mentioned, the way to diagnosing these children is pretty long and painstaking. It takes about seven years from symptom to diagnosis, and in that period the children see at least seven doctors.³⁵⁻³⁷ All this has a negative impact on the child and his family, representing emotional and financial burden on everyone, and negatively influencing and affecting the quality of life and prognosis of the child. The list of possible differential diagnoses (DD) of fetal syndromes is extensive.

Currently there are several types of online databases that can help in detecting and identifying certain patterns of anomaly in syndromes, sequences or associations. The most commonly used medical databases are: Online Mendelian Inheritance in Man (OMIM), POSSUM-web, London Dysmorphology Database (LDDDB) as part of a larger database: London Medical Database (LMD). Some of the larger databases combined their data to enrich them, allowing users a faster and more accurate search. As part

of the new FDNA (The Facial Dysmorphology Novel Analysis) database, LMD database access is integrated in combination with a brand new technology called Face2Gene.³⁵⁻³⁷ Namely, with the help of the Face2Gene database, genetic search facilitates detection of dysmorphic facial features and recognizes human malformations from face photos to present a list of the most commonly used syndromes.³⁷ Patient symptoms, features and genomic data are analyzed in the network of thousands of genetic experts around the world, providing scientific insights to improve and accelerate diagnosis and therapy. Notwithstanding, all of these network databases are based mainly on the data, symptoms and features found in newborns and children, and are not directly relevant to the recognition of syndromes in the antenatal period. *Phenotype online database*³⁸ is a database for specialists dealing with prenatal detection of syndromes using ultrasonic technology so that all prenatal sonographic markers and features are involved in data analysis. Data is easily accessed, ultrasonic markers and symptoms are combined, anomaly list that may still appear within the diagnosis is obtained, and some features that are identified in the parents can even be included.³⁸

Clinical application of 3D ultrasound in the antenatal detection of fetal syndromes.

The systematic approach (according to guidelines) to fetal assessment by two-dimensional (2D) ultrasound is still a gold standard and should always be followed to avoid mistakes. When evaluating structures such as the face or brain, the advanced 3D / 4D US modalities gives a whole range of additional information that cannot be obtained by 2D technique.^{3,16}

Systematic review of 525 articles on 3D / 4D ultrasound by Goncalves et al.⁴², found that 3D US provides additional diagnostic information for diagnosing facial anomalies, particularly facial clefts, neural tube defects and skeletal malformation. Merz and Welter⁴³ examined a large group of 3472 fetuses with 2D and 3D ultrasound intended to detect fetal anomalies. The total number of detected defects was 1012. Comparing the 2D and 3D techniques, 3D US showed superiority with 60.8% of detected anomalies, which concerned more favorable visualization of target areas in different views (e.g. Multiplanar, Surface View).^{43,44}

Only in recent years high-frequency probes and high-resolution displays called High-Definition Live (HDlive) technology have revolutionized the quality of sonographic imaging. 3D HDlive mode of display uses the advantages of 'shadowing effects' to enhance the visualization of the desired details in the image.⁴⁴ Unlike the conventional 3D surface display that uses a fixed virtual light source and reflects light from the surface of the skin, HDlive modality calculates the spread of light through the skin and tissue.^{10,12,16,31,44} Shadows are created where the light was moved through the dense tissue. The virtual light source can be easily changed and directed from any angle and manipulated in this way to improve segmentation of the tissue structure, define precise contours and highlight important clinical details.^{10,44} This is suitable for observing the surfaces, especially in the face area.⁴⁶⁻⁵⁰ Any suspicious surface or malformation can be shown and investigated much better than conventional 2D ultrasound.

Changing the virtual light angle, it can be perfectly adjusted to highlight something and thus gain a depth perception in the visualization of an area of interest that may be anomalous. Transparent (translucent) effect is obtained if the light source is located behind the object.^{44,49} Improved smoothing performance is obtained by applying volume-speckle reduction imaging (V-SRI) on high-quality multi-planar 3D / 4D images using volume (voxel) compared to traditional single-slice imaging (pixels).

The 3D HDlive mode can be used successfully throughout the entire pregnancy.¹⁶ In the first trimester, normal and abnormal development of embryos and fetuses can be monitored and evaluated to the finest details. Only few years ago, new applications in 3D ultrasound called HDlive Silhouette (Flow) and Flow (Flow) were launched. HDlive Silhouette revealed the clinical significance of simultaneous imaging of internal morphology through the outer surface in a transparent manner (Figure 1,2).

This helps in determining the exact localization and volume of internal structures that may be hyperechoic as bone, or hypoechoic structures (Figure 2 A-D) such as cysts.^{49,50} HDlive Flow technology adds more spatial resolution to conventional angiogram. Combining both techniques at the same time (HDlive Silhouette and

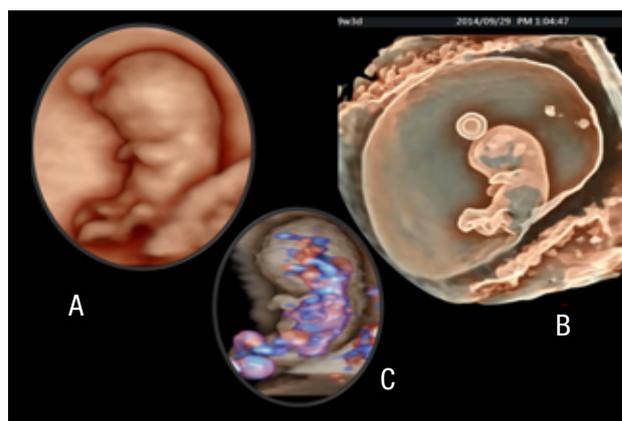


Figure 1. A-C

1A, B: 3D HDlive Silhouette fetal presentation at 9 + 3 gestational weeks. With the different intensity of Silhouette mode, we get more or less transparent picture in "see through fashion".

1A: The surface details.

1B: See differences in the details of the previous picture (A), see the details of the internal organs filled with fluid, such as a ventricular system in the brain, an egg yolk, etc.

1C: 3D HDlive Silhouette and Flow showing the same fetus as above. Facing the details of early fetal circulation in the brain, heart and umbilical cord.¹⁶

Flow) can display the exact location of the vascular structure within the body and organs and accurately determine the direction of the vascular flow (3D HDLive bidirectional power Doppler) (Figure 1C). These two new applications allow visualization of blood circulation within the fetus, the various parts of the fetal brain and lung flow.^{49,50} Using different color shades of the skin by the HDlive application, brought a great news as it gives the impression of a living fetus with even more realistic and impressive imaging illustrations.^{16,49-51} Many of the above-mentioned innovations in 3D / 4D ultrasound applications are particularly useful in prenatal detection and visualization of fetal anomalies and discrete details.⁵²⁻⁵⁵

Together with this sonographic diagnostic tools, we should also mention something new that will greatly assist in accelerating postnatal recognition of rare syndromes. The fascinating combination of science, research and new technologies is jointly implemented in a new research program titled 'Give Face Syndrome'. Facial Dysmorphology Novel Analysis (FDNA) is a new technology that facilitates the detection of dysmorphic features and recognizable patterns of human malformations in newborns, children and adults, to provide comprehensive and updated neurogenetic references available to everyone online.^{36,37,55}



Figure 2 A-D

A-D: The same fetus at 9 weeks of gestation. Note the difference in imaging details.

A: VCI mode- sagittal image of the fetus- increased nuchal translucency (NT)

B-D: 3D HDlive Silhouette images with the increasing silhouette mode from B to D. Ending up with the transparent view. Notice the increased accumulation of the fluid in the neck area and the cystic structures within: Cystic hygroma coli.¹⁶

Syndromes featuring primarily craniofacial anomalies

In this section, it will be described various syndromes featuring primarily craniofacial anomalies and their associated defects that can be detected by standard 2D ultrasound techniques, and demonstrate how the recognition is enhanced by advanced 3D / 4D techniques in order to increase the accuracy of prenatal diagnosis.

As mentioned earlier, we know more than 300 syndromes associated with some type of facial anomaly: most commonly: with cleft lips and / or palate, micrognathia and hypoplasia of the maxilla and the face (Goldenhar syndrome, Treacher-Collins, Pierre-Robin sequences as part of syndrome, Apert syndrome, van der Woude syndrome)^{2,4,8,9} These syndromes can be divided by various features, such as orofacial cleft, craniosynostosis, pharyngeal arch abnormalities and simply facial dysmorphism. From embryologic point of view, the face is made up of five facial prominences that surround the future mouth and all meet in one point called the philtrum, a small recess above the upper lip.^{5,6,56,57}

How to distinguish what is normal and what is not?

Although we all have the same basic features, we also have our own recognizable features. There is evidence that the human brain (fusiform gyrus) has a specialized mental module devoted to processing facial recognition features. Researchers in various parts of the world work on understanding how fusiform gyrus mechanisms follow information: how we recognize faces and interpret their various facial expressions. Fetuses with dysmorphic characteristics are diagnosed, as noted earlier, according to the criteria to be met, which are also used in postnatal assessment. These include: head shape and closure of the skull bones, facial asymmetry, hyper / hypotelorism of the eyes, inclined eyes (mongoloid, antimongoloid position (Figure 3), low set or abnormal ears, micro or retrograde jaw confirmed by measuring Jaw-Index,⁵⁸ cleft lips and / or palate.⁵ In the process of face evaluation, we must also consider ethnic variations and normal differences. For example, epicanthal fold may be normal for Asiatic Asian and non-Asiatic people, but can be considered as a dysmorphic feature of syndromes such as Down, Turner, Noonan,



Figure 4
3D HDlive surface rendering, US imaging of the fetus at 14 weeks of gestation. Notice: subtle and small paramedian cleft lip only.¹⁶

Williams and Fetal Alcohol Syndrome etc. Different nose forms and shapes is another example (Mediterranean, African, Asian, Latin and Caucasian).

The paramedian cleft lip (CL), cleft palate (CP) or combination of the two-cleft lip and palate (CLP) are one of the most common fetal facial anomalies and one of the most common anomalies of the fetus at all.

This can be an isolated finding (Figure 4) in less than 50% of the cases or in combination with associated anomalies as part of diverse syndromes. If this is an isolated finding, it can be elegantly repaired and reconstructed with a professional cranio-maxillofacial surgeon engagement.

It can be distinguished: a bilateral complete lip and palate cleft that can easily be identified even in the first trimester (Figure 5,10), and on the other hand, a unilateral complete cleft of the lips and palate, or an incomplete cleft of the lip that has only subtle indications and can easily be overlooked (Figure 9). The median facial clefts are the most severe anomalies, regularly part of some serious and complex sequences or syndromes, such as holoprosencephaly, Patau (trisomy 13) (Figure 6,7,12), Edwards syndrome



Figure 5
3D HDlive surface rendering of the fetal face at 23+3 weeks of gestation. Notice: bilateral cleft lip and palate (CLP). Because of the protrusion of inner maxillary segment under the nose, anomaly is easily detected.¹⁶

(trisomy 18) and Aicardi syndrome.⁶⁰ Trisomy¹³ is the commonest chromosomal abnormality associated with alobar holoprosencephaly (fetal face: cyclopia, cebocephaly, flat nose, facial hypoplasia and lip clefts) (Figure 6,7,12). Nevertheless, it is to be remembered that 75% of fetuses with holoprosencephaly have a normal karyotype! (Figure 8,10) Unfortunately, most of the fetuses with CL, CP or CLP have a high incidence of chromosomal abnormalities and other related syndromic anomalies.⁶¹

Van der Woude Syndrome (VdW) carriers have up to 50% of the cases facial clefting. VdW syndrome is autosomal dominant mode of inheritance which accounts for about 2% of all cases with CL and CP.¹⁶ An partial one-sided small defect CL can easily be overlooked by the usual 2D US (Figure 4)¹⁶.

Three dimensional multiplanar display and HDlive surface view is a better method for detecting all forms of clefts and facial malformation. Bilateral CL may sometimes be missed because it does not change the face symmetry. The two-sided complete CL and CP, on the other hand, will most likely be detected due to the protrusion of the inner maxillary segment below the nose (Figure 5,10), which is an obvious and unusual mass when looking at the face profile.⁵

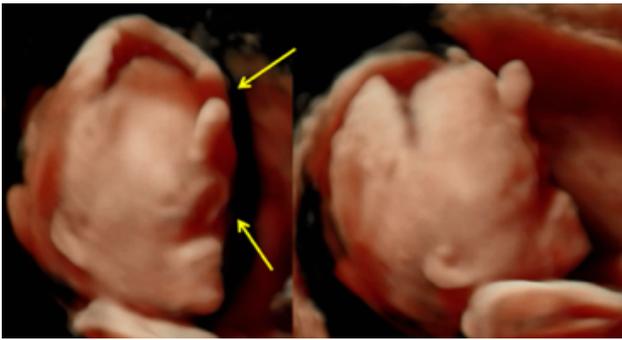


Figure 6
3D HDlive surface rendering, the same fetus from the front and profile. Notice. Abnormal shape of the head, wide open sutures of the skull, anomalies of the midline of the fetal face: cyclopia (lower arrow), proboscis (upper arrow) and holoprosencephaly and low set ears are seen. All features of trisomy¹³- Patau syndrom.¹⁶

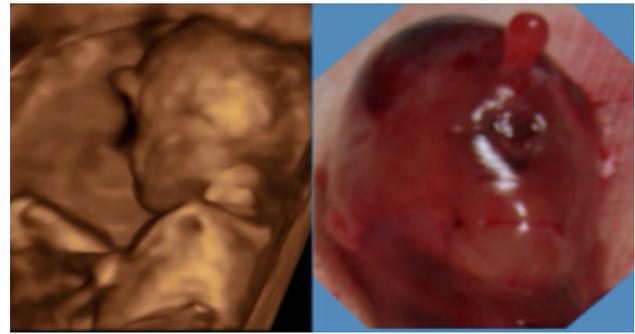


Figure 7
Image on the left: 3Dsurface rendering, profile: notice: proboscis!
Image on the right: the same fetus after miscarriage:notice similarities with the prenatal imaging (proboscis, cyclopia)



Figure 8: 3D HDlive surface rendering of the fetus at 13 weeks of gestation. Notice abnormal shape of the head, holoprosencephaly, bilateral CLP, microphthalmia, low set ears. But NORMAL karyotype!

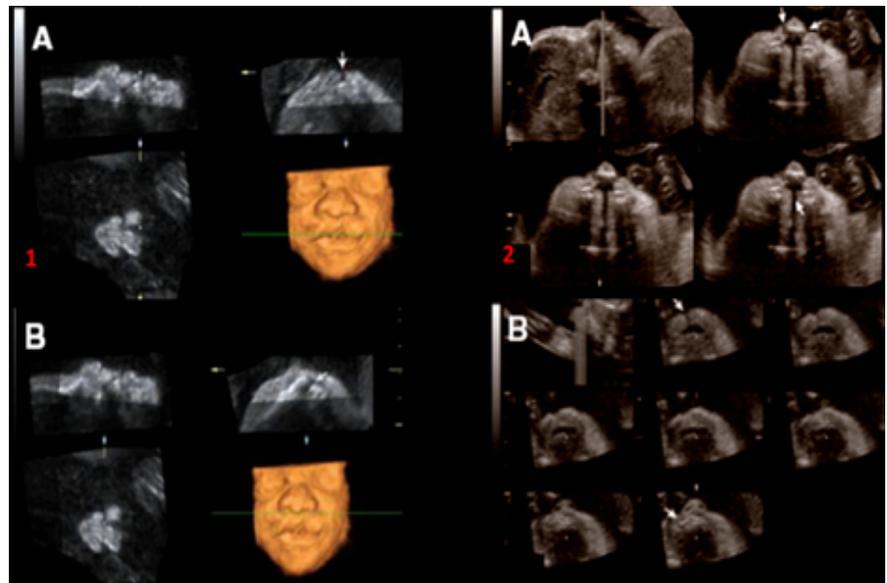


Figure 9: 1A: 3D Multiplanar imaging, small, only subtle paramedian cleft lip seen on this image but, 1B: movement of the green dot across the mandible, small alveolar defect becomes evident.
2A, 2B: A: Bilateral CLP; detected by tomographic ultrasonography imaging technique (TUI)
B: Isolated cleft lip Izolirani – integrity of the fetal palate can be obtained by TUI imaging method

When checking for cleft of the lip, 3D HDlive techniques are very useful. For detection of cleft palate, the use of tomographic ultrasonography (TUI) (Figure 9,10) allows better determination of the extent of the cleft in relation to other structures.¹⁶

Mandibular anomalies (agnathia, micrognathia, retrognathia) (Figures 11-15) have been described in more than 100 different syndromes.⁶² It appears to be very common as isolated anomaly, but also commonly as part of heterogeneous syndromes. By 2D US abnormal profile (Figure 11, 12) is first to be detected. Abnormal fetal pro-

file is noted! With the application of different 3D US modalities, it is possible to investigate in detail and to get a complete impression on the fetal appearance and possible existence of other orofacial anomalies.

Pierre-Robin Sequence (PRS)

It is characterized by a triad of orofacial anomalies consisting of retrognathia, glossoptosis and median cleft of the soft palate. Mandibular hypoplasia (Figure 11) is a primary deficiency that occurs early in pregnancy between the 7th and 11th week of pregnancy. The tongue maintains high in the oral cavity, which subsequently prevents

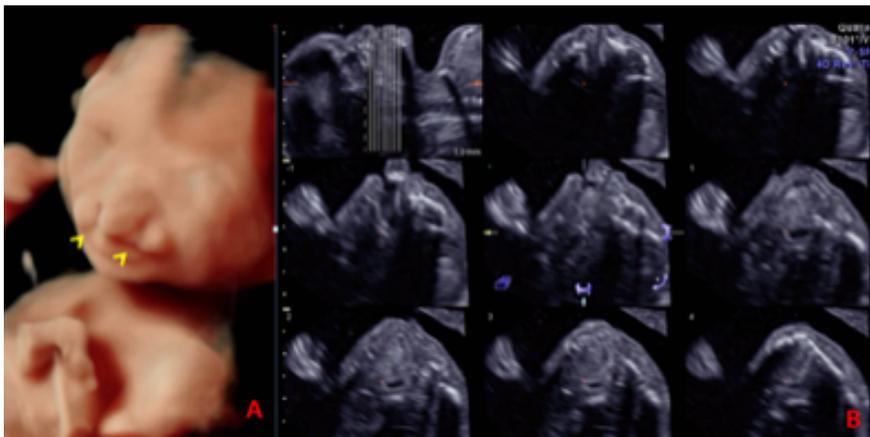


Figure 10:

3D HDlive surface imaging: bilateral CLP; fetus with multiple anomalies but: NORMAL KARYOTYPE!

B: TUI display –notice: the extent of the cleft !¹⁶

the normal tongue placement and prevents the soft palate from fusing.^{16, 63} Prenatal diagnosis of micrognathia in PRS can be set very early in the first trimester of pregnancy, by using 3D ultrasound and its applications.^{64,65} Micrognathia can be quantified by "Jaw-index" = the ratio of the AP mandible and BPD diameter. If the ratio is <0.23 , a micrognathia diagnosis⁵⁸ can be set. More than 40 PRS syndromes have been described, most common of which are Stickler's syndrome (SS) and 22q11.2 deletion syndrome (Di George Syndrome)¹⁸⁻²³.

Pooh and Kurjak published the sequence of images of mandibular hypoplasia and slow development of jaw in case of PRS during pregnancy.¹⁶ Serial 3D imaging can be used to clearly document the mandibular growth over a few weeks.¹⁶ Accelerated compensatory growth of the lower jaw is expected during the 1st year of life, and adjustment of the child's profile can be expected from the 3rd-6th year of life.^{54, 66-69}

Isolated PRS (without any other associated malformation) occurs in about 50% of cases, however, in the second half of the cases PRS is part of the syndrome. The clinical manifestations of syndrome depend on the persistence and severity of related anomalies.⁵ The nature of these anomalies is diverse, most commonly anomaly of the 1st pharyngeal arch, various chromosomal disorders (DiGeorge syndrome), collagenopathy or syndromes associated with the use of toxic substances in pregnancy, such as alcohol (fetal syndrome alcohol FAS), etc. In the study of 115 cases of PRS patients, as expected, 54% had an isolated isolate finding. (5%), facial and hemifacial microsomia (3%), other defined (3.5%) and undefined. Other syndromes: Stickler (18%), Velocardiofacial syndrome (7%), Treacher-Colins syndrome (9%)²².

Facial dysmorphism usually derives from a combination of migration disorders and inadequate formation of facial mesenchym (especially when it is associated with disorders of the 1st and the 2nd pharyngeal arch).^{61, 67}

Goldenhar syndrome (GS)

Synonyms are: Hemifacial microsomia or oculo-auriculo-vertebral syndrome (OAV). This is a combination of migration disorder and inadequate formation of facial mesenchym (disorders of the first and second throat arch).^{61, 67} GS is characterized by a wide range of main and associated features that can differ in the severity from one to the other case (Table 1).

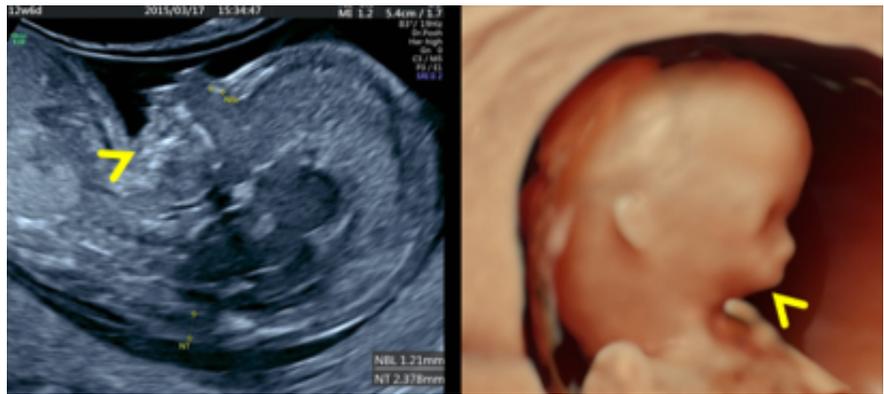
Table 1: Main features of Goldenhar syndrome

Goldenhar syndrom	GS
Asymetric (unilateral) hypoplasia of the face	70-85%
Mandibular hypoplasia- PRS	
"Skin tags" around the ear	
Hypoplasia of the ear	
Malformation/ microphthalmia of the eye	
Unilateral cleft lip/ palate	
Anomaly of vertebrae (hemivertebrae)	
CHD	
CNS anomalies- Corpus Callosum Lipoma	
Malformations of gastrointestinal system	

The classic feature of GS is asymmetric (mostly unilateral) facial hypoplasia. (Figure 13.14) Fetuses with GS have major anomalies such as unilateral mandibular hypoplasia involving the temporomandibular joint, multiple pre-auricular skin tags (Figure 13,14) around ear, ear hypoplasia/ aplasia and/ or eye malformations (anophthalmia and microphthalmia) and verte-

Figure 11

A: left image: 2D midsagittal view of the fetus, notice: abnormal fetal profile and shape of the head, increased NT and prominent micrognathia (arrow).
 B: right image: the same fetus as in the left image, 3D HDlive surface rendering modality. uz notice: all mention above and detail of the low set ears,¹⁶

**Figure 12**

3D HDlive surface rendering: notice: cleft lip and palate (CLP) and hypoplasia of the mandible. Case of Trisomy 13 (Patau syndrome at 21 gestational weeks)



bral anomalies (Typically, these malformations are one-sided (70-85%) and give an asymmetric facial appearance. There have been various theories about the emergence of this syndrome. Some authors assumed that the problem could be a one-way blood flow disorder (ischemia) to the 1st and 2nd pharyngeal arch that could occur during the time period between the 4th and 8th week of pregnancy.⁷⁰Wang et al.⁷¹ analyzed data from a large register of congenital anom-

alies in Spain and established the association between diabetic mothers and the increased risk of their children born with GS syndrome. It is assumed that poorly controlled mother's diabetes interfere with the migration of cells into the cephalic portion of the neural ridge causing this syndrome.⁷¹

PRENATAL DIAGNOSIS

The first sonographic indicator to detect this syndrome may be the discovery of facial asymmetry due to hemifacial microsomy (Table 2, 3) or a small detail such as a very typical periauricular skin pendant (tag) (Figure 13, 14). This is most commonly diagnosed in the second and third trimesters of pregnancy. With 3D surface rendering, more details can be visualized. Unilateral craniofacial anomaly, (cerebral hemisphere hypoplasia), eye (micro / anophthalmia), low set ears with various malformations, face (soft tissue asymmetry), kidney (hydronphro-

sis) etc. Using 3D HDLive imaging technology, even small facial details or other parts of the body can be visualized in a very detailed way, which can be of great help in counseling the parents. Differential diagnosis should be based on Treacher's syndrome (TCS), Hellerman-Streiff syndrome, Delleman's syndrome, Nager's syndrome, Townes-Brocks syndrome).

Genetic counseling: GS occurs rarely and sporadically, but for the first-degree relatives the possibility of repetition (RR) is estimated at

2%. Due to the complexity of the clinical picture, multidisciplinary approach to problem is important.^{2,4} The combination of micrognathia along with low set ears is a common occurrence in many syndromes.

Treacher-Collins syndrome (TRC)

The discovery of bilateral symmetrical facial hypoplasia, periauricular tags in combination with micrognathia, may be part of Treacher-Collins (TCS) or some other syndrome such as Nager or Miller syndrome.

TCS is the disorder of the first and second pharyngeal arch. This is a congenital disturbance of craniofacial development induced by mutations in the long arm of chromosome 5 (5q32) of TCOF1 gene.⁷² It is depicted by bilateral symmetric oto-mandibular dysplasia (Figure 15). Furthermore, there is downward slanting of a palpebral fissure, lower eyelid coloboma, lack of medial eyelashes, middle and outer ear malformations, and conductive hearing loss. The frequency of TCS is estimated at 1: 50,000 live births per year. The genetic trait is autosomal

Table 2: Ultrasoundassessment of fetal face by 3D US in the 1st trimester of pregnancy (modified) according to Merzu et al.⁵⁹

Anatomy (3 orthogonal planes)	Section	
3D Surface rendering	Sagittal view	PROFILE: forehead, nose, lips, chin
3D Transparent rendering	Parasagittal view	Nasal bone yes/no Image angle 45°
3D Maximum mode imaging	Coronal view	Forehead, orbits, both nasal bones, maxilla and mandible
	Transverse view	Forehead, orbits, both nasal bones, maxilla and mandible
Biometry	Sagittal view	FMF angle (>85°)
Stored images		Face profile with the nasal bone FMF if needed

Table 3: Assessment of the fetal face by 3 D US in the second and third trimesters. (modified) according to Merzu et al.⁵⁹

Anatomy (3 orthogonal planes)	Section	
3D Surface rendering	Sagittal view	PROFILE: forehead, nose, lips, chin Nasal bone yes/no
3D Transparent rendering	Coronal view	- forehead with metopic (= frontal) suture – orbits with lenses (symmetry) – lips – palate (3 D surface rendered image and transparent (= maximum intensity) image
3D Maximum mode	Transverse view	- Orbits with the lenses - palate (transparent) - maxilla - mandible
Biometry	Sagittal view	- nasal bone - FMF angle - inferior facial angle (IFA)
	Coronal view	- orbital diameter – - inner orbital distance - outer orbital distance
	Transverse view	- orbital diameter – - inner orbital distance outer orbital distance maxilla width – mandible width or Jaw index
Stored images		- profile with nasal bone coronal or transverse view with orbits – coronal view

Figure 13: First two images- prenatal (left): 3D surface rendering. Notice preauricular tag. Second two images- postnatal (right): postnatal images of the baby with Goldenhar syndrome: notice hemifacial hypoplasia and microphthalmia.⁴



Figure 14 Postnatal images of the baby with Goldenhar syndrome. Notice Hemifacial hypoplasia with microphthalmia, periauricular tags and sinuses, external ear deformity hemihypoplasia of the mandible.⁴



dominant in 40% of cases with variations of expression. The remaining 60% are the result of new mutations. Cranio-skeletal hypoplasia develops due to insufficient number of neurons as a result of the death of the neuroepithelial progenitor cells.⁷³ The onset of defects occurs very early between the 4th and 8th week of embryonic development.

Prenatal diagnosis:

Prenatal ultrasound TCS diagnosed mainly in the second trimester of pregnancy⁷³⁻⁷⁵ but with sophisticated 3D applications and HDlive technology Pooch reported detection in the 1st trimester of the pregnancy.¹⁶ With the combination of existing anomalies, the established suspicion of this syndrome is very important to informing geneticists who can order the gene sequencing added to the usual amniocentesis panel (AC) to confirm a diagnosis that would otherwise be missed.⁷⁵

Apert syndrome (AS)

Apert syndrome shows autosomal dominant mode of inheritance. There is a congenital mutation in the FGFR2 gene, chromosome 10q26.13.⁷² This syndrome is also associated with the advanced age of the fathers. The syndrome has several characteristic features that can be recognized during a routine ultrasound examination. Triad of signs to remember would be: strawberry shaped head, flat face, 'mitten-like' hands (like baby gloves) (Figure 16)¹⁶

This syndrome is characterized by:

- Craniosynostosis: is a skull disorder caused by the early fusion of one or more skull bones (early fusion of the sutures). Otherwise, more than

180 different syndromes are known, which include craniosynostosis in the diagnosis. Changing shape of the cranial vault varies, depending on the fused sutures, so that the compensating growth occurs in dimensions that are not limited by the fusion. Apert syndrome occurs in 4.5% of cases of craniosynostosis (Figure16)

- In the case of Apert syndrome, premature fusion of bicoronal suture (metopic suture) occurs and consequently brachycephalic and acrocephalic shaped head forms appear. In other words, an abnormal flathead skull, frontal bossing, mid-face hypoplasia (flat face), ocular hyperthelormism, and swelling of the eyelids ("puffy eyes") can be detected. (Figure 16)

- Sonographic assessment: Combination of conventional 2D US techniques with 3D maximum mode (for bony structure), 3D surface rendering and 3D HDlive surface mode can be used.

- Mild ventriculomegaly can be detected represented by 3D inversion mode or, if available, with the latest HDLive silhouette display.

- Agenesis of corpus callosum (AGCC) can best be detected by 3D surface imaging in the central (midsagittal) plane with the additional visualization of the 3D sonoangiogram (3D HDlive bi-directional power Doppler) showing the anomaly: the absence of pericallosal artery.

- Very specific for fetuses with Apert syndrome is a defect at the extremities called 'Mitten-like hands / feet': syndactily of the second, third and fourth finger (soft and bone tissue) in combination with a broad thumb that has the appear-



Figure 15: 3D surface rendering of the fetus with Treacher Collins syndrome. Notice: typical facial dysmorphism: bilateral symmetric otomandibular dysplasia with the hyperplasia of the soft tissue of the face. Low set ears.⁴

ance of a child's gloves). (Figure 16)

Pooh et al.⁵⁴ published a prenatally detected case of a fetus with Apert syndrome by using 3D ultrasound diagnostics. Correlation was performed between prenatal 3D USV image anomalies with identical postnatal appearance (Figures 16)^{16,54}. 3D US images could be used when communicating with the parents for better understanding of the extent of abnormalities of the face, skull and extremities. Differential diagnosis should include other syndromes with craniosynostosis such as: Carpenter, Crouzon, Pfeiffer and Seethre-Chotzen syndrome.⁵ The important difference is that there is no syndactyly for the fingers and toes.⁵

Genetic counseling: AS has autosomal dominant inheritance, but in most cases it is a case of mosaicism. When the mutation is "de novo", the risk of repetition is unlikely, but if one of the parents is the gene carrier, the recurrence risk is 50%!⁷⁶

In order to confirm the diagnosis prenatally, it is necessary to talk to the parents and to offer invasive prenatal testing. Children with Apert syndrome will need multiple surgical procedures to improve their quality of life. A multidisciplinary approach is required.^{2,4}

Some of the sonographic signs and their association with syndromes

The frontal bossing (Figure 15, 16, 18) may be a

typical finding in Apert syndrome (Figure 15.16) with achondroplasia (autosomal dominant disease with rhizomelic shortening of extremities) (Figure 17), in Russell-Silver syndrome (poor growth, asymmetric IUGR of the skeleton with normal head size).

Asymmetry of the fetal skull, except for a variety of craniosynostosis which may or may not be associated with syndromes, can also be found in a fetus with:

Amniotic Band syndrome: because of the rupture of the amnion, which occurs very early in the first trimester of pregnancy, the amniotic band causes a great variety and severity of destructive fetal malformations depending on the fetal parts that come into contact and become trapped in it. When they affect the skull, it is possible to detect asymmetric anencephaly, encephalocele, facial clefts and micrognathia. Other abnormalities that were also found were limb flaps, constriction rings and limb amputations.

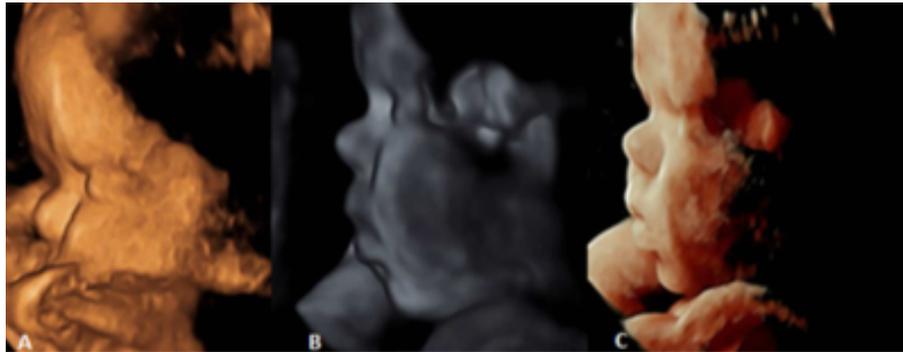
Other abnormalities of the skull detected by ultrasound are microcephaly and macrocephaly.

Microcephaly denotes a group of disorders characterized by a small head and is usually associated with abnormal neurological findings and mental disorders.⁵ Microcephaly usually also means microencephaly because the size of the head usually determines the size of the brain. Fetuses with prenatal susceptibility to microcephaly have a head circumference (HC) of more than 3 standard deviations below the average for gestational age.⁷² The diversity of associated anomalies found by ultrasound depends largely on the etiologic factors that cause microcephaly. Precise etiology in most cases of microcephaly is still unknown. However, this is related to numerous chromosomal abnormalities syndromes such as: Cornelia de Lange, DiGeorge, Wolf-Hirschhorn, Cri du Chat, trisomy 13 and 9, Fetal alcohol syndrome and exposure to some toxic substances (drugs, chlorophene, methotrexate MTX, phenylalanine), mothers' malnutrition, exposure (of pregnant woman) to certain infections during pregnancy such as rubella, toxoplasmosis, varicella, cytomegalovirus (CMV). There are reports of new causes such as exposure (during pregnancy) to Zika virus and unwanted pregnancy outcomes such as microcephaly, other brain, and eye anomalies and increase in loss of pregnancy.⁷⁷

Figure 16: Combination of prenatal and postnatal images of the same baby with the Apert syndrome. Prenatal images with 3D surface mode imaging. Notice the similarities of the images: prenatal and postnatal facial dysmorphism, abnormal shape of the scul, frontal bossing hyperthelorism and „mitten like hands/ feet“.⁴



Figure 17: A-C: 3D surface rendering of the 34 gestational week fetus, face- profile. Achondroplasia was suspected. Notice different methods of surface rendering. Frontal bossing and depressed nasal bridge.



Zika Congenital Syndrome is generally characterized by cerebral atrophy which may interfere with formation and with the neuronal migration during early cerebral embryogenesis.⁷⁸ Other features of this syndrome are the following: expressed microcephaly, lysencephaly, microphthalmia, contractures, and arthrogriposis^{72, 77,78} Viruses such as CMV or Zika have been shown to attack brain cells, particularly neural progenitors, infecting and destroying the primary stem cells (radial glial cell) of the brain, and therefore missing new neuronal ‘daughters’. The severity of depends largely on the time of infection during pregnancy.^{63-65, 79} Microcephaly is mainly a result of a small cerebral cortex. In addition, the infection can cause scars and calcifications in the brain tissue which can be depicted by ultrasound. Infection should be confirmed by a real rev. by polymerase transcription reaction (rRT-PCR)⁷⁹ Recent research from the endemic region of Brazil has shown that although some babies are born with normal size of head, postnatal development of microcephaly may occur, as well as significant neurological disorders leading to arthrogryposes, conditions leading to deformity of joints and disabilities.⁷⁸

Abnormalities such as periventricular and intraparenimal calcification, ventricular hypertrophy, secondary cerebral atrophy, cerebellar hypoplasia and cortical abnormalities are seen and detected much earlier than the microcephaly itself.⁷⁹

When needed, in addition to the standard ultrasound examination by 2D US, 3D/ 4D US may be used to enhance the accuracy. Fetal neurosonography with 3D advanced US techniques should be included in prenatal assessment if abnormalities are suspected. Different displays of 4D US can be used to evaluate the fetal dynamics and functionality of some organs and different organ systems.

Kurjak Antenatal Neurodevelopmental Test (KANET) can be used to evaluate fetal brain function between 28 and 38 gestational weeks.⁸⁰⁻⁸⁵ KANET (Figure 25) can be very useful tool, easily performed and used to detect the fetuses at risk for neurological impairment. If score of the test is borderline or abnormal, this test should be repeated at intervals of every 2 weeks until delivery. Prenatal results can be compared with the neonatal ones. Fetuses that are found to be at risk should be followed-up during at least first 2-3 years of life, as suggested by pediatricians, to be able to exclude the neurological damage to same extent and cerebral palsy (CP). Clinical usefulness of the KANET test was suggested by many authors over the past decade, and the exact data of the meta-analysis are underway, nevertheless, preliminary reports are promising.

During the routine ultrasound examination, abnormalities of fetal kidneys and bladder can be detected (Figure 18,19) and abnormalities of the anterior abdominal wall in the form of ompha-

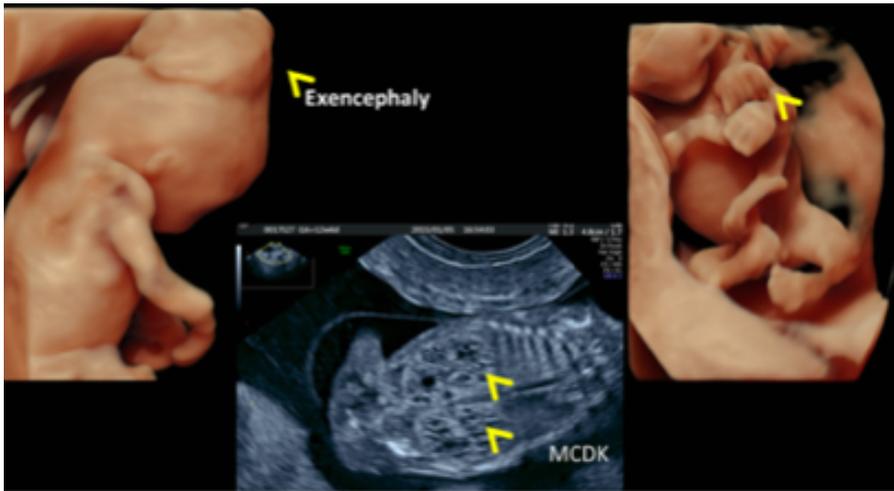


Figure 18: Typical triade of findings: Encephalocele(80%), Renal cystic dysplasia-bilateral MCDK (95%); Polydactyly(75%); Lethal syndrome: Meckel Gruber Syndrome!¹⁶

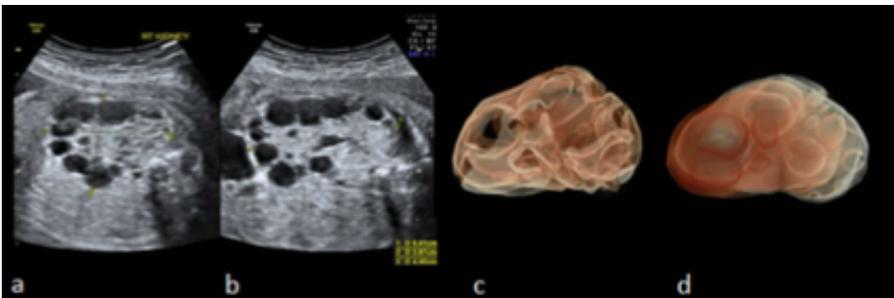


Figure 19
A, b: Multicystic Dysplastic Kidneys: (MCDK) in a fetus of 26 gestational weeks.
C, d: 3D HDlive Silhouette imaging: extraction of the volume of the MCDK kidney^{16,4}

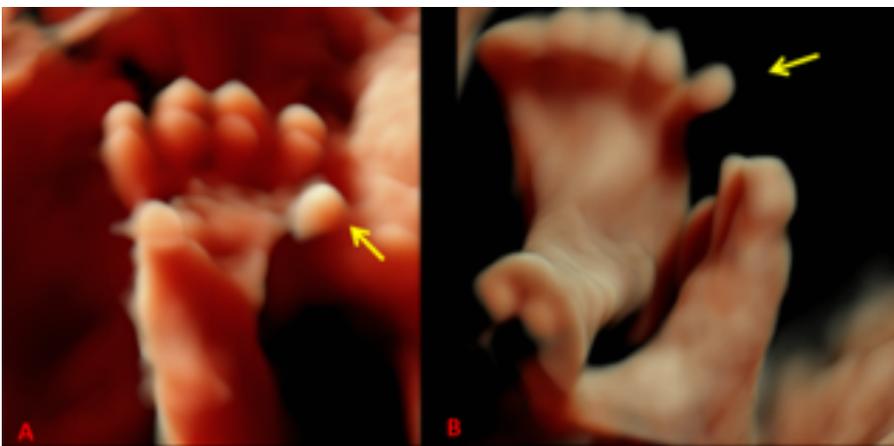


Figure 20
3D HDlive surface rendering of the fetal hand and the feet. Notice the polydactily in both cases! Fetus with Meckel Gruber Syndrome.

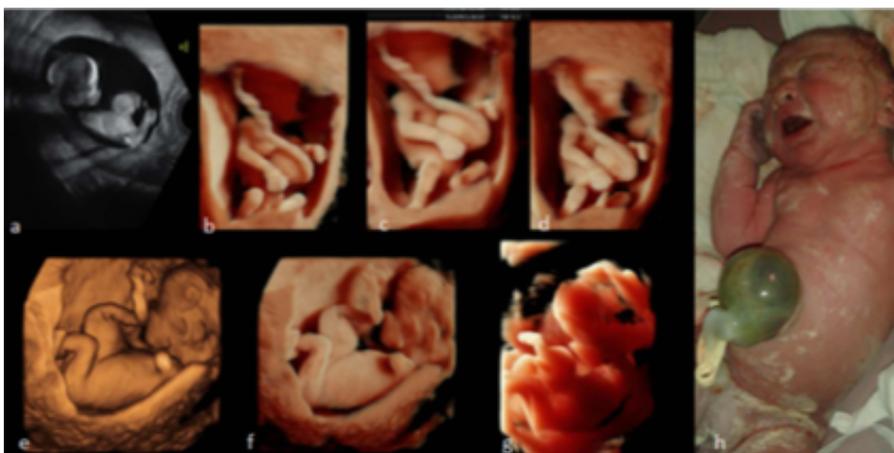


Figure 21:
Sequence of images with a: 2D US: fetus with the omphalocele in the second trimester,
b-g: 3D HDlive modality in the assessment of the fetus. Notice the difference in image illumination and angle of imaging. Notice the similarities of imaging with postpartum image.⁴

locele (Figure 21). It can be seen a wide range of structural and functional abnormalities.

Dysplastic kidneys with multiple cysts (Multi-cystic dysplastic kidney - MCDK) (Figure 18,19) that vary in size can be found as an anomaly in some very severe syndromes. Because MCDK is dysfunctional, if found bilaterally, it indicates a lethal outcome and is often associated with Meckel Gruber's syndrome (Figure 18,19) with autosomal recessive inheritance.⁸⁶ Specifically, a triad of anomalies is found: occipital encephalocele, MCDK (Figure18) and polydactyly (Figure 20). A newborn dies in the first few days of life due to lung hypoplasia and kidney failure. Detection of occipital encephalocele in the first trimester is easier because of a better examination and normal amount of amniotic fluid. Later in pregnancy, there is a progressive oligohydramnios which can be the cause that encephalocele is missed. Special attention should be paid to the evaluation of both fetal kidneys because the normal ultrasound finding of a kidney excludes lethal Meckel Gruber syndrome!

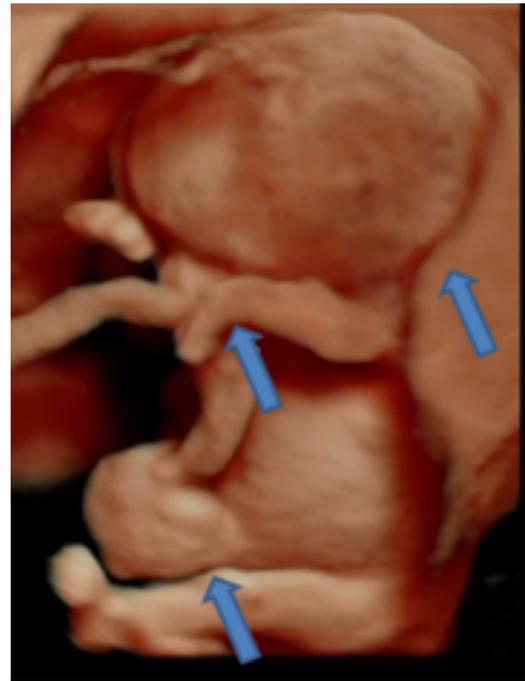


Figure 22: 3D HDlive surface image of the fetus with Trisomy 18. Notice the triade of the anomalies: omphalocele, contracted wrists/hands and hygroma colli (arrows)¹⁶

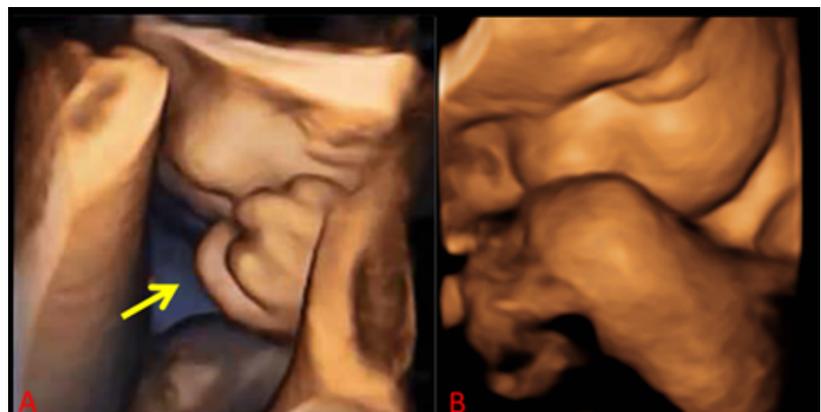


Figure 23: 3D surface rendering; trisomy 18 (Edwards syndrome) in 2 cases. Notice the clenched haand (arow) and overlapping fingers in both.



Figure 25: 3D/ 4D HDlive fetal assessment by KANET test: Notice open eyes of the fetus at 28 gestational weeks.⁴

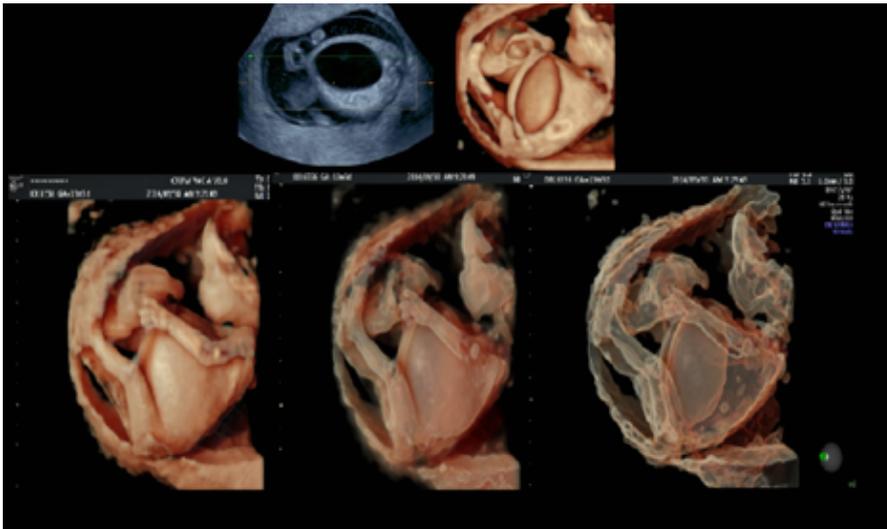


Figure 24
Sequence of images. From 2D to 3D surface and Silhouette display with increasing Silhouette intensity. Notice: Megacystic bladder in the fetus with Prune Belly syndrome.^{16,2}

CONCLUSION

The 3D ultrasound technique with different visualization and manipulation capabilities of stored volume, provides a unique opportunity for a detailed view of normal and abnormal fetal development. If facial anomaly is suspected, this technique will help in the evaluation and gained images will give some answers to question about severity and extent of anomalies. Particularly handy tool may be when communicating the neonatologist, pediatrician, plastic and reconstructive head and neck surgeon and especially when consulting with the parents of the child. However, as in any imaging technique (ultrasound, MSCT, MRI), you need to know the dome and limitations of 3D / 4D rendering and to be aware of possible artifacts and traps.

From the first trimester to the next, as soon as it becomes possible to detect congenital anomaly by prenatal ultrasound, the question arises- what can and should be done. Many ethical dilemmas present at the time.^{2,4} Contemporary medicine faces some major problems when it has the ability to prolong life of severely sick baby with potentially lethal congenital syndromes.

Taking specific ultrasonic diagnostics into account, the idea is to find the balance between the advantages and limitations of sonographic assessment. At the same time, it should be possible to optimize recommendations with the expectations of parents of potentially seriously ill baby. Given the complexity of prenatal diagnosis

of syndrome, everything involved in the process is also complex. This includes conformation of prenatal diagnosis postnatally and determination of the short and long term prognosis if possible to assist parents who are facing a baby with syndrome.^{2,4} It is essential to point out the necessity of complex, lifelong and costly multi-disciplinary care for severely ill baby.

All the aforementioned, 3D / 4D US techniques promise to improve the accuracy of clinicians in detection of fetal abnormalities and detecting fetal syndromes as early as possible. There are many advantages in prenatal detection of fetal syndromes already described, but there is also a great room for improvement. Since new 3D / 4D ultrasound technology becomes more available in everyday clinical practice, the clinician should remain well-informed, well trained and monitor new diagnostic capabilities. Continuous education is necessary. In this way, the number of fetal abnormalities and syndromes detected prenatally will probably increase over time.⁴ Auxiliary tools such as network databases ("online databases") that integrate all the necessary information should be included and used for better diagnostic precision.^{2,4,87}

CONFLICT OF INTERES

The authors declare no conflict of interest.

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CASE REPORT

Primary Cardiac Hodgkin Lymphoma – Case Report

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ABSTRACT

Primary cardiac tumors are rare, with an incidence of 0,056%. The most of primary cardiac tumors are benign tumors, only 10% are malignant, and 1% from malignant tumors are primary cardiac lymphoma (PCL). From the clinical standpoint, PCL is a non-Hodgkin lymphoma presenting as cardiac disease in rightsided chambers in elderly population. It has been presented young man with Hodgkin PCL, that has been successfully treated with transplantation of the hematopoietic stem cells, leading to stable remission of the disease that has been maintained for 12 years.

Key words: primary cardiac lymphoma, Hodgkin lymphoma, transplantation of the hematopoietic stem cell.

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INTRODUCTION

Primary cardiac lymphoma (PCL) is very rare disease. One percent of primary heart tumors are lymphomas, and from the clinical standpoint, that are mostly a non-Hodgkin lymphomas. Systemic lymphoma with cardiac involvement has been reported in about 20% of cases¹⁻². In most of cases the tumor mass is intrapericardial². The mean age at presentation of PCL is approximately 60 years, with a male gender and the rightsided chambers predominance¹. The gold standard in diagnostic of PCL is computed

tomography scan, and it is considered superior to echocardiography in the detection and demarcation of tumor. In treatment of PCL the conventional chemotherapy and its combination with radiation have been reported as the treatment of choice which use leads to longer survival rate³. Hodgkin's lymphoma of any localization, in young adult patients, can achieve excellent outcome with high dose of chemotherapy and autologous stem cell transplant treatment⁴.

CASE HISTORY

A 35-year-old man has been presented. The disease started in 2006, February, manifested in dry cough and hemoptyses. He was originally treated with the antibiotic therapy, whose application was not followed by the improvement. By the cardiac ultrasound imaging on February 27th, 2006, the 4 x 3.5 cm diameter mass was verified in the right ventricular outflow tract, which infiltrated the ventricle wall and reached the pulmonary artery and almost fully obstructed it (Figure 1). On the same day, he was hospitalized in the Coronary Unit of the Cardiovascular Clinics of the Clinical Center in Banja Luka

with the assumed development of pulmonary thromboembolism. The fibrinolytic therapies were ordinated, after whose application the hemoptyses appeared again. Then, the computed tomography of the chest was performed, which indicated the consolidation of the pulmonary parenchyma in the medial segment of the middle pulmonary area to the right along with a somewhat bigger corresponding hilus. The patient was sent to the Urgent Care Clinics of the Military Medical Academy (MMA) in Belgrade on February 28th, 2006, from where, on March 2nd, 2006, by the direct relocation, the treatment

continued at the Cardiac Surgery Clinic of the MMA with the assumed development of the primary cardiac tumor, and aimed at surgical treatment. On the same day, after the patient's relocation, the surgery - the medial sternotomy was performed with the biopsy of the right ventricle tumor, whose extempore analysis indicated the Hodgkin's lymphoma II/IV AaCS. The treatment continued further at the Hematology Clinics of the MMA, where, on March 17th, 2006, the first dose of chemotherapy was ordained according to the ABVD protocol (Doxorubicin/Bleomycin/Vinblastine/Dacarbazine). He received eight cycles of polychemotherapy according to the ABVD protocol, after whose application the disease remission occurred, which retained until 2007, January. The early disease relapse was manifested in the manifestation of general symptoms being characteristic of the lymphoproliferative ailments with the appearance of the subfebrile temperatures, overnight sweating and cough. In the ambulatory conditions, the growth of the acute reactants of inflammation was recorded, and the cardiac ultrasound imaging determined the tumor in the right ventricle again. The first early relapse of the disease was treated with the chemotherapy according to the BEA-COPP protocol (Bleomycin/Etoposide/Adriamycin/Cyclophosphamide/Vincristine/Procarbazine/Prednisone) that was received in four cycles. The first cycle of this chemotherapy was utilized as the mobilization protocol aimed at collection of primary stem cell hematopoiesis. Upon completion of chemotherapy treatment, the patient was presented to the consilium for transplantation of the hematopoietic stem cells (HSC), which indicated further treatment with the high dose chemotherapy along with the support to the autologous HSC. In the pre-transplantation phase, on May 15th, 2007, the conditional protocol was applied according to the BEAM scheme (Carmustine/Etoposide/Cytarabine/Melphalan).

Transplantation of the HSC was performed on May 21st, 2007, when in the suspension of 250 ml of the previously collected HSC or 7.2×10^8 HSC/kg were returned to the patient. Since then the disease has been in a stable remission. On two occasions, the PET-CT scan was performed, by which the complete metabolic inactivity of the disease was recorded as well as the cardiac ultrasound did.

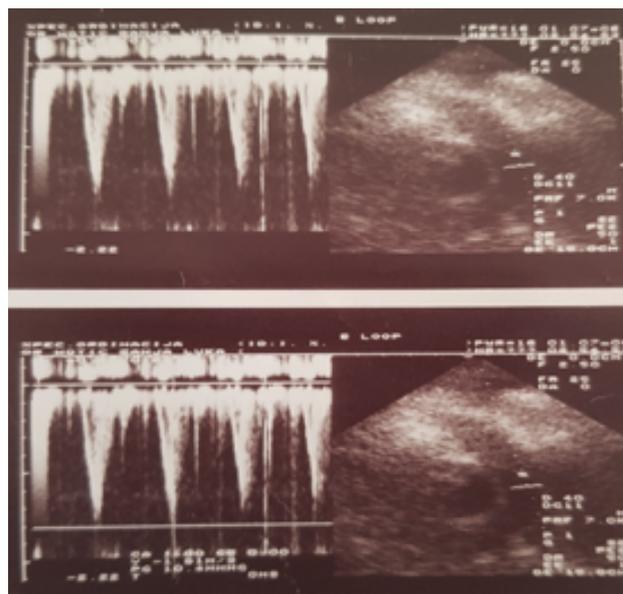


Figure 1. Ultrasound picture of primary cardiac lymphoma: The mass involving right ventricle of hearth. (Source: History of patient's illness)

CONCLUSION

Here has been presented the case of the Hodgkin PCL, which was unsuccessfully treated with the conventional polychemotherapy. So far, not many cases of the primary Hodgkin lymphoma of the heart have been described, which have been treated with the transplantation of the HSC. In the presented case, the stated therapeutic protocols along with the transplantation of the stem cells led to the stable remission of the disease that has been maintained backwards for 12 years.

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Medical Writing for Non-Native English Speakers: Help for Usage of Articles

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ABSTRACT

Many non-native English speakers, especially those whose mother language has no articles (like Slavic languages) make mistakes when they speak or write English. This may sound rather rough to English-speaking listeners or readers. To improve their command of the language and the usage of articles, individuals for whom English is a second language should review the usage of articles from time to time. We prepared a short text on this subject and also included exercises from the medical publications.

Key words: articles, definite article, indefinite articles, zero article, omitted article, non-native English speakers, exercises.

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INTRODUCTION

Many non-native speakers, especially those whose mother languages have no articles, sometimes have difficulty speaking standard English. This is particularly true of Slavic languages that lack articles as a part of speech. As a result, this may sound rough to native English speakers. More importantly, writing that contains frequently misused or lack of articles is harder to understand and less appreciated. Medical articles and reports that suffer from this condition

are troublesome to medical journal editors, reviewers and readers alike. For that reason, individuals who speak English as a second language should review the use of articles from time to time. While doing such a review, we prepared a short text to help our colleagues appraise their facility with this subject in both speaking and writing. We also included exercises from the medical publications.

ARTICLES, GENERAL

Three short words in the English language, *a*, *an*, and *the*, are used as articles. Indefinite articles (*a/an*) are used when the speaker or writer is talking about a non-specific member of a group, while a definite article (*the*) is used for a specific member(s) of a group. Articles are basically adjectives, meaning they can be used as limiting adjectives¹ that precede a noun or nounphrase and determine the noun or phrase's use to indicate something definite (*the*) or indefinite (*a, an*). An article might stand alone or be used with other adjectives. For example, one would say a

road, or a brick road, but to make it specific, one would say *the* yellow brick road.

Proper nouns (name of person, place, or thing) usually require no article, but some, like the *Danube river* or *the United Kingdom*, should have a definite article. Common nouns are words used to name general items rather than specific ones, eg. Common noun: I really want to live in a **big city**. Proper noun: Of all places I have lived, Banja Luka was the best. Common nouns can be divided into two categories, count-

able and uncountable. Countable nouns are those that refer to something that can be counted. They have both singular and plural forms, eg. cat/cats, man/men. Most nouns come in this category. A smaller number of nouns do not refer to things that can be counted, and they do not regularly have a plural form; these are uncountable nouns. Examples include rain, wine, milk, wood. Uncountable nouns can't be preceded by "a" or "an". Many abstract nouns are typically uncountable, eg. truth, humour, love.

Choosing article is one of the most confusing parts of English grammar. Many languages have

no articles and other use articles very differently than English language. To master the use of articles one should listen native speakers, as much as he/she can, and practice. The process of choosing article starts with identifying noun. The articles "a" and "an" are indefinite articles. They are used with a singular countable noun when the noun referred to is nonspecific or generic. The article "the" is a definite article. It is used to show specific reference and can be used with both singular and plural nouns and with both countable and uncountable nouns. In order to help non-native speakers in the use of articles, Table 1 shows the basic rules for their use.

Table 1: Basic rules of choosing article

Type of noun	Singular/ Plural	Use of the articles for definite or indefinite nouns*
Proper (specific) nouns**		None
Common nouns***		
Countable	Singular	For definite noun use "the" For indefinite noun use "a" or "an"
	Plural	For definite noun use "the" For indefinite noun None
Uncountable		For definite noun use "the" For indefinite noun None

*Definite nouns are specific nouns. In the sentence "The dog bit me" we are referring to a specific dog. "The dog" is an example of a definite noun with the proper article. However, when we are referring to unknown or unidentified dog, we shall use an indefinite "A dog bit me." "A dog" is an example of an indefinite noun with a proper article.

**Proper nouns include names of persons, places, or things. They usually require no article; there are exceptions to this rule including the names of countries that contain words like Republic, Kingdom, Union, Empire, or names of some geographical regions, oceans, or rivers.

***Common nouns are words used to name general items rather than specific ones, eg. Common noun: To live in a big city. Proper noun: I have lived in Banja Luka. Countable nouns are those that refer to something that can be counted. They have both singular and plural forms, eg. cat/cats, man/men. A smaller number of nouns do not refer to things that can be counted, and they do not regularly have a plural form; these are uncountable nouns.

Examples include rain, wine, milk, wood. Uncountable nouns can't be preceded by "a" or "an".

DEFINITE ARTICLE

The word the is a definite article that is used when a speaker is talking about a specific member of a group. The noun being modified can be either singular (*the house*) or plural (*the houses*) as well as reference to count—the number of things. For example, *the bicycle* was stolen today, or *the fog that appeared last evening caused many accidents*. A definite article is used only when its noun is obvious to both speaker and listener. It points to a definite object that is so well understood that it does not need description (e.g., *the letter is here* is shortened from *the*

letter that you expected is here); the same is true for something that is about to be described (*the sights of Sombor*) or something that is important (*the medal of Karađorđe*).

A noun or noun phrase is specific when both speaker and listener know which specific place, person, or thing is being talked about. In such a case, the definite article is used with singular and plural countable and uncountable nouns. Example: *The milk you spilled is all over the floor in the room*. A noun is also specific when



it represents something unique, where there is only one such thing or person: *The president* was right. A noun or noun phrase can be made specific by context.² Example: Who are you? I'm *the nurse*. (A patient in a hospital asked this question when he first awakened after anesthesia.)

Certain adjectives can make a noun represent something unique. Examples of such adjectives are *right, wrong, first, only*, and the superlative forms of adjectives. Examples: *The best solution* is to stop talking. Sometimes it's the *only solution*.

The definite article (*the*) is used with the names of some countries, such as those that are plural or include a "political" word for instance *Republic, Kingdom, Union, or Empire*. Example: *The United Kingdom* is a big naval power. *The Federative Socialist Yugoslavia* disappeared from the map. *The United States of America* is very interesting country. Nonetheless, with an abbreviated form, such as *Made in USA*, this country's name is used without the definite article.

The names of some geographical features and regions are preceded by the definite article. Example: *The Adriatic Sea, The Atlantic (Ocean), The Black Sea, The Mediterranean (Sea), The Suez Canal, The Danube River, The Mississippi River, The Rio Grande River, The Volga River, The Balkans, The Middle East (The Near East), The North Pole, The Orient, The Sahara, The Alps, The Andes, The Caucasus Mountains, The Pyrenees, The Urals*. Also, the names of specific airplanes, ships, and hotels often use the definite article. Example: *The Concorde, The Titanic, The Grand Hotel*.

A noun is often indefinite the first time a speaker mentions it. However, it is usually definite after the first mention. Example: Should they be allowed to camp close to *an unspoiled Danube beach*? *The beach* will be spoiled. I bought *a bicycle* last month. *The bicycle* was stolen yesterday.

For successful communication in writing, short titles tend to be more attractive. Therefore Hall³ suggests that definite articles can usually be dispensed with. [He also recommends discarding excessive adjectives, and 'noun salads' (a string of nouns masquerading as adjectives to form clumsy phrases like "community hospital liaison nurse activity analysis") in order to achieve a more accurate and interesting title.]

Indefinite articles are *a* and *an*. They are used only with singular countable nouns (a cat but not "a rice"). Remember, a countable noun is anything that can be counted. The indefinite articles point to nonspecific objects, things, or persons that are not distinguished from other members of a class. They may be singular (*a doctor*), or uncountable (*a multitude*), or generalized (*A dog is a common household pet* is a general statement about any and all dogs.)³

The choice of "a" or "an" word depends on the sound of the word it precedes. "A" comes before any word beginning with a **consonant sound** regardless how word is spelled. So, in addition to words that begin with consonants, this includes the words that begin by the following sounds: (ju), as in the words "user" or "European", and (wa) as in the word "one". Example: *a man, a dog, a United Nation, a one-pound weight, a historic occasion, a user*. "An" should be used before words beginning with a **vowel sound** to modify a nonspecific, singular countable noun. This includes vowels and, as well, the words that begin with a silent "h" as in "herb" or "honor." Thus, we use *an honor, an herb, an elephant*. This kind of indefinite article (*an*) makes pronunciation easier and clearer.

In early English "an" meant one and was the only form used. The "n" slowly disappeared before words with consonant sounds, and indefinite article "a" was formed. Because it is difficult to say *a idea* or *a episode*, the indefinite article "an" remained. Thus, it is much easier to pronounce an idea, an episode, and all other words that need the indefinite article. It is important that before words that begin with vowels that have a consonant sound an indefinite article "a" must be used (e.g., a United Nation, a one-pound weight, a user).

For the abbreviate form of the title a medical doctor (MD), the indefinite article "an" is used. Example: When an MD came in, all students became silent. MD is pronounced /em di/.

The indefinite article (a/an) should be used with indefinite singular countable nouns. Example: It is brutal to harm or displace **a** species.

ZERO ARTICLES

Certain nouns require neither an indefinite nor definite article before them. Some usages call for no article at all or an article implicitly present, such as one before a plural countable nouns or uncountable nouns.¹ Example: Although both new and washed bottles are stacked nearby, the extract is poured into new bottles only (*the* is implicit before *new bottles*); Environmentalists are against developments (plural countable noun); Environmentalists are against *development* (uncountable noun).

Zero, or no article usually occurs in idiomatic references to time, illness, transportation, personal routines, and meals. Example: *by sunset, has cancer, travel by train, go to bed, make breakfast.*

OMITTED ARTICLE

The absence of an article may alter a sentence's meaning. Example: The meaning of the news brought us little comfort (we weren't comforted) changes if an article (**a**) is inserted before *little*: *...the news brought us a little comfort (...we felt somewhat comforted).*

ARTICLES AND GENERIC NOUNS

A noun is used generically when it represents all members of a class of persons, places, or things. One may use *the, a/an* or no article with generic nouns in order to generalize, classify, or define. Example: Teachers are devoted to their profession (generalization); *A monkey* is a primate (classification); *A car* is a four-wheeled vehicle (definition).

For some singular generic countable nouns, the definite article is used. They fall into three main categories: inventions, musical instruments and animal species. Example: The wheel is one of most important inventions. Ana plays the violin. The Siberian tiger is endangered.

There are four ways of classifying or defining with generic countable nouns.²

PRACTICE

Part One

Fill in the blanks with the correct form of the articles **a**, **an**, and **the**. Use **o** if no article is needed. The following text presents several segments from the paper entitled *Conflicting interests involved in the process of publishing in biomedical journals*.⁴

Authors submit manuscripts according to acceptance criteria for ¹_____ specific journals. Editors, with help of reviewers, assess ²_____ manuscripts and make final decisions on publication. ³_____ main goal of the editor is to fulfill the needs of his readers, providing ⁴_____ most current and relevant information by proper presentation and interpretation of research data. It is well known that participants in ⁵_____ publication process, authors, peer reviewers, and editors, sometimes have potential financial interests or ⁶_____ other concerns related to ⁷_____ articles under consideration. This brief discussion on ⁸_____ conflicting interests of all participants in the publishing process may help readers to understand what can be done to provide better evaluation of manuscripts and increase ⁹_____ credibility of published articles.

Journal editors play ¹⁰_____ major role in the publishing process, including notation of ¹¹_____ potential conflicts of interests of ¹²_____ authors, peer reviewers, journal's editorial board members, and publishers. Any editor, or any member of ¹³_____ executive board, who has conflicts of interests relating to articles under consideration should absent himself from ¹⁴_____ editorial decisions.

Peer-review is a process of critiquing ¹⁵_____ manuscript before publication. The word "peer" means "¹⁶_____ person of the same rank, or ¹⁷_____ person who is ¹⁸_____ member of ¹⁹_____ same group as another". ²⁰_____ role of ²¹_____ manuscript assessor (reviewer or referee) is that he/she advises ²²_____ editor on ²³_____ originality, quality and suitability of ²⁴_____ manuscript for publication and provides written feedback that will be transmitted to ²⁵_____ authors. ²⁶_____ ideal reviewer is as knowledgeable as ²⁷_____ author(s) on the subject, and he should also be familiar with ²⁸_____ goals and rules of the manuscript review.

When ²⁹_____ first periodical journals, *Le Iovrnal des Sçavans* (January 5, 1665) and *Philosophical Transactions of the Royal Society* (March 6, 1665) were introduced in Paris and London, respectively, ³⁰_____ peer review process did not exist, but 66 years later ³¹_____ Royal Society of Edinburgh published ³²_____ first peer reviewed collection of medical ³³_____ articles. Development of ³⁴_____ peer-reviewed process over ³⁵_____ next two centuries and beyond followed various paths to ensure ³⁶_____ quality of scientific information. It has not been always accepted that peer reviewing is necessary, but over time most researchers agree that ³⁷_____ peer review system is a necessary tool for publishing.

³⁸_____ objective critique of a scientific manuscript is ³⁹_____ essential element of ⁴⁰_____ peer review assessment. Current scientific peer-review system is not perfect, but it is ⁴¹_____ required step in ⁴²_____ editing process of ⁴³_____ majority of biomedical journals. Peer review system has gradually developed to ⁴⁴_____ present day, where experts in ⁴⁵_____ field examine ⁴⁶_____ scientific quality and determine ⁴⁷_____ novelty of ⁴⁸_____ study, clarity of presentation, ethical validity, and technical quality of ⁴⁹_____ manuscript. By ⁵⁰_____ end of ⁵¹_____ 20th century, ⁵²_____ majority of ⁵³_____ medical journals used ⁵⁴_____ peer-reviewed system. ⁵⁵_____ reviewers recommend acceptance, rejection, or revision. ⁵⁶_____ editor then communicates with both reviewers and authors in order to improve ⁵⁷_____ manuscript before he makes ⁵⁸_____ final decision on publication.

From ⁵⁹_____ beginning of ⁶⁰_____ peer-reviewed system, there have been pros and cons, and many discussions propose improvements, such as blinded reviewers or authors, unmasking ⁶¹_____ identity of ⁶²_____ reviewer to co-reviewer, open review process or even elimination of ⁶³_____ review process. ⁶⁴_____ recommendations of ⁶⁵_____ reviewers, in ⁶⁶_____ any journal published in ⁶⁷_____ so called "small scientific community" especially if it is published in ⁶⁸_____ local language, sometimes may be either uncritically positive or negative, but that is no reason to avoid this step in ⁶⁹_____ publishing.

Part Two

Fill in the blanks with the correct form of the articles *a*, *an*, and *the*. Use *o* if no article is needed. The following text presents a segment from the paper entitled *Severe non-opioid induced pruritus following spinal block*.⁵

Case Presentation: ⁻⁶⁹_____ 57-year-old, 122 kg, 6'1" male, had ⁻⁷⁰_____ past medical history of hypertension, glucose intolerance, benign prostatic hypertrophy, and right-sided Bell's palsy. This patient was admitted for cystolithotripsy under spinal anesthesia. Subarachnoid block produced ⁻⁷¹_____ prompt onset of anesthesia with satisfactory sensory blockade to ⁻⁷²_____ level of ⁻⁷³_____ tenth thoracic dermatome. Propofol infusion was maintained for sedation, and ⁻⁷⁴_____ Ramsay scale of 5 was obtained. The patient tolerated ⁻⁷⁵_____ procedure with no untoward events and was transferred to ⁻⁷⁶_____ Post Anesthesia Care unit. Approximately 60 minutes after arrival in ⁻⁷⁷_____ recovery room, ⁻⁷⁸_____ patient complained of intense itching confined to soles of both feet. He reported ⁻⁷⁹_____ onset of this discomfort soon after he awoke in ⁻⁸⁰_____ OR. Upon evaluation, ⁻⁸¹_____ patient had no motor block and sensory recovery to ⁻⁸²_____ level of ⁻⁸³_____ L3 dermatome. Treatment of ⁻⁸⁴_____ present severe pruritus was initiated with ⁻⁸⁵_____ intravenous lidocaine. Ten minutes later ⁻⁸⁶_____ patient reported no relief (verbal analog scales 10/10). His discomfort was so great that physical restraint was required to keep him from getting off ⁻⁸⁷_____ bed and excoriating his feet. Subsequently, IV diphenhydramine controlled his agitation but had no effect on ⁻⁸⁸_____ severe itching. Subhypnotic propofol infusion was started with ⁻⁸⁹_____ total infusion time of thirty minutes. ⁻⁹⁰_____ itching subsided dramatically after five minutes and after 20 min was no longer present. At that time, there were ⁻⁹¹_____ complete resolution of sensory and motor block and ⁻⁹²_____ patient was discharged from ⁻⁹³_____ recovery room and advised to pursue ⁻⁹⁴_____ work up for diabetic neuropathy and to seek follow up in ⁻⁹⁵_____ pain clinic if symptoms recurred. In ⁻⁹⁶_____ mean time patient had another cystoscopy procedure under spinal anesthesia and had ⁻⁹⁷_____ exactly ⁻⁹⁸_____ same course in ⁻⁹⁹_____ Post Anesthesia Care unit. ⁻¹⁰⁰_____ only difference was that prior to ⁻¹⁰¹_____ placement of subarachnoid block he received ⁻¹⁰²_____ IV fentanyl as ⁻¹⁰³_____ premedication.

Part Three

Fill in the blanks with the correct form of the articles *a*, *an*, and *the*. Use *o* if no article is needed. The following text presents several segments from the book entitled "How to write a paper"³ and "Statistical methods for anesthesia and intensive care."⁶

Abstracts. After ⁻¹⁰⁴_____ title, ⁻¹⁰⁵_____ abstract is ⁻¹⁰⁶_____ second most read part (frequently ⁻¹⁰⁷_____ only other red part) of paper, and so is likely to ⁻¹⁰⁸_____ basis on which ⁻¹⁰⁹_____ work is judged by uncritical readers. It is also ⁻¹¹⁰_____ first part of ⁻¹¹¹_____ paper that an editor reads carefully, and it may provoke ⁻¹¹²_____ choice of references. Like ⁻¹¹³_____ title, ⁻¹¹⁴_____ abstract will reward time spent on it and should be short, intelligible, informative, and interesting. It should be ⁻¹¹⁵_____ digest of ⁻¹¹⁶_____ whole paper and contain its essence. It should consist of four basic parts, which can vary individually in length. These should describe succinctly (a) why what was done was done; (b) what was done; (c) what was found; and (d) what was concluded. ⁻¹¹⁷_____ permissible length may be defined by the journal in question, but 200 words is a good average target that should be exceeded only in exceptional circumstances. ⁻¹¹⁸_____ Vancouver Group suggests a maximum of 150 words for ⁻¹¹⁹_____ unstructured abstracts and 250 for fully structured formats. The process takes time. Remember, ⁻¹²⁰_____ text that is easy to read is usually hard to write.

Statistical methods. 'Statistics' is ⁻¹²¹_____ science of collecting, describing and analyzing data that are subject to random variation. It consists of two main areas: (i) descriptive statistics, whereby ⁻¹²²_____ collection of data is summarized in order to characterize features of its distribution, and (ii) inferential statistics, whereby these summary data are processed in order to estimate, or predict, characteristics of another (usually larger) group.

Before ⁻¹²³_____ research study is undertaken it is important to consider the nature of ⁻¹²⁴_____

observations to be recorded. This is an essential step during _____ planning phase, as _____ type of data collected ultimately determines _____ way in which _____ study observations are described and which statistical tests will eventually be used.

At _____ most basic level, it is useful to distinguish between two types of data. _____ first type of data includes those that are defined by some characteristic, or quality, and are referred to as _____ qualitative data. _____ second type of data includes those that are measured on numerical scale and are referred to as quantitative data.

Because qualitative data are best summarized by grouping the observations into categories and counting _____ number in each, they are most often referred to as categorical (or nominal) data. Examples of categorical data: 1. Gender (male, female), 2. Type of operation (valvular, coronary artery, myocardial, pericardial, other), 3. Type of _____ ICU admission (medical, surgical, physical injury, poisoning, other), 4. Cardiovascular adverse events (acute myocardial infarction, congestive cardiac failure, arrhythmia, sudden death, other). _____ simplest way to describe categorical data is to count the number of observations in each group. These observations can then be reported using absolute count, percentages, rates or proportions.

If there is _____ natural order among categories, so that there is _____ relative value among them, then _____ data can be considered as ordinal data. Although there is _____ semi-quantitative relationship between each of _____ categories on an ordinal scale, there is not _____ direct mathematical relationship. For example, _____ pain score of 2 indicates more pain than a score 1, but it does not mean twice as much pain, nor is _____ difference between _____ score of 1 and 0 equal to _____ difference between _____ score of 3 and 2.

For ordinal data, _____ numerical scoring system is often used to rank _____ categories by _____ non-numerical record (A, B, C, D; or +, ++, +++, +++++). _____ numerical scoring system does, however, have practical usage, particularly for _____ convenience of data recording and eventual statistical analysis. These observations can be described by _____ absolute count, percentages, rates or proportions. Ordinal data can also be summarized by _____ median value and range.

Examples of ordinal data: 1. Pain score (0=no pain; 1=mild pain; 2= moderate pain; 3. severe pain; 4. unbearable pain); 2. Preoperative risk (ASA* I/II= low risk; ASA III= mild risk; ASA IV= moderate risk; ASA V= high risk).

Quantitative data are more commonly referred to as numerical data; these observations can be subdivided into discrete and continuous measurements. _____ observations that are counted are discrete numerical data and observations that are measured are usually continuous data. Examples of numerical data: episodes of myocardial ischemia (discrete), body weight (continuous), creatinine clearance (continuous), cardiac index (continuous), respiratory rate (discrete/continuous), post-tetanic (discrete).

Part Four

Fill in the blanks with the correct form of the articles **a**, **an**, and **the**. Use 0 if no article is needed. The following text presents the abstract from the paper entitled "*Seven decades of angiotensin (1939-2009)*".⁷

Two research groups in both North and South America independently discovered that renin released a novel vasopressor agent. The Argentine group named it hypertensin, and called its plasma protein substrate hypertensinogen. The group from the United States named it angiotenin. In 1958, Braun Menendez and Irvine Page suggested that _____ peptide should be named angiotensin. The combined name eventually became commonly used to avoid linguistic confusion. Research scientists and physicians today acknowledge that studies of _____ renin-angiotensin system (RAS) have greatly improved our understanding of several diseases. Certainly, medical practice profited significantly from _____ synthesis and application of numerous pharmaco-

logical agents that antagonize either¹⁵⁸ the biosynthesis or pharmacological responses of endogenously generated angiotensin II. Ultimately, discovery of¹⁵⁹ renin-angiotensin system led to many studies that resulted in therapies for vascular disease. This article briefly reviews research related to¹⁶⁰ discovery of angiotensin and indicates¹⁶¹ importance of additional studies related to¹⁶² RAS.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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ANSWER KEY

Part One

1.0, 2.the, 3.The, 4.the, 5.the, 6.0, 7.the, 8.0, 9.the, 10.a, 11.the, 12.0, 13.the, 14.0, 15.a, 16.a, 17.a, 18.a, 19.the, 20.The, 21.the, 22.the, 23.the, 24.a, 25.the, 26.An, 27.the, 28.the, 29.the, 30.the, 31.The, 32.the, 33.the, 34.the, 35.the, 36.a, 37.An, 38.an, 39.the, 40.a, 41.the, 42.the, 43.the, 44.the, 45.the, 46.the, 47.the, 48.a, 49.the, 50.the, 51.the, 52.0, 53.the, 54.The, 55.The, 56.the, 57.A, 58.the, 59.the, 60.the, 61.a, 62.the, 63.The, 64.the, 65.0, 66.the, 67.a, 68.0.

Part Two

69.A, 70.a, 71.a, 72.the, 73.the, 74.a, 75.the, 76.the, 77.the, 78.the, 79.the, 80.the, 81.the, 82.the, 83.0, 84.the, 85.0, 86.the, 87.the, 88.the, 89.a, 90.The, 91.the, 92.the, 93.the, 94.a, 95.the, 96.the, 97.the, 98.the, 99.the, 100.The, 101.the, 102.0, 103.a.

Part Three

104.the, 105.the, 106.the, 107.the, 108.the, 109.the, 110.the, 111.a, 112.the, 113.the, 114.the, 115.a, 116.the, 117.the, 118.the, 119.0, 120.0, 121.the, 122.a, 123.a, 124.the, 125.the, 126.the, 127.the, 128.the, 129.the, 130.The, 131.0, 132.The, 133.the, 133.the, 134.0, 135.The, 136, 137a, 138.the, 139.a, 140.the, 141.a, 142.a, 143.the, 144.a, 145.the, 146.a, 147.a, 148.the, 149.a, 150.A, 151.the, 152.an, 153.the, 154.0.

Part Four

From 155. to 162.the.



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- a) The title should be concise but informative, while subheadings should be avoided;
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- c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;
- d) Contact data of the corresponding author.

2. Abstract and key words

The second page should contain the title of the article and a structured abstract (250-300 words for original articles). In short, clear sentences the authors should write the Background/Aim, major procedures – Methods (choice of subjects or laboratory animals; procedures, methods for observation and analysis), the obtained findings – Results (concrete data and their statistical significance), and the Conclusion. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles Introduction, Case report, Conclusion). Below the abstract Key words 3-5 key words should be provided that describe the topic of the article. The key words should be selected from Medical Subject Headings (MeSH).

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rentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical ones. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethics Committee for the tests in humans and animals.

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Examples of references:

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