Publications of presented papers in the Scientific Programme of:

Joint Forum:
12th South-East European Conference of Chemotherapy, Infections and Cancer and 32th Annual Assembly of International Medical Association Bulgaria (IMAB)

20-23 October 2022,
Congress Center, Hotel Calista, Mineral Bath Resort Stara Zagora, Bulgaria

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Journal of IMAB - 2022; vol. 28(Supplement 12 SEEC & 32 IMAB); Section Medicine.
ISSN: 1312-773X; DOI: https://dx.doi.org/10.5272/jimab.2022Supplement1
Characterization of allergies – gender, age and blood group structure in a reproductive population from Southern Bulgaria

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Abstract

Allergies are a large group of diseases characterized by complexity and diversity. Their expression depends on various genetic components and environmental factors.

Purpose: The aim of the study is to characterize allergies and their potential relationships with the gender, age and blood groups among the reproductive population in Bulgaria.

Material and methods: Individuals, between 18 and 59 years are included in the study. They voluntarily have filled out a questionnaire, giving disease information after being diagnosed by a physician. Blood type (AB0 and Rh) have been determined in laboratory conditions by a routine technique. A software package SPSS, version 22.0 has been used for the statistical analysis.

Results: Among the participants, the manifestation of allergies in both sexes is similar with some difference in the age category over 44. Allergies occur most often in individuals with blood group A. No differences were found concerning among people with Rh+ and Rh- blood groups.

Conclusions: The male sex in combination with the age over 44 years and the blood group A for both sexes could be accepted as risk factors for the manifestation of the allergies in the studied population

Keywords: allergies, gender, age, AB0, Rh blood group

Introduction

Hyperactivity of the immune system due to the insertion of allergens into the living body has been known as an allergic reaction. Some substances, such as pollen grains, insects' venom, house dust mite, foods, and medicines, can induce allergic responses. Allergic diseases are a complex and diverse heterogenous group, relatively common in all parts of the world among all ethnic groups. Their expression depends on the interaction of the genotype with a variety of environmental factors [1]. Twin studies provide strong evidence for allergy heritability, such as 95% for asthma, 91% for allergic rhinitis and 84% for atopic dermatitis [2]. The present study aims to characterize the manifestation of allergies according to gender, age and blood group in a reproductive population from Southern Bulgaria.

Material and methods

Individuals, aged between 18 and 59 years are included in the study. They voluntarily have filled out a questionnaire, giving disease information (allergy) after being diagnosed by a physician. Blood type (AB0 and Rh) have been determined in laboratory conditions by a routine technique. A software package SPSS, version 22.0 has been used for the statistical analysis.

Results

Among the participants in the study 20.6% indicated suffering from some kind of allergy. The gender distribution is: 20.5% of the women and 20.8% of the men (p = 0.943) – Fig. 1. The age distribution is presented in Table 1. The occurrence of allergies in representatives of both sexes up to the age of 44 years is similar (p = 0.924). In the age category over 44, a certain difference is reported (p = 0.214). The results show that in 11.9% of women and in 17.6% of men, there is a manifestation of the disease. When comparing the blood group affiliation of the individuals in the context of the disease, the results show, that allergies are most common in individuals with blood group A - 24.0% and least common in those with blood group AB - 14.7% (p =0.394) – Table 2.

No differences were found in the frequency of allergies between people with Rh+ and Rh- blood groups (Fig.2).
Table 1. Age structure in dependence on both genders

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Gender</th>
<th>Number</th>
<th>Allergy</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Up to 44 years</td>
<td>Male</td>
<td>N 38</td>
<td>140</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>% 21.3</td>
<td>78.7</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>N 93</td>
<td>325</td>
<td>418</td>
</tr>
<tr>
<td></td>
<td>% 22.2</td>
<td>77.8</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>N 131</td>
<td>465</td>
<td>596</td>
</tr>
<tr>
<td></td>
<td>% 22.0</td>
<td>78.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Over 44 years</td>
<td>Male</td>
<td>N 6</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>% 17.6</td>
<td>82.4</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>N 10</td>
<td>74</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>% 11.9</td>
<td>88.1</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>N 16</td>
<td>102</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>% 13.6</td>
<td>86.4</td>
<td>100.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Distribution of allergies depending on AB0 blood type: (+) expression; (-) lack of expression; * percentage according to the blood group type; ** percentage according to the manifestation of allergy

<table>
<thead>
<tr>
<th>AB0 Blood group</th>
<th>Allergy</th>
<th>Total **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>0</td>
<td>N 36</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>% 18.4</td>
<td>81.6</td>
</tr>
<tr>
<td>A</td>
<td>N 58</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>% 24.0</td>
<td>76.0</td>
</tr>
<tr>
<td>B</td>
<td>N 22</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>% 18.2</td>
<td>81.8</td>
</tr>
<tr>
<td>AB</td>
<td>N 14</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>% 14.7</td>
<td>85.3</td>
</tr>
<tr>
<td>Total **</td>
<td>N 130</td>
<td>524</td>
</tr>
<tr>
<td></td>
<td>% 19.9</td>
<td>80.1</td>
</tr>
</tbody>
</table>
Figure 2. Distribution of allergies in both Rh blood types: (+) expression; (-) lack of expression

Discussion
Kurukulaaratchy et al. (2011) [3] and Nadhim (2022) [4] commented that boys and men were more prone to allergic diseases and reactions, which was not confirmed as a general trend in the present study, but only for the age group over 44 years. To date, only a few studies have reported a relationship between ABO blood groups and susceptibility to allergic diseases such as allergic rhinitis, atopic dermatitis, asthma and food allergies [5],[6],[7],[8]. Some authors reported possible relationship between the disease and the blood type 0 [9],[10],[11],[12]. In contrast, Moses et al. (2015) [13], Gangopadhyay et al. (2006) [6] and Abid (2015) [1] commented that people with blood types A and B are at a higher risk of developing allergic dermatitis. Our study confirms those results, regarding blood type A.

Conclusions
Based on the results, we can conclude that there are some predictors for manifestation of allergies: 1) the male sex in age over 44 years, 2) blood group A for both sexes. Further studies focusing on allergies would contribute clarifying the mechanisms of their manifestation and may help in prevention activities for the patients’ health improvement.

References:


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Examine of gait parameters in post-traumatic conditions (material and methods)

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Abstract
The examination and diagnosing the gait is decisive when analysing the pathological condition for making up a long-term complex rehabilitation program for patients who have suffered traumatic damage to the lower limbs with the presence of contractures. The purpose of this communication is to indicate the criteria for determining the contingent of observed patients by groups (age, sex and trauma) and the parameters for research (methods of registration) of the gait. The contingent to be included in the study are patients who sought physiotherapy and rehabilitation treatment at the "Dr. G. Stranski" UMBAL Pleven after a traumatic condition of the lower limbs, who use aids (two underarm crutches) when walking. Clinical methods consist in examining gait parameters, which are spatial (stride length; step length; step width; angle of lateral deviation of the foot) and temporal (stance time and swing time; cadence; stride time and step time; single support time and double support time; gait speed). Clinical, sociological and statistical methods are applied. Performing a comparative analysis of the obtained results, we will have grounds to determine the severity of lower limb injuries and their importance for restoring the patient's normal locomotion (without the use of aids).

Keywords: gait parameters, lower limb injuries, locomotion

Introduction
For humans, the main mode of locomotion in the environment is walking. It is a natural motor act and through it the human body movement is carried out in the environment, in which the whole motor apparatus participates [1]. It is carried out mainly by the supporting function of the lower limbs and the contraction of their muscles [2]. In the presence of damage affecting the lower limbs, changes in gait parameters occur, thereby the normal walking is disrupted [3]. The examination and diagnosis of the gait is decisive when analyzing the pathological condition, in order to make up a long-term complex rehabilitation program for patients who have suffered traumatic damage to the lower limbs with the presence of contractures.

The purpose of this communication is to indicate the criteria for determining the contingent of observed patients by groups (age, sex and trauma) and the parameters for research (methods of registration) of the gait.

Material
The object of the study are patients suffered trauma in the lower limbs, who are divided into groups depending on age („up to” and „past” 65 years), gender (gait differs in both genders) and localization of the trauma (in the area of: hip joint – fracture of pelvic bones, fractures of the femoral neck, trochanter and diaphysis; knee joint – ligamentous and meniscal damage; fractures of the patella, condyles, tibial plateau; ankle and foot complex – distortion and luxation; fractures of malleolus, tarsal bones and calcaneus).

The subject of the study are relationships, dependencies and factor interactions and comparisons between variables for recovery of gait in patients after trauma of the lower limbs, followed from the beginning of rehabilitation until their recovery.

Contingent for inclusion in the study are patients who sought physiotherapy and rehabilitation treatment after a lower limb injury requiring the use of two underarm crutches. To register the obtained results, an
individual file card has been developed, in which the data from the initial, ongoing and final examinations are entered.

In compliance with the requirements of Ordinance №14 of 27.09.2007 on the „Conditions and procedure for conducting therapeutic and non-therapeutic scientific research on human beings”, a clinical „Patient Card” has been prepared for informed consent, which he familiarizes himself with and signs.

Research methods

- Clinical research methods consist of:

1. Gait parameters: main characteristics for determining gait parameters are two main groups: spatial and temporal, which can be registered with appropriate apparatus research (Fig. 1) [4].

   ![Figure 1: Spatio-temporal characteristics of gait obtained by a specialized G-WALK apparatus](image1)

   - Spatial characteristics include: stride length; step length (1/2 step); stride width; the angle of lateral deviation of the foot (Fig. 2).

   ![Figure 2: Determining the length of step and stride](image2)

   - Temporal characteristics include: stance time and swing time; cadence (the frequency of steps); stride time and step time; single support time and double support time; gait speed [5]. Spatio-temporal indicators provide basic information about the quantitative characteristics of the gait (Fig. 3).
Figure. 3 Temporal characteristics of walking phases

1. **Goniometry:** The volume of movement in the injured joint is measured according to the classic SFRT method, and the results are reflected in the patient's individual file card at the beginning and the end of the rehabilitation process.

   - **Statistical methods**
     The survey data is going to be processed with statistical computer program SPSS and MS EXCEL. Parametric statistical tests are going to be applied to test hypotheses for normal distribution and non-parametric statistical tests for non-normal distribution. Significance of results for inferences and conclusions was determined at $p < 0.05$.

   - **Sociological methods:** an interview and documents research

**Conclusion**

At the end of the study, we expect to establish the time required to restore normal gait without the use of aids in the different groups of injuries of the lower limbs, as well as to track the recovery of the individual parameters of the gait by temporal and spatial characteristics. Performing a comparative analysis of the obtained results, we will have grounds to determine the severity of lower limb injuries and their importance for restoring the patient's normal locomotion (without the use of aids).

**References:**

Study of the ratio between alcohol content in whole blood and blood plasma for the needs of forensic toxicology

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Abstract
The purpose of the work is to investigate the correlation between the alcohol content (for both ethanol and methanol) in whole blood and blood plasma and to evaluate the margins for possible differences in concentration. The measured mean difference for ethanol is 9.90% (range: 3.30 - 14.4%); for methanol the corresponding figures are 8.20% (range: 2.70 - 11.2%). For practical needs (e.g. for recalculations in forensic toxicology) estimated alcohol content in whole blood is approximately 10% less than measured in blood plasma/serum. The difference may vary from sample to sample, however at confidence level 99.9% it is not expected to exceed 20% for both ethanol and methanol.

Key words: alcohols, ethanol, methanol, whole blood, blood plasma

Introduction
Determination of the blood alcohol content is of a great practical importance – both for clinical work (diagnostic and treatment of acute intoxications) and forensic investigations (prosecution in civil and criminal trials) [1-3]. Reference levels for alcohol concentration in blood matrices are routinely defined for whole blood only. However, there are numerous cases when samples of whole blood are not presented, yet blood plasma (or serum) samples are available. That may happen by many reasons: mistakes in blood sampling/shipping; using inappropriate blood vials for collection/transportation; delay or mismatch of analysis type orders; inhomogeneity of the samples; or by staff incompetence/negligence. In such cases the alcohol content is measured in available blood plasma/serum sample and the obtained result is used for toxicological conclusions [4, 5]. It is known yet, that alcohol content in whole blood and blood plasma/serum are not numerically identical [6], therefore interchanging their values is unacceptable, as it leads to inaccuracy of some extent, usually close to 10% [5-8]. Whereas the error of such magnitude is not dramatically important in clinical diagnostics, it may be of a major significance in medico-legal investigations (e.g. knowing the exact value is crucial for judging the type and weight of the penalty for drunken drivers, Tabl. 1). Therefore it appears vital for the forensic toxicologist to be able to produce reasonable estimations for whole blood alcohol content using data from blood plasma/serum analysis as well as to know the limiting margins of possible deviation. As serum and plasma ethanol levels have been reported to be merely identical [9], the study is carried concerning blood plasma matrices only (most often presented ones).

Table 1. Types of penalty for driving under the influence of alcohol in Bulgaria.

<table>
<thead>
<tr>
<th>Whole Blood Ethanol, ‰</th>
<th>Driver penalty type/weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0,50</td>
<td>N/A</td>
</tr>
<tr>
<td>0,50 – 0,80</td>
<td>Administrative offence (light fine)</td>
</tr>
<tr>
<td>0,80 – 1,20</td>
<td>Administrative offence (heavy fine)</td>
</tr>
<tr>
<td>&gt; 1,20</td>
<td>Criminal offence (imprisonment + fine)</td>
</tr>
</tbody>
</table>

Materials and methods
Blood samples are taken from the patients of the Clinic for Intensive Treatment of Acute Intoxications and Toxicoallergies, Naval Hospital – Varna, Military Medical Academy, within the course of their due treatment. The collection of samples was made using a 4 mL vacuum tubes containing 6.0 mg NaF and 12.0 mg Na₂EDTA (BD Vacutainer®, grey caps). Determination of alcohol content in biological matrices was done by means of gas-chromatography, using Agilent 7890B GC System, coupled with Flame ionization detector and Agilent 7697B Headspace sampler. For each measurement a 100 μL blood matrix sample was used. 1-Propanol (Merck, extra pure) was used as internal standard. Calibration of the analytical method was done using Medidrug® analytical standard solutions of ethanol, provided by MEDICHEM Diagnostica GmbH & Co. KG, Germany, as well as progressive aqueous dilutions of methanol (Merck, extra pure), made in-house. Deionized water (0.067-0.100 μS cm⁻¹, TKA™ Pacific water purification system) was used. Agilent OpenLAB (ChemStation edition, rev. C.01.05) software was used for chromatographic data acquisition and processing. The method validation parameters for both methanol and ethanol are: quantitation limit LOQ = 0.02‰ (g/L), linear range 0.02-6.00‰ (g/L), and linearity r² = 0.9998.

54 Random whole blood samples taken from patients with acute ethanol intoxication were analyzed for determination of ethanol content. After that samples were centrifuged @4000 rpm for 10 minutes and separated blood plasma was analyzed again. For each of the pairs, the difference in ethanol concentration was calculated. 20 More alcohol-free samples were spiked beforehand with known quantity of methanol and consequently analyzed for methanol content analogically.

Results and discussion

Ethanol concentration range was found to be 0.25-4.83‰ (g/L) for the original whole blood and 0.29-5.10‰ (g/L) for the corresponding blood plasma matrices. The Shapiro-Wilk statistical test performed showed a normal distribution of alcohol content, relative difference with mean value 9.90% (min 3.33%, max 14.4%) and standard deviation of 2.89.

Methanol concentration range was 0.21-4.23‰ (g/L) for the original whole blood and 0.23-4.62‰ (g/L) for the corresponding blood plasma matrices. The Kolmogorov-Smirnov statistical test performed showed a normal distribution of alcohol content, relative difference with mean value 8.20% (min 2.68%, max 11.2%) and standard deviation of 2.18.

It could be easily seen that for every single whole blood – blood plasma sample pair, the alcohol concentration in blood plasma is greater than in whole blood; there is no exceptions observed. Such phenomenon is expected and its explanation is simple: alcohols are water-soluble substances, therefore their phase distribution favors the water-rich matrix. Blood plasma contains more water than whole blood [5, 10]; hence the alcohol content ratio blood plasma to whole blood is greater than 1. The measured relative concentration difference is close to 10% both for methanol (mean value 8.20%) and ethanol (mean value 9.90%).

The determined concentration differences could be used in alcohol content recalculations, when the only sample available is blood plasma/serum, yet the whole blood alcohol content is needed (C represents the alcohol concentration given in ‰ (g/L):

For ethanol: \[ C_{\text{Whole blood}} = 0.901 \times C_{\text{Blood plasma/serum}} \]

For methanol: \[ C_{\text{Whole blood}} = 0.918 \times C_{\text{Blood plasma/serum}} \]

It should be always kept in mind, that the conversion quotients in the expressions above are not exact values, as they possess a statistical origin; that is, they were determined by averaging an array of experimental values, and can somewhat vary from sample to sample with no direct method available for their precise
prediction [5]. As in real samples the exact values may remain unknown, the proposed recalculation
gives an estimated alcohol content. However, the probable deviations are expected to fall within particular
margins that can be evaluated a priori regarding the confidence level required, as it is shown in Tabl. 2.

**Table 2.** Prediction intervals for relative alcohol concentration difference.

<table>
<thead>
<tr>
<th>ETHANOL</th>
<th>METHANOL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confidence level (α)</td>
<td>Relative concentration difference</td>
</tr>
<tr>
<td>95.0 % (± 2 SD)</td>
<td>4.12 – 15.7%</td>
</tr>
<tr>
<td>99.7 % (± 3 SD)</td>
<td>1.23 – 18.6%</td>
</tr>
<tr>
<td>99.9 % (± 3.3 SD)</td>
<td>0.36 – 19.4%</td>
</tr>
</tbody>
</table>

The upper margins (the bigger numbers in the Tabl. 2) are of a great importance to medico-legal
investigations, as they represent the most possible values (corresponding to largest expected error
in estimation of whole blood alcohol content, done by recalculation). It could be summarized as: "Alcohol
content in a whole blood sample cannot be less than 20% of alcohol content in corresponding blood plasma
sample, with a chance better than 1000:1".

**Conclusion**

The correlation between alcohol content in whole blood and blood plasma has been investigated. It has been
shown that even in cases when whole blood sample is not available but blood plasma/serum is presented
instead, analysis of alcohol content still can be performed and the obtained results used for making valuable
medico-legal conclusions. It has been demonstrated that alcohol concentration in blood plasma samples is
always greater compared to the correspondent whole blood matrix (valid both for ethanol and methanol).
The difference is quantitatively described along with the estimation of the deviation margins. Observed
values are in very good agreement with published data.

**References:**

5. Jones AW, Tilson C. Distribution ratios of ethanol and water between whole blood, plasma, serum, and erythrocytes: Recommendations for interpreting


Method of filling existing defect of the tibial bone during total knee arthroplasty

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2. Department of Orthopaedics and Traumatology, Faculty of Medicine, Medical University Varna

Abstract
Purpose: The aim of the present study is to share our experience and midterm results of applying bone cement with screw augmentation as a method for treatment of tibial bone defects in total knee arthroplasty.

Material and methods: The present study include 12 patients undergoing primary total knee replacement for arthritis of the knee joint with bone deficiency in the proximal tibia. The technique of augmentation with bone cement and screws support was used. Eleven patients had an intermediate bone defect in proximal tibia (5–10 mm) and one was with large bone defect more than >20 mm.

Results: Clinical and functional follow-up of the patients was carried out for a period of two years. The American Knee Society (AKS) Score was used to assess functional outcomes. Results were reported as excellent in six of the patients, good in four and satisfactory in two patients.

Conclusion: This technique is a valuable alternative to other known methods, especially when the surgeon has limited access to them due to logistics or financial issues.

Key words: proximal tibial defect, cement-screw augmentation, total knee arthroplasty

Introduction
Total knee arthroplasty (TKA) is an effective surgical procedure in patients with advanced knee osteoarthritis. Proximal tibial defects can be considered as one of the most challenging complications encountered during primary or revision total knee replacement. Bone defects hinder the correct orientation of the implants and eventually lead to malalignment of the components and disturbance of the axis of the lower limb which determines the survivorship rate of the implants [1,2,3]. Bone deficiency in proximal tibia may be manage through cement augmentation alone or with screw reinforcement, metal augments, tantalum, allografts or autografts. Lateralization of the tibial component is also option in severe varus deformities [4,5,6].

Purpose
The aim of the present study is to share our experience and midterm results of applying bone cement with screw augmentation as a method for treatment of tibial bone defects in total knee arthroplasty.

Material and Methods
The present study is retrospective (2019-2022) and include 12 patients undergoing primary total knee replacement for arthritis of the knee joint with deficiency in the proximal medial tibia. The technique of augmentation with bone cement and screws support was applied. Average age was 65 years (57-78). Gender distribution: seven women and five men, average body mass index 34. Eleven patients had an intermediate bone defect in proximal tibia (5–10 mm) and one was with large bone defect more than >20 mm (fig.1).

Fig. 1 Severe osteoarthritis of the knee joint with a massive bone defect in proximal tibia
In nine patients, the defect was a result of advanced osteoarthritis of the knee joint with varus deformity. The other three patients were operated after aseptic loosening of unicodylar medial arthroplasty. After joint arthrotyomy, the tibial plateau was sufficiently visualized and the size of the existing tibial defect (depth and width) were determined. These defects were classified as central or peripheral. In a varus knee deformation usually the periphery (postero-medially) was involved (fig. 2).

First step was to clean vigorously the granulation tissue in the area of the available medial tibial defect until cancellous bone is reached. Then a minimal tibial bone resection was performed. Holes were drilled in the defect area with a 2.7 mm drill and 3.5 mm screws (length 30-35 mm) were placed in the cancellous and cortical areas of the defect. The screws should be perpendicular to the defect so that they do not interfere with the wedge and pegs of the tibial component when it is positioned on the resected bone surface. The bone cement that is used for fixation of the tibial component filled the existing bone defect that was beforehand augmented with screws (fig. 3).

**Fig. 2** Postero-medial defect in proximal tibia

**Fig. 3** Augmentation with bone cement-screw reinforcement (a-p and lateral view)

**Results**

Clinical and functional follow-up of the patients was carried out for a period of two years. The American Knee Society (AKS) Score was used to assess functional outcomes. Results were reported as excellent in six of the patients, good in four and satisfactory in two patients. Average functional score improved significantly from 46 to 90 (p<0.005). During the follow-up period there were no secondary procedures, no subsidence or wear of the implanted tibial component was observed. Control x-ray images showed radiolucent lines in four patients without signs of progression or osteolysis for the follow up period (fig. 4).
Discussion
Severe arthritis of the knee joint is often associated with proximal tibial bone loss. The location of the defect is usually posteromedial especially in varus osteoarthritis with chronic anterior cruciate deficiency [7]. Originally, Freeman et al. reported the technique of bone cement with screw augmentation for the treatment of tibial bone defects in primary total knee arthroplasty [8]. There are a number of publications regarding the application of this technique for small defects of no more than 5 mm in depth. However, more and more studies focus on successful early and mid-term follow-up results after usage of this technique in patients with relatively larger defects (up to 20 mm in depth) of the tibial plateau [9,10]. In 1986, Ritter et al. popularized this technique and later reported satisfactory results in treatment of relatively larger tibial plateau defects (from 5 to 20 mm deep) [9]. Lotke et al. reported two failed cases of 59 total knee arthroplasties where bone cement with screw augmentation were used for management of proximal tibial defects. Furthermore, they report that this technique can be applied to tibial defects ranging from 10 to 20 mm in depth with a satisfactory end result [11]. Zhao et al. investigated the optimal angle of screw insertion in cement-screw technique to repair tibial defect in total knee arthroplasty. They found that screws perpendicular to the upper surface achieve better stability than those parallel to the proximal cortical bone of the tibia. If two vertical screws cannot be performed, one vertical and one oblique is also acceptable [12]. Strong limitations of the present study are the relatively small group of patients, the short follow up period and the lack of a control group.

Conclusion
The technique of bone cement with screw augmentation is reliable option for management of moderate defects in proximal tibia in patient undergoing total knee replacement. It is safe, relatively easy to perform and affordable. Our results correlate with those of other authors. This technique is a valuable alternative to other known methods, especially when the surgeon has limited access to them due to logistics or financial problems.

References:


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Asymmetry in nociceptive responses to cannabinoid receptor ligands microinjected into hippocampal CA1 area of bulbectomized rats

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The endocannabinoid system is involved in neuropsychiatric disorders such as depression. Olfactory bulbectomy (OBX) in rodents is a widely used model of depression. **Aim:** To study the effects of cannabinoid (CB) receptor agonist HU 210 and CB1 receptor antagonist SR 141716A microinjected unilaterally into the CA1 hippocampal area on nociception of rats with model of depression. Olfactory bulbectomized (OBX) rats were used as an experimental model of depression. Cannulas were implanted bilaterally, into the right (R) and left (L) CA1 area. HU 210 (5 µg) or SR 141716A (3 µg) were injected into the R or L CA1 area on the 15th day after OBX. The nociceptive withdrawal threshold was evaluated by using Randall–Selitto paw pressure test. **Results:** Microinjection of HU 210 into the R-CA1 significantly increased the pain threshold (antinociceptive effect) in both OBX- and sham-operated rats, while in the R-CA1 SR 141716A exerted a hyperalgesic effect, compared to the respective controls. The CB ligands showed similar effects in the L-CA1 of the OBX rats, and the nociceptive responses were more pronounced in the R-CA1. Our results point to an involvement of CB1 receptors in the mechanical nociception of bulbectomized rats. The right CA1 area is predominantly implicated in the nociceptive responses to the CB ligands in both sham-operated and OBX rats, suggesting an asymmetry in the distribution of CB1 receptors in the left and right CA1 hippocampal area.

**Introduction**

The hippocampus is a structure of the limbic system, with a key role in learning, memory and spatial navigation. Studies reveal that it is also involved in modulation of pain processing, although not much is known about the exact mechanisms. The hippocampus shows structural and functional abnormalities in depression, while pain and depression are often comorbid states. The endocannabinoid system (ECS) consists of endocannabinoids, cannabinoid receptors and the enzymes for synthesis and degradation. Two types of cannabinoid (CB) receptors have been identified - CB1 and CB2 [1]. CB1 receptor is expressed predominantly in the brain, while CB2 is found in the peripheral tissues. Numerous reports demonstrate the involvement of the brain CB1 receptors in the regulation of nociception [2]. CB1 receptors are expressed in the major components of the brain analgesic system (PAG and RVLM), but also in the hippocampus, which has been suggested to play role in both chronic pain and depression [3]. Research on the central pain-modulatory effects of the cannabinoids has focused mainly on brain stem, while no data are present about the effects of CB ligands administered into the hippocampus. The aim of our study was to examine the effects cannabinoid receptor ligands (CB agonist HU 210 and selective CB1 antagonist SR141716A) microinjected unilaterally, in the left or right CA1 hippocampal area on the nociceptive responses in a rat model of depression - olfactory bulbectomy.

**Materials and methods**

**Surgical procedures.** Bilateral olfactory bulbectomy (OBX) and bilateral implantation of guide cannulas were performed as described previously [4]. HU 210 (5 µg), SR141716A (3 µg) or saline were microinjected unilaterally, into the right (R) or left (L) CA1 hippocampal area. The experimental animals (male Wistar
17  

rats), were divided into 6 groups: OBX rats, injected into the R- or L-CA1 with HU 210 or SR 141716A or saline and sham-operated (SO) controls, injected into the R- or L-CA1 with drugs or saline.

**Assessment of mechanical nociception.** The changes in the nociceptive response were determined by the Randall-Selitto paw pressure test. A gradually increasing pressure was applied to the hind paw. When the animal displayed pain by withdrawing the paw or vocalization, the load applied was determined in arbitrary units (AU) as a pain threshold.

**Statistical analysis.** Two-way ANOVA between subject factors: drug (saline, HU-210 or SR141716A) and side of injection (left and right) was used to analyze the data obtained after unilateral microinjections. Data were further analyzed by post hoc Student-Newman-Keuls test.

**Results and discussion**

ANOVA for the effects in SO rats revealed significant effects for “drug” (F_{2,35} = 58.796, P <0.0001), “side” (F_{1,35} = 9.2035, P<0.005) and “drug” x “side” interactions (F_{2,35} = 18.820, P < 0.0001). Microinjection of HU-210 into the right CA1 of SO rats increased pain threshold (P<0.001), while SR141716A decreased it (P < 0.03), as compared to the SO-saline controls (Fig 1A). HU-210 applied in the R-CA1 increased the pain threshold as compared to the left side (P <0.001). There were no significant effects of the drugs in the L-CA1 (P-NS) as compared to the SO-saline controls (Fig 1A). ANOVA for the effects in OBX rats showed significance for “drug” (F_{1,35} =128.3882, P<0.001), “side” (F_{1,35} = 7.599, P <0.009) and “drug” X “side” interactions (F_{2,35} = 26.331, P < 0.001). OBX increased of the pain threshold as compared to the SO controls (Fig 1 A, B). Both right side (P < 0.001) and left side (P <0.03) injections of HU-210 increased pain threshold, while SR141716A decreased it in both R-CA1 (P < 0.001) and L-CA1 (P <0.001), as compared to the respective OBX-saline controls (Fig 1B). The antinociceptive effect of HU-210 was more pronounced in the R-CA1 as compared to the L-CA1 (P<0.001) (Fig 1B), same for the pronociceptive effect of SR141716A (P<0.01) (Fig 1B).

**Fig 1 A**

![Analgesymeter](image)

**Fig 1 B**

![Analgesymeter](image)

**Fig. 1 A, B.** Effects of CB ligands HU 210 (5 µg) and SR141716A (3 µg), microinjected unilaterally, into the left or right CA1 hippocampal area on nociception in sham-operated, SO (A) and OBX (B) rats. n = 6, "P ≤ 0.05, ""P ≤ 0.01, *** ≤0.001; oooP ≤0.01, oooP ≤0.001. Asterisks depict comparisons of pain threshold (AU) in drug-treated OBX/SO rats vs. the respective OBX/SO saline-treated controls. Circles depict comparisons of R- and L-side effects.

The microinjection of the CB ligands in R-CA1 showed opposite effects in both OBX- and SO- rats: HU 210 increased the mechanical pain threshold (antinociceptive effect), while SR 141716A exerted a
hyperalgesic effect, compared to the controls. Our results support the data concerning the involvement of hippocampus in the modulation of acute pain signaling [5]. We demonstrated lateralization in the nociceptive responses in both SO and OBX rats: the responses in R-CA1 of OBX rats were more pronounced as compared to the left side, while the drugs significantly affected the pain threshold only upon right-side administration in the SO group. The disturbances of the ECS functioning, following the bulbectomy [6] might contribute to the different side-dependent effects in SO and OBX rats. The observed right-side asymmetry in the effects of the CB ligands is in accord with the reports about pain lateralization in different brain structures [7].

**Conclusion**

The right CA1 area is predominantly implicated in the nociceptive responses to the CB ligands in both OBX- and sham-operated rats, suggesting an asymmetry in the distribution of hippocampal CB1 receptors. Our observations might contribute to the understanding of the left–right lateralization during pain processing in the CNS.

**References:**

Diagnosis and treatment of chronic viral hepatitis C in risk groups

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Abstract
Background: Chronic Hepatitis C (HCV) has a 170 million worldwide burden and is the second leading cause of liver cirrhosis and hepatocellular carcinoma. A challenge for hepatologists is diagnosis and treatment of risk groups. The discovery of direct acting agents, an undisputed breakthrough of modern medicine, has changed the course of disease. The aim of the study is to evaluate and compare the frequency, clinical course, therapeutic response of patients with chronic viral hepatitis C in high-risk groups. Results: Treatment-ready 13 incarcerated individuals - non-responders to interferon therapy and with two positive results for Anti HCV antibodies in the last 6 months, all males aged 24-49 years, mean 31.15. 7 patients completed therapy with a sustained virological response. Conclusion: The high efficiency of modern antiviral therapy reduces the progression of liver disease and mortality, as well as transmission of HCV infection in patients in risk groups.

Introduction
Transmission of hepatitis C is through blood and sex, as well as vertical transmission - from the mother to the fetus. Drug addicts, injecting drug users and men who have sex with men are particularly at risk [1, 5]. Extremely important for the diagnosis of hepatitis C is the screening test of liver transaminases - AST, ALT, as well as Anti HCV antibodies [2, 4]. Hepatitis C (HCV) is an enveloped, single stranded RNA virus capable of causing acute and subsequent chronic infection [1] affecting mainly but not only the liver [3]. Chronic HCV has a 170 million worldwide burden and is the second leading cause of liver cirrhosis and hepatocellular carcinoma [6, 7, 9]. The discovery of direct acting agents, an undisputed breakthrough of modern medicine, has changed the course of disease. Natural progression has given way to treatment with a near-to-100% success and a WHO Program for worldwide elimination of HCV as a public health threat by 2030 [5, 8]. Screening of high-risk groups is very important to achieve HCV elimination and prevent transmission.

Risk patients who most often do not reach treatment:
• Patients with a low level of education
• Drug addicts
• Patients with mental illness
• Immigrants
• Uninsured patients
• Prisoners
• Patients with alcohol dependence

Results
Treatment-ready 13 incarcerated individuals - non-responders to interferon therapy and with two positive results for Anti HCV antibodies in the last 6 months, all males aged 24-49 years, mean 31.15. The average value of the viral load in the studied patients was 645728 IU/ml, predominant genotype 1. Staging of the patients showed a predominant number with fibrosis F1 – 6; fibrosis F2 – 4; fibrosis F3 – 2 and one patient refused biopsy – with unknown fibrosis (Fig.1). Adhering to the requirements of the Consensus for the treatment of chronic viral hepatitis C, documents have been prepared for antiviral therapy with direct-acting...
agents for all patients. Successfully completed the treatment with a lasting virological response - 7 patients; three patients are awaiting approval of therapy and two have an unknown outcome of therapy due to missing follow-up examinations.

**Fig 1.** Staging of the patients

**Discussion**

Screening of high-risk groups is very important to achieve HCV elimination and prevent transmission. High-risk groups for testing are: drug addicts, people with HIV, children born to mothers with HCV, people with sexual partners with HCV, people with piercings and tattoos, people who take drugs through the nose, recipients of contaminated blood products or invasive procedures in healthcare facilities with inadequate infection control, prisoners or ex-prisoners [1].

There are barriers to HCV treatment in the prison system: insufficient medical staff on prison grounds, Additional administrative resources are needed to accompany prisoners to hospital, short sentence lengths and lack of continuity for patient follow-up, Transfers within prisons and other time-consuming activities (e.g. court appearances) leading to compromised follow-up [3, 5, 6].

The most suitable regimen for HCV treatment in prison should meet several conditions: high efficacy (>95%), short duration, simple to administer regimen e.g. once daily by mouth, well tolerated e.g. minimal DDIs [1].

The benefits for prisoners of timely diagnosis and adequate antiviral treatment are numerous: It reduces the progression of liver disease and mortality, as well as the transmission of HCV infection, facilitates treatment in prisons for short sentences, facilitates the expansion of physician care for HCV, access to treatment, e.g. through health centers, addiction clinics, prisons, a clean needle exchange program and primary care.

**Conclusion**

Screening of high-risk groups is very important to achieve HCV elimination and prevent transmission.

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Malaria prevention in Varna district

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Abstract

Malaria is a protozoan infection caused by parasites (plasmodium malariae) and spread through biting the human blood. Most common are the genus Anopheles mosquitoes. In different parts of the globe, the circulation of microorganisms, vectors, infected humans and animals represents a global threat to public health and requires anti-epidemic measures at national and international level.

The aim is to study imported malaria in Varna region and Border Health Control at Port of Varna for 2017-2020, using RHI Varna’s reports and analyzes of registered acute infectious morbidity; programs and regulatory documents. Epidemiological analysis, documentary, and graphic methods are used.

Varna region is among the high-risk regions of Bulgaria. Border Health Control is provided at the port, due to risk of infection transmission. Such type of control on the Bulgarian Black Sea coast is done at permanent Border Checkpoints located at the Port of Varna and the Port of Bourgas. Health inspectors from the Regional Health Inspection are 24/7 on duty.

The Border Health Control’s features at the Port of Varna for protection against imported malaria by Bulgarian and foreign citizens returning or arriving from endemic countries have been studied. There is a potential danger of malaria being imported into the Varna region, so it is necessary the laboratories in the city to continue the research. The relative share of Bulgarian citizens for the studied period is nearly 30 times smaller than that of foreign citizens. Varna passengers are only 7-8% of Bulgarian citizens and a decreasing trend is observed.

Key words: Parasitosis, borderline health control, malaria, mosquito

Introduction

Malaria is a protozoan infection caused by parasites (plasmodium malariae) and spread through biting (entering the human blood) by mosquitoes of the genus Anopheles. In different parts of the globe, the circulation of microorganisms, vectors, infected humans and animals represents a global threat to public health and requires anti-epidemic measures at the national and international level [1]. The disease has a characteristic natural endemicity in areas with certain climatic and geographical features that favor its rapid spread (suitable biotopes, turnover of plasmodia and anopheles, etc.) About 40 percent of the Earth's population lives in such areas. Malaria continues to be a major health problem for many of the countries in Africa, Asia, and Central and South America. [1] Globally, there were nearly 229 million cases of malaria in 2019 in 87 malaria-endemic countries, which is a slight reduction from 238 million in 2000. 95% of all malaria cases worldwide are concentrated in 29 countries. Nigeria (27%), Democratic Republic of Congo (12%), Uganda (5%), Mozambique (4%) and Niger (3%) account for about 51% of all cases worldwide. Until the 70s of the 20th century, malaria was endemic in our country, mainly along the Black Sea and riverside regions. As a result of significant hydro-ameliorative measures and widespread disinsection measures, the eradication of the disease was achieved. Today it is found only imported and is subject to epidemic surveillance aimed at preparing for anti-epidemic protection of the population.

Today, 55 years after the certification of Bulgaria by the WHO as free from malaria, the existing regulatory framework [2] helps to maintain the elimination, despite the international conditions for its spread and the available climate-geographical and social prerequisites. [3,4,5]. Increased migration flows to Europe in the
recent years have raised concerns about the effectiveness of surveillance and control of imported pathology and the potential risk to the health of the local population, which is a priority for ECDC [6,7]. According to data from the National Center of Infectious and Parasitic Diseases, a total of 22,668 Bulgarians and foreigners were examined for imported parasitic diseases during the period 2014-2020. A total of 87 cases of imported malaria were registered in the country for the period 2014-2020, 47% of which are Bulgarian citizens and 53% are foreign citizens. According to the type of causative agent, the highest relative shares among them are tropical malaria (59%) and malaria vivax (37%). Cases of imported malaria caused by other representatives of Plasmodium species are registered relatively rarely (1%-3%). [8]

**Aim**
To study the state health control carried out by inspectors of the RHI at the port of Varna for travelers arriving or returning from malaria endemic countries for 2017-2020. To study the general practitioners’ work to eradicate malaria in the Varna region.

**Materials:**
Data from registration of acute infectious morbidity, reports and analyses, studies of RHI-Varna and Bulgaria, programs, regulatory documents were collected, summarized and analyzed.

**Methods:**
The study used data summary, epidemiological analysis, documentary, statistical and graphical methods. The study was retrospective in nature from official medical records.

**Results**
Varna region is the 3rd in Bulgaria in terms of population (474,334 people) or 6.5% of the country's population, and the 12th in terms of area. It occupies a territory of 3819.5 square km, which represents 3.44% of the country's territory. There are 159 settlements - 11 cities and 148 villages, distributed in twelve municipalities. The city of Varna with its port is the main gateway to the country by sea, with a very wide range of international connections. The border health control on the Black Sea coast in Bulgaria is carried out by permanent border checkpoints at the port of Varna with nine terminals and Burgas with seven terminals. Health inspectors from RHI are on duty 24 hours a day. During the ships’ inspections, data is taken on preventive measures - carried out disinsection, chemical prophylaxis, medical ship logs are checked for registered cases with symptoms suspicious of malaria, instructions are given to prevent the disease when staying in risky countries. The detention or suspension of ships, fines and gaps in the border regime are monitored. [9,10,11]. We present the moored ships from countries with malaria in Varna for 2017-2020.

![Number of ships and passengers - in total and from restricted areas, with health control carried out at the port of Varna for 2017-2020.](image)

A slight tendency of decrease in the number of ships from 1,822 in 2017 to 1,740 in 2020 was found. The number of passengers are also decreasing from 28,536 people in 2017 to 25,456 people in 2020 (Fig.2) Passengers coming from malaria areas are with a relative share of 41.26% for 2017, 32.56% for 2018, 30.72% for 2019 and 29.3% for 2020. Of the passengers who have crossed the boundary in 4 years (107,840...
people), 33.59% are from malaria areas. The passengers coming from malarious countries - both Bulgarians and foreigners who passed health control at the port of Varna for 2017-2018, are mainly foreigners. 

The relative share of Bulgarian citizens was 3.82% in 2017, 3.24% in 2018, 1.6% in 2019 and 2.34% in 2020. The relative share of foreign citizens is high - over 96.8% for 2017, 96.78 for 2018, to 98.39% for 2019 and 97.65 for 2020. The total number of people who crossed the boundary was examined, as well as how many are Bulgarians and how many of them are from Varna. 

Among all Bulgarians in the four years (2017 - 2020), the passengers from Varna have a relative share of 8.88% (2019), 7.09% (2018) to 2.35% (2017). In 2020, it is 0%, as due to the COVID-19 pandemic, passenger traffic is sharply reduced. (Fig. 3) There are seven medical-diagnostic laboratories for medical parasitology in the city of Varna. The number of Bulgarians and foreigners examined for malaria in the parasitological laboratory of the RHI Varna for 2017-2020 is relatively small.

In the conditions of a globally connected world, the surveillance of imported malaria and the prevention of its local spread, the active activity of control bodies from the RHI is leading. Specialist parasitologists in the medical-diagnostic laboratories, the municipalities and the treatment network, the coastal and naval services are involved in the ever-changing aspects of the disease and effective collaboration between them. The study found that there is control - there is also a registration of the tested persons who are negative. There is one positive, diagnosed by a clinical case in the laboratory "St. Marina" in 2017, returned from Cameroon. [5]

Although not everyone is examined in the RHI-Varna (there are 6 more specialized diagnostic parasitological laboratories), in reality the system works because for these 4 years there is no delay in the registration of imported cases with malaria and there are no registered local cases due to imported ones, as there are in some other European countries. The GPs’ activities, who are engaged in continuous training and specific projects, were also studied, such as:

◊ To recommend prophylaxis before, during the stay and after returning to Bulgaria to patients going to malarious countries, according to WHO recommendations;

◊ To record the year of return of patients arriving from countries with endemic malaria;

◊ To carry out medical surveillance for a period of three years from the last stay in a country with endemic malaria;
◊ Refer for examination in a specialized parasitological laboratory the persons with unclear temperature status and/or evidence of anemia and hepatosplenomegaly, who have visited countries with widespread malaria,
◊ To consult the patient with a specialist parasitologist;
◊ Do not prescribe "antimalarials" before taking the blood samples

Taking the preparations, confirming the cases at the National Center of Infectious and Parasitic Diseases, registering the sick and referring them for hospitalization and treatment are the duties of the medical parasitologists in the specialized medical-diagnostic laboratories (according to Ordinance 17).

**Conclusions**

1. The border health control carried out at the port of Varna to protect our and foreign citizens arriving or returning from endemic countries from the importation of malaria was studied. A slight downward trend was established in the number of ships and passengers arriving.
2. The relative share of Bulgarian citizens for the studied period is nearly 30 times smaller than foreign citizens - from 0.14% (2019), 0.27% (2018), 1.20% (2017) to 2.34% (2020).
3. Varna passengers are only 7-8% of all Bulgarian citizens and there is a decreasing tendency - from 2.35% (2017), 7.09% (2018) to 8.88% (2019). No Varna passengers are registered in 2020 due to a sharp decrease of the passenger flow during the COVID-19 pandemic.
4. The modern fight against malaria, also based on border health control, results in the eradication of malaria in the Varna region.

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The place and the role of psychiatrist during Covid pandemic

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Summary: The corona virus pandemic seriously affected mental health, resulting in increasing prevalence of psychiatry disorders. The results of a lot of international studies and also a few study results in Serbia support this data. Mental health consequences have continued after the recovery, leading to persistent depression, anxiety, neurocognitive impairment etc. Future studies are important for controlling psychiatry manifestations and suggesting strategies to prevent them as well as improving quality of life in general population due to global pandemic impact.

Key words: covid, psychiatry, mental health

The coronavirus (COVID-19) pandemic has serious mental consequences, leading to psychiatric morbidity in patients, health care workers and in the general population. (1) The data from the recent studies suggest that there has been an increased prevalence of anxiety, stress, poor sleep quality, obsessive-compulsive symptoms, and depression among the general population during the pandemic. COVID-19 can also impact the CNS directly and result in delirium, cerebrovascular events, encephalitis, unspecified encephalopathy, altered mental status, or peripheral neurologic disorders. (2) The systematic review conducted by Preti at all reported severe anxiety symptoms and insomnia in health care workers. Especially female gender, being a nurse and working in intensive care unit were risk factors for worsening psychological outcome. (3)

The SARS-COV-2 pandemic – link of systemic infection to neuropsychiatric diseases

The SARS-COV-2 enters the body through various routes and causes systemic and tissue inflammation. Systemic inflammation compromises the blood-brain barrier (BBB) and floods the brain with pro-inflammatory factors and cytokines. The combination of systemic inflammation, hypoxia resulting from respiratory failure and neuroinflammation may trigger or exacerbate psychiatric diseases. (4) It is possible to assume an increased incidence of mental pathologies as an unwanted sequelae. Patients with COVID-19 could present with a wide range of neuropsychiatric symptoms, which result from systemic inflammation, CNS effects of cytokines, infection of neural cells by SARS-COV-2, neuroinflammation, glial dysfunction or aberrant epigenetic modifications of stress-related genes. Minimizing the relevance of psychiatric symptoms could be a potential danger by assuming that sometimes “an abnormal reaction to an abnormal situation is a normal behavior”. (5-7)

COVID outbreak and challenges for mental health systems

During COVID 19 outbreak mental health institutions faced several problems specific for them. Firstly, psychiatry in- patients had been more vulnerable to COVID infection due to crowded living conditions in hospitals, taking part in group activities and sharing dining and bathroom species. Their disordered mental state, lack of insight, being unable for self-care diminishes the efforts in conducting protective measures. (8) On the other side, the psychiatrist presented with limited clinical competence in infectious diseases, but in the lack of health workers, in Serbia, they were mobilized to treat COVID patients as well as to supervise psychiatry patients in COVID hospitals and departments.

The psychiatry disorders and COVID pandemic – Serbian results

The Serbia also recognize the problem and several studies had been conducted. The cross sectional survey had been conducted where 20% of the subjects reported at the time of survey that they had been infected
with the SARS CoV-2 virus. A total of 3.2% of subjects reported that they had been hospitalized because of it (fig 1.) The most common stressors were related to household infection and lack of personal protective equipment at the workplace. (9) In the same study, the distress during the pandemic was expressed by the severity of depressive and anxiety symptoms. Depressive symptoms were assessed by PHQ-9 and showed that mild depression is reported by one fifth of those surveyed. Moderate is reported by 3.9%, moderate to severe by 1.2%, and severe by only 0.7%. A total of 74.0% of the respondents do not report any symptoms of depression. Anxiety were assessed with GAD-7 questionaries’ and the results show that mild intensity of anxiety symptoms is reported by 13.2%, moderate is reported by 1.8%, and strong by 1.2% of those surveyed. The majority of participants do not report symptoms of anxiety (83.8%).

The second study assessed epidemic and mental health status in general adult population in Serbia. The results are shown on Figure 2. and suggests that stress, anxiety, and depression were prevalent in the Serbian population during COVID 19 and the quarantine measures and lockdown. Uneasiness to COVID-19 news, the feeling of helplessness, likeliness of impending death, and presence of COVID-19 symptoms were significantly associated with depression, anxiety, and stress levels. Current smoking status was associated with a higher risk of depression and stress. Fear of disease and religious beliefs were associated with higher anxiety and stress scores. Students had a higher level of depression and stress, while older age was protective against anxiety and stress. The presence of chronic disease was associated with higher stress levels, while good health was associated with lower levels of depression. Higher socioeconomic status was significantly associated with lower levels of depression, anxiety, and stress. (10)

<table>
<thead>
<tr>
<th>Threatening events</th>
<th>%</th>
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<tr>
<td>Infection, disease Covid 19 among household members</td>
<td>34,5</td>
</tr>
<tr>
<td>Lack of personal protective equipment at the workplace where such equipment is needed</td>
<td>28,2</td>
</tr>
<tr>
<td>Obligation to stay in self-isolation for a certain period of time</td>
<td>27,5</td>
</tr>
<tr>
<td>SARS-CoV-19 infection (with a positive test), with or without hospital treatment</td>
<td>20,1</td>
</tr>
<tr>
<td>Death of a loved one due to Covid-19 infection</td>
<td>14,3</td>
</tr>
</tbody>
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Figure 1. Stressors associated with risk of SARS-CoV-2 infection
Long Covid-19 associated with Psychiatry Disorders

COVID-19’s mental health consequences have continued even after hospital discharge. The main risk factors were female gender and previous psychiatry disorder. The exact cause of these psychological effects is not known yet, as it could be the direct action of the virus on the CNS, or indirect effects via systemic inflammatory responses to the virus, or a result of psychological stressors such as being infected, stigma, and the experience of being in the Intensive Care Units (11). The persistent symptoms of long COVID-19 appear to affect cognitive and physical function, health-related quality of life, and participation in society (12) Therefore, early interventions are important for controlling the rising psychiatric manifestation of COVID-19, to minimize the possibility to develop neurocognitive impairments. Also, it is important to improve quality of life in those infected. Since, it is not yet determined what really happened during and after Covid in our brain, future studies would be of great value to address this issue.

Conclusion

There has been an evident risk among COVID-19 survivors of higher incidences in mental health disorders, including anxiety, depression, stress and adjustment disorders, opioid and other substance use disorders, neurocognitive impairment, and sleep disorders. The priority for the societies should be to tackling mental health disorders in post-COVID era.

References:


Postexposure prophylaxis of rabies in Dobrich region (2015-2020)

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Abstract
Rabies is an acute infectious disease that affects mammals and humans, a classic zoonosis. It occurs with severe nervous disorders, manifested by agitation, paresis and paralysis. If the infected person is not vaccinated with rabies vaccine within a few hours after the bite, a painful death may occur.

The aim of the study is to present the registered persons who suffered injuries from wild and domestic animals and applied rabies immunization in Dobrich region for 2015-2020.

In the district of Dobrich for 2015-2020 a total of 751 people were registered, injured by domestic and wild animals, and the dynamics is upward. With performed emergency prophylaxis are (29.16%) and (70.83%) are non-immunized persons. Injuries caused by stray dogs and pet dogs occupy the largest relative share (59.47% stray dogs, 33.92% domestic dogs). The main epidemiological indicators of the conducted emergency prophylaxis for rabies of people who have suffered accidents with animal injuries are analyzed - place of residence, sex, age, occupation, place of exposure, day of immunization and vaccine doses.

Key words: rabies, injuries, bites, emergency prophylaxis, dogs.

Introduction
Rabies is an acute infectious zoonosis that affects mammals and humans. It is a viral disease that occurs with severe nervous disorders, manifested by agitation, paresis and paralysis. Natural reservoir and source of the disease are wild animals – wolves, jackals, wild dogs, wild cats, foxes, rodents and birds. Of the domestic animals, dogs and cats are the most important. The virus is contained in the salivary glands and saliva, as well as in some internal organs (spleen, kidneys). In rare cases, it can be found in blood or milk (1). Stray dogs are a link between wild and domestic animals. Between 10 and 20 cases of killed animals suffering from rabies are registered in our country every year, and peaks of the disease are possible in some years.

The mechanism of transmission of the infection is the mechanism of the outer coverings - skin and mucous membrane. Infection occurs directly through the bite of healthy individuals by an animal suffering from rabies, in which the virus enters the wound with the saliva.

If the infected person is not vaccinated with rabies vaccine within a few hours after the bite, a painful death may occur.

Purpose
The purpose of the study is to present registered persons who suffered injuries from wild and domestic animals and applied rabies immunization in Dobrich region for a period 2015-2020.

Materials and methods
We performed a retrospective analysis (period 2015-2020) of post-exposure prophylaxis (emergency) against rabies and administered doses of Verorab vaccine in Dobrich region using data from Regional Health Inspectorate - Dobrich, Ministry of health and National Center of Infectious and Parasitic Diseases - Sofia and data from the National system for control of post-exposure prophylaxis.

Results and discussion
In the period 2015 - 2020, no cases of rabies in humans were registered in Dobrichka region, but 751 persons were registered who suffered injuries after being bitten by pets, domestic or wild animals. The dynamics being upward from 108 cases (in 2015) to 178 (in 2019), after which there was a downward trend until
reaching the initial level (110 cases). The summarized information about the immunized persons shows that 219 persons (29.16%) have undergone emergency prophylaxis and 532 persons (70.83%) are the non-immunized persons (Fig. 1).

![Figure 1. Relative share of immunized and non-immunized against rabies after injury from animals in Dobrich district for the period 2015-2020.](image)

The results show that only one-third of patients after injury from domestic or wild animals received a post-exposure rabies vaccine. The gender distribution of the immunized persons (219 immunized persons during the considered period) is 128 persons (41.55%) of male sex and 91 persons (38.44%) of female sex (41.55%) Males are more often at risk of exposure than women and the proportion of men in cases is higher. These results may be related to occupational or behavioral factors due to which men have more frequent contact with animals. These results are also confirmed by the data presented in other studies (2, 3, 4, 5).

In Fig. 2 are presented data related to 219 immunized persons for the period 2015-2020. The relative share of immunized person reaches 50.68% in the age group 20-59 years, 22.83% is the share of persons over 60 years, followed by 7-14 years (14.6%), 15-19 years (5.93%). The small age groups have a small relative share - 1.36% for 0-1 years and 4.57% for 4-6 years.

![Figure 2. Immunized patients against rabies by age in Dobrich district for the period 2015-2020.](image)

The affected municipalities in the district, in which there are registered incidents of injury to people, are 8 with 6 cities: Dobrich, Dobrichka, Balchik, Kavarna, Shabla, Tervel, General Toshevo, Krushari. There are 215 settlements in the district. During the considered period, the analysis shows a predominant involvement and respective immunization of urban residents - twice as much 69.4% against 30.59% for rural residents. The distribution by years shows an even course without significant fluctuations.
Fig. 3. Distribution of persons immunized against rabies depending on the type of animal that caused the injury (Dobrich district for the period 2015-2020).

Distribution of injured persons depending on the type of animal that caused the injury in Dobrichka region shows that for the period 2015-2020 it was mainly dogs (59.47% stray dogs, 33.92% domestic dogs), followed by cats - 3.64%, foxes - 1.32%, rats - 0.82% and bats - 0.44% (Fig. 3). The trend shown in our study is also described by a large part of the studies in the world - namely, that stray dogs are the main cause of injuries to people as a result of bites. In a number of studies, cases of rabies in humans were caused in the highest percentage after being bitten by stray dogs.

Immunization against rabies depends on the timely initiation of the immunization schedule already on the day of the incident (bite). For 2019-2020, immunization started in 37% of cases on the first day, 30.3% on the second, 21.21% between 4 and 10 days, after which they significantly decreased to 6% on the third day and 1.51% after 10 days.

<table>
<thead>
<tr>
<th>Year</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4-10 day</th>
<th>After 10 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2019</td>
<td>V 18</td>
<td>GG 0</td>
<td>V 10</td>
<td>GG 5</td>
<td>V 8</td>
<td>GG 1</td>
</tr>
<tr>
<td>2020</td>
<td>7</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>0</td>
<td>20</td>
<td>6</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>%</td>
<td>37</td>
<td>0</td>
<td>30,3</td>
<td>9,09</td>
<td>21,21</td>
<td>1,51</td>
</tr>
</tbody>
</table>

Figure 4. Immunization against rabies in Dobrich district (2019-2020). V- vaccine; GG – gamma globulin.

The implemented immunization schemes are mainly with the administration of 5 injections (on days: 0, 3, 7, 14 and 28) after the bite (incident) or 3 injections of rabies vaccine.

Conclusions
Injuries to people in Dobrichka region for 2015-2020 are 751 persons, with post-exposure prophylaxis for 219 persons (29.16%) and 532 persons (70.83%) are not immunized.
Injuries caused by stray dogs and pet dogs took the largest relative share (59.47% - stray dogs, 33.92% - pets). It is necessary to increase the health culture and responsibility of society to reduce the population of stray dogs.

The distribution by age and place of residence shows that the patients with injuries for the studied period are mostly adults - 73% over 20 years and over 70% urban population.

Only one third of the patients were applied with a timely vaccine - on the first day after the injury incident.

References:
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Microbiological evaluation of artisanal milk products

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Abstract

Introduction In general, milk and milk products are rich in nutrients, supplying high-quality proteins, trace elements, vitamins and energy-rich fats. At the same time, this diverse composition of milk provides an ideal environment for the growth of various food-borne microorganisms and zoonotic agents. Aim The aim of this study is to give an overview of artisanal cheese production and its microbiology control in the European Union. Materials and methods We summarize sources of world literature data about artisanal and farmhouse cheese microbiological evaluation in European Union. Results Lactic acid bacteria (LAB) are an integral part of the composition of most dairy products. They are a heterogeneous group of microorganisms including strains of the genus Lactobacillus. The mesophilic lactic acid bacteria starter cultures as well as non-starter LAB including different Lactobacillus spp. are detected in the most samples. The mentioned studies are aimed at searching for the most common pathogens found in artisanal dairy products such as S. aureus, Listeria monocytogenes and Escherichia coli. Conclusion This review highlights the fact that artisanal and farmstead cheese products are associated with various microbiota and microbial. The majority of the traditional cheese samples in this paper complied with the microbiological criteria notifications on food safety. Keywords: artisanal, milk, dairy products, microbiota

Introduction
Historically, milk and milk products have been important components in the diet of Bulgarians and other communities in European Union (EU). In general, milk and milk products are rich in nutrients, supplying high-quality proteins, trace elements, vitamins and energy-rich fats. At the same time, this diverse composition of milk provides an ideal environment for the growth of various food-borne microorganisms and zoonotic agents. In theory, the microbiological quality of milk at the time of milking from a healthy animal is expected to be safe for human consumption. However, in the subsequent stages of its processing, it can be easily contaminated by food-borne microorganisms and pathogens from a variety of sources, including animal feces, soil, air, feed, water, equipment, animal skins, and humans. Thus, the spread of pathogens and microorganisms that deteriorate the microbiological composition of milk and milk products depends on many factors. These factors include health status of the dairy herd, level of hygiene in the dairy farm environment, milking and pre-storage conditions, available storage facilities and technology, farm management practices, geographic location and season. In addition to microbial hazards, milk and milk products may also contain chemical contaminants introduced mainly through the environment, animal feed, animal husbandry and others. Safety and production in the dairy food chain are inextricably linked; from production through handling and processing to consumption [1]. Aim This review provides an overview of artisanal cheese production and its microbiology control in the European Union.

Materials and methods We summarize sources of world literature data about artisanal and farmhouse cheese microbiological evaluation in European Union.
Results
Cheese production is regulated within the European Union (EU) and all food business operators (FBOs) (i.e., producers), large and small, are currently regulated by a number of EU Food Hygiene Regulations. In addition to the prerequisites, cheese-making FBOs must implement and maintain procedures based on the principles of Hazard Analysis Critical Control Point (HACCP), which is a control system focusing on preventive measures (FAO/WHO, 2003; EC, 2004b) [2]. To ensure good hygiene practices in the production of artisanal cheese and other dairy products, the European Commission has published a guide to good hygiene practices. The guide covers questions about farm products that smallholders produce at home. This voluntary guide aims to be applicable to farmhouse and artisan dairy processors [3]. In terms of production methods, milk may be obtained from cows, sheep, goats, buffaloes, or domestic equids and may be pasteurized or unpasteurized. The main characteristic of this type of farm is that the milk there is usually processed by a local producer or in a facility where traditional foods are produced. The implementation of the manual aims to achieve the high standards and hygiene required for traditional processes [3]. The microbiological composition of different farm dairy products has been investigated in different products worldwide. The Lactic acid bacteria (LAB) are a main part of the composition of most dairy products. They are a heterogeneous group of microorganisms including strains of the genus *Lactobacillus*. These bacteria have beneficial health effects and are a major factor in dairy technology. When administered in adequate amounts and with good viability, probiotics maintain the balance and composition of the gut microbiota, increase resistance to pathogens, and protect the host's gut from several disorders [4]. In addition, LAB play the role of starter microflora in fermented and raw foods, which implies the safety of most species and their classification generally recognized as safe. In addition to their proven positive health effects, LAB improve the taste, smell, and texture of products and serve as bioprotectors that naturally extend their shelf life [5]. This is due to the rich enzyme system through which they carry out their metabolic processes on the one hand, while on the other they synthesize organic acids, hydrogen peroxide, diacetyl, bacteriocins and other biologically active metabolites. According to the Food and Agriculture Organization and World Health Organization (FAO and WHO) guidelines, probiotic effects are usually strain-specific. This requires extensive research and characterization of the strains for subsequent use as probiotics [6].

Artisanal and farmstead production of regional cheeses and milk products are a part of the cultural heritage of many countries in Europe. This also leads to the wide variety of dairy products and technologies characteristic of each region of Europe.

Combining traditional cheese making and good product safety is a challenge, since several human pathogens pose a hazard in the products. One of them, *Staphylococcus aureus* is an important foodborne pathogen that can be transmitted via milk to cheese. Some strains can produce staphylococcal enterotoxin during growth in foods and cause food poisoning. Environmental conditions during the initial phase of cheese making are close to optimal for growth of *S. aureus* [2].

Rosengren, shares results from the study examined Swedish small-scale artisan cheese production and relevant pathogens, with the aim of formulating advice on microbiologically safe production. A survey of fresh and short-time ripened cheeses monitored the levels of *S. aureus*, *Listeria monocytogenes* and *Escherichia coli*. Detected levels of the three pathogens were reasonable in most cheese samples tested. *E. coli* and enterotoxigenic *S. aureus* were frequently found in raw milk cheeses, sometimes at high levels but *Listeria monocytogenes* and staphylococcal enterotoxin were not found. The *S. aureus* isolates were mainly of animal biotype and two-thirds were enterotoxigenic. The isolates tested were able to grow and produce staphylococcal enterotoxin in the presence of moderate concentrations of undissociated lactic acid.
Staphylococcal enterotoxin was produced continuously during incubation and high concentrations were found long after *S. aureus* levels had peaked [2].

Freitas and Malcata, summarized information on the microbiological composition of cheeses manufactured with ovine milk and coagulated by plant rennet in Portugal. *Leuconostoc* was an important genus, which increased in concentrations from $10^5$ to $10^7$ cfu/g of cheese at 0 day to approximately $10^8$ cfu/g by 20 days of ripening. The most frequently identified species of LAB were *Lactococcus lactis*, *Lactobacillus casei* spp. *casei*, *Lactobacillus casei* spp. *pseudoplanterum*, *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus curvatus*, *Leuconostoc dextranicum*, *Leuconostoc mesenteroides*, and *Leuconostoc lactis*. The concentration of coliforms decreased throughout ripening and ranged from $10^7$ to $10^9$ cfu/g of cheese at 0 day to $10^6$ to $10^7$ cfu/g by 20 day. The concentration of yeasts and fungi increased more than 1-log cycle during the first week and then decreased slightly to $10^6$ cfu/g [7]. According to Mimoso et al., the total mesophilic microflora in “Azeitaõ” cheese reached values in the range of $10^9$ to $10^{10}$ cfu/g of cheese, increasing in the first week of ripening [8].

The findings of Frece et al. give an important insight into the fermentation and microbial ecology of the cheese in lambskin sacks in Croatia. As in the traditional production of cheese in lambskin sacks raw cow's or sheep's milk is mostly used. They tested 39 samples of raw cow's and sheep's milk, curd, ripened cheese (15, 30 and 45 days) and lambskin sacks for native microbial population. Two-thirds of the milk, curd and cheese samples had higher counts of staphylococci and enterobacteria than permitted by regulations. They found *Escherichia coli* in sheep's milk and cheese, and yeast and mold in both types of milk and cheese. But not a single sample had *Salmonella* and *Listeria monocytogenes*. The results show that *Staphylococcus xylosus* prevailed in lambskin sacks. Despite the high incidence of *S. aureus*, even in the final product, staphylococcal enterotoxin was detected in only two sheep's cheese samples. Part of their results about microbiological evaluation of the cheese in lambskin sacks are given in Table 1 [9].

**Table1.** Results of microbiological analyzes and detection of staphylococcal enterotoxins in the samples of ripened cheese in lambskin sacks.

<table>
<thead>
<tr>
<th>(microorganism) N(log CFU/g)</th>
<th>Cheese Sampling collection I</th>
<th>Cheese Sampling collection II</th>
<th>Cheese Sampling collection III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total aerobic bacteria</td>
<td>4.4±1.1</td>
<td>4.0±1.7</td>
<td>4.9±1.9</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>1.0±0.3</td>
<td>0.8±0.2</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>&lt;LD</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1.8±0.8</td>
<td>1.9±0.8</td>
<td>1.9±0.6</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>&lt;LD</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>&lt;LD</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>Molds and yeasts</td>
<td>1.1±0.8</td>
<td>&lt;LD</td>
<td>&lt;LD</td>
</tr>
<tr>
<td>LAB</td>
<td>5.4±1.0</td>
<td>4.9±1.1</td>
<td>5.4±1.4</td>
</tr>
</tbody>
</table>

* Detection of staphylococcal enterotoxins negative

*not satisfactory criterion (≤2 log CFU/g), **not satisfactory criterion (≤5 log CFU/g), LD=limit of detection

Gori et al. present results from study of the microbiota of four Danish surface-ripened cheeses produced at three farmhouses and one industrial dairy. The cheese core microbiota of the farmhouse cheeses consisted of the mesophilic lactic acid bacteria starter cultures *Lactococcus lactis subsp. lactis* and *Leuconostoc mesenteroides* as well as non-starter LAB including different *Lactobacillus* spp. The cheese from the industrial dairy was almost exclusively dominated by *Lb. paracasei*. The surface bacterial microbiota of all
four cheeses were dominated by *Corynebacterium* spp. and/or *Brachybacterium* spp. *Brevibacterium* spp. was found to be subdominant compared to other bacteria on the farmhouse cheeses. They do not detect *Brevibacterium* spp. on the cheese from the industrial dairy, even though *B. linens* was used as surface-ripening culture. In mentioned study, Gram-negative bacteria identified as *Alcaligenes faecalis* and *Proteus vulgaris* were found on one of the farmhouse cheeses. The surface yeast microbiota consisted primarily of one dominating species for each cheese. For the farmhouse cheeses, the dominant yeast species were *Yarrowia lipolytica*, *Geotrichum* spp. and *Debaryomyces hansenii*, respectively, and for the cheese from the industrial dairy, *D. hansenii* was the dominant yeast species. Additionally, *Streptococcus thermophilus* was present in the farmhouse raw milk cheese analyzed in this study [10]. Beev et al. investigated that milk pasteurization for white-brined cheese production leads to a severe reduction of microorganisms in milk in Bulgarian local farms. Thus, after pasteurization the total number of microorganisms decreases from 480,000 to 810 cfu/cm³, *Salmonella* spp. from 800 to 2 cfu/cm³ and *E. coli* from 4000 to 0 cfu/cm³. Ripening processes lead to a drastic reduction of cheese microflora with prevalence of specific lactic microflora (lactobacilli and lactococci) on the 45th day and complete annihilation of *E. coli* and *Salmonella* spp. These changes in the cheese microflora made the final product safe for consumption [11].

**Conclusion**

This review highlights the fact that artisanal and farmstead cheese products are associated with various microbiota and microbial. In conclusion, the microbiological quality and hygienic status of traditional cheeses produced in European Union (EU) countries are favorable. The majority of the traditional cheese samples in this paper complied with the microbiological criteria notifications on food safety.

**References**

The predictable role of adma and oxidative stress in endothelial dysfunction—a pilot study

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"Faculty of Pharmacy, University ‘’ Prof Paraskev Stoyanov’ Varna

Abstract
The aim of the study was to examine the plasma levels ADMA, MDA and lipid markers in people with metabolic syndrome as well as in clinically asymptomatic people. We further studied whether such elevation of ADMA as marker of endothelial dysfunction was correlated with oxidative stress and conventional risk factors.

According to modified ATP III criteria the participants (52 females, 8 males) of the study were divided in four group: G1, n=19 people with MetS; G2, n=12 peoples with genetic predisposition and risk for MetS; G3, n=15 people with unhealthy life style and risk of MetS and Control group n=14). The study revealed significant differences between people groups with and without anamnestic data of MetS in terms of ADMA, MDA and lipid markers. Plasma levels of ADMA and MDA in G1 were significantly higher (p<0.001) compared to these of G3. The levels of lipid markers (TG, TChol and LDL-C, HDL-C) in G1 were close to the control. The levels of ADMA significantly correlated with plasma MDA levels. These results suggest a link between plasma ADMA and MDA as indicator of oxidative stress and their role as predictive biomarkers for of endothelial dysfunction and cardiometabolic risk.

Key words: ADMA, oxidative stress, endothelial dysfunction. metabolic syndrome

Introduction
Asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthase (eNOS) is marker of endothelial dysfunction (ED) and cardiometabolic risk [1] Endothelial dysfunction, characterized by decreased bioavailability of nitric oxide (NO) is one of the major factors in the pathogenesis of MetS [2]. In addition, increased ADMA has been also linked to the presence of hypertension, type 2 diabetes, obesity as components of metabolic syndrome [3-4]. However, it is unknown whether elevated ADMA plasma concentrations may be considered simply as a marker for c CVD or whether increased ADMA levels per se may predispose to the development of vascular disease [5]. Plasma ADMA concentration is elevated in hypercholesterolemia, leading to ED and producing proatherogenic changes of endothelial cell [6]. Recent evidence suggests that oxidative stress (OS) induced by LDL or by TNF-alpha, increases ADMA and decreases NO bioavailability [7-8].

The aim of the study was to examine the plasma levels of ADMA, MDA and lipid markers in individuals with and without anamnestic data for MetS. We further studied whether such elevation of ADMA as marker of ED was correlated with OS and conventional risk factors.

Material and methods
Sixty people (52 females, 8 males) aged from 20 to 73 years were included in the study. According to NCEP-ATP III criteria for the MetS participants of the study were divided in four group: G1, n=19 people with MetS; G2, n=12 peoples with genetic predisposition and risk for MetS; G3, n=15 people with unhealthy life style and risk of MetS and control group (n=14) clinically healthy individuals who volunteered to participate in the study.

Plasma lipid parameters (TChol, LDL-C, HDL-C and TG) were measured by routine methods. ADMA plasma concentrations were determined using ELISA assay kit (DLD Diagnostica GMBH, Germany) after it was validated locally. Plasma lipid peroxidation was assayed by MDA levels detected by thiobarbituric acid (TBA) reactivity.
Data were analyzed statistically by one-way analysis of variance (ANOVA) and expressed as mean ± SEM. A value of $P < 0.05$ was considered statistically significant. Additionally, the correlation analysis was used. The statistical procedure was performed with GraphPadInStat software.

**Results**

The study revealed significant differences between people groups with and without anamnestic data of MS in terms of ADMA, MDA and lipid markers. Plasma levels of ADMA and MDA in G1 were significantly higher ($p<0.001$) compared to those of G3. The levels of lipid markers (TG, TChol and LDL-C, HDL-C) in G1 were close to the control. The levels of ADMA significantly correlated with plasma MDA levels. Strong negative correlation was found only between ADMA and HDL ($-0.367, p<0.004$).

**Table 1. Change in Plasma Lipid markers (TG, HDL-C, LDL-C, TChol)**

<table>
<thead>
<tr>
<th></th>
<th>Controls N=14</th>
<th>G3 N=15</th>
<th>G2 N=12</th>
<th>G1 N=19</th>
<th>Controls N=14</th>
<th>G3 N=15</th>
<th>G2 N=12</th>
<th>G1 N=19</th>
</tr>
</thead>
<tbody>
<tr>
<td>TChol mmol/L</td>
<td>4.25±0.1</td>
<td>4.52±0.1</td>
<td>5.43±0.1</td>
<td>5.79±0.2</td>
<td>2.34±0.1</td>
<td>2.27±0.1</td>
<td>1.92±0.0</td>
<td>1.52±0.0</td>
</tr>
<tr>
<td>P values vs. C</td>
<td>ns</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td>-</td>
<td>ns</td>
<td>ns</td>
<td>=0.0387</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>-</td>
<td>=0.0006</td>
<td>=0.0003</td>
<td>=0.0002</td>
<td>-</td>
<td>-</td>
<td>ns</td>
<td>=0.0004</td>
<td>-</td>
</tr>
<tr>
<td>LDL-C mmol/L</td>
<td>2.34±0.1</td>
<td>2.64±0.2</td>
<td>3.48±0.1</td>
<td>3.78±0.3</td>
<td>0.70±0.0</td>
<td>0.99±0.1</td>
<td>1.05±0.1</td>
<td>1.99±0.1</td>
</tr>
<tr>
<td>P values vs. C</td>
<td>ns</td>
<td>&lt;0.0001</td>
<td>=0.0005</td>
<td>=0.0501</td>
<td>ns</td>
<td>ns</td>
<td>=0.0276</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>-</td>
<td>=0.0062</td>
<td>=0.0066</td>
<td>=0.0001</td>
<td>&lt;0.0001</td>
<td>ns</td>
<td>-</td>
<td>=0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Figure 1.** Change in Plasma Levels of ADMA and MDA in Control and G1, G2 and G3 groups * $P<0.02$; **$P<0.01$; ***$p<0.001$. 

$P(DISJUNIONN)0.0001$
Discussion
The study aimed to evaluate 1. whether plasma levels of ADMA, MDA and lipid markers differ between groups of adult individuals with and without anamnestic data for MetS. 2. the association between plasma AMDA and oxidative stress markers as well as with lipid indicators and conventional risk factors. 3. Possible role of ADMA and MDA as predictable (predictive) markers for of ED in people without significant increases of lipids and history of MetS.

The study revealed significant differences between people groups with and without anamnestic data of MetS in terms of ADMA, MDA, lipid markers. Levels of lipid markers, ADMA and MDA were higher in people with anamnestic data for MetS than in persons with a risk without known CVD. Some studies report a significant association between increased ADMA levels, hypercholesterolemia and ED in people with CVD [2] while others did not find any relationship [9].

Cardiovascular factors increased vascular superoxide production (O2-) which may activate PRMT and reduce activity of DDAH1. It is suggested that oxidative stress may stimulate ADMA production and/or inhibit ADMA degradation that lead to increased ADMA levels, inhibition of eNOS and reduction of NO availability [7]. A possible mechanism for reducing eNOS activity is the inhibitory effect of free radicals and cytokines on the expression of this enzyme in the vascular endothelium [10]. It is suggested that OS may stimulate ADMA production and/or inhibit ADMA degradation that lead to decrease inhibition of eNOS and reduction of NO availability [10]. The superoxide anion radicals in turn react with the NO radical, thus reducing the bioavailability of NO in favor of the formation of ONOO – and ED.

ADMA is present in the cytoplasm and acts as a competitive inhibitor of NO synthase, thereby increasing the risk of ED (reduced endothelium-dependent vasodilatation, tendency to thrombus formation and remodeling of the vascular wall and the development of atherosclerosis. It is well established that hypercholesterolemia and increased ADMA and MDA levels lead to endothelial dysfunction, a key factor in development of proatherogenic changes of endothelial cell and vascular events including stroke and myocardial infarction. Hypercholesterolemia may disturb the function or regulation of DDAH1, thereby leading to intracellular accumulation of ADMA and increased OS [11].

The present study showed that ADMA, as marker of endothelial dysfunction and MDA, as marker of OS and lipid peroxidation were increased in people without hyperlipidemia and age close to people with MetS. Probably other factors besides hyperlipidemia could lead to increased levels ADMA and MDA, too.

Based on the present findings, we established possible role ADMA and MDA as predictive biomarkers for of ED and cardiometabolic risk. A limitation of the study involves the relatively small covered persons. Our further studies would be directed to the cellular-molecular mechanisms of changes in ADMA and MDA with emphasis on transcription factors Nrf2, increasing the expression of antioxidant enzymes such as HO-1 and the protection of endothelial cells in CVD.
Conclusion
Our findings in this study show the importance of plasma ADMA and MDA in the pathogenesis of endothelial dysfunction in CVD and make it possible to use these markers in screening for the risk of early atherosclerotic vascular change.

References
Microbiologic evaluation of novel metronidazole derivatives

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Abstract
The development process of searching for new antimicrobial drugs includes global research efforts. The aim of the present scientific work is to evaluate antimicrobial properties of two new metronidazole derivatives through agar agar and broth dilution assays. The results identified the novel amide derivatives as more potent antibacterial against Bacteroides fragilis ATCC25285 in compared to the parent metronidazole.

Keywords: metronidazole, antimicrobial, amide derivatives, synthesis, identification

Introduction
Antibacterial resistance is a global problem, and it affects million people all over the world. Antibiotics are the most effective treatment of all bacterial infections. Many of the bacteria, however, have become resistant to the antibiotics used in the clinic, which has led to the need for new agents to control pathogenic microorganisms [1]. Despite advances in medicine, we remain vulnerable to infections with limited or non-standard therapies. There is a need for continuous research to detect and control infectious agent, as well as to develop newer functional antimicrobial agents [2]. The prevailing view is that the current antibiotic discovery model is not delivering new agents at a rate that is sufficient to combat present levels of antibiotic resistance [3]. Aim The aim of the present scientific work is to evaluate antimicrobial properties of two new metronidazole derivatives.

Materials and methods
DMSO (®, ≥99%, Sigma-Aldrich); 0,9% Sodium chloride (Baxter); Bacteroides fragilis ATCC 25285 – lyophilized strains 25285 (Mecconti, MicroSwabs®); Metronidazole 5 mcg MT – antibiotic discs; Mueller-Hinton broth - agar (HiEncap); Sabouraud dextrose agar – growth medium in capsules (HiEncap); Wilkins-Chalgren agar – petri dish, 90 mm (HiEncap); Infusion Agar – growth medium (HiEncap), granulated; Soyabean Casein Digest Agar -growth medium; (HiEncapTM); Brain heart broth – growth medium in capsules (HiEncap); Brain Heart Infusion broth - growth medium; (HiEncap). In our study we used Bacteroides fragilis ATCC 25285 (Mecconti, MicroSwabs®, provided by Ridacom). To activate the lyophilized strain, the manufacturer's instructions were followed using an anaerobic culture medium Wilkins-Chalgren agar (HiMedia®) and an anaerobic Gas Pack system. Wilkins-Chalgren agar was enriched with vitamin K1 (0.1% of the total dry mass). The incubation of samples during the stability and microbiological testing were performed at thermostat Memmert loading models 30-1060 (Atmosafe). A densitometer (Cell density meter model 40, Fisher scientific) was used to measure the optical density.

Two new amide metronidazole derivatives-Ethyl 4-((2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide) benzoate (MT2) and Butyl 4-((2-(2-methyl-5-nitro-1H-imidazol-1-y1) acetamide) benzoate (MT3), non-described in literature, were synthesized and structurally characterized by IR, NMR spectroscopy and TLC, HPLC chromatography methods [4]. The synthesized compounds were tested for in vitro antimicrobial activity against reference bacterial strain Bacteroides fragilis ATCC25285. The obtained results were compared with the activity of the reference drug metronidazole. The disk diffusion and minimal inhibitory concentration evaluating methods were used.
Results
The two novel amide derivatives-MT2, MT3 and 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid-MT1 were tested for in vitro antibacterial activity by following described methods. Kirby-Bauer disk diffusion susceptibility test for determining the antimicrobial sensitivity of \textit{B. fragilis} ATCC25285. We made dense seed of 0.5 MF standardized bacterial culture on Mueller – Hinton agar (HiMedia®). After the surfaces of the culture media have dried, sterile filter discs were placed and soaked with the appropriate concentration of the test compound (metronidazole M and three derivative compounds - MT1, MT2 and MT3). Each sterile filter disk (HiMedia®, provided by Ridacom, Bulgaria) was placed for five seconds in the appropriate test solution, sufficient for complete irrigation. Controls are set for the two diluents, which are used for the preparation of suspensions of the active substances - saline and methanol. All samples were made in triplicate. The media was placed with the lids upside down in an anaerobic jar loaded with Gas Pack system and an anaerobic indicator strip [5].

Minimum inhibitory concentration determination (MIC). For the testing of the MIC of metronidazole and its derivatives to \textit{B. fragilis ATCC25285} we used Brain Heart Infusion broth (HiEncapTM). To remove oxygen from the liquid, we worked with freshly sterilized nutrient media, which were immediately cooled in a water jet. Since in standard preparation of serial dilution with stirring of each subsequent suspension with a pipette the liquid is overly aerated, we have developed our own methodology as follows: 1) The initial amount of liquid medium in each tube was 0.75 ml; 2) The active compounds are dissolved, so that in 0.25 ml volume to contain a concentration that when added to 0.75 ml liquid culture medium to have a desired concentration - respectively 2,4,25, 50,100 µg / ml; 3) 0.1 ml standardized per densitometer bacterial culture of \textit{Bacteroides fragilis} ATCC25285 was added to each tube; 4) All solution surfaces are immediately coated with liquid paraffin. The solutions were cultured for 48 hours at 35°C without further providing anaerobic conditions. Bacterial culture, antibiotic, and sterile fluid control sets were also established. After 48 hours of cultivation, the result was reported by visually determining the turbidity of the test solutions. The lowest concentration at which no visible multiplication of cultured bacteria is observed (turbidity of the solution is not observed) is defined as the minimum inhibitory.

Minimum bactericidal concentration (MBC) determination. After determining the MIC of the tested solutions from all tubes, in which no visual turbidity was reported, bacterial seeds were made on agar medium according to the following technique: 0.1 ml of the suspensions were transferred on Wilkins - Chalgren agar and distributed evenly with a Drigalski spatula. The media were cultured in anaerobic jar using a Gas Pack system and an anaerobic indicator strip. The device is placed in an incubator at 35°C and the results are reported at the 48th hour. The lowest concentration at which bacterial growth is inhibited to 99.9% is reported as the minimum bactericidal concentration.

Study of the antimicrobial activity of Metronidazole, MT1, MT2 and MT3 against \textit{B. fragilis ATCC25285} via disk diffusion method. Solutions were prepared with dilutions of Metronidazole, MT1, MT2 and MT3 corresponding to 2, 4, 25,50,100 µg/ml. Depending on the type of diluent, the samples are divided into two groups, one using sterile saline as the diluent and the other using methanol. Methanol is a better solvent for the tested active compounds, but its proven antimicrobial effect requires the preparation of a large number of controls to differentiate the demonstrated antimicrobial efficacy. The results of the reported antimicrobial activity of Metronidazole, MT1, MT2 and MT3 against \textit{B. fragilis ATCC25285} via disk-diffusion method are shown in Table 1.
### Table 1. Antimicrobial activity of Metronidazole, MT1, MT2 and MT3 against *B. fragilis ATCC25285* determined via disk-diffusion method (zones of inhibition in mm).

<table>
<thead>
<tr>
<th>Concentrations of NaCl solutions</th>
<th>µg/ml</th>
<th>2</th>
<th>4</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
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<tbody>
<tr>
<td>Metronidazole</td>
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<td>-</td>
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<td>-</td>
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<td>MT1</td>
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<td>-</td>
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<tr>
<td>MT2</td>
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<td>MT3</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentrations of CH&lt;sub&gt;3&lt;/sub&gt;OH solutions</th>
<th>µg/ml</th>
<th>2</th>
<th>4</th>
<th>25</th>
<th>50</th>
<th>100</th>
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<tbody>
<tr>
<td>Metronidazole</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>MT1</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>MT2</td>
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<tr>
<td>MT3</td>
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<td>+</td>
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</tbody>
</table>

Legend: - no zones of inhibition

No zones of inhibition were observed in the nutrient media with active compounds dissolved in sodium chloride, bacterial growth was abundant. The reasons may be lack of antimicrobial activity of the test compounds or low activity of saline as a solvent. When we tested the antimicrobial activity of Metronidazole and MT1 dissolved in methanol, inhibition of bacterial growth was also not observed. Bacterial growth is abundant and dense. The lack of zones of inhibition may be due to a lack of antimicrobial activity of the test compounds or a poor manifestation of the antimicrobial efficacy of the compounds dissolved in methanol. According to other authors [6], it is possible for methanol to actively evaporate from the agar medium during the long period of crop cultivation and to influence the expression of zones of inhibition. At three of the MT2 concentrations - 25, 50 and 100 µg/ml, inhibition zones were reported, 9.7 mm, 11 mm and 11.4 mm, respectively. Zones of inhibition are also observed in MT3, but their diameters were too small and therefore the antinicrobial efficacy is unsatisfactory. According to EUCAST, 2020, criteria for disk-diffusion testing of anaerobic susceptibility have not yet been defined, which means that there are currently no standards by which we can determine whether the reported effectiveness of the three concentrations of MT2 are satisfactory.

### Determination of minimum inhibitory concentration of Metronidazole, MT1, MT2 and MT3 against *B. fragilis ATCC25285* by a modified method.

### Table 2. Determination of MIC of Metronidazole, MT1, MT2 and MT3 against *B. fragilis ATCC25285*

<table>
<thead>
<tr>
<th>Concentrations of NaCl solutions</th>
<th>µg/ml</th>
<th>2</th>
<th>4</th>
<th>25</th>
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<tbody>
<tr>
<td>Metronidazole</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>MT1</td>
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<td>+</td>
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<tr>
<td>MT2</td>
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<tr>
<td>MT3</td>
<td></td>
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<td>+</td>
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<tr>
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<th>2</th>
<th>4</th>
<th>25</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>MT1</td>
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<td>+</td>
<td>+</td>
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<td>MT2</td>
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<tr>
<td>MT3</td>
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</tr>
</tbody>
</table>
Legend: + bacterial growth is observed; - lack of bacterial growth

Solutions with dilutions of Metronidazole, MT1, MT2 and MT3, corresponding to 2, 4, 25, 50, 100 µg/ml, were prepared. Again, sterile saline and methanol are used as diluents. After preparing the samples with B. fragilis ATCC25285, following the modified procedure for MIC determination (described in the section of this text Materials and Methods), the following results were observed, described in Table 2 and Fig. 2. For sodium chloride solutions, antimicrobial activity of MT2 (MIC 25 µg/ml) was reported. When determining the MIC of methanol solutions for Metronidazole, MT2 and MT3, antimicrobial activity is reported as follows: Metronidazole - 25 µg/ml, MT2 - 2 µg/ml, MT3 - 2 µg/ml. In MT1, no antimicrobial activity against B. fragilis ATCC25285 was observed in any of the concentrations. There were no indications of bacterial growth in the control of methanol in liquid culture medium and standardized bacterial culture. 

Determination of minimum bactericidal concentration of Metronidazole, MT1, MT2 and MT3 against B. fragilis ATCC25285. After determining the MIC of the test solutions, from all tubes without visible growth, we transferred 0.1 ml of the suspension into Wilkins–Chalgren agar. After 48 hours of incubation, the following minimum bactericidal concentrations were obtained: Metronidazole - 50 µg/ml; MT2 –25 µg/ml; MT3 - 25 µg/ml. During the determination of MIC in liquid medium, the absence of bacterial growth was reported in the methanol controls. From these solutions 0.1 ml of the suspension was transferred into solid media. After the incubation period, bacterial growth was observed in all three testing agar media. This indicates that the active compounds MT2 and MT3 show antimicrobial activity, as methanol alone does not show complete inhibition of bacterial growth. For its part, however, methanol visibly contributed to the better solubility of the tested active compounds, comparing the results with the same compounds dissolved in 0.9% NaCl.

Conclusion

Two new metronidazole derivatives were successfully synthesized and characterized. Their antibacterial properties were evaluated by agar and broth dilution assays. The obtained results show that the introduction of an amide group in the structure of metronidazole derivatives leads to the appearance of stronger antimicrobial activity against B. fragilis compared to metronidazole. The new derivatives can be tested for antibacterial activity against another anaerobic microorganism.

Acknowledgements

This study was financially supported by the Fund “Science” of Medical University of Varna–Project number 19026/2020.

References

Rare case of undifferentiated renal sarcoma-case report

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1) Clinic of Urology, Medical University-Varna, Bulgaria. Multiprofile hospital for active treatment "Sveta Anna-Varna"
2) Clinic of Urology, Medical University-Varna, Bulgaria. Multiprofile hospital for active treatment "Sveta Anna-Varna"

Abstract: We present a rare case of undifferentiated (unclassified) sarcoma of the kidney discovered incidentally during prophylactic ultrasound examination. The diagnosis of “renal mass” was confirmed with CT-scan but the final diagnosis was established after the surgical removal of the tumor. Despite the repeated operations combined later with chemotherapy the patient died 13 months after the initial diagnosis.

Keywords: Fibrosarcoma of the kidney, unclassified sarcoma of the kidney, Vimentin, CD 34, CD 99, CD68, CD10, Ki 67.

Introduction
Primary sarcomas of the kidney are rare; the reported incidence is 1 to 3% of all malignant renal neoplasms [1, 2]. They are most commonly present in the fifth decade of life, usually in men. 50% of them are leiomyosarcomas; liposarcomas are the second most common type. Here we present a rare case of fibrosarcoma which later was reclassified as undifferentiated (unclassified) sarcoma with aggressive growth and poor prognosis.

Case Presentation
The patient-a 55-years old man-had no symptoms, on prophylactic US-examination a mass was found near the left kidney. During the physical examination nothing abnormal was detected. From the laboratory tests the level of hemoglobin was 100g/l.-the other tests were normal. The CT-scan revealed that from the upper pole of the left kidney arose heterogenous rounded mass (140/122/132 mm). Centrally, it was fluid-equivalent, peripherally-with parenchymal components. The latter significantly increased their density after contrast-enhancement. The image corresponded to renal cell carcinoma. The only concomitant disease was arterial hypertension.

The treatment included radical nephrectomy, adrenalectomy and para aortic lymphatic dissection. The pathological report stated the tumor consisted of spindle cells with moderate amount of cytoplasm and large round nuclei (granular to hyperchromatic), small nucleoli. High mitotic index was found-27 mitoses per 10 fields (400x magnification level). There was moderate to pronounced nuclear polymorphism, with scattered single lipoblast and polynuclear cells (FFCCSG grade score 2+3+1=6). Extensive necrotic fields with infarction were found in <50% of the tumor. The ureter was without tumor involvement and so were the left suprarenal gland and the paraaortal lymph nodes. Immunohistochemistry was also performed-CKAE 1/3 was without expression and EMA- also without expression. Vimentin -with pronounced cytoplasmic expression diffusely, in all cells. S100- without expression. SMA - without expression in the tumor cells, expression in the smooth muscle cells of the blood vessels. Desmin- without expression. CD 34- moderate granular cytoplasmic expression in the tumor cells and in the endothelial cells. CD 99- moderate irregular cytoplasmic expression in the tumor cells, diffusely in the whole tumor. The tumor was described macroscopically as 17 cm mass, so stage T4 (> 15 cm) was determined. The pathological conclusion was: fibrosarcoma of the kidney (originating from the renal capsule).

After the operation a PET-CT scan was performed and it showed no signs of metastases. Nevertheless 6 months later the patient complained of dull pain in the left abdomen-below the ribs. During the physical
examination a firm painless mass was palpated in the left abdomen. The laboratory was as follows: hemoglobin 112 g/l, white blood cells 18.5, platelets 268, creatinine 152, ASAT 88, ALAT 41, GGT 149, C-reactive protein 183.1, uric acid 522, O₂-saturation was 90%. On the chest X-ray no mass lesions were found in lung parenchyma, there was pleural effusion on the left side reaching 5-6th intercostal space anteriorly. The patient was again operated - retroperitoneally (on the place of the previously removed left kidney) a large mass (15/20 cm) was found. The mass was resected and local involvement of the paravertebral muscles was found. Also splenectomy, partial gastrectomy and resection of the tail of the pancreas were performed because of local tumor involvement. Part of the left diaphragm was also resected after which a lung involvement from the tumor was found-atypical resection of the affected portion of the lung was performed, after that pleural drainage was inserted and the diaphragm was sutured. Lymph node dissection was done-para aortal masses (10/10cm total volume) of enlarged lymph nodes were found and removed. End-to-side esophagogastrostomy was performed.

The pathological report stated that atypical cells were present, with oval or elongated form, with vesicular nuclei with nucleoli and eosinophilic cytoplasm. Also found were numerous giant polynuclear cells, numerous mitoses, extensive zones with coagulation necrosis and hemorrhage. At the periphery of the tumor there was a thin fibrous layer infiltrated from the atypical cells. The tumor cells were found in the resected parts of the lung, visceral pleura, diaphragm, spleen, pancreas, stomach and the lymph nodes. The immunohistochemistry showed weak cytoplasmic expression of CD 68 in 20% of the mononuclear cells; diffuse cytoplasmic expression of CD 10 and expression of Ki 67 in the nuclei of more than 70% of the tumor cells. The pathological conclusion was tumor characteristics nearest to undifferentiated (unclassified) sarcoma.

The postoperative period was relatively uneventful. 1 month after the second operation new PET-CT was done-it revealed local recurrence of the tumor, metastases in the mediastinal lymph nodes, single osteolytic metastasis in the left acetabulum. The patient began chemotherapy with epirubicin. 1 month later he was admitted in a surgical department with the symptoms of nausea and vomiting. From the laboratory tests the level of hemoglobin was 107g/l., leucocytes 15.5, creatinine 160 μmol/l, C-reactive protein 296.5 mg/l, ASAT - 38.1 U/l; ALAT - 26.2 U/l; GGT - 181.3 U/l. CT scan was performed with the following result: pleural effusion on the left with a layer thickness of up to 21mm. Liver - hypodense lesions in both lobes up to 30 mm in diameter, with the appearance of metastates. On the place of the left kidney a parenchymal lesion with lobulated outlines was visualized, increasing its density along the periphery after contrast – enhancement, hypodense centrally, with dimensions 100/60/100mm. Multiple lesions of different caliber with similar characteristics bilaterally in the abdominal cavity. Enlarged paraaortal lymph nodes up to 25 mm in size. Because the patient was unable to take food orally a decision was made to create a feeding enterostomy (jejunostomy). The postoperative period was uneventful – the feeding through the stoma was successful. Unfortunately, the patient died 1 month after the last operation – due to progression of the disease.

**Discussions**

As mentioned the incidence of renal sarcomas is very low and surgeons generally lack experience in treating them. The most common presenting symptoms are flank or abdominal pain, palpable mass and weight loss – these symptoms are similar to those seen with large renal cell carcinomas. Preoperatively sometimes is possible to suspect a sarcoma on the basis of the CT-scan – when the tumor arises from the renal capsule or renal sinus and when the tumor is hypovascular or avascular on angiograms. Also renal sarcomas do not appear to have a propensity for extension into the renal vein or the inferior vena cava [3]. In our case sarcoma
was not quite obvious from the CT scan. So the final diagnosis was made after the pathological examination of the removed kidney.

Pathologically renal sarcomas are typically of renal capsular origin. 50% of them are leiomyosarcomas [4], the other 40-50% are fibrosarcomas, liposarcomas or hemangiopericytomas. Our patient initially was diagnosed with a fibrosarcoma. Later a local recurrence was excised and another pathologist (more experienced) suggested the diagnosis undifferentiated (unclassified) sarcoma.

All sarcomas (including those of the kidneys) are surrounded by a pseudocapsule that is often infiltrated with cancer cells. There is no recognizable border between normal and infiltrated tissue. Thus wide local excision (often with adjacent organs) gives the best chance for cure [5]. Local recurrences are common and require repeat resection to prolong survival. According to the literature the lungs, lymph nodes and liver are the most frequent sites of metastases [6] – with no significant difference among the histological subtypes. Chemotherapy is generally with disappointing results – anthracyclines have been tried (traditionally doxorubicin [7]). In our case epirubicin was used with no measurable effect on tumor size. The patients usually die of disease progression within 6 months after surgery – our patient survived 13 months which is a fairly good result. A survey from a high-volume center presents an overall 1-, 3-, and 5-year survival rates as 86.3%, 40.7%, and 14.5%, respectively, with median survival 28 months. The median survival after recurrence is 10 months (range 4-24) and that after metastasis 8 months [8].

**Conclusion**

Renal sarcomas are highly malignant tumors which very often present at advanced stage. Surgery is the main (or the only one?) curative treatment but it is very difficult to achieve durable results even when reoperations are done.

**References:**


**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.
New possibilities for treatment and recovery of women’s intimate zone

Mariya Angelova
Medical Faculty of Trakia University – Stara Zagora, Department of Obstetrics and Gynecology

CO2RE Intima treats vagina, vulva and vaginal opening

Introduction
1. Remodels the vaginal connective tissue with single use internal applicator.
2. Alleviates dyschromia, dystrophy of the vulva, improves the texture of the skin and remodels the connective tissue of the vaginal opening with hygienic single use external applicator.
3. The surgical multiple use applicator is used for excision or incision, ablation or coagulation of the external tissue of the vulva.

CO2RE – fully equipped CO2 device
4 modes: light, middle, deep, fusion for fractional renewal of the surface.
Classical mode for complete renewal of the surface.
Surgical mode for incision.

Materials and methods:

<table>
<thead>
<tr>
<th>Application</th>
<th>Symptoms</th>
<th>Target patients</th>
<th>Reasons for seeking therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rejuvenation of the labia</td>
<td>Dissatisfaction with the appearance of the vulva (flaccid skin, pigmentation, texture)</td>
<td>All ages (flaccid skin due to advanced age, exactly like the face)</td>
<td>Beauty, comfort, quality of life, sexual satisfaction</td>
</tr>
<tr>
<td>Tightening of the vagina</td>
<td>Loose vagina has a negative impact on sexual life</td>
<td>Women in postpartum period</td>
<td>Sexual satisfaction, intimacy</td>
</tr>
<tr>
<td>Stress incontinence</td>
<td>Micturition during increase of intraabdominal pressure</td>
<td>Mostly women in postpartum period and menopause</td>
<td>Comfort, quality of life</td>
</tr>
<tr>
<td>Vaginal atrophy</td>
<td>Dryness, pruritus, burning sensation, dyspareunia</td>
<td>Up to 50% of women in menopause</td>
<td>Comfort, quality of life, sexual satisfaction</td>
</tr>
<tr>
<td>Dryness after radiotherapy</td>
<td>Excessive dryness</td>
<td>Patients after chemotherapy</td>
<td>Comfort, quality of life</td>
</tr>
<tr>
<td>Rejuvenation</td>
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</tbody>
</table>

What is it? Treatment of flaccid skin, texture, pigmentation changes
When does it occur? It is age-related, childbirth may be a contributing factor. Pigmentation changes may be due to hormonal factors.
Relaxation of the vaginal canal
Condition: Vaginal relaxation syndrome
What is it? Stretching to the borders of elasticity, causing laxity and reduced sensitivity.
When does it occur? After vaginal birth, rarely after surgical intervention.

Urinary incontinence
Condition: urinary incontinence; types: stress, imperative or mixed. Usually after vaginal birth, also with low estrogen levels; varies from slightly irritating to invalidating state.
When does it occur? Before menopause – after vaginal birth;
After menopause – at low estrogen level.

Urinary stress incontinence
Two types of incontinence: imperative incontinence and stress incontinence
(Questionnaire for Urinary Incontinence Diagnostics – QUID)
Do you leak urine even in small drops, do you lose control of your bladder?
Rarely, from time to time, often, most of the time, all the time
1. When you cough or sneeze
2. When you bend to pick up something
3. When you walk fast or jog or exercise
4. Do you feel such strong and discomflicting need to urinate that you leak urine (even in small drops) or you lose control of your bladder before you reach the toilet?
5. Do you have to rush to the toilet, because you feel a sudden, strong urge to urinate?

Genitourinary syndrome in women in menopause (Vulvovaginal atrophy)
Vulvovaginal atrophy (VVA), atrophic vulvovaginitis, atrophic vaginitis, genitourinary syndrome in women in menopause.
What is it? Vaginal atrophy is caused by a low level of estrogen formation. The lower amount of estrogen makes the vaginal tissues thinner, dryer, less elastic and more vulnerable.
When does it occur? It is related to a lower formation of estrogen: surgically induced menopause (hysterectomy), chemically induced (chemotherapy, radiation), natural menopause.
A multifocal study informs about 64,7%-84,2% objective signs, exhibited 1-6 years after menopause.

Atrophy of the mucous membrane
Lower estrogen levels are related to the following symptoms of vaginal atrophy: reduced lubrication, vaginal dryness (lack of humidity of the vagina), irritation of the vagina and the vulva, inflammation, vulvar pruritus (itching).
Urinary: frequency, urgency and incontinence, pain or burning sensation when urinating
Prevalence: 40% of women in menopause have symptoms
>60% of breast cancer surviving patients in menopause inform about vaginal atrophy symptoms
>20% of breast cancer surviving patients before menopause.
Only 20-25% of the affected women seek treatment

For whom is this procedure suitable?
The majority of the population rejects surgical interventions when cosmetic procedures is a first choice. The recovery time after the intervention is key in our contemporary life. There is no longer a recovery period.
Method 1: in this case the impulses are applied linearly, as the applicator is withdrawn one centimeter at a time. After that the applicator is reinserted at the next position clockwise and the procedure is repeated.
Method 2: in this method the impulses are applied clockwise at the same depth before retracting the applicator by one centimeter. The applicator is withdrawn by one centimeter and the same procedure is repeated.
CO₂ laser is the gold standard in tissue remodeling
Ablation if changes in the texture and pigmentation are present, collagen regeneration, elastitin regeneration, detailed documenting of histological results, neocollagenesis is prolonged, neovascularization, fractioned techniques allow greater depth of penetration.

**Mechanism of action – external applicator**
The superficial and deep derma are warmed in order to induce contraction of collagen and denaturation; induction of growth factors, the old collagen fibres are replaced with new ones which are histologically more compact and better organized.

Suitable patients: women after vaginal birth, women in menopause, women looking for improvement of the vaginal relaxation, pigmentation in the area of the vulva, dryness, pruritus, painful intercourse.
Counterindications: Surgery in the vaginal or pelvic area in the last 12 months, presence of sheet, past history for genital herpes, idiopathic vaginal bleeding, urinary tract infection, pelvic infection, active malignant disease or past history for malignant condition in the past five years, significant concomitant diseases, i.e. heart disease, diabetes, autoimmune diseases, immunodeficiency-related disorders, or other concurrent diseases for which the physician thinks may influence the therapy or the treatment process, use of anticoagulants or immunosuppressants drugs, use of systemic corticosteroid therapy in the previous 6 months, past history for problematic wound healing, pregnancy.

**Preparation for the procedure**
Recent PAP smear, no found injuries or bleeding in the internal vaginal area, informed consent, evaluation of the muscles of the pelvic floor.

**After therapy**
Make an evaluation of the mucous membrane after the therapy – slight reddening is normal. A water-based lubricant may be applied. Maintain the area clean and wash carefully with warm water and mild cleansing detergent.
The patient must not have an intercourse for 7 days. No vaginal wash or tampons should be used for 7 days. If there is any pain, fever or unusual discharge, the physician must be informed immediately. A second check-up must be done in 7 days.

**Therapy protocol**: three procedures with four weeks in-between, supportive therapy session after one year.

**Labioplasty – current methods**
Shaping linear contours 53%, the dark edges may be removed according to the wish of most patients (97%). The natural colour of the edges may be preserved, if desired.
Technically more difficult to perform well.
Study results: efficacy, evaluation of the patients over a 12-week monitoring:
81% of the patients inform about an improvement in their sexual satisfaction
94% of the patients inform about vaginal rejuvenation
100% inform about satisfaction from the treatment
94% would recommend the procedure.
Micronutrient deficiencies following bariatric surgery - strategies for nutritional care

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Abstract
Obesity causes serious health problems and is officially recognized as a disease nowadays by multiple professional bodies. The main reason behind this is the complexity of body-weight regulation, increased morbidity and mortality associated with obesity, and the substantial burden on public health. Micronutrient deficiency is a common complication of metabolic changes in obese patients and malnutrition together with fat excess is very often detected among them. Guidelines for the treatment of obesity include diet therapy, respective energy restriction, lifestyle modification, behavior therapy, and pharmacotherapy if the previous approaches have acted insufficiently to reach a 5%-10% loss of body weight. Weight-loss surgery (bariatric surgery) has proven to be the most effective therapy for people with severe degrees of obesity and related comorbidities but this approach can exacerbate some previously existing micronutrient deficiencies, if not properly managed.

The purpose of this review was to focus the attention on some deficiencies of micronutrients among obese patients considered for bariatric surgery and post-bariatric and to outline the main approaches to reduce some nutritional risks of this intervention through long-term supplementation and monitoring.

Key words: obesity, bariatric surgery, micronutrient deficiencies management

Introduction
Obesity is a chronic, relapsing, and progressive disease associated with serious complications and comorbidities. Obesity and overweight are public health problems affecting more than 1.9 billion people worldwide based on most recent WHO reports [1]. Over 39% of adults aged 18 years and over were overweight in 2016, and currently 13% of the world population is obese in both developed and developing countries. Several countries worldwide reported a drastic increase in the prevalence of obesity with a progression of morbid obesity over the last 30 years, probably due to multifactorial reasons - urbanization, increasingly sedentary lifestyle, and nutritional transition to processed foods and high-calorie diets. The analyses show that the problem of obesity affects not only adults but children as well and being obese in childhood increase the risk of morbid obesity later in life [2]. If this trend continues, by 2030 an estimated 20% of the world’s adult population will be obese and with multiple comorbidities [3]. Based on the complexity of body-weight regulation, increased morbidity and mortality associated with obesity, and the substantial burden on public health, obesity was officially recognized as a disease by many professional bodies, including the World Obesity Federation [4]. The increasing prevalence of obesity and related comorbidities worldwide prompt effective strategies for both treatment and prevention. Guidelines for the management of obesity include diet therapy, respective energy restriction, behavior therapy for lifestyle modification, and pharmacotherapy if the previous approaches have been insufficient to reach a 5% -10% loss of body weight. For people with severe degrees of obesity and related
comorbidities weight-loss surgery (bariatric surgery) together with life-style modifications has proven to be the most effective therapy. The progressive increase of the prevalence of morbid obesity is likely to place bariatric surgery among the leading treatment methods. In spite of clinical benefits, this approach, if not properly controlled, can cause several complications such as micronutrient deficiencies which deserve careful consideration.

**Micronutrient deficiencies of obesity**

Obesity is an element of co-occurring of several known cardiovascular risk factors, including insulin resistance, dyslipidemia and hypertension, but also some micronutrient deficiencies are higher in obese individuals compared to normal-weight people [5]. The most important factor for obesity related malnutrition is a poor diet with highly processed foods, refined carbohydrates, and low on fruit, vegetables, and dairy which can also contribute to the low intake of some vitamins and minerals [6]. Excessive fat mass can impact the bioavailability and utilization of micronutrients and increase physiologic demands of some vitamins and minerals which are underestimated by dietary reference intakes (DRI) intended for the general population. Even more, micronutrient deficiencies (vit E, beta carotene, Se and Zn) were included among the risk factors favoring the development of obesity in the most recent ESPEN Micronutrient guideline [7]. In patients with obesity, particularly grade III, along with energy imbalance, deficiencies of a number of micronutrients appeared, such as vitamin D, magnesium, phosphorus, iron, and vitamin A, which are not always identified and compensated in a timely manner. Deficiency of vitamin D is very common and was confirmed among obese persons in Bulgaria as well [8]. All this put obese patients, candidates for bariatric surgery at higher pre-operative risk of micronutrient deficiencies and of deficiency-related complication after the surgery.

**Bariatric surgery and micronutrient deficiencies**

Bariatric procedures are generally classified into restrictive procedures, in which the stomach’s capacity is greatly reduced, malabsorptive procedures, in which malabsorption is the primary driver of the weight loss, or a combination of restrictive and malabsorptive elements. Nutritional deficiencies can develop as a consequence of reduced intake and/or malabsorption of nutrients and are more commonly seen after malabsorptive or mixed procedures in comparison to the restrictive procedures [9]. Other causal factors include pre-existing deficiencies, post-surgery food intolerance, changes in taste and eating patterns and non-adherence to dietary and supplement recommendations. Nutritional deficiencies can present with a wide spectrum of clinical and laboratory manifestations, depending on the specific micronutrients that are involved, the severity of deficiency, and the duration of the non-compensated deficiency status and can cause several serious health issues. [10]. Because of the seriousness of these complications a nutritional screening both before and after surgery is strongly recommended.

**Micronutrient deficiency management in bariatric surgery**

The pre-operative nutritional state is a critical factor for success of the surgery and the long-term patient’s health. Criteria for selecting bariatric surgery as a weight loss strategy are based on body mass index (BMI) of the patient as well as the presence of comorbidity. Assessment of nutritional
status with a special focus on nutrition deficiencies should be considered for all patients - candidates for bariatric surgery. All patients must undergo a preliminary nutritional evaluation, including micronutrient measurements, before any bariatric procedure. Whole-blood thiamine levels may be considered in patients before bypass procedures [11]. It should also be recommended to perform pre-Sleeve Gastrectomy (SG) correction of any deficiencies of vitamins and minerals and replenish the respective body stores to prevent the early postoperative micronutrient deficiencies. Lifelong multivitamin-mineral supplementation is recommended to avoid complications such as anaemia, poor bone health or neurological complications in all patients experiencing any bariatric procedures [12]. A regular monitoring plan should be established and individualized depending on the patient's nutritional status in progress to avoid any deficiencies.

**Conclusion and Recommendations**

Good as well as lasting results of bariatric intervention can be ensured by: preoperative assessment of micronutrient status and preliminary corrections of any deficiencies, life-long adequate supplementation and regular monitoring of nutritional status after the intervention focused on micronutrients.

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Possibilities of kinesitherapy in the complex treatment of crohn's disease: presentation of a clinical case

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Abstract

Purpose: to implement and evaluate a complex therapeutic program in a patient with Crohn's disease.

Contingent: A 31-year-old man diagnosed with Crohn's disease turns to the University Center of East Medicine at the Medical University of Varna because of pain in the spine and abdomen, insomnia, chronic fatigue and frequent headache. Despite the medical treatment and diet prescribed by the gastroenterologist, the patient continues to have complaints that affect his work activity and are the cause of frequent absences from work due to temporary inability. The team of the University Center of East Medicine applied a complex program, including methods of treatment and secondary prevention of the disease. Methods: laboratory tests - fecal calprotectin, C-reactive protein; highly specialized diagnostic activities – colonoscopy; sociological method - the generic questionnaire for assessing the quality of life (WHOQOL-BREF), validated for Bulgaria by V. Petkov (1999).

Results: At the end of the therapeutic course, the patient reported a significant reduction in fatigue, increased activity in daily life and ability to work. The positive dynamics in the patient's condition directly correlates with the tests showing the presence of an inflammatory process in the body. Fecal calprotectin and C-reactive protein were as follows: 214 mg/g and 1.36 mg/l.

Conclusions: The progressive-relapsing nature of Crohn's disease necessitates a constant search for effective ways to deal with the disease. Early diagnosis, correct therapeutic behavior and cooperation from the patient are the basis for coping with the disease.

Key words: Crohn's disease, complementary medicine, physiotherapy, quality of life, disability

Introduction

Crohn's disease is an autoimmune relapsing inflammatory disease that affects different areas of the digestive system. The disease is characterized by a variety of symptoms, with the most common localization in the terminal ileum and proximal colon, but it can also affect other parts of the body, including the skin, joints and eyes [1, 2]. The etiology of the disease is not fully understood, but bacterial contamination, weakening of the immune system, and genetic predisposition are considered potential risk factors. Crohn's disease worsens the quality of life, leads to temporary and permanent disability and is characterized by high mortality. For these reasons, the disease is a serious challenge that provokes the clinical and research interest of medical professionals worldwide [3, 4].

Therapeutic behavior in patients with Crohn's disease is aimed at lifestyle changes with avoidance of risk factors such as smoking and alcohol, systemic drug treatment, adherence to a strict diet, as well as inclusion of appropriate physical activity [5]. Drug treatment and diet are key to the health of Crohn's patients, aiming to increase remission of the disease, reduce relapses, the number of hospitalizations and operative interventions. Despite progress in the treatment of the disease, the frequency of surgical interventions remains high (75-80%) [6]. The variety of symptoms, adverse reactions, and complications of drug treatment necessitate an expanded therapeutic approach to patients suffering from Crohn's disease.
The aim of the study is to implement and evaluate a complex therapeutic program in a patient with Crohn's disease.

Material and methods
A 31-year-old man for years complained periodically of chronic fatigue, alternating diarrhea with constipation, insomnia, pain in the spine, knees, headache, impaired concentration and memory. The severe abdominal pain was the reason for the emergency hospitalization of the patient in the gastroenterology clinic at “Saint Marine” - Varna in March 2021. Colonoscopy and biopsy revealed Crohn's disease of the large intestine. Examination of fecal calprotectin (FCP) was highly elevated > 1000 mkg/g, as well as C-reactive protein > 12 mg/l. During hospitalization, therapy includes infusions of saline solutions, antibiotics and antispasmodics. After discharge from the hospital, medical treatment with Salofalk-500 mg and a probiotic was prescribed. A suitable diet was prepared and a consultation with a specialist kinesitherapist was recommended for conducting motor therapy program.

The team of University centre of East Medicine applied a complex therapeutic program that includes acupuncture and moxibustion, therapeutic massage, general strengthening exercises, breathing exercises, stretching and auto-stretching, bicycle ergometry. The course of the therapeutic program lasted 2 weeks, as each procedure having a total duration of 60 minutes. The results achieved before and after the complex therapeutic program were evaluated by examination of fecal calprotectin, C-reactive protein and colonoscopy. For bio-psycho-social assessment of the achieved results was applied the generic questionnaire for assessment of the quality of life (WHOQOL-BREF), validated for Bulgaria by V. Petkov (1999).

Results and discussion
As a result of the applied methodology, on the third procedure the patient feels an improvement in the general condition, tone and motivation. On the fifth procedure, a reduction in pain symptoms in the spine and abdomen was observed, with positive dynamics in the quality of sleep. At the end of the therapeutic course, the patient reported a significant reduction in fatigue, increased activity in daily life and ability to work. The positive dynamics in the patient's condition directly correlates with some of the laboratory tests showing the presence of an inflammatory process in the body. Faecal calprotectin and C-reactive protein were as follows: 214 mkg/g and 1.36 mg/l. The data in the table.1, which show the change in the quality of life, testify to the effectiveness of the complex treatment, taking into account the greatest change in domains “physical health” and “psychological health”.

Table 1. Overall and domain-specific assessment of quality of life survey data with WHOQOL-BREF

<table>
<thead>
<tr>
<th></th>
<th>Overall score</th>
<th>Domain „physical health“</th>
<th>Domain „psychological health“</th>
<th>Domain „social relationships“</th>
<th>Domain „environment“</th>
</tr>
</thead>
<tbody>
<tr>
<td>before</td>
<td>62</td>
<td>16</td>
<td>12</td>
<td>9</td>
<td>25</td>
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<tr>
<td>after</td>
<td>84</td>
<td>24</td>
<td>19</td>
<td>12</td>
<td>29</td>
</tr>
</tbody>
</table>

The main method of treatment in patients with Crohn's disease is drug therapy, which depending on the stage and activity of the disease, includes anti-inflammatory drugs, corticosteroids and immunomodulators to increase the period of remission, reduce relapses and slow the progression
of the disease [7]. Drug therapy entails the inevitable side effects such as headache, diarrhea, nausea, vomiting, which further worsen the condition [8]. Diet is essential to delay the development of the disease, including and increasing periods of remission. Therapeutic nutrition, specifically exclusive enteral nutrition (EEN), has been shown to be as successful in achieving remission in children with Crohn's disease as steroids, but with significantly fewer adverse effects [9]. Otherwise, if not follow strictly the diet prescribed by the doctor, there is a high probability of worsening symptoms, and frequent hospitalizations. Studies have also highlighted the leading role of lifestyle change by avoiding risk factors such as smoking and alcohol. Scientific evidence suggests that smoking is strongly associated with the progression of Crohn's disease, due to its multiple negative effects on physical and psychological health, suppression of medication and risk of relapse. Other research pay attention at alternative methods of treatment in the adjunctive therapy of this disease. Acupuncture with an anti-inflammatory effect and moxibustion are most often applied to increase immunity and improve the general condition, concentration and memory [10, 11, 12].

The possibilities of supporting and preventing Crohn's disease through physical exercises are increasingly being discussed. It is believed that physical activity in the form of gymnastics and dosed walking is a useful component in the concomitant therapy of this disease. Although there is currently no definitive evidence for specific motor programs in Crohn’s disease, a number of authors emphasize the positive effects of exercise on systems and organs, including the gastrointestinal tract. They affect the physical and psycho-emotional level by improving the immune system, nutritional status and muscle trophic [13, 14, 15].

In our research, similar to other scientific studies, we applied curative and prophylactic strategies to combat the disease. In support of the above statements, we believe that in order to be more effective treatment, it is necessary to include kinesitherapeutic approach. Kinesitherapy combines a variety of means such as manual techniques, physical exercises, elements of sports and provides the possibility of dosing these loads. Kinesitherapeutic methods affect the physical and psycho-emotional tone, increase immunity, reduce complaints from the musculoskeletal system, lead to the prevention of complications and improve the quality of life [16, 17].

Conclusion
Drug therapy and diet, as well as lifestyle, are the major therapeutic methods in Crohn's disease approach. Kinesitherapy, sports, acupuncture and moxibustion should be accepted as a good option in complex treatment. The specificity of the course of the disease is associated with temporary incapacity and disability, which necessitate the search and development of additional therapeutic strategies.

References;
De novo design of a PCR-RFLP assay for detecting the leptin receptors single nucleotide polymorphism in hypothalamic nuclei

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Abstract
Obesity in adulthood is associated with many comorbidities, such as cardiovascular diseases, type 2 diabetes, and cancers. The central melanocortin system is very important for the regulation of energy homeostasis and controlling body weight. The hypothalamic leptin-melanocortin regulation pathway involves signaling interactions between leptin (LEP), leptin receptors (LEPR), and melanocortin receptors (MC4R). The rs1137101 polymorphism of the LEPR gene is one of the polymorphisms with the highest allele frequency and is associated with increased body weight and high LEP levels due to impaired LEPR intracellular signaling. The aim of the current study is to design specific primers that amplify the region of LEPR containing rs1137101. DNA was isolated from peripheral blood leukocytes. The primers used for gradient PCR were de novo designed by Primer 3. The resulting 346 bp PCR fragment of the LEPR gene was visualized on a 2.5% agarose gel. The expected band size of 346 bp was detected only within the range between 45 °C to 58.6 °C. Within the tested temperature range, 53.8 °C and 56.2 °C were the optimal temperatures that showed lack of non-specific amplification, so 55 °C was chosen as appropriate annealing temperature for the tested primer pair.

Keywords: single nucleotide polymorphism, leptin receptors, obesity, gradient PCR, hypothalamic leptin-melanocortin regulation pathway.

Introduction
The high prevalence of obesity and type 2 diabetes is a leading public health problem of the 21st century. The body mass index (BMI) of children and adolescents has increased globally over the past decades [1]. Overweight children and adolescents have an increased risk of becoming overweight adults [2]. Obesity in adulthood is associated with several comorbidities, including but not limited to cardiovascular diseases, type 2 diabetes, and different forms of cancer [3, 4]. Various genetic, metabolic, behavioral, and environmental factors play a key role in the development of obesity [5]. Changes in BMI are related to energy balance, which is maintained via a dedicated homeostatic system. Regulation of energy homeostasis and control of body weight are mediated by the melanocortin system [6]. The hypothalamic leptin-melanocortin regulation pathway involves signaling interactions between leptin (LEP), leptin receptors (LEPR), and melanocortin receptors (MC4R) [7]. LEP is a peptide hormone secreted by unilocular adipocytes. It binds to LEPR expressed on neurons in several hypothalamic nuclei such as nucleus arcuatus and nucleus ventromedialis. Hormone-receptor binding stimulates post-translational cleavage of pro-opiomelanocortin (POMC) to melanocyte stimulating hormone, and further signaling through MC4R [6, 8]. Scientific data regarding the relationship between polymorphisms in LEP and LEPR genes and human obesity are still controversial. The rs1137101 polymorphism of the LEPR gene is one of the polymorphisms with the highest allele frequency and it is associated with increased body weight and high LEP levels due to impaired LEPR intracellular signaling [9, 10]. The aim of the current study was to design a new in-house assay for LEPR rs1137101 genotyping by PCR-RFLP. The goal of the experiment was to amplify a 346 bp fragment of interest from the LEPR gene.

Materials and methods
Genomic DNA was isolated from peripheral blood leukocytes using column-based DNA extraction (QIAmp DNA Blood Mini Kit, QIAGEN, USA). DNA was quantified using NanoDrop™ One (ThermoScientific,
USA), and diluted to a final working concentration of 5 ng/µL. The primers used for the PCR-RFLP assay were *de novo* designed by Primer 3 by using the default parameters. The following primers were chosen for DNA amplification: Forward primer: 5'-GCTCTTTATTTTTCAATATAGGC-3'; Reverse primer: 5'-ATCATTACAGTGTAAAGCAAA-3'. Primers were purchased from Metabion International (Germany).

Optimal primer annealing temperature was chosen using gradient PCR ranging from 45 °C to 65 °C on qTOWER³G (Analytik Jena, Germany) PCR thermal cycler. DNA was amplified in a total volume of 20 µL with 35 cycles of PCR using 0.3 µM of each primer, 200 µM dNTPs, 0.5 U of HotBegan™ Hot Start Taq (Canvax, Spain) and 1 µl of DNA (5 ng/µL) as a template. Ten microliters of each PCR sample were resolved on a 2.5 % agarose gel, and PCR products were visualized with ethidium bromide staining and UV illumination. Cycling conditions were 5 min at 94 °C, 30 s at 94 °C, 30 s at 45.0 °C to 65.0 °C (gradient PCR), 45 s at 72 °C and a final extension at 72 °C for 5 min for 35 cycles.

**Results**

Using the gradient function of the qTOWER³G, a gradient of 45.0 °C to 65.0 °C was set. Twelve PCR reaction tubes containing identical reaction mixture and DNA template were loaded across the block. The expected band size of 346 bp was detected only at temperatures ranging from 45 °C to 58.6 °C (fig. 1).

![Fig.1 Experimental determination of optimal annealing temperature. 1) 45 °C; 2) 45.5 °C; 3) 46.9 °C; 4) 49.1 °C; 5) 51.4 °C; 6) 53.8 °C; 7) 56.2 °C; 8) 58.6 °C; 9) 60.9 °C; 10) 63.1 °C; 11) 64.5 °C; 12) 65 °C. M, molecular weight marker HyperLadder™ 25 bp (Bioline, Germany).](image)

In the range of temperatures from 45 °C to 51.4 °C the band with the expected size of 346 bp was detectable on the agarose gel, together with an additional band of larger size. When using 63.1 °C, 64.5 °C and 65 °C as annealing temperature, specific amplification was not detectable on the gel. Temperature of 58.6 °C appears to suppress the annealing of the primers and lower quantity of the PCR product was seen on the agarose gel. Within the tested temperature range, 53.8 °C and 56.2 °C were the optimal temperatures that showed lack of non-specific amplification, without inhibition of the specific amplification, so for further experiments 55 °C was chosen as preferred annealing temperature.

**Discussion**

Primer annealing optimization involves lowering of the reaction temperature to allow binding of the primers to their complementary DNA. The optimal annealing temperature should be optimized depending on the PCR results. If there is not amplification or the quantity of the amplified product is low, lowering of the
annealing temperature in increments of 2-3°C might be necessary. If non-specific products of amplification are present the annealing temperature should be raised in increments of 2-3 °C [11, 12].

In our study, the calculated annealing temperature by Primer3 was 51.3 °C, and the calculated Tm of the forward and reverse primers was 55 °C and 52 °C, respectively. The calculated annealing temperature is a starting reference temperature for primer annealing. In this experiment, annealing temperature of 51.4 °C resulted in a band of high intensity as visualized on agarose gel, but non-specific product with larger length was also visible.

Optimization of the annealing temperature of the designed primers was done by gradient thermal cycler. Using gradient PCR to determine the appropriate annealing temperature saves time and is a cost effective approach [12]. Temperature gradient of 20 °C was set and all PCR reactions were tested in one PCR run, with different annealing temperatures. Using this approach, the optimal annealing temperature for the designed primers was in the range between 53.8 °C to 56.2 °C.

Conclusion
Within the tested temperature range, annealing temperature of 55 °C was chosen as appropriate due to the absence of non-specific products on the agarose gel.

Acknowledgements: This study was carried out with financial support of Medical University – Pleven through the University Grants Commission (Project No. D1/2021)

References:


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Distribution of Covid 19 Infection Among HIV Positive Individuals in the Centre for Diagnosis and Treatment of HIV / AIDS in the Infectious Diseases Clinic, Part of the University Hospital "Prof. Stoyan Kirkovich" AD Stara Zagora

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Abstract The population of people living with HIV (PLWH) is known to be at a higher risk of the new SARS-CoV-2 infection. The aim of our study is to determine the immunological status against SARS-CoV-2 of HIV+ patients treated in the center at the Clinic of Infectious Diseases with HIV/AIDS activities at the University Hospital, “Prof. St. Kirkovich“ - AD Stara Zagora. A total of 41 out of a possible maximum of 51 patients aged between 23 and 58 years were studied, with an average age of 39.9±9.23 years. Four of them (9.75%) were women. Clinical examinations, epidemiological, laboratory and immunological studies were performed. The chemi-luminescent CLIA method was used to determine the levels of virus-neutralizing antibodies against SARS-CoV-2. Most patients received antiretroviral therapy and had an undetectable viral load, with a mean CD4+ cell count of 463.70 ± 241.64. A possible Covid-19 infection was reported by 9 of the patients (21.95%). Ten (24.39%) were vaccinated of whom 7 (17.07%) had an adequate protective titer of virus-neutralizing antibodies against SARS-CoV-2. In all HIV+ patients infected with SARS-CoV-2, the disease was mild, with most of the patients who had been vaccinated or ill showing good protective antibody titers. However, patients with low levels of CD4+ cells and higher than 20 copies/ml viral load, had failed to produce adequate protection. Vaccination is currently the only form of specific prophylaxis against the disease and is highly recommended for PLWH.

Keywords: Anti-SARS-CoV 2- antibodies; HIV+ individuals, co-infection

Introduction: The new coronavirus infection has spread around the world rapidly, infecting millions of people, leading to significant disruptions of daily life, and causing an unprecedented number of deaths in recent years. Furthermore, the subsequent socio-economic and psychological damage will be difficult to overcome for a long time. At this stage of the pandemic, scientists efforts to create a vaccine have been successful, however those to target the virus with a specific etiological agent are still ongoing. The susceptibility of people living with HIV (PLWH) to SARS-CoV-2, as well as the risk of severe disease and adverse outcome from Covid-19, is still largely unknown. The altered immune system and constant use of antiretroviral therapy (ART) in this group of patients makes them pathognomonic and at increased risk for SARS-CoV-2. The antiretroviral medication Lopinavir/Ritonavir has been used experimentally to treat Covid-19, however it has proven to be unsuccessful.

The aim of our study is to analyze the status of PLWH relating to the new coronavirus infection. To study the presence and affinity of antibodies against SARS-CoV-2 and to test patients’ attitude towards specific vaccine prevention.
Materials and methods: In the Clinic of Infectious Diseases at the University Hospital "Prof. Dr. Stoyan Kirkovich", Stara Zagora 51 patients with HIV infection were treated and on-going observations were performed. For the purpose of the study, all were invited via telephone call to visit the Infectious Diseases Clinic with 41 patients accepting the invitation (80.39%). Their age at the time varied between 23 and 58 years, $\bar{x}$ 39.9±9.23 and a male predominance of 37 (90.24%). The patients were interviewed if they previously had a SARS-CoV-2 infection and/or vaccination. Clinical examinations, routine laboratory, immunological and virological tests were performed to determine the level of HIV viral load. CLIA chemiluminescent kits were used to detect antibodies against SARS-CoV-2. The above mentioned kits determine the amount of IgG antibodies against the antigen of the S1 receptor-binding domain of the Spike protein of SARS CoV-2. The results were processed using the methods of the static program SPSS, V. 21.

Results: Upon analysis only 9 (21.95%) of our 41 patients reported a past Covid-19 infection. Their reported clinical manifestations included the following: a mean temperature of 37.8°C ± 0.73; severe fatigue and cough. In 8 of the 9 (19.51%) Covid-19 patients a radiological examination of the lungs was performed. In 2 of the patients, a mild interstitial pneumonia was found, however it quickly began to improve. Seven (17.07%) of the participants reported they had a lack of taste and 3 (7.33%) had a loss of their sense of smell. Another 3 (7.33%) reported short-term diarrhea with up to 4 or 5 bowel movements per 24 hours and abdominal pain and 2 (4.87%) participants reported vomiting. In our patients the level of CD4+ cells ranges from 130 to 1283, $\bar{x}$ 463.70 ± 241.64 cells/mcL, CD8+ from 200 to 2233, $\bar{x}$ 653.62±367.01 cells/mcL. Eighteen patients (43.90%) have a CD4+ level below the laboratory reference values - $\bar{x}$ 220±90 cells/mcL). In 31 of the participants in our study, the HIV viral load was undetectable. The remaining 10 patients had a VL of 222 to 871,000, $\bar{x}$ 1506.81 ± 264.98. Three were recently admitted and started ART at the clinic, while the remaining patients did not have good adherence to therapy. The presence of SARS-CoV-2 antibodies was tested in all 41 patients. The values established a range from 0.12 to 209.50, $\bar{x}$ 37.64±18.26 AU/ml. According to the instructions of the kits used, the antibody titer at values above 33.8 AU/ml was considered protective. Ten (24.4%) of the patients were vaccinated, 3 of whom had an incomplete vaccination course. The most commonly used vaccine is BNT162b2 in 6 patients, mRNA-1273 in 3 patients and JNJ-78436735 in 1 patient. One of our patients became infected after their first BNT162b2 vaccine dose. From the patients examined, 12 (29.26%) had a protective antibody titer - they had values above 33.8 AU / ml. The remaining 29 (70.73%) were below the required minimum; ergo they had no protection. Of the 9 patients, who had a previous Covid-19 infection, 4 had no protective immunity. Two of them suffered from COVID-19 in the interval 8-10 months before their inclusion in the study, whereas in the other two patients with no protective immunity low levels of CD4+ respectively 130 and 32 copies/mcL were present. A protective titer of anti-SARS-CoV-2 antibodies was not detected in any of the HIV+ participants with a viral load level above 20 copies/ml; p = 0.019. The mean values of the detected antibodies in the vaccinated and unvaccinated are presented in Table 1. Value of SARS-CoV-2 antibodies in study participants with and without vaccine

<table>
<thead>
<tr>
<th>COVID-19 vaccination status</th>
<th>Number of people</th>
<th>Average value of anti-SARS-CoV-2 Ab</th>
<th>Min.</th>
<th>Max.</th>
<th>SD</th>
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<tr>
<td>Vaccinated</td>
<td>10</td>
<td>94,78</td>
<td>0,43</td>
<td>209,50</td>
<td>71,76</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>31</td>
<td>19,20</td>
<td>0,12</td>
<td>163,90</td>
<td>39,27</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>37,64</td>
<td>0,12</td>
<td>209,50</td>
<td>58,26</td>
</tr>
</tbody>
</table>
When asked what their attitude was towards the Covid-19 vaccines, 15 (36.58%) of our patients gave positive answers, 23 (56.09%) said they were strongly against, 3 (7.31%) had no opinion. **Discussion:** Common symptoms of Covid-19 are fever, cough, headache, shortness of breath, fatigue, loss of taste or smell, and gastrointestinal symptoms such as diarrhea, anorexia, nausea, and abdominal pain [1]. The data on the expression of clinical symptoms of Covid-19 in our, albeit small number of HIV+ patients, coincide with those reported in the literature. Recent studies on the incidence of Covid-19 in PLHIV are contradictory. Some researchers believe that these people are at greater risk of infection than others. Leading from this 86% of HIV+ patients receiving ART have such a beneficial effect from the therapy that their viral load is reduced to undetectable levels, so it is reasonable to believe that HIV+ people who carry out and adhere to ART are not considered immunocompromised [2]. However, according to some authors, PLWH have a 24% higher risk of SARS-CoV-2 infection and a 78% higher risk of death from Covid-19 compared with the rest of the population [3]. Other authors are of the opinion that Covid-19 standardized incidence rate is lower in PLWH than in the general population [4]. In our 9 patients the disease was mild. The clinical manifestations of Covid-19 are thought to be influenced by host factors such as male gender, older age and related diseases, as well as virus-related factors such as viral kinetics and viral load [5]. In our study, there was no clear age-related difference in the severity of Covid-19. In addition to HIV and Covid 19, two patients also have chronic hepatitis B, three have hypertension, and one is being treated for syphilis. We did not find a difference in the magnitude of fever and the duration of clinical symptoms compared to those without coinfections. Within this sample, male patients represented a significantly larger number and this coincides with the data from the literature [6]. Inflammatory changes in the lung, which is a major target in the course of Covid-19 such as bronchitis and pneumonia, can lead to acute respiratory distress, acute respiratory failure and even death. The extra pulmonary clinical manifestations of Covid-19 may affect many other systems, such as the cardiovascular, digestive system, urogenital and nervous system [7]. All our patients had fever and fatigue. In 15 (36.58%) of the patients there were symptoms as ageusia, anosmia, vomiting and diarrhea. Patel RH, et al. found an unexpectedly high recovery rate [8]. This contradicts the common notion of higher Covid-19 morbidity and mortality in immunocompromised patients. They noted that patients in stages 3 and 4 of HIV with lower CD4+ counts showed milder symptoms of Covid-19 [8]. Among the 9 HIV+ patients who survived the disease, 3 were in the first stage, 5 in the second and 1 in the third stage. Patients with a more severe Covid-19 disease course, showed a lower number of T lymphocytes (CD4+, CD8+) and B lymphocytes. Calza et al. found that HIV-positive patients with CD4+ counts below 258 cells/mcL recovered fully [9]. The explanation lies in the inability of the compromised immune system in HIV+ individuals to provoke a strong cytokine storm against the SARS-CoV-2 infection, which is at the root of the severe clinical course and possible fatal outcome [10]. The results of the present study show that HIV+ patients with lower CD4+ counts showed milder clinical symptoms and recovered faster after Covid-19 than others. However, no protective titer of antibodies against SARS-CoV-2 was detected in them. According to the literature, PLWH who are not being treated or are virally suppressed are at greater risk of severe Covid-19 [11]. Both HIV-1 and SARS-CoV-2 infection share CD4+ T cell loss in association with disease outcome and immunodeficiency [12]. A SARS-CoV-2 infection in HIV+ individuals is thought to alter the progression and prognosis of Covid-19 [13], however we did not report a similar trend. In all 9 of the Covid-19 patients, the outcome of the disease was favorable. There was no statistically significant difference in CD4, CD8 and VL between those who recovered from Covid-19 and those who did not have the infection, as well as between vaccinated and unvaccinated individuals. In 3 out of 10 patients who were vaccinated in which protective immunity had not been developed, CD4+ levels were below the lower limit of the reference values—respectively 239; 256 and 327 cells/mcL. We found a direct correlation between
CD4+ levels and the presence of a protective antibody titer against SARS-CoV-2. Viral load above 20 copies/ml correlates with a lack of protective antibody titer. There was a statistically significant difference between the two groups of patients - vaccinated and unvaccinated - p <0.005 in terms of the amount of antibodies against SARS-CoV-2. Covid-19 has had an undeniably negative impact on many aspects of everyday life. The coronavirus pandemic seriously disrupts communication between HIV+ patients and health professionals. Furthermore, healthcare attention, resources and staff are being diverted to the fight against COVID-19. The impact of this on the lives of people living with HIV is manifested in various aspects, such as the way they receive care for their condition and their antiretroviral drugs. Concerns about stigma may delay the diagnosis of COVID-19 and delay medical care. Many of our patients have been receiving antiretroviral therapy by courier since the beginning of the pandemic. The lack of regular contacts between the physicians and the patients affects their psychological state, deepens their sense of social isolation and disrupts the usual rhythm of routine examinations.

Conclusions: Overall, in 9 patients out of 41 included in this study, the Covid-19 infection was mild without requiring hospital treatment. Two had radiologically proven mild interstitial pneumonia. Patients with Covid-19 HIV + with CD4 levels below the reference limit did not show a more severe clinical course of the disease, however they had failed to produce protective antibody titers. Ten patients were vaccinated, of which one patient with an incomplete course of vaccination became infected with SARS-CoV-2. Less than half of our HIV+ patients had a mostly positive attitude towards Covid 19 vaccines. From now on, to show the benefits of SARS-CoV-2 vaccination, more research and good education of HIV patients should be done. It is hoped that a more targeted vaccine with fewer side effects will be implemented in the near future to improve the delivery of medical services, prevention and treatment of CHD and provide a more comfortable environment from which patients and medical staff can benefit.

References:
Epidemiological study of measles in the Bulgaria and Varna region for 2017-2022

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¹) Department Hygiene and Epidemiology, Faculty Public health, Medical University Varna, Bulgaria
²) Department Microbiology and Virology, Faculty Medicine, Medical University Varna, Bulgaria

Abstract
In recent years in Bulgaria, the risk of measles importation and spread remains high, especially among some ethnic communities with a high percentage of unvaccinated persons against measles. The purpose of our research is to conduct a retrospective study of the incidence of measles in Bulgaria and in the Varna region for the period of 2017 - 2021 and to assess the factors influencing the resurgence of the disease in the country and the low immunization coverage of children with the MMR vaccine.

Key words: measles, epidemiology, Bulgaria, vaccination

Introduction
Measles is a highly contagious, potentially fatal, but vaccine-preventable disease caused by a paramyxovirus (measles virus, MV) that can easily spread through airborne respiratory droplets or by direct contact with upper respiratory tract secretions of infected individuals (Kaić & Tešović, 2019). Humans as the only reservoir for the virus and the symptoms include fever, maculopapular rash, and at least one of cough, coryza, or conjunctivitis, although vaccinated individuals can have milder or even no symptoms (Hübschen et al., 2022). The global measles vaccination program has been extraordinarily successful in reducing measles-related disease and deaths worldwide (Gastañaduy PA et al., 2021), but the number of reported worldwide measles cases has increased by 79 per cent in the first two months of 2022 compared to the same time last year. It is a worrying sign of an increased risk for the spread of the highly contagious virus and other vaccine-preventable diseases (Unicef, 2022). The World Health Organization (WHO) and UNICEF describe the current condition for measles outbreaks as a perfect storm. Pandemic-related disruptions, increasing inequalities in access to vaccines. In 2020, 23 million children missed out on all basic childhood vaccines. That is the highest number seen since 2009 and 3.7 million more than in 2019 (WHO, 2022).

Purpose
To conduct a retrospective study of the incidence of measles in Bulgaria and in the Varna region for the period 2017-2021 and to assess the impact of the Covid-19 pandemic on the immunization coverage of children with the MMR vaccine.

Materials and methods
Epidemiological data from the reports of RZI-Varna, the National Center for Infectious and Parasitic Diseases in the Republic of Bulgaria, ECDC and WHO were used.

Results
In the pre-pandemic years from 2017 to 2019, the epidemic process of measles in Bulgaria took place sporadically and in the form of limited epidemic outbreaks, due to the "importation" of the infection by Bulgarian and foreign citizens who resided and became ill in other European countries (Table 1, 2 and 3).
Table 1. Number of people infected with measles in Bulgaria and the Varna region in the period 2017-2021.

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Bulgaria</td>
<td>165</td>
<td>13</td>
<td>1231</td>
<td>257</td>
<td>0</td>
</tr>
<tr>
<td>Varna region</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Incidence (1/100,000) of measles in the Varna region and Bulgaria for the period 2017-2021.

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>Bulgaria</td>
<td>2,32</td>
<td>0,18</td>
<td>17,59</td>
<td>3,7</td>
<td>0</td>
</tr>
<tr>
<td>Varna region</td>
<td>0</td>
<td>0</td>
<td>1,06</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

The two outbreaks of measles that occurred in 2017 are limited geographically in three regions of Bulgaria: one blast in Plovdiv and Pazardzhik (n=161), and the second in Montana (n=4). The first cases in the regions of Plovdiv and Montana were "imported" by persons who fell ill in Germany and Romania and returned to the country. The cases were laboratory confirmed with proven measles virus genotype B3 MVs/Dublin.IRL/08.16. In the course of the epidemic outbreak in Plovdiv and Pazardzhik, hospital-acquired measles infections and new family outbreaks among the public occurred. The majority of sick persons (89%) are of Roma origin. The disease mainly covers children aged 0 to 9 years, infants without immunity against measles due to incomplete immunization age, unvaccinated children and children with incomplete reimmunization (table 3) (NCIPD, 2018).

In 2018 imported into the country measles cases (n=18) are of Bulgarian citizens who resided in European countries Greece, Italy, Portugal and Great Britain, and foreigners from Ukraine and Russia who entered the country with temporary work visas for the summer season under the Bulgarian Black Sea resorts. The immunization status of the patients – 3 persons are unvaccinated, two have two doses of measles vaccine and 8 have an unknown measles vaccination status. The majority of patients (76.92%) are over 15 years old. (NCIPD, 2019).

In Bulgaria, we observe a recovery of the endemic spread of measles in 2019, which continues until March 2020, when the first cases of Covid-19 are announced in the country and anti-epidemic measures are imposed. At the beginning of February 2019 two epidemic outbreaks occurred in the Blagoevgrad and Sofia city regions, caused by two genotypes of measles virus B3 and D8, circulating in the countries of the European continent. The disease spread quickly and by the end of the year, it covered 15 districts in the country, 1231 cases of measles were registered, which determined the incidence rate at 17.59 per 100,000. Hospital outbreaks were found in children's wards, affecting children and medical staff. Of the sick, 38.76% were unvaccinated and 21% had an unknown immunization status for measles vaccination. 36.39% were vaccinated with 1 dose and 3.76% with two doses. (NCIPD, 2020).
**Table 3.** Relative share by age group of measles patients in the Republic of Bulgaria and for the period 2017-2021.

<table>
<thead>
<tr>
<th>Years</th>
<th>&lt;1</th>
<th>1-4</th>
<th>5-9</th>
<th>10-14</th>
<th>15-19</th>
<th>20-29</th>
<th>&gt;30</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>36</td>
<td>47</td>
<td>24</td>
<td>12</td>
<td>13</td>
<td>13</td>
<td>20</td>
<td>165</td>
</tr>
<tr>
<td>2018</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>13</td>
</tr>
<tr>
<td>2019</td>
<td>178</td>
<td>394</td>
<td>365</td>
<td>66</td>
<td>83</td>
<td>117</td>
<td>1231</td>
<td>1231</td>
</tr>
<tr>
<td>2020</td>
<td>36</td>
<td>67</td>
<td>50</td>
<td>22</td>
<td>7</td>
<td>30</td>
<td>45</td>
<td>257</td>
</tr>
</tbody>
</table>

In the Varna region, during the epidemic rise of measles in the country in 2019, 5 cases were registered, distributed in the age groups 1-4 years (n=2), 5-9 years. (n=1) and 20-29 years. (n=2). The patients are unvaccinated or of unknown immunization status. (RHI, analyses)

The pandemic spread of Covid-19 led to the cessation of the epidemic spread of measles in Bulgaria and in the Varna region after March 2020. The disruptions in the health services of the population had an impact on the immunization coverage of those subject to the MMR vaccine, which reached values for the country below the critical 90% (Table 4).

**Table 4.** Immunization coverage (%) with MMR 1 (at 13 years old) and MMR 2 (at 12 years old) in Bulgaria for the period 2017-2021.

<table>
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<tbody>
<tr>
<td>MMR 1</td>
<td>93,8%</td>
<td>92,9%</td>
<td>96,1%</td>
<td>88,3%</td>
<td>88,7%</td>
</tr>
<tr>
<td>MMR 2</td>
<td>91,6%</td>
<td>87,4%</td>
<td>92,9%</td>
<td>84,5%</td>
<td>85,8%</td>
</tr>
</tbody>
</table>

**Discussion**

After introduction into the immunization programs of the global initiatives for the prevention and elimination of measles by the WHO member countries, in the period 2000-2018. MCV1 immunization coverage increased globally from 72% to 86%. Annual measles incidence has decreased by 66% and measles deaths have decreased by 73% (WHO, 2022). However, the number of measles cases in 2018 increased by 167% worldwide compared to 2016, and the estimated global death rate from measles has increased since 2017. (Patel MK, Dumolard L 2019).

In the US, 2019 saw the highest number of cases reported since 1992, with measles disease tending to spread and cause outbreaks in US communities where there are unvaccinated populations (CDC, 2022).

We observe a similar trend among the countries of the WHO European Region. According to ECDC, 14,600 (28.3 pmp) measles cases were reported in 2017; in 2018 – 17,822 (34.4 pmp); in 2019 – 13,200 (25.4 pmp); in 2020 we have data for the first half of the year – 1917 cases (ECDC, 2022). According to the ECDC, however, in 2020 and 2021, monthly reported measles cases were at their lowest levels in the time frame provided, which may have been influenced by the increased social distancing measures implemented due to the COVID-19 pandemic. In 2020, Romania had the highest rate of measles in Europe with over 50 cases per million population (pmp). This was followed by Bulgaria with approximately 35 pmp cases and Russia where there were 7.54 pmp cases (Statista. 2022).

In Bulgaria in 2019 a "revival" of the measles infection is established, assuming its endemic and nosocomial spread in the country, which further contributes to the intensity of the epidemic process. The unsatisfactory
level of immunization coverage with both MMR 1 and MMR 2 regimens, especially in the Covid-19 pandemic years, refusal of immunization, population migration and vaccine supply difficulties put at risk the maintenance of measles infection elimination on a national scale.

**Conclusion**
The spread of measles in Bulgaria in the pre-pandemic years from 2017 to 2020 characterized by limited epidemic outbreaks and epidemic spread in 2019. The patients are mainly persons of Roma origin, unvaccinated or with incomplete immunization schemes with the MMR vaccine.
The accumulation of a non-immune population in Bulgaria and a deepening migrant and refugee crisis hide a serious risk of a resumption of the epidemic spread of measles in the country and will prevent the achievement of the desired goal - elimination of measles in the future.

**References:**
A Case Report of a Co-Infection with SARS-CoV-2 and Rhinovirus at the Beginning of the Pandemic in Bulgaria

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2) Clinic of Infectious Diseases, University Hospital “Prof. Dr. Stoyan Kirkovich” AD - Stara Zagora, Bulgaria

Abstract: The evaluation of the impact of co-infections was greatly hampered during the peaks of the COVID-19 pandemic. Retrospective studies of cases, taking into account the presence of more than one respiratory pathogen, could reveal the mechanisms of pathogen interactions. Case report: The laboratory findings, clinical management, and the outcome in a patient with co-infection of SARS-CoV-2 and rhinovirus in the beginning of the pandemic in Bulgaria, are reported in this case report. The patient had undergone a coronary bypass in the past and suffered non-insulin-dependent diabetes mellitus. After more than 2 weeks of influenza-like symptoms, the patient sought medical attention. No clearance of either coronavirus or rhinovirus infection was observed within the two months of hospital stay. The patient passed away after 58 days in hospital. Conclusions: The lethal outcome is largely due to the poor premorbid terrain and the existence of a co-infection.

Keywords: COVID-19, rhinovirus, co-infection

Background

In Bulgaria, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in the beginning of March 2020. Since then, till the end of November 2022, the number of cases has reached 1,287,035 and 38,039 associated deaths were recorded [1].

Because of the public health emergency and the fast spreading of the novel virus, in many areas of the world, including Bulgaria, testing for SARS-CoV–2 negatively affected the availability of diagnostic testing supplies and the diagnostic capacity for other respiratory viral pathogens. Therefore, the majority of laboratories performed SARS-CoV–2 testing with priority. In many clinical settings, in case of a positive SARS-CoV–2, further testing for other respiratory viruses was discouraged. As a result, many viral infections considered minor and mild like those caused by rhinoviruses, remained undiagnosed and the role of possible co-infections could not be assessed.

We performed a single institution retrospective study of SARS-CoV-2 positive patients who presented to the Clinic of Infectious Diseases at the “Prof. Dr. Stoyan Kirkovich” University Hospital, Stara Zagora. All nasopharyngeal swabs, collected from March to May, and from September to November, 2020 (the period of active circulation of rhinoviruses), were re-tested for the presence of rhinovirus RNA via RT-PCR technique. A total of 5 patients out of 533 positive COVID-19 patients were identified to have co-infection with rhinovirus. The aim of this case report is to present the patient characteristics, laboratory findings, and the lethal outcome in one of the patients with co-infection of SARS-CoV2 and rhinovirus, in whom no clearance of either coronavirus or rhinovirus infection was observed within the two months of his hospital stay. This clinical case highlights the importance of establishing a diagnosis based on the clinical findings.
and the patient's history bearing in mind the possibility of a co-infection with other respiratory viruses, which may have an aggravating effect on the clinical course.

**Case Presentation**

A 61-year-old male patient presented at the Clinic of Infectious Diseases at the "Prof. Dr. Stoyan Kirkovich” University Hospital in Stara Zagora in the beginning of the COVID-19 pandemic in Bulgaria. The patient has suffered influenza-like symptoms 15 days before hospital admission with complaints of progressive shortness of breath, bilateral chest pain, fever with chills, muscle and joint ache, unproductive cough and rise of body temperature to 39°C in the last two days before the hospital visit.

The patient had undergone a coronary bypass 18 years ago and suffered non-insulin-dependent diabetes mellitus (NIDDM).

On presentation, he had a temperature of 36.9°C and blood pressure of 100/60 mmHg. The rapid test for IgG antibodies against SARS-CoV-2 was found positive. Physical examination revealed a significantly damaged general condition with signs of intoxication, hyperemic throat and moist wheezing in the right lung. Following the hospital’s testing guidelines at the time, blood samples for influenza and nasopharyngeal swab for SARS-CoV-2 test were taken and sent to laboratory. The patient tested positive via reverse–transcriptase–polymerase-chain–reaction (RT–PCR) for SARS-CoV-2. IgM antibodies for influenza A and B were tested via ELISA and were both in “gray zone”. The nasopharyngeal swabs were negative for pathogenic bacteria and candida.

Laboratory tests at admission detected plasma glucose 14.16 mmol/l, serum creatinine 132.0 umol/L, C-reactive protein 153.3 mg/L. Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were slightly evaluated. Chest radiograph revealed bilateral pneumonia.

The patient was treated with a double antibiotic combination of ceftriaxone and oral azithromycin, hydroxychloroquine, corticosteroids in moderate doses (considering the premorbid diabetes), anticoagulants, and symptomatic agents.

Blood sugar was monitored on every 6 hours and fast-acting insulin was administered according to an approved schedule.

However, the patient’s symptoms worsened. The blood-gas analysis (Table 2) at the 6th day of his hospital stay showed 39% oxygen saturation, which prompted his admittance to the Intensive Care Unit. There, further nasopharyngeal swabs were taken and subjected to microbiological and virological tests. The swabs were tested negative for pathogenic bacteria and candida, and positive for SARS-CoV-2, again. Subsequent laboratory tests showed moderate leukocytosis with severe lymphopenia, followed by an anemic syndrome. After 5 days of oxygen supplied by mask, the blood-gas results deteriorated, showing acute respiratory failure which required artificial pulmonary ventilation.

As the blood glucose concentration remained above the reference range for a prolonged period of time, the patient developed severe diabetic ketoacidosis and acute renal failure. Inflammation markers - LDH (lactate dehydrogenase) and C-reactive protein (CRP), showed active inflammation and tissue disintegration. The third round of respiratory samples taken for microbiological and virological testing confirmed the presence of SARS-CoV-2 RNA again and the absence of pathological bacterial or fungal growth.

The patient passed away after 58 days in the hospital and 52 days in ICU. At the time of his death results for rhinovirus testing were still pending. Retesting of the collected swabs proved co-infection with SARS-CoV-2 and rhinovirus in all the three samples, taken on 1st, 6th, and 14th day of his hospital stay. As the main cause of death, we assumed that it was a severe COVID-19 on the background of poorly managed diabetes, and the negative impact of the co-infection.
Table 2. Dynamics of blood-gas analysis results. Light gray – days after ICU admission and oxygen mask. Dark gray – the period after patient was put on artificial ventilation.

<table>
<thead>
<tr>
<th># Day in hospital</th>
<th>Sat O2 % [range:95-100]</th>
<th>pCO₂, mmHg [range:35-45]</th>
<th>pO₂, mmHg [range:75-100]</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>39.00</td>
<td>36.30</td>
<td>24.20</td>
</tr>
<tr>
<td>11</td>
<td>99.20</td>
<td>45.80</td>
<td>208.00</td>
</tr>
<tr>
<td>12</td>
<td>98.00</td>
<td>38.10</td>
<td>90.50</td>
</tr>
<tr>
<td>14</td>
<td>50.10</td>
<td>51.10</td>
<td>28.70</td>
</tr>
<tr>
<td>17</td>
<td>98.00</td>
<td>40.20</td>
<td>106.00</td>
</tr>
<tr>
<td>58</td>
<td>70.70</td>
<td>26.70</td>
<td>32.50</td>
</tr>
</tbody>
</table>

Discussion

There are a few reports worldwide of patients co-infected with SARS-CoV-2 and other respiratory viruses. According to these reports the effect on disease severity and outcomes in co-infected patients are very controversial [2, 3]. Several studies describe co-infection with multiple respiratory pathogens in children. These co-infections may contribute to the severity of acute viral respiratory disease [4, 5]. Little is known about the effect of co-infections with more than one viral pathogen in adults. Multiple studies reported rhinoviruses as the most frequent or among the most frequent respiratory viruses co-infecting COVID-19 patients [6, 7, 8]. To our knowledge this is the first report of co-infection with SARS-CoV-2 and rhinovirus in Bulgaria. In the country, the diagnostics of rhinoviruses remains poor, primarily because the infection is assumed to be mild and self-limiting. Nevertheless, in patients with underlying premorbidity the clinical manifestations and pathogenesis of rhinovirus infections may be severe and similar to COVID-19.

In the case presented here, the co-infection of rhinovirus and SARS-CoV-2 was diagnosed post mortem and was not considered during treatment, although a potential possibility of a co-infection was discussed. Moreover, the detection of both viruses simultaneously in the patient's samples was maintained over a prolonged period of time, which was described previously [9].

A high glucose concentration has been shown to be an independent predictor of death and morbidity in COVID-19 [10]. Despite constant monitoring and treatment, blood sugar levels remained high in the presented case.

Multiple factors contributed to the patient’s cause of death including the severe viral pneumonia, which could be a result of the co-infection, poorly managed diabetes and delayed professional medical help.

Conclusion

In conclusion, since the future of the current SARS-CoV-2 pandemic is unknown a close surveillance and investigation of the co-incidence and interactions of SARS-CoV-2 and other respiratory viruses should be widely available to improve patient management and optimize healthcare resources and to understand the interaction between SARS-CoV-2 and other viruses.

Acknowledgments:

This work was supported by project KP-06-H23/10 of the Bulgarian National Science Fund, Ministry of Education and Science, Sofia, Bulgaria

References:

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Epidemiological analysis of acute intoxications that passed through the Department of Toxicology, Naval Hospital- Varna over an 8-year period

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Abstract

**Purpose:** Acute drug intoxications are one of the most common critical cases in emergency departments and toxicology clinics and require a lot of resources and lead to thousands of hospitalizations worldwide. The prevalence of acute poisoning varies by religious, cultural and geographical context and is dynamic.

**Aim:** The aim of the study was to analyze the acute drug intoxications relating to age and sex, etiological distribution of acute drug intoxications and following the dynamics of acute drug intoxications.

**Material/methods:** The object of the study was 1100 patients with acute drug intoxications that have passed through the Department of Toxicology, Naval Hospital- Varna for the period 2010-2018. Statistical and documentary methods were used.

**Results:** The average age of the patients with acute drug intoxications was 42.91 years. The ratio of women to men was 3:1. Depression was the most common comorbidity. The most commonly overdosed drugs were cardiovascular, benzodiazepines, neuroleptics, antidepressants and anxiolytics. A positive trend was observed in reducing the number of acute drug intoxications with the lowest frequency in 2018.

**Conclusion:** acute drug intoxication is a serious problem observed mainly among young people and requires increased control when prescribing psychotropic drugs, greater psychological support from family, relatives and medical specialists.

**Keywords:** analysis, drug overdose, epidemiology, intoxication, mortality

Introduction

Acute drug intoxications are one of the most common life-threatening cases in emergency departments result of the advancement of the economy, industrialization, the change in lifestyle and daily stress. A drug overdose requires a lot of resources and leads to many hospitalizations worldwide. Many cases of poisoning are associated with high morbidity and mortality. The prevalence of acute poisoning is dynamic and affected by factors such as education, culture, religion and geographic area. The most commonly overdosed drugs are antidepressants, neuroleptics and cardiovascular drugs [1].

The aim of the study was to analyze the acute drug intoxications relating to age and sex, etiological distribution and following the dynamics of acute drug intoxications which are registered in the Department of Toxicology, Naval Hospital- Varna. A better understanding of poisonings will help develop more effective educational and treatment strategies that would reduce morbidity and mortality from acute drug intoxications.

Materials and methods

The object of the study is 1100 patients with acute drug intoxications that have passed through the Department of Toxicology, Naval Hospital- Varna. The study is retrospective and covers an 8-year period. Access to the medical documentation is provided with the permission of the Ethics Committee of the Naval Hospital- Varna. An analysis was made of the following indicators obtained from the medical documentation: age, sex, comorbidity, etiological distribution of acute drug intoxications and cause of hospitalization. The statistical analysis was performed using the statistical functions in “Excel 2016”, software package “Statistica 7.0”. For all statistical analyzes performed, an acceptable level of confidence
level P <0.05 is assumed, divided into three ascending classes: P <0.05, P <0.01 (high significance) and P <0.001 (very high significance).

Results
Statistical analysis showed that acute drug intoxications are most common among the working population, namely those aged up to 45 years. The average age of the patients was 42.91 years. For the studied period, 186 suicide attempts of persons under 18 years of age were registered (16.9%). The total number of intoxications in people over 18 years old is 914 (83.09%). Acute drug intoxications were more frequent among women (73.45%), (p<0.01). The ratio of women: men was 3:1. Attempted suicide was the most common cause of overdose 91.18%, (p<0.01), followed by taking drugs by mistake (3.72%), toxic effects of drugs (3%) and other reasons (2.09%). Depression was the most common comorbidity - 44.89% (p<0.01) followed by arterial hypertension (13.9% (p<0.01) and schizophrenia 9.48% (p<0.01). Intoxications with drugs that have cardiotoxic effects prevailed (59.63%). The drugs that were most often overdosed were cardiovascular, benzodiazepines, neuroleptics, antidepressants and anxiolytics. A total of 124 patients overdosed on cardiovascular medications, with the highest frequency of calcium channel blockers (52.42%), followed by beta-blockers (50%) and cardiac glycosides (8.06%). A total of 532 patients overdosed on drugs affecting the central nervous system, with the highest frequency of intoxications with benzodiazepines (57.89%), neuroleptics (26.69%), selective serotonin reuptake inhibitors (11.65%), flupentixol/melitracen combination (6.01%) and tricyclic antidepressants (3, 95%). In 444 patients, mixed intoxications with antiepileptics, anxiolytics, non-steroidal anti-inflammatory drugs, alcohol, organophosphorus compounds, anti-infectious and anti-allergic drugs were found (40.36%). For the period 2010-2018, 61 deaths were registered (5.54%). However, there is a positive trend in reducing the number of acute drug intoxications with the lowest frequency in 2018 (93 patients), compared to 2010, when 142 drug intoxications were registered.

Discussion
The study found that acute drug intoxications were most common among the working population and were more common among women. The most common cause of drug overdose is the suicide attempt. Similar results have been observed in other countries [2]. The study found a high incidence of acute drug intoxication among young people. A high number of suicide attempts among young people has been recorded in other countries as well [3]. Depression is the most prevalent comorbidity in the patient population studied. Psychiatric diseases leading to suicide attempts were also registered by other authors [4,5]. The use of benzodiazepines, neuroleptics and selective serotonin reuptake inhibitors in psychiatric disorders is the reason for the high frequency of suicide attempts with them. The situation is similar in the USA, where benzodiazepines and opioid analgesics were most often used for suicide in the period 1999-2017 [6]. The incidence of poisoning with tricyclic antidepressants is the lowest, since nowadays they are no longer a first-line treatment for depression [7]. The use of selective serotonin reuptake inhibitors is constantly increasing and reflects the increase in their prescriptions [8]. Intoxications with them are 5.63%. According to our results, the most common are intoxications with cardiovascular drugs, namely calcium channel blockers. The situation is similar in the USA [9], and in Sweden, suicide attempts with calcium blockers have a frequency of 1.1 suicides per 1000 persons per year [10]. Their widespread use makes them readily available as a means of suicide.

Conclusion
Acute drug intoxication is a serious problem. The most commonly overdosed drugs are cardiovascular, benzodiazepines, neuroleptics, antidepressants and anxiolytics.
Persons under 18 years are a risk group for suicide and training of psychologists and doctors in schools for suicide prevention at this age is needed.

For the working population, early screening and treatment of depression can be an important factor in preventing suicide attempts. The development of strategies to deal with interpersonal conflicts as well as policies limiting the amount and type of drugs purchased by teenagers would be beneficial in preventing drug overdoses.

References

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The influence of information sources on COVID-19 vaccine acceptance in Bulgaria

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Medical University-Varna

Abstract

This study examines the associations between preferred information sources, risk perception, and COVID-19 vaccine acceptance in Bulgaria. In April 2022, a cross-sectional study was conducted on a convenience sample of 1,200 Bulgarian residents using a self-administered online questionnaire. Bivariate statistical analyses revealed significant differences in information source choices between vaccine acceptors and non-acceptors. Obtaining information from health professionals, health officials, scientific publications, traditional media, and communities increased the odds of accepting vaccination. Respondents relying on credible sources were also less concerned about vaccine safety. The results did not show significant associations between social and Internet news media use, risk perceptions, and vaccine uptake. Providing accurate information based on scientific facts and dispelling misinformation have the potential to overcome vaccine hesitancy; thus, communication is an essential tool in promoting vaccination.

Keywords: COVID-19, vaccine, information source, vaccine acceptance, risk perception

Introduction. The COVID-19 pandemic ushered a new wave of misinformation and fake news. The World Health Organization warned of an “infodemic” - an abundance of inaccurate or even dangerous information from non-health actors [1]. Lack of knowledge about the vaccine and contradictory and ambiguous messages from community leaders and experts contributed to hesitancy. Misinformation has become a significant barrier to achieving coverage targets [2]. The COVID-19 pandemic saw excessive reliance on digital communication. Social media and other non-traditional communication channels disseminated anti-vaccination messages and misinformation [3, 4], which influenced vaccine attitudes and increased vaccine hesitancy [5].

The aim of study is to examine the associations between preferred information sources, risk perception, and COVID-19 vaccine acceptance in Bulgaria.

Materials and Method. In April 2022, a cross-sectional study was conducted in a convenience sample of 1,200 adults residing in Bulgaria. The majority of respondents were reached via social media and the authors’ networks using a snowball technique. A self-administered online questionnaire was used to collect data on vaccine attitudes, perceived risks and benefits of the COVID-19 vaccine, use of various information sources, and sociodemographic variables. Participants were questioned about their use of various sources for acquiring information about the vaccine. We use four-point scale ranging from “very often” to “never”, which was dichotomised for the analyses into “yes” and “no” responses on the use of each source. Bivariate statistical analyses (chi-square tests) were employed to examine the associations between information sources, risk perceptions, and acceptance of the COVID-19 vaccine.

Results Out of the 1,200 participants who completed the survey, 872 (62.7%) were female. A majority of the respondents (78.0%) had a university degree. About 59% had already had at least one dose of the COVID-19 vaccine, and 25% had received a booster. Medical specialists were the most preferred source for information (65.1%), followed by family and friends as a trusted source for 62.6% of the respondents. Sixty percent of the participants obtained information about the vaccine through specific digital channels (Internet news media and health authorities’ websites), whereas 42.6% used social media outlets. The bivariate
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analysis found significant differences in information source choice and use between vaccine acceptors and non-acceptors (i.e., refusers and hesitators) as shown on Figure 1.

**Figure 1.** Associations between information source use and COVID-19 vaccine acceptance

![Graph showing associations between information source use and COVID-19 vaccine acceptance](image)

The odds of COVID-19 vaccine acceptance were two times greater for respondents who used information provided by health authorities ($p<.001$). Participants who sourced information from medical specialists were almost twice less likely to be vaccine-hesitant ($OR=1.92, p<.001$). Those relying on information from scientific publications, traditional media, communities, and pharmacists were also more inclined to accept the vaccine. Relatives and friends, as well as social media, were preferred by vaccine non-acceptors. However, there were no significant associations between the use of social media platforms and Internet news media and vaccine uptake. We also examined the relationship between information sources and the perceived risk of the vaccine, hypothesising that access to accurate information may affect risk perceptions. Descriptive statistics revealed that participants relying on online news, social media or relatives and friends had higher risk perceptions compared to those obtaining information from medical specialists, health authorities, scientific publications or traditional media. However, the bivariate analysis did not show a significant association between the use of social and other non-traditional media outlets and the perceived vaccine risk (Table 1).

**Discussion.** The relationship between the information source, vaccine risk perceptions, and vaccination acceptance has been largely validated by our study. Respondents who acquired information from reliable sources were less concerned about vaccine safety and assessed the likelihood of adverse events to be low. Consistent with other studies, health professionals seem to be the most reliable and trusted source of information with the potential to increase vaccine uptake [6]. Information provided by health officials and traditional news channels also improved the probability of vaccine acceptance [7]. A growing body of research demonstrated the adverse effect of information provided through social media platforms on public attitudes towards the COVID-19 vaccine and the pandemic in general [4, 7]. Unlike other studies, we did not find a significant association between social media usage and increased risk perceptions. Limitations: The sampling technique employed precluded a representative sample of the Bulgarian population, and the use of an online questionnaire resulted in the underrepresentation of some subgroups, including the elderly, those with lower levels of education, and most notably, the unvaccinated.

**Conclusion.** Lack of confidence in COVID-19 vaccine safety and effectiveness, combined with poor communication, posed a significant obstacle to the COVID-19 vaccine uptake in Bulgaria. Misinformation and false claims undermined vaccine acceptance and further eroded confidence in health authorities and
Communication has never been more apparent as a tool to address vaccine hesitancy and promote health-protective behaviour. Neglecting communication at the outset of vaccination programs can impede vaccine uptake and encourage antivaccination propaganda. Effective communication based on credible information and reliable sources can be instrumental in encouraging vaccine uptake and overcoming vaccine hesitancy.

### Table 1. Perceived risk of COVID-19 vaccine by information source users

<table>
<thead>
<tr>
<th>Information source</th>
<th>N</th>
<th>Perceived risk of vaccine, %</th>
<th></th>
<th></th>
<th></th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>low</td>
<td>medium</td>
<td>high</td>
<td></td>
<td></td>
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<tr>
<td>Medical specialists users</td>
<td>670</td>
<td>51.8</td>
<td>21.6</td>
<td>26.6</td>
<td>21.8</td>
<td>&lt;.001*</td>
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<tr>
<td>non-users</td>
<td>324</td>
<td>38.0</td>
<td>21.9</td>
<td>40.1</td>
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<tr>
<td>Pharmacists users</td>
<td>336</td>
<td>49.4</td>
<td>25.6</td>
<td>25.0</td>
<td>9.84</td>
<td>0.007*</td>
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<tr>
<td>non-users</td>
<td>658</td>
<td>46.2</td>
<td>19.8</td>
<td>34.0</td>
<td></td>
<td></td>
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<tr>
<td>Health authorities’ websites users</td>
<td>612</td>
<td>53.3</td>
<td>22.5</td>
<td>24.2</td>
<td>36.3</td>
<td>&lt;.001*</td>
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<tr>
<td>non-users</td>
<td>382</td>
<td>37.7</td>
<td>20.4</td>
<td>41.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scientific publications users</td>
<td>622</td>
<td>52.4</td>
<td>21.4</td>
<td>26.2</td>
<td>21.6</td>
<td>&lt;.001*</td>
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<tr>
<td>non-users</td>
<td>372</td>
<td>38.7</td>
<td>22.3</td>
<td>39.0</td>
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<td></td>
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<tr>
<td>Traditional media outlets users</td>
<td>550</td>
<td>50.5</td>
<td>23.3</td>
<td>26.2</td>
<td>13.3</td>
<td>0.001*</td>
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<tr>
<td>non-users</td>
<td>444</td>
<td>43.2</td>
<td>19.8</td>
<td>36.9</td>
<td></td>
<td></td>
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<tr>
<td>Internet news media users</td>
<td>603</td>
<td>46.1</td>
<td>22.6</td>
<td>31.3</td>
<td>0.994</td>
<td>0.608</td>
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<tr>
<td>non-users</td>
<td>391</td>
<td>49.1</td>
<td>20.5</td>
<td>30.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social media platforms users</td>
<td>434</td>
<td>44.0</td>
<td>21.9</td>
<td>34.1</td>
<td>4.17</td>
<td>0.124</td>
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<tr>
<td>non-users</td>
<td>560</td>
<td>49.8</td>
<td>21.6</td>
<td>28.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relatives and friends users</td>
<td>619</td>
<td>43.9</td>
<td>24.9</td>
<td>31.2</td>
<td>11.4</td>
<td>0.003*</td>
</tr>
<tr>
<td>non-users</td>
<td>375</td>
<td>52.8</td>
<td>16.5</td>
<td>30.7</td>
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<td></td>
</tr>
<tr>
<td>Communities users</td>
<td>476</td>
<td>52.1</td>
<td>21.0</td>
<td>26.9</td>
<td>9.65</td>
<td>0.008*</td>
</tr>
<tr>
<td>non-users</td>
<td>518</td>
<td>42.9</td>
<td>22.4</td>
<td>34.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**References:**

1. World Health Organization. Working together to tackle the “infodemic”. News release. 29 June 2020. [Internet]

Cell mediated immunity in COVID-19 patients evaluated by Interferon Gamma Release Assay (IGRA)

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Abstract

Aim. SARS-CoV-2 infection can trigger extremely severe inflammatory reactions and elicit an immune response, combining both arms of the immune system – humoral and cellular immunity. The acquisition of the latter is crucial to obtaining a prolonged cell-mediated immunological memory. In this study we aimed to evaluate the cell-mediated immunological response in blood in the first 6 months after SARS-CoV-2 infection.

Material/Methods. To investigate the longevity of T-cell response in convalescent patients we implemented an evaluation of both humoral and cellular immune responses during the 1st, 3rd, and 6th month after symptoms onset. By performing an Interferon Gamma Release Assay (IGRA) we evaluated in dynamics the T-cell response to in vitro stimulation with SARS-CoV-2 peptides. The anti-SARS-CoV-2 IgG levels were assessed via Enzyme Linked Fluorescent Analysis (ELFA).

Results. Results showed that the humoral response is exceedingly declining after the first month post symptoms onset as this occurrence persists to deteriorate in the following months. Whereas the T-cell response is maintained up to six months after initial infection with the viral pathogen, although the amount of secreted interferon gamma (IFN-γ) is gradually declining.

Conclusion. This study underlines the importance of obtaining cell-mediated immunity to preserve a prolonged viral defense wall.

Key words: COVID-19, IGRA, interferon gamma, cellular immunity, SARS-CoV-2.

Introduction

Humoral and cell-mediated responses are both required to manage SARS-CoV-2, an infectious viral agent that has caused over two million deaths to date. Substantial interest exists in learning how cellular immune responses operate to reduce acute infection and protect against reinfection. The antibody response to SARS-CoV-2 is defined by reactions to various viral proteins, including the spike, nucleocapsid, and membrane antigens. The humoral response is usually more frequently evaluated when investigating the body’s immunological condition post-encounter with certain infectious agents. It is known, however, that antibodies decline after clearance from the initial infection and their reliability is not abiding (1). T-memory cells, on the other hand, contribute to the accumulation of long-lasting cellular immunity (2).

Due to the immense general interest in exploring the longitude of cell-mediated immunity to SARS-CoV-2, we conducted this study in an attempt to shed light on this popular topic.

Materials and Methods

Study design. In this prospective observational cohort study, cellular and humoral SARS-CoV-2 specific immune responses were evaluated in patients with natural infection, confirmed by positive PCR test result. A total of 190 patients were included - 67 males and 123 females, respectively, aged between 22 and 95 years (M = 50.4, SD = 17.7). Samples were collected between April 2022 and October 2022 and classified into three groups, based on sample acquisition: Group 1: less than 30 days post symptoms onset (PSO), Group 2: 1–3 months PSO, and Group 3: 6–8 months PSO.
IGRA for SARS-CoV-2 testing. Interferon-gamma release assay (IGRA) for the evaluation of SARS-CoV-2 specific T-cell response was performed using the QuantiFERON® SARS-CoV-2 Two Plate ELISA Kit (Qiagen). The stimulus in the kit is Ag3 – a pool, consisting of S, M, N peptides and other parts of the SARS-CoV-2 genome. The amount of released interferon-gamma was measured via ELISA according to manufacturer’s protocol. Absorbance was measured on Microplate reader 800 TS (BioTek). Results interpretation (positive, negative, intermediate) was made as per manufacturer’s instructions.

Virus-specific antibodies testing. Levels of SARS-CoV-2 Receptor-binding domain-specific IgG were measured using the VIDAS® SARS-CoV-2 IgG II Kit (bioMérieux), according to manufacturer’s instructions. Testing was performed on an automated benchtop immunoanalyzer VIDAS® (bioMérieux), based on the Enzyme Linked Fluorescent Assay (ELFA) technology using patients’ sera, previously isolated from whole peripheral blood. Interpretation of the results and their conversion into binding antibody units per milliliter (BAU/ml) was performed as per manufacturer’s instructions.

Statistical analysis. All statistical analyses were performed using GraphPad Prism version 9.0. Comparison between patients’ groups was performed using the Mann-Whitney U test, independent unpaired t test, or one-way ANOVA test, as appropriate. P values of <0.05 were considered significant.

Results
Following the SARS-CoV-2 infection, IFN-γ secretion was detected early in 12 of the subjects in the 1st group of analysis (<30 days PSO), although the rest of the subjects reacted negative (n=10) or intermediate (n=3). The peak, however, was measured in the 2nd group (1-3m PSO), reaching 1 IU/ml on average (range, 0 to 2.72 IU/ml) (Fig.1). In only one of the samples in this group no IFN-γ was detected. Results in the 3rd group showed a non-significant decline of IFN-γ secretion after stimulation, compared to the results in the second group of analysis. 6-8 months PSO, reactivity was still detected with levels of 0.5 IU/ml on average (range, 0 to 1.9 IU/ml), with only two samples being negative for IFN-γ secretion.

Between the 14 – 30th day PSO accumulation of RBD-specific antibodies is detected, peaking at 460 BAU/ml (Fig.2).

Fig.1: Levels of secreted INF-γ (IU/ml) in three groups of analysis: 14 – 30 days, 1-3 months, and 4-8 months PSO (ns= p>0.05)

Fig.2: Levels of anti-SARS-CoV-2 IgG (BAU/ml) in three groups of analysis: 14 – 30 days, 1-3 months, and 4-8 months PSO

In the second group, a slight decline in the level of virus-specific antibodies is shown, measuring at 380 BAU/ml on average. Data in the last group of analysis (6-8 m PSO) display a statistically significant
reduction of RBD-specific IgG levels. In comparison to the first two groups, levels in the last period of sampling after symptoms onset diminished at 160 BAU/ml.

A correlation analysis was performed between INF-γ secretion after in vitro stimulation and RBD-specific antibodies. No correlation was found between both of the parameters.

**Discussion**

During the first weeks following infection, SARS-CoV-2 induces a fast B-cell response, with virus-specific IgM, IgG, and IgA antibodies production and neutralising potential. Antibodies against SARS-CoV-2 have no thoroughly researched protective role, although they are a plausible correlation of antiviral immunity, and the anti-RBD antibody levels coincide to plasma viral neutralizing activity. Considering this rapid antibody decay, post-acute phase of the infection, being almost exponential, it suggests that humoral immunity is not long-lasting at high antibody rates in people with mild form of COVID-19, who represent majority of the cases. Our findings of viral-specific antibodies drastically diminishing after the first month post-COVID-19 but persisting for 8 months in low amounts are consistent with studies published by other authors (1,3). Ibarrondo et al. describe a accelerated deterioration of anti-SARS-CoV-2 antibodies in moderate cases of COVID-19 (1). They report an antibody half-life of 90 days with exponential decline, which is in line with our findings.

Quality of the immunological memory response to SARS-CoV-2 will be crucial in inhibiting reinfection. Here, we assessed the longitude and magnitude of cellular immunity by measuring the INF-γ secreted by cells in whole peripheral blood in the presence of SARS-CoV-2- derived peptides. Related to the antibody values being present at 6-8 months PSO in tracible amounts after declining drastically, the INF-γ secretion is maintained at relatively high rates. Retention of cell-mediated immunity for such a long time has been documented by other groups of scientists (4,5). Almendro-Vázquez et al. (5) describe a persistent T-cell response 7 months PSO in combination with significantly lower levels of anti-viral IgG antibodies, consistent with our results. Zhang et al. (4) depict comparable results, where a positive correlation is made between disease severity and longitude of the immune response. In our study no correlation was found between antibodies and cell-mediated immunity dynamics, which is in line with Zuo et al. work (6).

The acquisition and maintenance of cell-mediated immunity and its assessment in clinical laboratories is important for several reasons. Cellular immunity against SARS-CoV-2 is becoming more volatile in the face of mutated SARS-CoV-2 variants with (humoral) immune breakout characteristics (7). Thus, the presence of detectable cellular immunity to SARS-CoV-2 should provide a reduced chance of illness development than those patients who do not possess perceptible cell-memory response. Considering this, examining cellular immunity against SARS-CoV-2 may be explanatory for clinicians about the patients’ level of protection against COVID-19.

**Conclusions**

Our study implies the convenience of the QuantiFERON IGRA assay for routine laboratories to assess cellular immunity against SARS-CoV-2. The main conclusion of this study is that in the long-term, testing of cell-memory immune response against SARS-CoV-2 may be a more reliable parameter than humoral immune responses for the evaluation of immunity in COVID-19. However, further research is required to validate this intriguing and important novel assay before it can be applied in routine diagnostics and for monitoring purposes.

**Acknowledgments**

The conduction of this study was supported by:
1. Synmed Bulgaria, who provided the QIAGEN QuantiFERON kit

References
Indicators for measuring the amount of antibiotics in hospitalized patients in Slovenia

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Abstract
Background. Irregular and uncontrolled consumption of antibiotics in hospitals, but also in the out-of-hospital environment, promotes the development of resistant mutants and leads to high costs and unwanted side effects. In recent years, multi-disciplinary cooperation has been recommended in this context. A set of 20 indicators for measuring the amount of antibiotics in hospitalized patients (IQMs) was examined in the international project DRIVE-AB.

Purpose. The purpose of our research was to estimate the amount of consumed antibiotics on the basis of IQM indicators – three indicators, IQM 1, IQM 2 and IQM 3 – in the period from 2014 to 2018 in Slovenian hospitals.

Methods. In the retrospective study that was done in the period from 2014 to 2018 in 12 Slovenian hospitals, we calculated the defined daily dose (DDD) per 100 bed days (IQM 1), DDD per 100 admissions (IQM 2) and DDD per 100 bed days/case mix index (CMI) (IQM 3).

Results. The indicators for antibiotic consumption in 12 Slovenian hospitals showed the following: mean value were: IQM 1 was 55.10; IQM 2 was 286, and IQM 3 was 26.78.

Conclusion. The IQMs can be a valuable tool to facilitate the interpretation of quantitative hospital antibiotics data.

Key words: defined daily dose, admissions, bed days, antibiotic consumption, cost

Introduction
Antibiotics are commonly overused and misused, which increases the emergence of resistant organisms, side effects and additional costs. To assess the appropriate use of antibiotics, many methods are available. The authors of the international DRIVE-AB (Driving Reinvestment in Research and Development and Responsible Antibiotic Use) project reviewed a list of 20 indicators for measuring the amount of antibiotics consumed in hospitalized patients – quantity metrics for antibiotic use in inpatients (IQMs). Twelve of them were selected as valid (1). The aim of the present study is to prove that indicators of antibiotic use have a value.

Methods
In the retrospective study that was done in the period from 2014 to 2018 in 12 Slovenian hospitals, we calculated the defined daily dose (DDD) per 100 bed days (IQM 1), DDD per 100 admissions (IQM 2) and DDD per 100 bed days/case mix index (CMI) (IQM 3). The number of admissions, the number of bed days (BD) and the total weight for infectious diseases were obtained from the collection of 'Hospital treatments of the same type' (SPP) at the National Institute of Public Health (NIPH) (2). Data on antibiotic consumption, defined daily dose (DDD), provided by hospital pharmacists of all selected hospitals, was entered into the Microsoft Office Excel 2010 database of the Clinic for Infectious Diseases and Febrile Conditions (3). The selected antibiotics were classified according to the index 'Anatomical Therapeutic Chemical (ATC) classification', antibiotics for the systemic treatment of bacterial infections (group J01), year 2019 (4). The CMI is the sum of the product of the number of admissions and the average weight (or the sum of the total weights) divided by the number of all admissions (5). The diagnoses of infectious diseases (which we selected according to the recommendations of the Center for Disease Control and Prevention) were re-coded from the American Classification of Diseases 9 (ICD-9) (6) to the International Classification of Diseases 10 (MKB-10) (7). For statistical analysis, we used the SPSS Statistics for Windows package, Version 21.0. For the analysis of the data, we used descriptive statistics.

Results
Table 1 showing average values of IQM 1, IQM 2, IQM 3 in selected Slovenian hospitals from 2014 to 2018
<table>
<thead>
<tr>
<th>Year</th>
<th>IQM 1</th>
<th>IQM 2</th>
<th>IQM 3</th>
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<tbody>
<tr>
<td>2014</td>
<td>51.77</td>
<td>272</td>
<td>23.25</td>
</tr>
<tr>
<td>2015</td>
<td>54.41</td>
<td>278</td>
<td>23.69</td>
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<td>2016</td>
<td>55.27</td>
<td>288</td>
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<tr>
<td>2017</td>
<td>57.38</td>
<td>300</td>
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</tr>
<tr>
<td>2018</td>
<td>56.69</td>
<td>293</td>
<td>23.25</td>
</tr>
</tbody>
</table>

Legend 1: IQM – inpatient quantity metrics, measuring antibiotic use in inpatients.

Table 2 showing average values and ranges of IQM 1, IQM 2 and IQM 3 for selected Slovenian hospitals from 2014 to 2018

<table>
<thead>
<tr>
<th>Hospitals</th>
<th>IQM 1 Mean (range)</th>
<th>IQM 2 Mean (range)</th>
<th>IQM 3 Mean (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH Nova Gorica</td>
<td>54.16 (37.12–62.65)</td>
<td>265 (253–288)</td>
<td>24.95 (22.10–28.21)</td>
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<tr>
<td>GH Brežice</td>
<td>56.02 (52.77–58.81)</td>
<td>276 (213–362)</td>
<td>34.60 (30.05–36.39)</td>
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<tr>
<td>GH Novo mesto</td>
<td>56.47 (52.92–59.91)</td>
<td>313 (283–350)</td>
<td>27.41 (25.32–28.53)</td>
</tr>
<tr>
<td>GH Celje</td>
<td>59.74 (56.50–62.65)</td>
<td>271 (238–297)</td>
<td>25.91 (25.11–27.33)</td>
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<td>GH Izola</td>
<td>60.44 (58.42–62.19)</td>
<td>258 (243–269)</td>
<td>32.42 (30.61–35.25)</td>
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<tr>
<td>GH Jesenice</td>
<td>56.52 (54.13–58.41)</td>
<td>239.91 (234–251)</td>
<td>30.32 (28.17–32.09)</td>
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<tr>
<td>UCC Ljubljana</td>
<td>63.24 (61.48–64.25)</td>
<td>340 (324–340)</td>
<td>23.10 (22.23–24.02)</td>
</tr>
<tr>
<td>GH Ptuj</td>
<td>46.80 (44.20–49.13)</td>
<td>250 (231–262)</td>
<td>25.25 (22.21–26.70)</td>
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<tr>
<td>UCC Maribor</td>
<td>59.27 (48.65–63.43)</td>
<td>340 (325–358)</td>
<td>20.95 (16.95–22.49)</td>
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<td>GH Murska Sobota</td>
<td>54.83 (50.39–61.11)</td>
<td>322 (293–362)</td>
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<td>GH Trbovlje</td>
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<td>279 (251–299)</td>
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<tr>
<td>GH Slovenj Gradec</td>
<td>51.27 (43.69–55.15)</td>
<td>250 (213–280)</td>
<td>28.60 (25.72–31.38)</td>
</tr>
</tbody>
</table>

Legend 2: IQM – inpatient quantity metrics, measuring antibiotic use in inpatients; GH – general hospitals; UCC – University Clinical Centre.

Discussion
Pokrajac T. study showed (8), the median values were 317.69 DDD per 100 admissions and 58.88 DDD per 100 bed days from 2004 to 2013 (2). IQM 1, IQM 2 and IQM 3 show an increasing trend for each year until 2017, with a slight
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...decrease in 2018. IQM 3 is DDD per 100 bed days / CMI and is the ratio of antibiotic consumption to an economic indicator of a comparable case study to estimate the severity of illness of hospitalised patients. In the hospitals studied, IQM 3 varies from 26.13 in GH Trbovlje to 34.60 in GH Brežice. We estimated that antibiotic consumption in relation to CMI is high in Clinical Centre Ljubljana and Clinical Centre Maribor. Among the general hospitals, GH Brežice, GH Izola, GH Jesenice, and GH Murska Sobota are notable, as IQM 3 is higher than in the other hospitals – i.e., the DDD per 100 bed-days is higher, which means that the antibiotic consumption is exceedingly high.

References

Synthesis and characterization of new metronidazole derivatives

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²Department of Pharmaceutical chemistry, Faculty of Pharmacy, Medical University of Sofia
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⁴Medical college of Varna, Medical University of Varna, Bulgaria

Abstract

Antimicrobial agents were discovered more than 100 years ago. However, the struggle with bacterial agents continues at present. The development process of searching for new antimicrobial drugs includes global research efforts. The aim of the present scientific work is to synthesize and structurally characterize two new amide derivatives of metronidazole. The chemical structures of the new compounds were confirmed by its IR and NMR spectral data.

Keywords: metronidazole, identification, synthesis, imidazole derivatives

Introduction

For decades, there has been active work on the synthesis, identification, and exploration of heterocyclic compounds. However, the imidazole nucleus is the main scaffold in molecules with various pharmacological properties such as antibacterial, antifungal, antineoplastic, antiviral, antidiabetic, etc. On the other hand, 5-nitroimidazole drug derivatives are the most efficient antimicrobial agents [1,2,3,4,5]. The most widely known agent from a group of imidazoles is Metronidazole – a drug that, for the past 70 years, is the most efficient in severe anaerobic infections. However, Metronidazole is becoming increasingly inefficient because of drug resistance comprised the microbial variability and long-term use of it [6]. Therefore, it is a suitable object for different chemical transformations and synthesis of modified metronidazole derivatives. The goal is to achieve better pharmacokinetic properties, reduced toxic effects, and extended biological activity.

Aim

The present study aimed to synthesize and characterize two new metronidazole amide compounds.

Materials and methods

2-methyl-5-nitroimidazole-1-ethanol (Fluorochem); Sulfuric acid (99.99% Chem Lab); Sodium bichromate (≥99.5%, Sigma-Aldrich); ethyl-4-aminobenzoate (98% Sigma Aldrich); butyl-4-aminobenzoate (≥98%, Sigma-Aldrich); Tetrahydrofuran (anhydrous, ≥99.9%, inhibitor-free Sigma Aldrich); methanol (for HPLC≥99.9%); N, N'-Dicyclohexylcarbodiimide (DCC) (Sigma Aldrich); ethanol (≥99.5%, for HPLC, Sigma-Aldrich); DMSO (®, ≥99%, Sigma-Aldrich); 0.9%. ¹H- and ¹³C-NMR spectra were recorded on Bruker AV600 and DRX250 spectroscopy using deuterated DMSO as a solvent at the Institute of Organic Chemistry with a Phytochemistry Centre at the Bulgarian Academy of Sciences.

Results

The amidation of metronidazole by two different amines was processed in the presence of N, N'-Dicyclohexylcarbodiimide (DCC). The synthetic procedure involves two steps, as illustrated on Fig 1. The first stage of the synthesis includes the oxidation of the initial Metronidazole in the presence of Jones reagent to obtain as an intermediate the corresponding 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid (MT1). In the next stage was applied the classical amidation approach of direct interaction of the initial MT1 with the corresponding ethyl 4-aminobenzoate and butyl 4-aminobenzoate in the catalytical presence of DCC to obtain the target ethyl 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide)benzoate (MT2) and butyl 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide) benzoate (MT3).
Figure 1. Synthesis of the targeted amide derivatives

Synthesis of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid-MT1. A 100 ml two-necked round bottom flask equipped with a magnetic stirring bar, combined with a reflux condenser, was charged with 5 g metronidazole, 5 ml sulfuric acid and 20 ml water. To the prepared solution was added solution of Na2Cr2O7 and the magnetic stirrer was switched on (500 rpm) for 24 hours. After reaction time was passed green colored solution was observed. The obtained green residue was recrystallized from a mixture of equal amounts (v/v) of tetrahydrofuran and ethyl acetate to afford 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid-MT1 acid as white solid product 4,22g dried over anhydrous sodium sulfate [7].

Synthesis of ethyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamido)benzoate- MT2.
The obtained MT1 from step one was charged in one necked round bottom flask equipped with a magnetic stirring bar and dissolved in hot 50 ml tetrahydrofuran initially. Then, 30 ml tetrahydrofuran was added obtaining a clear solution and the reaction mixture was cooled to room temperature. 4,54 g of DCC was added to the cold solution and a precipitate was formed. After five minutes 3,65 g of ethyl-4-aminobenzoate was placed into the reaction mixture too. Then, the magnetic stirrer was switched on (500 rpm) for 12 hours. The obtained product was filtrated and concentrated to dryness under reduced pressure [8]. The residue was crystallized from methanol to afford MT3 as light-yellow, crystalline mass-3,66 g; Mm=332,32. Physical properties: white to pale yellow crystalline mass powder, insoluble in water, freely soluble in methanol and DMSO.

Synthesis of butyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamido)benzoate-MT3.
The synthetic procedure is the same as the detailed described for MT2 and no subtle differences exist [7]. The obtained product MT3 was recrystallized from a mixture of equal amounts (v/v) of ethanol and water (1:1) to yellow crystalline mass-1,59 g. Mm = 360,37. Physical properties: white to pale yellow crystalline mass powder, insoluble in water, freely soluble in methanol and DMSO.

The structures of obtained products were identified by FTIR and 1H NMR, 13C NMR spectroscopy.

FTIR and NMR analysis of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetic acid-MT1. IR (cm-1): 3140, 3000, 1716 (-COOH), 1275, 1222 (νC=O), 1387 (vOH in COOH), 1541 (NO2), 1354, 1194 (C-Hr, C-Ns), 1472 (-CH3), 1145 (1,5-substituted imidazole); 1H NMR (δ, 600 MHz, DMSO): 2.41 [s, 3H, -CH3], 5.07 [s, 2H, -CH2-COOH], 8.06 [s, 1H, -CH2-COOH], 1275, 1222 (νC=O), 1387 (νOH in COOH), 1541 (NO2), 1354, 1194 (C-Hr, C-Ns), 1472 (-CH3), 1145 (1,5-substituted imidazole); 13C NMR (δ, 600 MHz, DMSO): 169.12, 151.88, 138.89, 133.43, 47.87, 14.05.

FTIR and NMR analysis of 2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate- MT2. IR (cm-1): 3242 (-NH-CO-), 1695 (νC=O), 1534, 1385,1115, 824 (NO2), 3067, 1469, 1430, 1099, 1026 (-C-Hs, -C-Hr, -C-Hw ), 1605, 1556 (C-Ns, C-Cs, C-C-Hb), 1556 (C-Ns), 1430, 1412, 1385, 1365 (C-Hr, C-Ns), 1307 (C-Os, C-Cs, C-Hw), 1514, 1193 (C-Hsc), 1149, 878 (C-Os, “breathing benzene vibrations” C-Hr), 850 (C-Hw benzene), 858, 824 (C-O-Cb, C-Ns, C-Hw “breathing benzene vibrations”), 764, 743, 695 (O=C-Ob, C-Hw), 1026, 987, 954, 794, 676 (C-C-Ob, N-C-Nb, C-N-Cb); 1H NMR (δ, 600 MHz, DMSO): 2.44 [s, 3H, -CH3], 5.23 [s, 2H, -N-CH2-CO-], 8.08 [s, 1H, -C=CH-N=], 10.86 [s, 1H, -CO-NH-], 7.71, 7.93 [d*, 2H], 2.28 [q, 2H, -CH2-CH2], 1.30 [t, 3H, -CH3]; 13C NMR (δ, 600 MHz, DMSO): 165.71*, 165.66*, 154.29, 143.27, 138.95, 132.90, 130.85, 125.10, 119.05, 60.99, 49.44, 14.69*, 14.24*.

FTIR and NMR analysis of butyl 4-(2-(2-methyl-5-nitro-1H-imidazole-1-yl)acetamide)benzoate-MT3. IR (cm-1): 3259,3054,1542 (-NH-CO-), 1642 (νC=O), 1530, 1383, 1113, 823 (NO2), 3259, 3112, 3054, 2932, 1463, 1426, 1058, 1010 (-C-Hs, -C-Hr, -C-Hw ), 1600, 1542 (C-Ns, C-Cs, C-C-Hb), 1605 (C-Ns), 1426 1406, 1383, 1361 (C-Hr, C-Ns), 1299 (C-Os, C-Cs, C-Hw), 1508, 1191 (C-Hsc), 1143, 893 (C-Os, “breathing benzene vibrations” C-Hr), 846 (C-Hw benzene), 859, 823 (C-O-Cb, C-Ns, C-Hw “breathing benzene vibrations”), 768, 741, 694 (O=C-Ob, C-Hw), 1020, 950, 768, 676 (C-C-Cb, N-C-Nb, C-N-Cb); 1H NMR (δ, 600 MHz, DMSO): 2.50 [s, 3H, -CH3], 5.29 [s, 2H, -N-CH2-CO-], 8.15 [s, 1H,-C=CH-N=], 10.93 [s, 1H, -CO-NH-], 7.77, 7.99 [d*, 2H]. 1.73 [m, 2H, -CH2-CH2], 1.46 [m, 2H, -CH2-CH2], 4.30 [t, 2H, -CH2-CH2], 0.98 [t, 3H, -CH3]; 13C NMR (δ, 600 MHz, DMSO): 165.75*, 165.66*, 152.49, 143.28, 138.95, 132.90, 130.85, 125.08, 119.07, 64.64, 49.44, 30.74, 19.22, 14.23*, 14.09*.

A careful assessment of the MT2 and MT3 spectral features shows significant differences compared to the parent structures. A major band observed at 3242 cm⁻¹ in the MT2 spectrum, is associated with the presence of a new amide
bond. The strong absorption band at 1695 cm\(^{-1}\) can rely on C=O (carbonyl group). The frequency of the nitro group is at 1534 cm\(^{-1}\). The oscillation in 1600 to 1000 cm\(^{-1}\) region, are consistent with C-N, C-C, C-C-H bonds. The set of bands in the region 3500-2700 cm\(^{-1}\) of MT3 spectra are very informative too for the presence of a secondary amide group. The absorptions occur as single bands at 3259 and 3054 cm\(^{-1}\). The peak at 1542 cm\(^{-1}\) is a confirmation for -NH vibration too. The oscillation of the nitro group is detected at 1530 cm\(^{-1}\). The observed absorption at 1642 cm\(^{-1}\) is assigned to C=O (carbonyl group). It is worth noting that the obtained data resulting of the formation of the target synthesis products. Despite the structural similarity, there are significant spectral differences that draw attention to the formation of new amide derivatives.

In addition, a complete proton \(^1\)H and carbon \(^{13}\)C nuclear magnetic resonance (NMR) spectral analysis of tested compounds was obtained. The identified differences point to a successful synthesis. One of the most significant signals is at 10,86 ppm and it demonstrates the proton of a new amide bond in MT2. The confirmation signal for the formed amide group in MT3 was detected at 10.93 ppm. The stated signals are not recorded at the parent structure and MT1. The \(^{13}\)C NMR analysis and obtained spectral data confirmed the identity of the test compounds too. The number of signals corresponds to the number of carbon atoms in the analyzed compounds. The significant differences observed in the samples lead to the conclusion that are made structural changes and obtained the target compounds.

**Conclusion**

In conclusion, we have synthesized two novel amide derivatives of metronidazole. The obtained compounds are characterized using FT IR and \(^1\)H and carbon \(^{13}\)C nuclear magnetic resonance (NMR).

**Acknowledgments**

This study was financially supported by the Fund “Science” of Medical University of Varna–Project number 19026/2020.

**References:**

High sensitivity 14-parameter flow cytometry for evaluation of minimal residual disease in childhood B-cell acute lymphoblastic leukemia – a pilot study

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2) Research Institute, Medical University of Plovdiv, Bulgaria
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Abstract: Purpose. Minimal residual disease (MRD) is a population of leukemic cells in the bone marrow or in the peripheral blood that is resistant to therapy and leads to relapse. In childhood acute lymphoblastic leukemia (cALL), early and late therapeutic response is assessed by monitoring of MRD. Evaluation of MRD is crucial both for predicting clinical outcomes and for choosing the optimal treatment strategy. The purpose was to evaluate the applicability of new markers for MRD and to define the sensitivity of 14-color flow cytometry (FC) panel.

Material/Methods. Materials include preparation of spiked-in samples with defined concentration of leukemic cells by the use of non-leukemic bone marrow aspirates and bone marrow of a child with B-cell precursor ALL (BCP-ALL), rich in blasts. Leukemic cells were added to non-leukemic samples to achieve the following expected blast levels: 1%, 0.1%, 0.01%, 0.001% and 0.0001% and were stained with the newly developed panel. Analysis of FC data was performed by classical manual approach and by automated methods using FlowJo software.

Results. Application of the 14 monoclonal antibody panel and the analysis algorithm allow detection of 0.001% blasts, which has a tenfold higher sensitivity than conventional flow cytometry.

Conclusion. The newly developed FC panel and analysis algorithm achieve sensitivity similar to that of the genetic methods. This would allow significantly earlier detection of MRD and appropriate therapeutic changes.

Key words: flowcytometry, leukemia, minimal residual disease

Introduction: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children, with peaks between 2 and 5 years of age. In childhood ALL (cALL), both early and late therapeutic response is assessed by monitoring of minimal residual disease (MRD). MRD is a population of leukemic cells in the bone marrow and less common in the peripheral blood that is resistant to chemo/radiation therapy and may lead to relapse [1,2]. These cells may be primary residual blasts before therapy or transformed secondary blasts. The evaluation of MRD is crucial both for predicting clinical outcomes and for choosing the optimal treatment strategy, especially in the pediatric setting where the balance between anti-leukemic efficacy and long-term toxicity is crucial [3-8]. The current focus is on detection of MRD as early as possible through the application of more sensitive, highly specific and reproducible approaches. Therefore, efforts are focused on the development of multiparameter flow cytometry (FC) [7-19]. The purpose of this study was to evaluate the applicability of some new markers for MRD and to define the sensitivity of 14-color FC panel.

Materials and Methods: Materials. In this study the sensitivity of the newly developed FC panel was assessed by preparation of samples with defined concentration of leukemic cells as follows: non-leukemic bone marrows of 6 children (routinely taken for other conditions) from the pediatric oncohematology center in St. George University Hospital in Plovdiv were mixed and served as non-leukemic background “control”; 1 bone marrow of a child with BCP-ALL at diagnosis, containing 80% blasts served as a source
of malignant cells. Calculated amounts of leukemic cells were added to the “control” sample in order to achieve the expected blast levels as follows: 1%, 0.1%, 0.01%, 0.001% and 0.0001%. Then samples were stained with the newly developed 14 color FC panel (table 1).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Clone</th>
<th>Fluorochrome</th>
</tr>
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<tbody>
<tr>
<td>CD58</td>
<td>1C3</td>
<td>BV355</td>
</tr>
<tr>
<td>CD45</td>
<td>2D1</td>
<td>BV480</td>
</tr>
<tr>
<td>CD34</td>
<td>BG12</td>
<td>PE-CF594</td>
</tr>
<tr>
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<td>H10A</td>
<td>PERCP-CY5.5</td>
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<td>SI25C1</td>
<td>PE-CY7</td>
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<td>CD22</td>
<td>S-H3-1</td>
<td>APC</td>
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<td>CD38</td>
<td>HB7</td>
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<td>2331 (F11N-1)</td>
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<td>PE</td>
</tr>
<tr>
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<td>AD2</td>
<td>BV421</td>
</tr>
<tr>
<td>CD304</td>
<td>U21-1283</td>
<td>BV605</td>
</tr>
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</table>

**Table 1.** 14 color FC panel

**Methods** for FC data analysis include two approaches. The first of them is the classical manual approach in which the acquisition of cells was performed on FACSaria III flow cytometer with DIVA software ver.8 (BD Bioscience). A two-page template was designed in DIVA software including 35 bivariate plots, based on the 14-color MRD panel. The second approach was automated and was performed with FlowJo software ver. 10.8.1 and its plugins: FlowAI, tNSE, UMAP, FlowSOM, Phenograph, Xshift, HyperFinder and ClusterExplorer.

The developed algorithm for automated FC analysis is thoroughly demonstrated (fig. 1).

**Fig. 1.** Sample analysis algorithm with FlowJo.
A) Bivariate plot showing a change in the quality of the collected events (on the left end are the bad events). The first step of cleaning is done by FlowAI plugin (B) which selects only the good events (C). D) According to FSC/SSC, a gate is placed, removing the “debris" in the lower left corner. E) A gate for exclusion of doublets and selection of single cells is placed. F) CD19/SSC gating selects CD19+ events involving the B-cell population. G) The 14 fluorescence parameters are reduced to a bivariate plot (dimensional reduction) with FlowJo's tSNE or UMAP plugins. Individual subpopulations are stained in different color. H) The individual subpopulations are then clustered with FlowSOM plugin. Additionally, Phenograph and XShift plugins can be used. I) Phenotypic characterization of each cluster is visualized using ClusterExplorer. At this stage, the combination of phenotypic markers determines the cluster corresponding to the MRD population.
**Results:** Cluster analysis with FlowSOM and ClusterExplorer is demonstrated where the phenotype of the leukemia population at diagnosis is analyzed (fig. 2). Relatively high expression of CD10, CD34, CD38 and CD58 and relatively low expression of CD20 and CD44 is observed. The result of the cluster analysis of the sample with 0.001% BCP-ALL blasts is presented as a comparison (fig. 3). A cluster with a profile similar to that at the diagnosis is presented. Among the events collected, 36 events have the BCP-ALL blast profile. The latter represent 0.0012% of all cells, which is close to the expected level of 0.001% blasts.

![Fig. 2. Phenotypic bone marrow clusters of a patient with BCP-ALL at diagnosis determined by FlowSom and ClusterExplorer.](image1)

![Fig. 3. Cluster analysis of the sample with an expected 0.001% blast cells by FlowSOM and ClusterExplorer.](image2)

The proposed 14-color panel and analysis algorithm enable the detection of 0.001% of BCP-ALL blasts in children, which has a tenfold higher sensitivity than conventional flow cytometry (table 2).

<table>
<thead>
<tr>
<th>Detected leukemic blasts</th>
<th>% Leukemic blasts placed</th>
<th>Total number of collected cells</th>
<th>Number of leukemic blasts</th>
<th>% Leukemic blasts</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>271</td>
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<tr>
<td><strong>0.001%</strong></td>
<td><strong>2927109</strong></td>
<td><strong>36</strong></td>
<td><strong>0.001236%</strong></td>
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<tr>
<td>0.0001%</td>
<td>3987292</td>
<td>0</td>
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</table>

**Table 2.** Percentage of blast cells detected by Cluster Analysis by FlowSOM and ClusterExplorer.

**Discussion:** Many recent studies, available elsewhere, discuss in details the different methods available today for the detection of MRD in cALL, as well as their sensitivity, advantages and disadvantages. They include FC, Real-Time Quantitative Polymerase Chain Reaction (RQ-PCR), Reverse transcription polymerase chain reaction (RT-PCR), Digital droplet polymerase chain reaction, Next Generation Sequencing. Although generally considered as less sensitive, FC has some remarkable features which make it applicable in most of the cases of cALL. In addition, it is relatively less expensive, fast (results are ready within 2-3 hours) and highly informative [9-11]. In 2019 Tembhare et al. published data from the most extensive study on the subject to date, demonstrating the application of a highly sensitive FC-MRD test in BCP-ALL. An easily reproducible 10-color FC-MRD panel with a high sensitivity of 0.0002% or 2 residual cells per 1x10⁶ cells is achieved. The panel allows the detection of minute MRD values in samples that would vote negative by the standard flow cytometry [12]. This outstands the technique of multiparameter FC for MRD as a highly sensitive approach, comparative to the genetic methods. Our study could not achieve Tembhare sensitivity, but reached a tenfold higher sensitivity compared to the currently used 8-color FC panel.

**Conclusions:** The developed 14-color panel and analysis algorithm has a tenfold higher analytical sensitivity (10⁻⁵) for the detection of MRD compared to the currently used 8-color FC panel (10⁻⁴) and similar sensitivity to that of the genetic methods available. The application of the panel would allow earlier detection of MRD in children with ALL and thus appropriate therapeutic measures could be taken. The approach described hereby has been tested for the assessment of MRD in childhood BCP-ALL and might...
also be applied for the development of multiparameter panels and analysis algorithms for the detection of MRD in other oncohematological diseases.

References:

Acknowledgments: The conduction of this study was supported by:
2. Project Contract № BG05M2OP001-1.002-0005 - Personalized Innovative Medicine Competence Center (PERIMED) EU, Operational Programme “Science and Education for Smart Growth” (OP SESG) 2014-2020
Fetal Echocardiography in the First Trimester of Pregnancy

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2) Maternal-Fetal Medicine, Plovdiv
3) SAGBAL Sheinovo, Sofia

Abstract

Objectives To review the recent evidence on first trimester screening and diagnosis of congenital heart diseases.

Materials and methods PubMed (Medline) searches of the current evidence-based publications concerning fetal echocardiography in the first trimester of the pregnancy.

Results Studies evaluating detection rates and the influencing factors of the fetal echocardiography in the first trimester of the pregnancy were identified (Table 1).

Conclusions First-trimester ultrasound examination of the fetal heart allows identification of more than half of fetuses affected by major cardiac pathology. First trimester screening should follow structured anatomical protocols and consider the introduction of outflow-tract views and color-flow Doppler, as this would improve detection rates of fetal cardiac pathology.

Key Words: Congenital heart disease, first trimester, fetal echocardiography

Introduction

Congenital heart defects (CHD) are the most common fetal structural anomalies affecting 8 per 1000 fetuses. Most of them (80%) occur in low-risk pregnancies [10] and half of them are major anomalies requiring surgery or are lethal. There is a growing trend to assess the fetal anatomy in earliest possible gestations because early diagnosis gives more time for diagnostic work up and patients choices as with improving technology and knowledge many clinical conditions are screenable and diagnosable that early. Considerable improvements in technology made possible early anomaly detection with increasing detection rates [1-5].

Objectives

To review the recent evidence-based data on screening and diagnosis of congenital heart diseases in the first trimester of the pregnancy.

Methods

Published English language literature concerning recent evidence-based data on first trimester screening and diagnosis of CHDs was retrieved through searches of PubMed (Medline).

Results

Table 1. Detection rates of CHD in the first trimester of the pregnancy.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Population</th>
<th>Protocol</th>
<th>DR %</th>
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<td>4CV-OT</td>
<td>79,63</td>
<td>11</td>
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<tr>
<td>Ebrashi</td>
<td>2019</td>
<td>Unselected</td>
<td>4CV-OT</td>
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<td>2022</td>
<td>Low-risk</td>
<td>4CV-3VT, High-risk</td>
<td>55</td>
<td>&gt;80</td>
<td>6</td>
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<tr>
<td>Karim</td>
<td>2021</td>
<td>Systematic review and Meta-analysis</td>
<td>Low-risk, High-risk</td>
<td>56</td>
<td>68</td>
<td></td>
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</tbody>
</table>

Discussion

The detection of CHD has improved substantially (Table 1.) in the first trimester of the pregnancy. The sensitivity of early cardiac screening in pregnancy varies around 50-60% due to natural history of CHD. In the first trimester we
can discriminate groups of CHD according to their detectability [1, 2]. The 11–14 weeks scan evolved from a scan for measurement of fetal NT and CRL to one which includes a basic checklist for examination of the fetal anatomy with the intention of diagnosing major abnormalities, in order the parents to have the option of earlier pregnancy termination. There is a high association between increased NT, tricuspid regurgitation, abnormal ductus venosus flow and cardiac defects in both chromosomally abnormal and normal fetuses [8]. The importance of the 11–14-week ultrasound exam beyond screening for chromosomal abnormalities is becoming more apparent in the era of non-invasive prenatal testing [2]. There is little international consensus as to how first-trimester cardiac anatomy assessment should be performed routinely. Currently first-trimester ultrasound guidelines recommend evaluation of the fetal heart beyond heart rate, position and the four-chamber view [7, 8]. Restricting the indications for first-trimester cardiac evaluation to high-risk [4, 5] categories will detect only a small proportion of cardiac defects because most of them occur in low-risk population. Even expanding first-trimester cardiac evaluation to those with risk-factors and abnormal ultrasound markers, such as thickened nuchal translucency, reversed a-wave in the ductus venosus and tricuspid regurgitation, will still miss almost 50% of major cardiac defects [6]. The criteria for good screening tests have been well-established by the World Health Organization and first-trimester fetal heart evaluation meets the components. In a review of first-trimester fetal echocardiography using both high-definition color flow Doppler and pulsed-wave Doppler ultrasound, Nemescu and colleagues [9] demonstrated that the safety indices were remarkably stable and relatively constant for all exams, with a thermal index (TI) and mechanical index (MI) well below the maximum levels recommended for practice and that a satisfactory assessment is possible within 3–4 min of exposure time, not only for experienced sonographers but also through the learning curve. Karim et al. [5] described the factors affecting screening performance: the imaging protocol used for examination was found to have an important impact on screening performance in both low- and high-risk populations, with a significantly higher detection rate observed in studies using at least one outflow-tract view or color-flow Doppler imaging.

Table 2. Simplified protocol for first trimester heart scanning

<table>
<thead>
<tr>
<th>Situs</th>
<th>Gray scale</th>
<th>Doppler</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four chamber view- axis, IVS, compare ventricular size, AV valves</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>Aortic outflow Origin from the LV</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>
Pulmonary outflow Origin from the RV

Three vessel trachea crossing of the outflow tracts, size , “V-sign” to left of the spine and trachea , flow in the same direction

Venous return Pulmonary veins entering left atrium, DV and cava veins

Factors responsible for false negative diagnoses are categorized in three groups: [4]: 1. Human errors (of image interpretation or scanning approach or both), 2. Technical factors (excessive color balance, wrong plane alignment), 3. Acoustic window impairment (maternal obesity and/or twins).

Conclusion

Many of congenital heart anomalies are detectable in the first trimester of pregnancy and it is recommended to use strict anatomical protocol. Early detection of CHD has numerous advantages. It enables early genetic testing, early decision-making about pregnancy continuation and earlier planning for appropriate management during and after pregnancy. Use of color Doppler is mandatory as well as liberal use of TV route is recommended. A normal first-trimester cardiac assessment may also provide reassurance to high-risk patients who might have a prior child with CHD. First-trimester cardiac evaluation cannot replace standard second-trimester exam, as there are many defects that evolve over time and will be best assessed later in gestation.

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Combination of the nutritional qualities of bulgarian yogurt and the immunostimulating properties of phycocyanin.

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Abstract
Purpose: The aim of the present study was to combine the nutritional qualities of Bulgarian yogurt with the immunostimulating properties of phycocyanin in order to design a new dairy product with potential functional characteristics.

Materials and methods: Yogurt samples with a fat content of 1.7% and 3.7% and the addition of phycocyanin extracted from Spirulina platensis were examined for the presence of some pathogens by microbiological methods according to Bulgarian State Standard (BSS), their color difference with the control was evaluated by a spectrophotometric method. A sensory analysis was conducted to assess smell, taste, consistency and color evaluation.

Results: The addition of phycocyanin led to decrease in the pH values of the new dairy product. All samples and controls were microbiologically clean. Color differences were observed between controls and samples after the addition of phycocyanin.

Conclusions: Bulgarian yogurt is a suitable product for phycocyanin enrichment, due to its traditionally high consumption. The current research recommend phycocyanin concentrations of 10% and 15% at 3.7% milk fat as suitable for obtaining a new lactic acid dairy product product.

Keyword: Bulgarian yogurt, phycocyanin, functional foods.

Introduction
Yogurt is one of the most popular food products, and its consumption is associated with a number of health benefits. There is a wide variety of lactic acid dairy products on the market and great demand [1]. Phycocyanin is one of the main pigments of Spirulina platensis. It can also be used as a natural colorant and as a food additive due to its high protein content and antioxidant activity. [2]. The peptides in phycocyanin possess a number of benefits for human health such as immunomodulatory, anti-inflammatory, anti-cancer and more ones [3]. Phycocyanin, a super functional ingredient from algae; properties, purification characterization, and applications. In recent years, a lot of emphasis has been placed on the development of multi-component foods, the introduction of additional substances or materials from different plant origin to improve or increase the beneficial health properties of certain foods. Some studies were devoted to the application of dry Spirulina platensis in dairy products [4-6]. Until now, dry extract from Spirulina platensis was not applied in dairy products, especially in Bulgaria. The aim of the present study was to combine the good nutritional qualities of Bulgarian yogurt with the immunostimulating properties of phycocyanin in order to design a new dairy product.

Materials and methods
Samples: Commercially available pasturized cow milk with 1.7 % and 3.7 % fat content were used for further experiments. Standardized milk with 1.7 % and 3.7 % fat was inoculated with 2 % Lactobacillus delbrueckii subsp bulgaricus and Streptococcus thermophilus (Genesis Laboratories Ltd., Sofia, Bulgaria). Phycocyanin was extracted from Spirulina platensis cultivated in a bioreactor in Varvara village, Bulgaria. The extraction was performed by ultrasonic irradiation in an ultrasonic bath operating at 40 kHz frequency and 30°C (IsoLab, Germany) in triplicate, then purification purified and lyophilization was performed. The cow milk was heated to 92 ± 2 °C and held at this temperature for 20 min. The temperature decreased to 42 ± 2 °C, then the samples were inoculated with yoghurt starter
culture in the amount of 2 %, as mentioned above. The samples were homogenized, and dry phycocyanin was added to samples, except the control. Then the samples were put into the plastic packagings with caps and then incubated at 42 ± 2°C. The fermentation process was performed. After coagulation, the samples were cooled down to 4 ± 2 °C and stored at this temperature [7].

**Methods: Active acidity** The pH of the samples was measured using a pH meter HANNA pH 211 (Mauritius) according to 02-52 from AACC.

**Color characteristics** The measurements of color were performed with Portable colorimeter model WR-10QC with light source D65, observer CIE10 standard observer according to the L*a*b* system.

**Microbiological test** Microbiological testing of controls and new yogurt products with addition of different phycocyanin content was performed according to BSS standards [8-12].

**Sensory analysis** To determine the sensory indicators (odor, color, taste and consistency) of the newly developed the new yogurt product with different concentration of phycocyanin from *Spirulina platensis*, a quantitative descriptive (descriptive) sensory profiling test was applied. The committee includes twelve trained panelists.

<table>
<thead>
<tr>
<th>Sample product</th>
<th>1</th>
<th>1.1</th>
<th>1.2</th>
<th>1.3</th>
<th>2</th>
<th>2.1</th>
<th>2.2</th>
<th>2.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh milk 1.7%</td>
<td>99.69</td>
<td>94.69</td>
<td>89.69</td>
<td>84.69</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fresh milk 3.7%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>99.69</td>
<td>94.69</td>
<td>89.69</td>
<td>84.69</td>
</tr>
<tr>
<td>Sourdough, ml</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>Phycocyanin, ml</td>
<td>-</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>-</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Total, ml</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Results**

pH in investigated samples was summarized in Table 2

<table>
<thead>
<tr>
<th>control</th>
<th>.1</th>
<th>.2</th>
<th>.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.45</td>
<td>5.00</td>
<td>4.74</td>
</tr>
<tr>
<td>2</td>
<td>5.53</td>
<td>4.96</td>
<td>4.70</td>
</tr>
</tbody>
</table>

For each of the two groups of yogurt products, the color difference between the samples and the control on the first and twentieth day after fermentation was calculated. The results are presented in fig. 1.

![Color differences between controls and yogurt with different concentrations of phycocyanin from *Spirulina platensis*](image1)

a) yogurt samples with 1.7% fat content b) yogurt samples with 3.7% fat content

**Figure 1.** Color differences between controls and yogurt with different concentrations of phycocyanin from *Spirulina platensis*.

**Figures 2** showed the results for the attribute odour from the sensory analysis.

![Sensory profile of odour of yogurt with different fat content and phycocyanin concentration (5, 10 and 15%) and the control control](image2)
The microbiological tests for the presence of coliforms, molds and yeasts, *L. monocytogenes* and *Salmonella* sp. according to the BSS methods used, showed their absence, both in the controls and in the samples with additives. The samples, regardless of the concentration of phycocyanin (5, 10 and 15%) do not exhibit antimicrobial activity against pathogenic microorganisms and saprophytic microorganisms *Candida utilis* ATCC 42402, *Saccharomyces cerevisiae*. Antifungal activity was observed with undiluted phycocyanin, with inhibition zone diameters of $9 \pm 0.58$ mm for *Aspergillus niger* ATCC 1015, $10 \pm 0.71$ mm for *Penicillium chrysogenum* ATCC 28089, and $10 \pm 0.58$ mm for *Aspergillus niger* ATCC 1015, respectively.

**Discussion**

The addition of phycocyanin to both lactic acid dairy samples resulted in a decrease in their pH (Table 2). Similar tendency was observed in yoghurts caused by addition of *S. platensis* powder in the *Spirulina* added yoghurts.

In samples with 1.7 % fat content, the addition of phycocyanin led to noticeable color differences with the control on the day of fermentation, which increased until the 20th day after fermentation. In the samples with 3.7% fat content, the opposite trend was observed (fig. 1). Addition of phycocyanin change the colour of yogurt in slight extent to acceptable greenish hue. However, the microalgae into the yogurt changed the color of this product to greenish or bluish based on the type and concentration of microalgae added. This characteristic was realized as an inappropriate sensory attribute (appearance) by the panelists [5].

The sensory indicators flavor and aroma provides information about the aroma-taste profile of the prepared yogurt samples with concentration of phycocyanin from *Spirulina Platensis* (5, 10 and 15%) at different milk fat content (1.7% and 3.7%). According to the panelists, the addition of phycocyanin increases and enhances the perception of the sour-milk taste of the products. Samples with 10% phycocyanin have a highly attractive, sour-milky odor and that of phycocyanin. As the fat content of milk increases from 1.7 to 3.7%, the intensity of perception of the smell indicator increases. For the low-fat milk (1.7%), tasters rated all three samples with 5, 10 and 15% phycocyanin as having an attractive consistency: homogeneous, thick and creamy structure. Close to the consistency of the control yogurt and with the highest values for the indicators homogeneous, thick and creamy structure was the sample yogurt encoded 2.3 with 15% phycocyanin with 3.7% fat content (fig. 2). It was reported that incorporation of dry *Spirulina platensis* from 2% to 8% in frozen yogurts, before incubation with the addition of 10% papaya pulp, was the more acceptable. Higher levels of Spirulina adversely affected sensory characteristics of frozen yogurt [5].

Samples with phycocyanin (5, 10 and 15%) did not show antimicrobial activity against pathogenic microorganisms and saprophytic microorganisms *Candida utilis* ATCC 42402, *Saccharomyces cerevisiae*, however they were active against fungi as *Aspergillus niger* ATCC 1015, *Penicillium chrysogenum* ATCC 28089 and *Aspergillus flavus*. Therefore, phycocyanin can influence infectious processes caused by normally non-pathogenic microorganisms in a host in which the mechanisms of natural and acquired resistance are not fully functioning.

**Conclusions**

Pathogens tested according to the given BDS standards are absent in all samples. Lowering the pH values does not affect the taste characteristics. The fat content of milk has a positive effect on the indicators of homogeneous, thick and creamy structure for samples of yogurt with phycocyanin addition 15%. We recommend phycocyanin concentrations of 10% and 15% at 3.7% milk fat content as suitable for obtaining a new sour-milk product.

**Acknowledgements:**

The study was conducted with the assistance of Project № 21001/2021 of the Science Fund of the Medical University of Varna, in 2021/2024.

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Factors for the spread of *T. gondii* infection among risk groups in the Stara Zagora region, Bulgaria

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2. Medical College, Trakia University-Stara Zagora, Bulgaria

Abstract

Toxoplasmosis is a cosmopolitan parasitic zoonosis caused by *Toxoplasma gondii*, with a wide range of seroprevalence in the human population. Humans get infections mainly by contact with the soil or accidentally ingesting water or food contaminated with Toxoplasma oocysts excreted in cat feces. Various surveys of *T. gondii*-infection have focused on people of different special groups, such as pregnant, immunocompromised persons, professions involved in agriculture, animal husbandry, food preparation.

This study aims to determine the most common risk factors for the spread of *T. gondii*-infection among some risk groups of individuals in Stara Zagora region, Bulgaria.

Patients and methods: A retrospective seroepidemiological case-control study was conducted among 365 individuals, of whom 175 (47.9%) were seropositive for *Toxoplasma gondii*. Antibodies of the IgM and IgG classes were determined by ELISA.

Results: Age range in the participants was among 7 and 72 years. The average age was 28.1 years ± 12.5, of the seropositive – 32.3 years ± 11.8. Five variables were found to be positively connected with *T. gondii*-infection (*p*<0.05) including living in urban areas, gardening or agriculture, undercooked meat consumption, contact with yard cats and knowledge about toxoplasmosis as a disease.

Conclusion: In the Stara Zagora region, Bulgaria among a certain contingent of persons, there is a higher seroprevalence for *T. gondii* infection, associated with irregularities in health behavior, and in keeping cats as pets, which increases public health risks for the spread of zoonotic infections.

Key words: toxoplasmosis, *Toxoplasma gondii*, risk factors, environment, zoonoses

Introduction

Toxoplasmosis is one of the most common cosmopolitan parasitic zoonoses in the world. The etiological agent, *Toxoplasma gondii* is a multiple host obligate intracellular protozoa with a wide range of seroprevalence in the human population. The definitive hosts are various species of the family *Felidae*, while intermediate hosts are a number of warm-blooded animal species, including humans. The seroprevalence of toxoplasmosis in human population varies widely: from below 5% to over 90%. The main routes of transmission vary and depends on human behavior in different communities [1,2]. The vertical spread of the *T. gondii*-infection may occur during a transplacental transmission from new infected naive pregnant woman and cause congenital toxoplasmosis. The horizontal transmission in humans is realized through indirect fecal-oral route – ingestion of mature oocysts, excreted in feline feces; or through alimentary route – ingestion of tissue cysts in raw or undercooked meat by the intermediate hosts.

In immunocompetent persons the acute *T. gondii*-infection is most often asymptomatic, or manifested with chorioretinitis, lymphadenopathy, fever, headache and myalgia. Latent toxoplasmosis has no characteristic clinical signs and infection is confirmed by the presence of antibodies or the reactivation of the immune system against the *T. gondii* antigens [3,4]. The infection, however, with some isolates of *T. gondii* may lead to acute severe disseminated toxoplasmosis with polyorganic dysfunction and chorioretinitis [5]. In immunocompromised individuals the reactivation of the latent infection may cause a fatal toxoplastic myocarditis, pneumonitis, encephalitis [6].

The etiological diagnosis of *T. gondii* infection is of key importance for monitoring, prevention and control of the disease. Conventional diagnostic methods are based on detecting specific classes of antigens or antibodies. IgA antibodies are produced earliest. IgM antibodies are discovered about a week post inoculation and remain for several months and years. The presence of IgG antibodies indicates *T. gondii*- infection, however, it does not inform about its onset [7,8].

The aim of this study is to determine the risk factors for the spread of the *T. gondii* infection among some risk contingents in Stara Zagora region, Bulgaria.
Materials and methods

Participants: A total of 365 patients were examined in a specialized office on parasitic diseases in the town of Stara Zagora. The participants in the study visited the office voluntarily due to clinical complaints or positive tests for carriers of antibodies against *T. gondii*.

Serological testing was performed in parasitology laboratories certified for immunodiagnostics. The sera of all 365 participants in the study were tested for specific IgG to *T. gondii* through enzyme-linked immunosorbent assay (ELISA), 116 were tested for specific IgM antibodies, and 26 also for IgG avidity.

The survey card for the epidemiological study on *T. gondii* infection was compiled the questions to clarify the main objective of the study.

For the statistical processing of the results, we used univariate logistic regression for analysing the link between the *T. gondii*-infection and the potential risks, as p-values < 0.05 were considered statistically significant; corrected OR; 95% CI. For data processing we used an MS Excel table.

Results

In the present study, 175 (47.9%) participants were seropositive for specific *T. gondii* antibodies. The sera of all participants were examined for specific IgG against *T. gondii*, 116 (31.8%) were tested for specific IgM antibodies, and 26 (7.1%) also for IgG avidity. Seropositive for specific IgG antibodies were 153 (87.4%), and for IgM antibodies were 89 (50.1%) persons. Patients with acute toxoplasmosis numbered 22 (12.6%). By sex, the seropositive levels of specific IgG *T. gondii* antibodies were respectively 138 (58.7%) among the females, and 37 (44.3%) among the males (p=0.06). The participants we divided into three age groups with varied seroprevalence, respectively: 7-19 - 22 (12.6%), 20-49 - 117 (66.9%) and 50-65+ - 36 (20.6%). Among children and teenagers, it was significantly lower (30.1%) compared to the adults (52.4%), (p<0.001) with clear tendency for a greater prevalence of the infection among the older participants. By location, the seroprevalence levels of anti-*T. gondii* IgG were respectively 114 (65.1%) the inhabitants of the regional center Stara Zagora, 7 (13.0%) - among the residents of the smaller towns in the region of Stara Zagora, and 54 (30.9%) - among the rural inhabitants.

Established possible risk factors for *T. gondii*-infection. In the study was found that five variables were found to be positively connected with *T. gondii*-infection (p<0.05): residence in urban areas, gardening or agriculture, consumption of undercooked meat, contact with yard cats, and knowledge about toxoplasmosis as a disease.

Discussion

Seroprevalence against *T. gondii*-infection in the world varies in wide ranges – from 1.82% among students in China [9], 4% in Korea, 23% among military in the Czech Republic [4], to 92% in Brazil [10]. In Bulgaria, between 20 and 51.5% of the population have antibodies against *T. gondii* [11,12]. The total distribution of the seropositive participants in our study was 47.9%, which corresponds to the data for the country. The level of seropositivity is higher among those living in smaller towns or rural areas versus those from the town of Stara Zagora (p=0.02; OR=0.56; 95% CI:0.4-0.9). In urban areas, the density of the stray cat population is higher, supported various cat caretakers. Under close to the natural conditions, the concentration of these parasitic pathogens is lower, and the chances for exposure are negatively related to the unfavorable results, which perhaps has a protective effect for toxoplasmic infection. Other studies, however, assert that rural people are exposed to much greater risk of infection with toxoplasmosis, because 95% of the domestic cats carry *T. gondii*, and they are abundant in rural and suburban areas [9, 13,14,15].

The seroprevalence levels of *T. gondii*-infection among children and teenagers are lower, compared to those of the adult participants in the study (p=0.000; OR=0.39; 95% CI:0.2-0.7). This is due to the increase in the population of immune individuals as they age, which is typical for infections creating permanent immunity. After their forties, between 30 and 90% of the population in Europe acquire seropositivity, which confirms the trend in our study [16,17].

Gardening or agriculture are also risk factors for *T. gondii*-infection – the proportion of immunized individuals engaged in these activities was significantly greater than that of individuals without specific IgG antibodies (p=0.000; OR=1, 86; 95% CI:1.2-2.9) which is similar to the results of a Chinese study [9].
We have found that the consumption of raw or undercooked meat is another risk factor for a *T. gondii* infection (p=0.01 corrected OR=1.85; 95% CI: 1.1-2.9). Various authors claim that the consumption of undercooked meat is a risk factor for toxoplasmosis, which is proven by our results, as well [18, 19].

The contact with cats has been proven as a risk factor in numerous seroepidemiological studies [4, 20]. In our study, the presence of yard cats as a risk factor for the spread of the infection (p=0.02; OR=1.64; 95%, CI:2.2-2.9). Of the 242 cat owners, 158 (60.1%) fed their pets an adapted diet that excluded raw meat, and this may have been the reason that cohabitation with cats was not dangerous for *T. gondii* infection.

More than half of the participants in the study – 194 (54.6%) had basic knowledge about *T. gondii* infection as a disease and the ways to prevent it. This group includes veterinary specialists and physicians, among whom the share of infested individuals is smaller than that of non-infected (p=0.02; OR=1.72; 95% CI:1.2-3.5). These results regarding awareness of toxoplasmosis show some similarity with another study of ours. [21].

**Conclusion**

This study shows that in the Stara Zagora region of Bulgaria, among a certain contingent of persons, the seroprevalence with *T. gondii* infection reaches the average rates for Bulgaria. *T. gondii* infection is a parasitic zoonosis, which is why it is impossible to eliminate the parasite in its reservoir and from the environment. Prevention in the field of public health should be directed against active transmission of the pathogen from known sources to humans. This includes limiting the consumption of undercooked meat, as well as increasing the health culture when raising pets. The irregularities in living a healthy lifestyle, and keeping cats as companion animals increase the public health risks for the spread of zoonotic infections.

**References:**


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Application of a combined methodology with tecar therapy for non-specific pain in the lumbosacral region.

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Abstract
Over the past 20 years, tecar therapy has proven its effectiveness and therapeutic effect. **Aim:** The aim of this study is to evaluate the long-term effect of tecar therapy in combination with mobilizations and specific exercises for patients with lumbosacral pain. **Methodology:** The study included 30 participants with persistent pain (more than 6 months), randomly divided in two groups – experimental (EG: n = 16) and control (CG: n = 14). Mean age 49.69 ± 4.56 years for the EG and 53.36 ± 4.34 for the CG. Merl d’Aubigne scale and assessment of the lumbar spine mobility was done before, after treatment and 3 months later. **Results:** The results show pain reduction and better lumbar spine mobility for the patients of the EG. The assessment done 3 months later show no significant changes. **Conclusions:** Combined therapy with Tecar presented a positive long-term effect for patients with chronic lumbosacral pain. **Key words:** chronic pain, tecar, lumbar spine

Introduction: Lower back pain is the number one cause of disability in the world. In a very large percentage of cases, the cause is unclear and the pain persists for more than 6 months. There are a number of therapeutic means to deal with this problem, but many times, despite their effectiveness, the pain recurs due to the daily stresses in the work environment or the household activities of the people suffering from this problem. Tecar therapy has proven its effectiveness in various studies in recent years. The application of resistive and capacitive electrical transfer leads to a strong reduction of pain and improvement at structural level of various tissues. It is widely used in a variety of clinical areas - orthopedics, traumatology, degenerative diseases and a number of others (Messina et al., 2022; Nakamura et al., 2022; Rodríguez-Sanz et al., 2021). There are also studies that follow the combination of tecar therapy with other means (Szabo et al., 2022). However, most of them are limited and follow patients in relatively short term. Manual therapy is a part of physiotherapy in which, through specialized manual techniques, better joint mobility and elasticity of soft and nervous tissues is achieved (Andreev et al., 2020; Mitova et al., 2020; Mitova et al., 2020). In the present study, we aimed to follow the effect of applying manual therapy, as an established and effective method used in physical therapy, and an additional technique in the therapy of muscle conditions such as TECAR therapy. Therefore, the aim of this study is to combine the conventional manual therapy with the relatively new Tecar approach to find a treatment plan that is effective in this kind of muscle diseases (Szabo et al., 2021).

Material and Methods: **Participants:** A sample of 30 participants were evaluated and met the inclusion criteria. They were randomly divided in two experimental (EG), and control group (CG). The EG consists 16 participants mean age (X ±SD) 49.69±4.56years. The CG was formed of 14 participants, mean age 53.36±4.34 years. **Test protocol and Instruments:** Merl d’Aubigne scale was used to assess the pain threshold. For the assessment of the lumbosacral mobility, we used goniometry and Schober test. The Roland-Morris Questionnaire was applied to assess the quality of life of the participants. **Procedure:** The duration of kinesitherapy procedures for patients in both groups was 35-40 minutes, four times a week, for a period of 4 weeks. Patients in the EG were given a specialized tecar procedure every other day for the entire treatment period with a duration of the procedure of 20-25 minutes. Tecar therapy (GIMA CR – 200, I-TECH) was done using the protocol program for LBP – resistive electric transfer – 15 minutes and
capacitive electric transfer – 10 min. combined with manual therapy and specific exercise. The CG received only tecar therapy. **Statistical analysis:** We used the primary statistics variables: arithmetic mean (±), standard deviation (Sd). In term to calculate statistically, significant differences we use Mann-Whitney posttest to compare independent quantitative variables.

**Results**

Mean values obtained of Merl d’Aubigne scale for the EG before specialized therapy were as follows: 4.5±0.63mm, and 3.79±0.8mm for the CG. After therapy results are 1.31±0.6 for the EG and 1.36±0.63 for the CG with no statistically significant differences (Mann Whitney, p>0, 05). The pain intensity decrease practically equal in patients of EG and CG (Fig. 1.).

**Figure 1.** Dynamic of the results of Merl d’Aubigne, Schober test and Goniometry (flex - ext) for EG and CG

Mean values of the Goniometry (flexion of the lumbar spine) for the EG, measured before after administered therapy were 30.3±6.18 and 53.13±3.09. For the CG obtained data before and after therapy were respectively: 26.4±4.57 before and 33.57±5.35 after therapy (Mann Whitney, p<0, 001). The results of the Schober test before procedures for EG were 1.38±0.5 and 3.94±0.25 at the end. For the CG data shows 1.71±0.47 before and 3.36±0.5, 4 weeks after therapy (Mann Whitney, p<0.005). The assessment is done 3 month later and data shows no significant differences for the patients of the EG. For the patients of CG the results are approaching the initial one as relapses are observed (Fig. 1.)

**Discussion**

Lumbosacral pain is a leading cause of disability worldwide. It affects a significant number of people and has a large burden on the health economy (Stoyanov et al., 2020). A complex and appropriate treatment approach is of utmost importance for the favorable outcome of this condition and reducing the risk of relapses. In the present study, we combined some specialized manual techniques with tecar therapy to manage pain and increase the mobility and functionality of the lumbosacral spine. The dynamics of pain and mobility of the lumbar region were monitored. Regarding pain, the results showed a strong reduction in EG, but also a retention of the results 3 months later. According to us and other authors, the rapid pain reduction is due to the application of capacitive and resistive electric transfer (Bretelle et al., 2020; De-Sousa et. Al., 2022). The pain is reduced already after the first procedures, which allows easier and painless application of manual techniques. In addition to the analgesic effect, the therapy also increases the elasticity of soft tissues (Valentini et al., 2021). This, in turn, provides additional mobility to the treated area. Based on the results obtained in CG, pain is also reduced, but the effect is much shorter-term. Pain in the lumbar region is often manifested as a consequence of a created muscle and joint imbalance of the structures. In response to the resulting instability and pain in the area, the structures increase their tension to stabilize and protect the area. This reduces the ROM and function of the lumbar spine. Manual therapy restores joint mobility and soft tissue elasticity. In order to maintain this positive effect over time, it is important to support
it with specialized exercises that ensure dynamic stability of the area and prevention of relapses. Tecar application, manual therapy and the specialized complex of exercises provide a longer-term effect – 3 months after therapy.

**Conclusion**

Pain reduction is significant in patients from both study groups. Regarding the mobility of the lumbosacral region, the results were significantly better in EG and show long term effect (3 months later).

**References:**


Study of the awareness and attitude of adults and elderly people regarding vaccines and vaccine preventable diseases

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Abstract
The increased susceptibility to infectious diseases in adults and elderly makes them an important target group for vaccination. Epidemiological data regarding vaccine-preventable diseases in the last decades of life emphasize the need to promote vaccination for them. The aim of our study was to research the awareness of adults and elderly people about vaccines and vaccine-preventable diseases, as well as their attitude towards vaccine prevention.

Materials and methods: survey, analysis of scientific literature, statistics from regional databases.

Results and discussion: A high relative proportion of the adults surveyed supported mandatory vaccines (78%). Almost all respondents (98%) shared the opinion that immunizations protect the individual and society from infectious diseases. According to 80% of respondents, vaccines are biological preparations that build active immunity against infectious diseases. 71% of those surveyed were not vaccinated with some of the recommended vaccines, and 19% believed that they were not safe and effective. The main reason why respondents are not vaccinated are worry about side effects (51%). 41% of respondents were not vaccinated against Covid-19. 46% of the respondents indicate that they need more information about vaccine prevention from specialists in the field.

Conclusion: A deeper knowledge and understanding of the optimal strategies to stimulate the immune system of the adults would have a huge impact not only in optimizing existing vaccines, but also in guiding the development of new ones, which are critically needed for the adults and elderly.

Key words: vaccine prophylaxis, Covid-19, awareness, attitudes, adults

Introduction
Immunization with modern vaccines is the safest and most effective way to protect against a number of infections. World practice shows that life-threatening infectious diseases have been eliminated or reduced to a minimum due to mass vaccination. Mandatory immunizations under current regulations are a guarantee of maintaining collective immunity at a high level. According to the current legislation, vaccines are mandatory for children, and adults must carry out the relevant re-immunizations. The attitudes of adults are extremely important for the effective implementation of immunoprophylaxis.

In just over a century, there has been a significant increase in human life expectancy due to improved living conditions, better medical care and a reduced risk of premature death [1]. As a result, the world's population is rapidly aging, with an increase in both the proportion and the absolute number of adults and elderly people, and hence the risk of a prolonged period of poor health in old age. The adults are a population that is vulnerable to a number of diseases and adverse effects of environmental risk factors. Age-related changes in the immune system contribute to an increased frequency and severity of infections in adults. Every year, more adults and elderly people worldwide suffer from serious health problems for which vaccines are available. This becomes a major social burden, as infectious diseases are often associated with long-term consequences such as impairments in daily activities, the appearance of disabilities or loss of independence, and the adults may need continuous assistance at high financial and psychological costs to public health system for families as well [2]. The increased susceptibility to infectious diseases in adults and elderly and epidemiological data regarding vaccine-preventable diseases in the last decades of life emphasize the need to promote vaccination and their attitudes are extremely important for the effective implementation of immunoprophylaxis. The aim of this study was to research the awareness of adults about vaccines and vaccine-preventable diseases, as well as their attitudes towards vaccine prevention.
Materials and methods
During the period July-August 2022, we surveyed 63 people from Stara Zagora over the age of 45. The survey included questions targeting the demographics of the respondents, their awareness of recommended vaccines, including against Covid-19, reasons for vaccine refusal and their personal contribution for protecting public health.

Results and discussion
Regarding the demographic data (gender and age distribution of the respondents), the men predominate - 67% (n=42), and the largest number of representatives is in the age groups 51-60 and 60-65 (n=18). A high proportion of those surveyed share the opinion that immunization protect the individual and society from infectious diseases and support mandatory vaccines (78%, n=49 and 98%, n=62, respectively) (Figure 1).

Figure 1. Your opinion FOR or AGAINST vaccines (mandatory and optional)

A large percent of the respondents are familiar with the fact that vaccines are biological preparations that build active immunity against infectious diseases (80%, n=51) and only 10% (n=6) think that vaccines provide ready protection against infectious diseases or are not aware of the relevant statement. A relatively large percentage of respondents were aware of the infectious diseases causing high mortality before mandatory immunizations were introduced to protect against them - tuberculosis, variola, poliomyelitis (78%, 73%, 68% respectively). (Figure 2).

Figure 2. Which infectious diseases do you think were the cause of high mortality before the introduction of mandatory immunizations?

Twenty four percent of the respondents (n=38) are not convinced of the safety and effectiveness of vaccines and almost the same percentage believe that the risks of recommended vaccines for adults are greater than their benefits (29%, n=18). Therefore, it is appropriate to increase health awareness among all communities based on reliable, scientifically proven facts about the benefits of vaccination. Seventy-one percent of respondents (n=45) were not vaccinated with any of the recommended adult vaccines – for example, influenza, pneumococcal, herpes zoster, hepatitis A/B or any of the recommended travel vaccines. The main reason why the small percentage (29%, n=18) of respondents undertook vaccination was to protect themselves and their relatives from infection (83%). Other reasons mentioned in a significantly smaller percentage (33% and 28%, respectively) of those surveyed are hope and calmness, and personal responsibility (Figure 3). The main reasons given by adults why they would not vaccinate with recommended vaccines are the worry of side effects (51%, n=23), the lack of sufficient information about the available vaccines (33%, n=15) and distrust in the healthcare system (36%, n=16) (Figure 4).
Vaccination is the most effective strategy for preventing a number of infectious diseases, facilitating healthy aging and guaranteeing the maintenance of collective immunity at a high level. Immunization for adults can reduce morbidity, mortality and cost to the country's economy. Despite the reduced immune response in the adult population, vaccination can provide valuable protection because prevention of diseases in these individuals is more effective than treatment [4]. In addition, vaccination can also play an important role in preventing serious complications after some infection diseases, such as pneumonia, cerebrovascular accident, cardiovascular disease [5, 6]. It is essential to develop vaccination strategies specifically tailored to the adults and elderly population, in terms of both vaccine composition and vaccination protocols, taking into account the aging immune system and inflammation, the two main features of aging. Factors that create negative attitudes toward vaccines and that could contribute to low vaccination coverage among the adults population include limited public awareness of vaccinations for adults, doubts about the reliability of vaccines, etc.

**Conclusion**

Vaccination hesitancy has been a concern worldwide for several decades, and the situation is even more controversial with recommended vaccines for adults. Immunization coverage rate in adults is very low. Health awareness of the adults about vaccine prevention against various infectious diseases would help to increase their motivation to
vaccinate, minimizing the risk of severe disease and mortality. To achieve a high immunization coverage in the adults and elderly, the application of a multidisciplinary approach is necessary.

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Options for ultrasonographic monitoring of the dynamic of abdominal pain in patients with Henoch-Schoenlein purpura

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Abstract
Injury of the gastrointestinal tract in Schönlein-Henoch disease is one of the most serious potential conditions. Common potential risks of serious late complications are: small bowel perforation, intussusception, gastrointestinal bleeding and others. Ultrasound diagnosis is effective as first-line screening method in children with an abdominal form of the disease (gastrointestinal symptoms). In the present clinical observation, we present our experience with children with Schönlein-Henoch-abdominal form, in which we studied the new aspects of the changes in the coagulation system, as well as the monitoring of abdominal involvement by ultrasound examination of the small intestines. The results of the ultrasound examination in these patients gives grounds for non-maintenance of the universal treatment with corticosteroids.

Key words—Henoch-Schoenlien, abdominal pain, ultrasound.

Introduction
Schonlein-Henoch vasculitis is the most common acute systemic vasculitis of childhood, affecting the skin, gastrointestinal tract, joints, and kidneys. The main clinical manifestation is purpura, without accompanying thrombocytopenia, with a symmetrical location on the glutes, both lower extremities, abdominal pain, arthralgia, blood in the stool, hematuria and/or proteinuria.[1, 2]

Increased production of IgA by the immune system in response to a mucosal-presented antigen, such as bacteria, viruses, or fungi, is thought to be the underlying mechanism. In the acute phase, there is an increased concentration of the provoked cytokines that cause the source of the endothelium and TNF-α is also detected.[3]

The overall incidence of affected children is estimated to be 3-26.7 per 100,000. [4, 5] The true incidence of Schönlein-Henoch disease is probably underestimated because cases are not reported in public and health registries. The first description of the disease was made by Scholein in Berlin in 1832, after which Henoch associated the appearance of the rash with gastro-intestinal manifestations, and later with renal involvement. [6, 7] In Bulgaria, the first general information about the syndrome was made by St. Dimitrov who had observed 32 children with Scholein-Henoch for the period 1942-1948.[8] In 1966 Liliana Basheva-Staneva published a monographic work, after which new information was missing for many years. [9] In 1996 G. D. Georgiev wrote the first scientific work focused on the treatment of the disease.[10]

Schonlein-Henoch purpura is usually diagnosed based on its clinical features—purpuric rash, colicky abdominal pain, arthralgia, hematuria, and/or proteinuria. In 10 to 40% of cases, gastrointestinal and/or joint symptoms may precede the skin rash, which is a mandatory diagnostic criterion, by up to two weeks. [11, 12]

Although the prognosis of Schonlein-Henoch purpura is generally good, involvement of the gastrointestinal tract can lead to one of the most serious potential complications such as: massive gastrointestinal bleeding, bowel infarction, perforation, intussusception, and peritonitis. Gastrointestinal manifestations of Schonlein-Henoch are associated with intestinal edema and intramural hemorrhage.

A bidirectional relationship between the inflammatory system and the coagulation system was demonstrated. The results of standard coagulation tests show that PT and aPTT are within ranges. [13] D-dimer and FDP's scores were less associated with disease’s activity than inflammatory markers such as WBC, ANC, and CRP. [14] Factor XIII, a fibrin-stabilizing factor, significantly decreases during the acute phase of the disease. [15, 16] Von Willebrand factor can be used as a specific marker of vascular damage and Schonlein-Henoch activity. [17]
Materials and methods

In the present clinical observation, we aimed to determine laboratory markers in the involvement of the gastrointestinal tract in Schonlein-Henoch disease and subsequent monitoring of echographic changes in the small intestinal wall. As a result, based on the obtained data, we can achieve a refinement of the therapeutic behavior and reduce the need for invasive surgical procedures. In addition to standard laboratory tests, levels of von Willebrand factor, f. XIII, D-dimer and fibrinogen, IgA, C3, C4.

Ultrasound follow-up was performed with a HITACHI ARIETTA 70 ultrasound machine, using a 5-7MHz high-frequency probe, in the morning. Normal intestinal loops have a stratified appearance on high-resolution ultrasound with the following 5 layers: mucosal surface with lumen (hyperechoic), mucosa (hypoechoic), submucosa (hyperechoic), muscularis (hypoechoic), and serosa (hyperechoic). [18] In healthy children, small bowel loops are compressible, show minimal vascularization, and have a wall thickness < 2.5 mm. An intestinal wall with a thickness equal to or greater than 3 mm is considered thickened. High-resolution ultrasound can detect changes in the intestinal wall in three degrees. Grade I defines the changes as an expression of infiltration of one or more of the surface layers concerning stratification. [19] In the absence of a pathological correlation in grade II, the abnormalities are more difficult to explain. Extravascularly, the blood has a different and heterogeneous reflectivity but has a mainly echogenic appearance. Most likely, the additional echogenic layer internal to the submucosa may be secondary, due to deep mucosal and submucosal bleeding. [20] This leads to the so-called pseudo-differentiation of the appearance. Such an unusual finding in the absence of coagulation disorder and trauma could be a strongly suggestive hemorrhagic vasculitis.

The established data for elevated von Willebrand factor concentrations are accepted as a reliable index of vascular damage. The von Willebrand factor does not play the role of an acute phase reagent but serves as a marker of vascular damage. Elevated values of vWF: Ag reflect the increased amount of circulating factor released from the endothelium due to vascular damage. Values return to normal when patients are in remission and remain above normal ranges with the continuation of the symptoms.

Fibrinogen, D-dimer, and FDPs levels are significantly higher during the acute phase of the disease. D-dimer and FDPs are more strongly associated with the clinical severity of the disease. [14] Therefore, they play an important role as indicators of the involvement of the gastrointestinal tract during the acute phase of Henoch-Schoenlein purpura.

Results

During the period 2018-2022, they were monitored, examined and treated at the UMBAL "Prof. Stoyan Kirkovich", Stara Zagora according to the generally accepted and up to now established diagnostic and therapeutic approach 15 children with Schoenlein-Henoch purpura. The standard diagnostic and therapeutic approach in Schonlein-Henoch cases and presenting with abdominal pain is to initiate corticosteroid therapy, and refinement of initiation, duration, and dosage has thus far been entirely empirical.

Based on the studies of Yang Y, Huang M, et. al for precision in the therapeutic plan in 9 children we examined the levels of vWF:Ag and factor XIII. In parallel, an ultrasound evaluation of the changes in the small bowel wall was performed. The following was established from the laboratory tests: (Table 1)

The level of vWF:Ag, which can currently be accepted as one of the most reliable criteria for the severity of vascular damage, in the studied clinical cases showed values from 95.6% to 181.40%, with an average value of 154.31%. A similar dependence is also found when evaluating the levels of factor XIII, values from 68% to 120%, with an average value of 90.8%.

From the performed ultrasound examinations of the small intestinal wall, a directly proportional relationship between the levels of f XIII and vWF:Ag and the degree of disturbance in the differentiation and the thickness of the small intestinal wall can be seen. Monitoring of sonographic changes was performed on the first, fifth and seventh days of the onset of abdominal pain, allowing to specify the need for corticosteroid therapy and its duration.

Table 1. Results from the undergone tests.
Figure 1. Ultrasound examination of gastrointestinal tract of patients with Schonlein-Henoch at the appearance of abdominal pain.

Discussion
The previous assessment of vascular changes, based only on the assessment of the D-dimer level, appears to be insufficiently informative by itself, and cannot be an indicator for the initiation of corticosteroid treatment. Therefore, this innovative diagnostic study based on changes in the levels of factor XIII and von Willebrand factor in parallel with the ultrasound examination of the small intestinal wall is an advanced approach to refine the start, duration and dose regimen of corticosteroid treatment. If the result of this treatment is not good, there is reason to supplement it by applying high doses of intravenous immunoglobulin G, cyclosporine, cyclophosphamide, dipyridamole, dapsone, etc.

Conclusion
The conclusions drawn so far give reason to assume that: the clinical, therapeutic and psychological effect of precision in the treatment of the abdominal form in Schonlein-Henoch disease will minimize the risk of potential surgical complications and interventions.

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Knowledge and beliefs about homeopathy: a cross-sectional survey among Bulgarian population

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Abstract

Homeopathy is one of the most widespread alternative methods of treatment in Bulgaria in the last 25-30 years. The aim of the research is to study and analyze the knowledge and attitudes of Bulgarians over the age of 18 regarding the application of homeopathy as a curative method in general medical practice. A cross-sectional survey among a sample of the general Bulgarian population was conducted during a 4-week period in April-May 2022. The data were collected using the Google Forms platform via an online questionnaire. A total of 508 completed responses were collected (women – 450, men – 58). The overwhelming number are familiar with homeopathy and have used it before for their own health problems (97% of women and 86% of men). A large number of those who have used homeopathy report an improvement in their health (88% of women and 74% of men). The majority of respondents believe that homeopathy is useful for health care (93% of women and 79% of men). Further representative studies are needed to determine the role of homeopathy as a complementary method in general medical practice.

Keywords: homeopathy, CAM, primary care, general medical practice

Introduction

Homeopathy is one of the most widespread alternative methods of treatment in Bulgaria in the last 25-30 years. Its use in many European countries, as well as worldwide, is increasing, despite the controversy regarding its effectiveness and scientificity. A number of countries include homeopathy in their publicly funded healthcare systems (UK, France, Italy, Germany, Switzerland, India, Pakistan, Brazil, and Mexico) [1]. In Bulgaria, in 2017, a study on homeopathy was conducted in four cities: Sofia, Varna, Veliko Tarnovo and Burgas. It defines a socio-demographic profile of chronically ill homeopathic patients: mostly women, with higher education, aged between 30 and 50 years. The sources of information about homeopathy, the reasons for choosing homeopathic treatment and the satisfaction with the obtained results are investigated. 60.5% of the surveyed persons stated that by using this method they improved their health. The message is that the future of successful chronic disease management lies in an integrative approach and the inclusion of alternative methods such as homeopathy in healthcare [2].

One of the goals of our research is to study and analyze the knowledge and attitudes of Bulgarians over the age of 18 regarding the application of homeopathy as a curative method in general medical practice.

Materials and methods

A cross-sectional survey among a sample of the general Bulgarian population was conducted during a 4-week period in April-May 2022. The target participants were Bulgarian adults aged 18 years and above. The data were collected using the Google Forms platform via an online questionnaire. A snowball sampling strategy was used to distribute the online questionnaire via social media (mainly Facebook, Viber). Participants were asked to share the questionnaire link to individuals in their social circles. These social media platforms were chosen because they are widely used among the Bulgarian population across sociodemographic characteristics. A total of 508 completed responses were collected (women – 450, men – 58). An informed consent was signed by all participants.

The analysis of the results is done with a software statistical package Jamovi ver. 2.3.18. A frequency analysis and the chi-square test of independence was used to evaluation. The confidence interval at the level of significance $p < 0.05$ provides information for assessing the accuracy of the analyzed indicators.

Results

A total of 508 completed surveys were received, of which women n=450 and men n=58. Several socio-demographic characteristics were examined: age, gender, religious affiliation, place of residence and monthly income of a family member. The most numerous age group among respondents of both sexes is between 35 and 54 (74.2% of women and 72.4% of men), majority (84%) live in a large city. The predominant religion is Christianity (92%). The monthly income was defined in the following categories: below BGN 850 – indicated by 31% of women and 21% of men; between BGN 851 and BGN 1,050 – 27.7% of women and 21% of men and over BGN 1,050 – respectively 41% of women and 58% of men (Table 1).
Table 1. Socio-demographic characteristics of respondents

<table>
<thead>
<tr>
<th>Demographic indicator</th>
<th>Women, %</th>
<th>Men, %</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
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<tr>
<td>18 - 34</td>
<td>8.9 %</td>
<td>12.1 %</td>
</tr>
<tr>
<td>35 – 54</td>
<td>74.2 %</td>
<td>72.4 %</td>
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<td>over 55</td>
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<td>15.5 %</td>
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<td>Residence</td>
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<td>Big city</td>
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<tr>
<td>Small city</td>
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<td>15.5 %</td>
</tr>
<tr>
<td>Village</td>
<td>1.1 %</td>
<td>3.4 %</td>
</tr>
<tr>
<td>Religious affiliation/beliefs</td>
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<td></td>
</tr>
<tr>
<td>Christianity</td>
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<td>82.8 %</td>
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<tr>
<td>Atheism</td>
<td>2.7 %</td>
<td>10.3 %</td>
</tr>
<tr>
<td>Others</td>
<td>4.0 %</td>
<td>6.9 %</td>
</tr>
<tr>
<td>Monthly income of a family member</td>
<td></td>
<td></td>
</tr>
<tr>
<td>under 850 lv.</td>
<td>31.3 %</td>
<td>21.0 %</td>
</tr>
<tr>
<td>851 – 1050 lv.</td>
<td>27.7 %</td>
<td>21.1 %</td>
</tr>
<tr>
<td>over 1050 lv.</td>
<td>40.8 %</td>
<td>57.9 %</td>
</tr>
</tbody>
</table>

Respondents were asked questions about their knowledge of the homeopathy method, its previous use and its results (Fig. 1, Fig. 2). Although the sample is not representative, the large number of those who answered positively is impressive – 97% of women and 86% of men said they used the method. It is possible that this high percentage was a result of the way the survey was distributed, suggesting that people who liked the method were more likely to participate in the survey. People aged 35-44 (34.8%) and 45-54 (36.2%) were the predominant group who used homeopathy. Those aged 18-34 are 8.2%, and over 55 – 16.3%. Knowledge of the method appears to be related to age ($p < 0.001$). Regarding religious affiliation, it is difficult to draw a conclusion about its influence regarding the choice of homeopathy as a treatment method.

The largest share of homeopathy users lives in a large city (84.6%). 14.2% live in a small town, 1.2% of them live in a village. No correlation was found between the place of residence and the use of homeopathy among the respondents ($p = 0.513$).

Fig. 1. Distribution according to previous use of homeopathic medicines ($p < 0.001$)

The relationship between the monthly income of a family member and the use of homeopathy was also investigated. A monthly income of less than BGN 850 was declared by 30.8%, between BGN 851 and BGN 1,050 by 27.7% and over BGN 1,050 by 41.5% of those who used homeopathy. Monthly income was not confirmed to influence the decision to use homeopathy ($p = 0.3$).

The relative share of those who believe that they had an improvement in their health condition after applying homeopathic treatment is also high (88% of women and 74% of men). Men are likely to be more critical, given the greater proportion of those who said they were unsure of the positive effect or that they had no improvement in their health – a total of 26%.
Figure 2 shows the opinion of the respondents on whether homeopathy brings additional benefits to health care. The majority – 93% of women and 79% of men – answered positively. Only 2% of women and 10% of men are not convinced of the usefulness of homeopathy. 5% of women and 10% of men hesitate.

Discussion
The present study confirms some of the findings of the 2017 study on homeopathy in Bulgaria. [2]. Predominantly those who seek homeopathy to solve their health problems are women, between 35 and 54 years old. The conclusions of a number of studies around the world are similar [3, 4, 5]. A smaller number of men participated in our study, but it is interesting that among them the proportion of those who used homeopathy, as well as those who declared that it helped them, was high.

The prevailing opinion that homeopathy is beneficial to health care in general is noteworthy. In other studies [6, 7, 8] it is found that patients expected their family physician to refer them to CAM, including homeopathy, to have updated knowledge about CAM, and to offer CAM treatment in the clinic based on appropriate training. It can be assumed that homeopathy could be part of an integrative approach in health care, given the increased number of people wishing to use it, as well as the large number of doctors who have completed a training course in homeopathy in Bulgaria [2].

Conclusion
The relative proportion of people who used homeopathic treatment as well as those whose health improved afterwards among the respondents is high. The majority of respondents believe that homeopathy is useful for health care. Further representative studies are needed to determine the role of homeopathy as a complementary method in general medical practice.

References

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Sialendoscopy as a therapeutic approach in sialolithiasis

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Abstract
Salivary glands are important anatomical structures for the otorhinolaryngologist. Sialendoscopy is a procedure used to examine the ducts of the glands. It is a minimally invasive method employed as a diagnostic and therapeutic technique in various salivary gland disorders of non-neoplastic character. The aim of this study is to determine the value of sialendoscopy as a method of choice for obstructive pathologies.

Material and methods: Prospective or retrospective study of adult patients who had undergone sialendoscopy or combination of endoscopy and open surgery were selected. The results were measured by symptoms free time, reduction of symptoms, the need of sialadenectomy or any other complications.

Results: As a result of this study we can say that people who had undergone sialendoscopy have a good clinical and therapeutic outcome. The ratio of needed surgeries is low and we see a stable decline in symptoms with long term free of disease period. 9 of 10 patients with obstructive salivary gland disease were observed for a period of a year and they had excellent outcome and free of morbidity time. The results are similar to these published in the literature worldwide.

Conclusion: As a conclusion we can say that sialendoscopy is effective, safe and minimally invasive procedure for patients with obstructive disease of the salivary glands. The ratio of complications occurred after the procedure are really low. The preservation of the gland is the major benefit of sialendoscopy. The method is now used largely for therapeutic and diagnostic purposes. It is efficient and precise for the management of salivary gland pathologies.

Key words: sialolithiasis, salivary glands, sialendoscopy, obstructive diseases, therapeutic approach

Introduction
Salivary stones, also called sialolithiasis, are small deposits of calcium and other minerals that form in the salivary glands. Larger stones can block the flow of saliva and cause the affected gland to swell. This disease is more common in men.

Eighty percent of salivary gland stones occur in the submandibular gland. Others can occur in the parotid and sublingual glands. [1]

Patients with sialolithiasis typically present with postprandial salivary pain and swelling. They may have a history of recurrent acute suppurative sialadenitis. On examination, bimanual palpation along the course of the duct may reveal the stone. Ultrasonography and non–contrast-enhanced computed tomography are accurate in detecting the stone. [2]
Conservative measures of treatment like massage of the gland, sialagogues, antiinflammatories, and where indicated, antibiotic medication most of the time should precede more invasive measures. Decisive parameters for the further management are size, location (distal duct, hilar region, intraparenchymal ductal system) and number of the stones. [3] Sialendoscopy is a good diagnostic tool for ductal pathology and unlike other radiological procedures findings of sialendoscopy correlate fairly with the symptomatology. [4]

Matherials and methods
The present paper is based on the clinical experience of the ENT clinic of UMHAT Burgas, Bulgaria including an analysis of the information on the topic in the world literature. All patients underwent computed tomography and sialendoscopy. If an obstructive disease was approved the diagnostic endoscopy was converted to therapeutic.

Results:
We evaluated 16 patients with obstructive salivary gland diseases. 12 of them had stones in the ductal system of submandibular gland and 4 of them in the parotid gland.

<table>
<thead>
<tr>
<th>Gender/Location</th>
<th>Submandibular gland</th>
<th>Parotid gland</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>10</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 1: Comparison between gender and location of the salivary stones.

The retrospective analysis shows that the disease was unilateral in all of the patients. A total of 16 glands were treated (12 submandibular glands and 4 parotids). According to gender patients are 13 male and 3 female. The age is between 24 and 96 years old (mean - 57 y). All patients were treated for lithiasic obstructive disease (100%) - in 12 patients (75%) it was in submandibular glands, 4 of patients with parotid stones (25%). 2 of patients had parotidectomy and 1 had right submandibular gland removed because of the localization of the stones.

Discussion
Obstructive sialadenitis is the most common non-neoplastic disease of the salivary glands, and sialendoscopy is increasingly used in both diagnosis and treatment. [5] Chronic sialadenitis is mainly caused
by salivary stones and it presents 50% of all the salivary gland diseases. Sialendoscopic techniques are relatively new and are increasingly used in the management of non-neoplastic diseases of the major salivary glands. Since histopathological studies suggest that salivary glands removed for sialolithiasis have normal glandular architecture, organ preservation should be the first goal of every treatment, reducing the morbidity of the open surgery.

Though there are more or less set treatment protocols for tumors of salivary glands, other pathologies like sialolithiasis and juvenile recurrent parotitis have been treated on arbitrary basis. Sialadenitis secondary to obstructive pathologies including sialoliths, strictures and ductal polyps, remains the most common disorder of the salivary gland.

Though it is an invasive procedure, morbidity associated with sialendoscopy is mostly minor and most of the time is temporary. [4]

![Stone extracted from right submandibular gland by sialendoscopy and a basket in our clinic.](image)

**Fig. 2:** Stone extracted from right submandibular gland by sialendoscopy and a basket in our clinic.

**Conclusion**

Endoscopic visualization of the ductal system is allowed by sialendoscopy and it developed the possibility for minimally invasive surgical approach to sialadenitis. This is an impressive achievement in the area of otorhinolaryngology and it could be a real benefit for the patients.

The technique has the advantage of diagnosing and treating the most common causes of obstruction in the same procedure. After spreading around the world this method the indications for open surgical approaches has been reduced. This leads to minimal chance of complications - facial or lingual nerve damage, scar and etc.

In obstructive salivary pathologies, sialendoscopy is currently being used largely for therapeutic purposes and its role as the first diagnostic tool of investigation is being evaluated. Availability of new miniaturised instruments for therapeutic purposes and enhanced optical resolution should increase the efficacy and precision of sialendoscopy in the management of salivary gland pathologies. [6]

**Acknowledgments:** None.

**Conflict of interest:** The Authors declare that there are no conflicts of interest involved in this manuscript.

**References:**


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Family environment as possible risk factor for development of molar incisor hypomineralization in children

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Department of Pediatric Dentistry, Faculty of Dental Medicine, Medical University – Plovdiv, Bulgaria

Abstract

**Purpose.** The aim of the study was to investigate the possible influence of socioeconomic status of the family, education of the mother and tobacco smoke exposure on the development of Molar Incisor Hypomineralization (MIH).

**Materials and Methods.** A cross-sectional study was performed in Plovdiv, Bulgaria among 1851 school children aged 6-10 years. A questionnaire was given to the parents to evaluate the impact of different environmental factors on the etiology of MIH.

**Results.** It was found that the prevalence of MIH was 9.2%. Among the MIH group 24.3% of the respondents confirmed smoking at home, while within the group without MIH this percentage was 14.2%. Within the MIH group 3.6% determined themselves as having a low, 85.6% average and 10.8% a high socioeconomic status, in the group without MIH the corresponding relative shares were 2.6%, 92.5% and 4.9%. It was found a statistically significant correlation between MIH and tobacco smoke exposure ($p<0.005$) and between MIH and socioeconomic status ($p<0.05$). Maternal education was not significantly correlated with MIH ($p>0.05$).

**Conclusions.** Smoking at home and socioeconomic status were found to be possible contributing factors in the etiology of MIH in children.

**Keywords:** children, MIH, socioeconomic status, tobacco smoke

Introduction

Molar Incisor hypomineralization is defined as a hypomineralization of systemic origin that affects one to all first permanent molars and is often associated with affected permanent incisors [1]. The etiology of MIH is believed to be multifactorial and still not enough clarified. It is widely accepted that postnatal factors can influence the occurrence of MIH during the first 4 years of life such as childhood diseases, medication, malnutrition, environmental pollution [2]. Many authors assume that children with poor general health and systemic diseases are more susceptible to developmental disorders of the enamel. These different factors theoretically may alter the ameloblast function due to direct influence of the disease or indirectly, due to hypoxia, hypocalcaemia, acidosis and disturbance in phosphate resorption [3]. While the association between postnatal medical problems and MIH is well established, there is not enough research, focusing on the effect of environmental pollution, urbanization, family environment and socioeconomic factors. The lack of sufficient knowledge and the controversy of the existing data motivated our study, which purpose was to investigate the influence of the maternal education, socioeconomic status of the family and tobacco smoke exposure at home on the etiology of MIH in children.

**Materials and Methods**

A cross-sectional study was performed in Plovdiv, Bulgaria. The schools participating in the study were randomly selected from all public schools in Plovdiv. 1851 children aged 6-10 years were examined by 5 calibrated specialists of Pediatric dentistry department. This was performed in the school’s classrooms using sterile dental kit and flashlight. Prior to the oral exam informed consent was given to the parents of the children and those who didn’t return signed paper were excluded from the examination. To assess the prevalence of MIH the European Academy of Pediatric Dentistry (EAPD) 2003 criteria for diagnosis were
used. A questionnaire was given to the parents to evaluate the impact of different environmental factors on the etiology of MIH. The socioeconomic status was estimated by themselves as low, average, or high and maternal education as primary, secondary or higher. There was a question “Do you smoke at home” with possible answers “yes” and “no”. All collected data was analyzed using statistic software package SPSS 17. Descriptive statistics and chi-square test of independence were applied to check the correlation between factors from the family environment and prevalence of MIH. A value of p<0.05 was considered statistically significant.

Results
One thousand eight hundred and fifty-one school children participated in our study, similarly, distributed in gender. The prevalence of MIH was found to be 9.2%. There was no significant correlation between gender and MIH $x^2(1) =0.538$, $p>0.05$;

From all the examined children 1580 returned filled questionnaire. The distribution of families with self-reported low, average and high socioeconomic status is presented in table 1. It was found statistically significant correlation between MIH and socioeconomic status $x^2(2)=7.712$, $p<0.05$.

Table 1. Socioeconomic status of the families within the MIH and healthy group

<table>
<thead>
<tr>
<th></th>
<th>Low</th>
<th>Average</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIH</td>
<td>4 (3,6%)</td>
<td>95 (85,6%)</td>
<td>12 (10,8%)</td>
</tr>
<tr>
<td>Healthy</td>
<td>37 (2,6%)</td>
<td>1323 (92,5%)</td>
<td>70 (4,9%)</td>
</tr>
</tbody>
</table>

It was found higher relative share of higher maternal education in the MIH group (61.9%), compared to the healthy group (68.4%), but these differences were without statistical significance $x^2(2)=2.154$, $p>0.05$.

The relative shares of families smoking at home is demonstrated in table 2. It was found a strong statistically significant correlation between smoking at home and prevalence of MIH $x^2(1)=8.315$, $p<0.005$.

Table 2. Tobacco smoke exposure within the MIH and healthy group

<table>
<thead>
<tr>
<th></th>
<th>Smoking at home</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIH</td>
<td>27 (24,3%)</td>
<td>84 (75,7%)</td>
</tr>
<tr>
<td>Healthy</td>
<td>207 (14,2%)</td>
<td>1250 (85,8%)</td>
</tr>
</tbody>
</table>

Discussion
Recent studies and meta-analyses show that peri- and postnatal factors are more likely to increase the odds of causing MIH than prenatal factors [4]. That motivated the focus in the current study. It is well established that socioeconomic status is important contributing factor, that may influences the overall quality of life, diet, education, access to medical care, general health. Despite the large number of studies regarding the prevalence and etiology of MIH, there is inconclusive data concerning the relationship between the socioeconomic status and prevalence of the disease [5,6,7]. A study in the UK found that children from high-income groups had a higher prevalence of MIH [8]. In contrast, a study in Brazil showed that children from rural areas had a higher prevalence of MIH than their urban counterparts [9]. In our study we also found higher prevalence of families with high socioeconomic status in the MIH group. A possible explanation may be a higher percentage of caesarean sections among them or more urbanized and thus more exposed to different kind of pollution settlements of such families. Some of the commonly suggested etiological factors for MIH have been associated with low maternal educational background, such as preterm birth and low birth weight [10]. A Mexican study among 686 children showed higher severity of MIH, but
not higher prevalence, associated with low maternal education [11]. In our study we found higher prevalence of higher maternal education in the healthy compared to the MIH group. The lack of statistical significance of the results might be attributed to the fact that all the participants were from the city of Plovdiv and the prevalence of mothers with average and higher education was very high, while the relative share of those with low education was under 2%. There is not enough knowledge about the impact of environmental pollution, such as dioxins and tobacco smoke on the development of MIH. The role of the dioxins is examined in very few studies and the results are conflicting [12,13]. In our research we found a strong statistically significant correlation between smoking at home and prevalence of MIH. This is consistent with the available data found in the scientific literature [14, 15]. Serious limitation of the current study is that we didn’t know the exact timing and duration of the influence of secondhand smoke upon the child, as the participants in the survey were simply asked “Do you smoke at home”. It would be much more valuable if that question was more specific. Nevertheless, we assume that smoking is a habit that usually starts early and persists throughout life. These results don’t allow us to draw precise conclusions but rather implicate the need for future more profound investigations in this field.

**Conclusion.** Socioeconomic status and smoking at home were found to be possible contributing factors for MIH in children. A full understanding of etiology and pathogenesis of this disease is needed to enable better awareness, management, and eventual prevention. This imposes the need to search for the whole spectrum of factors that may play role in the development of MIH.

**References:**


Effect of the new antiepileptic drug Lacosamide on cognitive performance in an experimental model of drug-induced amnesia

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Abstract

**Purpose** The present study was undertaken to assess the effect of a new anticonvulsant lacosamide (LCM) on cognitive functions in a model of drug-induced amnesia with Diazepam (DZP).  

**Materials and methods** Twenty two male adult rats were trained, divided into three groups (n=8): 1*st* group (controls) treated with saline p.o.; 2*nd* (DZP group) – with saline p.o. and DZP 2.5 mg·kg⁻¹ i.p.; 3*rd* (LCM group) – LCM 3 mg·kg⁻¹ p.o. and DZP 2.5 mg·kg⁻¹ i.p. All rats were trained on a device for passive and active avoidances Shuttle box. Learning sessions were performed for 5 consecutive days and on day 12 was the test for long-term memory traces.  

**Results** The DZP animals decreased significantly the number of avoidances, escapes and intertrial crossings during both learning and memory sessions compared to the controls. The LCM group increased significantly only the number of passive avoidances and intertrial crossings during the learning session and on the memory retention test compared to the DZP animals (p<0.05).  

**Conclusion** The dose of 3 mg/kg LCM improve cognitive performance and locomotor activity in a model of DZP-induced amnesia in rats.  

**Key words:** Lacosamide, Diazepam, learning, memory, shuttle box.

Introduction

Memory can be defined in many different ways according to its: (a) duration – short-term and long-term; and (b) stages – acquisition, consolidation and retrieval (Beracochea, 2006). Benzodiazepines (BZD) have been widely prescribed due to their anxiolytic, sedative, hypnotic, anticonvulsant and muscle relaxant effects. This group of drugs acts through facilitating the action of γ-amino butyric acid (GABA) leading to enhancement of the chloride influx through the GABAₐ receptor complex (Muñoz-Torres et al. 2011). Moreover, it is well known that BDZs cause cognitive and psychomotor impairment (Muñoz-Torres et al. 2011). Diazepam (DZP), which represents a BZD derivative drug, is widely used in experimental models of drug-induced amnesia. It can affect the performance and brain activity in different tasks, ranging from those that demand low cognitive processes to others that involve higher cognitive load such as attention, learning and memory (Beracochea, 2006).

In addition, it is shown that cognitive impairment is frequently observed in people with epilepsy and this can be a result not only of the disease but medication treatment as well (Liguori et al. 2018). Lacosamide (LCM) is a new antiepileptic drug (AED) which reduce seizure frequency in patients with uncontrolled partial-onset seizures (Ben-Menachem et al., 2007). LCM represents a new class of AED which enhances the slow inactivation of voltage-gated sodium channels and prolonged the hyperpolarization (Biton, 2012). The clinical and experimental data about the effect of LCM on cognitive performance is still scarce. The present study aimed to assess the effect of LCM treatment on cognitive performance in a model of drug-induced amnesia with DZP in rats.

**Material and Methods**  
Male Wistar rats (150-160 g) were used in the study (n=24). They were housed in cages and fed standard rat chow and water *ad libitum*. The rats were maintained at an ambient temperature of 21-25° C with a 12/12-h dark-light cycle. The experimental protocol was approved by the Bulgarian Food Safety Agency.
The rats were divided into three groups (n=8): 1st group (controls) was treated with saline p.o. and saline i.p.; 2nd (DZP controls) – treated with saline p.o. and DZP (2.5 mg·kg\(^{-1}\)) i.p.; and 3rd (LCM group) – LCM - 3 mg·kg\(^{-1}\) p.o. and DZP (2.5 mg·kg\(^{-1}\)) i.p. Diazepam was injected intraperitoneally half an hour after oral administration of lacosamide. The learning and memory functions of the rats were assessed half an hour after the administration of DZP.

Cognition was assessed with a test for active and passive avoidances - Shuttle box (Ugo Basile, Italy). The learning session was held for 5 consecutive days and consisted of 30 trails daily, each consisting of a 6-seconds light and sound stimulation (670 Hz and 70 dB), followed within 3 seconds by foot electrical stimulation through the grid floor (0.4 mA). Each session was followed by 12 seconds of rest. A retention test for long-term memory traces was performed on the 7th day after the last training. The behavioral parameters that were measured are: number of avoidances (number of correct responses on conditioned stimuli); number of escapes (number of unconditioned responses), and number of intertrial crossings.

Statistical analysis
Data are presented as mean ± standard error of the mean (SEM). Statistical evaluation was done by one-way ANOVA followed by Tukey’s post-hoc test. The intergroup differences were assessed by a paired Sample t-test. The level of significance was set as p < 0.05.

Results
The control rats increased the number of avoidances on days 3, 4, and 5 from the learning session and on the memory retention test compared with day 1 of the trial (resp. p<0.01). The LCM group significantly increased the number of avoidances on days 4 and 5 from the learning session (p<0.01, p<0.001, resp.) and on the memory retention test (p<0.01) compared with day 1 of the trial (Fig. 1). The DZP group decreased significantly the number of avoidances on days 2 (p<0.05), 3, 4, and 5 (p<0.01, resp.) of the learning session and on the memory retention test (p<0.01) compared to the control animals.

No change was observed in the number of escapes in the control and LCM groups during both sessions in comparison with day 1 (Fig. 2). The animals with the DZP model significantly decreased the number of escapes during all days of the learning session (p<0.01, resp.) and on the memory retention test (p<0.05) in comparison with the control animals. The rats treated with LCM significantly increased the number of escapes on days 3 (p<0.001), 4 (P < 0.01), and 5 (p<0.01) of the learning session and on day 12 (p<0.001) in comparison with the DZP group.
Figure 2. Effect of lacosamide (LCM) treatment on the number of escapes in a model of DZP-induced amnesia. \&p<0.05, \&&p<0.01 in comparison with the saline group of the same day; #P < 0.01, ##p<0.001 in comparison with the DZP group of the same day.

The DZP group significantly decreased the number of intertrial crossings on days 2, 3, and 4 of the learning session (p<0.05, resp.) and during the test for long-term memory traces (p<0.01) (Fig. 3). The LCM group increased significantly the number of intertrial crossings only on days 3 and 4 of the learning session (resp. p<0.05) and during the retest on day 12 (p<0.01).

Figure 3. Effect of lacosamide (LCM) treatment on the number of intertrial crossings in a model of DZP-induced amnesia. \&p<0.05, \&&p<0.01 in comparison with the control group of the same day; #p<0.05, ##p<0.01 in comparison with the DZP group of the same day.

Discussion
The results of the present study demonstrate that diazepam induced active and passive learning and memory deficits which are in accordance with other authors (Beracochea, 2006). Lacosamide administration to animals with DZP-induced amnesia led to a slight increase in the active learning abilities in the active avoidance test while the drug significantly improved the passive learning abilities as well as the formation of long-term memory traces. Treatment with lacosamide managed to restore the suppressed by DZP locomotor activity. Data about the effect of lacosamide on cognition are still contradictory. Our results are in agreement with other authors who have found that the drug improves cognitive performance in epileptic patients assessed by the validated EpiTrack system (Liguori et al. 2018). In addition, it produces favorable effects on executive functions in comparison with other AED such as carbamazepine (Barker-Haliski et al. 2016). Moreover, the long-term cognitive effects of LCM are similar to those of lamotrigine (Helmstaedter and Witt, 2013). In contrast, some evidence revealed that LCM can negatively affect attention in naïve rats and lead to cognitive dysfunction in healthy individuals (Higgins et al. 2010, Meador et al. 2016).
In conclusion, these results suggest that LCM improves cognitive performance in a model of DZP-induced amnesia. The cognitive effects of AEDs are of particular concern because they are the major therapeutic modality for the control of seizures. The assessment of the potential adverse cognitive effects of AEDs in different animal models would be of benefit in optimizing therapy.

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Helmstaedter C, Witt. JA. The longer-term cognitive effects of adjunctive antiepileptic treatment with lacosamide in comparison with lamotrigine and topiramate in a naturalistic outpatient setting. Epilepsy Behav. 2013, 26(2), 182-187.
Factors Associated with Increased Risk of Depression in International Medical Students

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Abstract
During their academic studies, medical students experience high degrees of depression. Our survey aimed to investigate the sociodemographic, academic, and lifestyle factors that affect the mental health of international medical students and raise their risk of developing depression. First and second year medical students completed sociodemographic questionnaire, Beck Depression Inventory (BDI), University Stress Scale (USS), and Perceived Stress Scale (PSS). The findings indicated a 20.1% probable depression among medical students. Depression positively correlated with academic stress, and perceived stress respectively. Although there was a moderate relationship between academic achievement and depression among medical students throughout the first two years of their education, this relationship does not seem to be solely due to the medical university experience. Positive correlations between BDI scores on one hand, and the self-reported financial status, frequency of exercise, and alcohol use were also present. We also found a complicated interplay of factors, such as self-assessed financial status and frequency of exercise that may affect the development of depression among the international medical students. Additionally, depression was explicitly related with females and the overall perception of “being unhealthy or sick”.

Keywords: depression, medical students, stress, mental health

Introduction
Many international students develop depression during their medical education. Among medical students, many studies have indicated that the prevalence of depressive disorders was more than 20% - considerably higher than rates reported in general populations. [1,2] International students trained in the English course at the Medical University of Varna, Bulgaria, have to adapt to the foreign country's cultural, linguistic, and social differences and the specifics of the educational system, which may increase the risk of developing depression. Our study aimed to investigate the factors associated with the increased risk of developing depression in international medical students.

Materials and methods
The survey included 363 1st and 2nd-year international medical students at the Medical University of Varna, Bulgaria. The participants commenced after receiving approval from the University research ethics committee. The volunteers completed a sociodemographic questionnaire, Beck Depression Inventory (BDI), University Stress Scale (USS), and Perceived Stress Scale (PSS).

The BDI is a 21-item self-report rating inventory that assesses depression-related attitudes and symptoms. The overall scores can be evaluated as follows: 17-20 - borderline clinical depression; 21-30 - moderate depression; 31-40 - severe depression; over 40 - extreme depression [3]. The USS assesses the stress level of university students. The overall intensity of the stress can be evaluated - a score ≥ 13 is predictive of psychological distress. [4]. PSS measures the degree to which situations in one's life are appraised as stressful [5].

Statistical analysis: Differences between students with and without depression were tested by the Chi-Squared test. A p-value of 0.05 was interpreted as significantly different. Spearman correlation analysis (rho) tested the association level between depression scores, study-related factors, lifestyle factors, and sociodemographic factors. All analyses were performed using jamovi v.2.3.21.

Results and discussion
363 1st and 2nd-year medical students were prospectively assessed for depression with BDI. More than half of our sample (54.3%, n=197) included female respondents aged between 20 and 26 (88.4%). About 85% of the respondents reported that they did not smoke, whereas 54% reported not drinking alcohol. More than 60% reported sleeping between 6-8 hours, whereas about 53% spent, on average, between 2–4 hours a day studying (Table 1).
Table 1. Demographic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male (N)</th>
<th>Female (N)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>166</td>
<td>197</td>
<td>54.3%</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-23 years</td>
<td>153</td>
<td>168</td>
<td>42.1%</td>
</tr>
<tr>
<td>24-26 years</td>
<td>33</td>
<td>9.1%</td>
<td></td>
</tr>
<tr>
<td>27-30 years</td>
<td>6</td>
<td>1.7%</td>
<td></td>
</tr>
<tr>
<td>31-35 years</td>
<td>3</td>
<td>0.8%</td>
<td></td>
</tr>
<tr>
<td>Alcohol consumption/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Don’t drink</td>
<td>196</td>
<td>54%</td>
<td></td>
</tr>
<tr>
<td>Light – one drink (glass, can) or less</td>
<td>151</td>
<td>41.6%</td>
<td></td>
</tr>
<tr>
<td>Moderate – more than one drink (glass, can)</td>
<td>16</td>
<td>4.4%</td>
<td></td>
</tr>
<tr>
<td>PSS score</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>0 - 13</td>
<td>77</td>
<td>21.2%</td>
<td></td>
</tr>
<tr>
<td>14 – 26</td>
<td>241</td>
<td>66.4%</td>
<td></td>
</tr>
<tr>
<td>27 - 40</td>
<td>45</td>
<td>12.4%</td>
<td></td>
</tr>
<tr>
<td>USS score</td>
<td></td>
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</tr>
<tr>
<td>Not at all</td>
<td>195</td>
<td>53.7%</td>
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</tr>
<tr>
<td>Sometimes</td>
<td>168</td>
<td>46.3%</td>
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<td>BDI score</td>
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<tr>
<td>1 - 10</td>
<td>223</td>
<td>61.4%</td>
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<td>11 - 16</td>
<td>67</td>
<td>18.5%</td>
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<tr>
<td>17 - 20</td>
<td>31</td>
<td>8.5%</td>
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</tr>
<tr>
<td>21 - 30</td>
<td>30</td>
<td>8.3%</td>
<td></td>
</tr>
<tr>
<td>31 – 40</td>
<td>9</td>
<td>2.5%</td>
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</tr>
<tr>
<td>Over 41</td>
<td>3</td>
<td>0.8%</td>
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</tbody>
</table>

The incidence of probable major depression during the first two years of medical school (scores above 17 points) was 20.1% (1st year - 10.5% vs. 2nd year - 9.6%, X²=1.52, (Δf=1), p=0.218). The result is close to the average level in other universities worldwide [1] (Tables 1,2). The presence of any psychiatric disorders in the family was 7.2 % (Table 2).

Table 2. Psychiatric disorders in the family

<table>
<thead>
<tr>
<th>Relatives diagnosed</th>
<th>No depression</th>
<th>Borderline and above</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>N 239</td>
<td>47</td>
<td>286</td>
</tr>
<tr>
<td>%</td>
<td>65.8%</td>
<td>12.9%</td>
<td>78.8%</td>
</tr>
<tr>
<td>Yes, in family</td>
<td>N 31</td>
<td>21</td>
<td>52</td>
</tr>
<tr>
<td>%</td>
<td>8.5%</td>
<td>5.8%</td>
<td>14.3%</td>
</tr>
<tr>
<td>Yes, second degree</td>
<td>N 20</td>
<td>5</td>
<td>23</td>
</tr>
<tr>
<td>%</td>
<td>5.6%</td>
<td>1.4%</td>
<td>6.3%</td>
</tr>
<tr>
<td>Total</td>
<td>N 290</td>
<td>73</td>
<td>363</td>
</tr>
<tr>
<td>%</td>
<td>79.9%</td>
<td>20.1%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

According to the data obtained from the PSS scale, 66.4% of the students showed moderate levels of stress and 12.4% - high level of stress (Table 1). The results are close to the averages for several other countries in Europe [6] and North America [7]. Depression among 1st and 2nd-year medical students was moderately related to school performance and indicators. Positive associations were found between BDI and USS scores (rho=0.472, p=0.001) and BDI and PSS scores (rho=0.474, p=0.001). Our results confirm the finding that stress and depression are positively correlated, as has been noted in earlier investigations. [8].

Our analysis showed that the rate of depression during medical school does not appear to be a result of the medical school experience alone. Results from our Spearman correlation analysis show a complex combination of factors that may influence the nature of depression among medical students predisposed to it. Weak but positive correlations were found between BDI scores and the self-evaluated financial status (rho=0.165, p=0.002) and BDI scores and the
frequency of exercising (rho=0.182, p=0.001), acknowledging the likelihood for hopelessness and depression among economically poorer students as well as among the ones less active in sport (Table 3).

The analysis also indicates a positive correlation between high levels of stress and alcohol consumption (rho=0.248, p=0.001). That can also be considered an indirect factor influencing the nature of depression among international students at Medical University – Varna (Table 3).

### Table 3. Correlation between depression scores, study-related factors, and lifestyle factors

<table>
<thead>
<tr>
<th>Study-related factors</th>
<th>BDI score</th>
<th>USS score</th>
<th>PSS score</th>
<th>Hours spent</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI score</td>
<td>Spearman's rho</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>USS score</td>
<td>Spearman's rho</td>
<td>0.472</td>
<td>***</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;.001</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PSS score</td>
<td>Spearman's rho</td>
<td>0.474</td>
<td>***</td>
<td>0.364 ***</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lifestyle factors</th>
<th>BDI score</th>
<th>USS score</th>
<th>PSS score</th>
<th>Hours spent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Spearman's rho</td>
<td>-0.100</td>
<td>0.248 ***</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.056</td>
<td>&lt;.001</td>
<td>—</td>
</tr>
<tr>
<td>Self-evaluated financial status</td>
<td>Spearman's rho</td>
<td>0.165 **</td>
<td>0.071 -0.050</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>0.002</td>
<td>0.178</td>
<td>0.347</td>
</tr>
<tr>
<td>Exercise score</td>
<td>Spearman's rho</td>
<td>0.182 ***</td>
<td>-0.003</td>
<td>0.089 -0.045</td>
</tr>
<tr>
<td></td>
<td>p-value</td>
<td>&lt;.001</td>
<td>0.952</td>
<td>0.092</td>
</tr>
</tbody>
</table>

**Note.** *p < .05, **p < .01, ***p < .001

### Table 4. Correlation between depression scores and sociodemographic factors

<table>
<thead>
<tr>
<th>BDI score</th>
<th>Gender</th>
<th>Age</th>
<th>Religion</th>
<th>Relationship</th>
<th>Self-evaluated health</th>
<th>Self-reported illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spearman's rho</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>p-value</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

| Gender | BDI score | Spearman's rho | — | — | — | — |
|        | p-value    | 0.003 | — | — | — | — |

| Religion | BDI score | Spearman's rho | — | — | — | — |
|          | p-value    | 0.381 | 0.992 | <.001 | — | — |

| Relationship | BDI score | Spearman's rho | — | — | — | — |
|              | p-value    | 0.079 | 0.092 | 0.240 | *** | — |

| Self-evaluated health | BDI score | Spearman's rho | — | — | — | — |
|                       | p-value    | 0.131 | 0.081 | <.001 | 0.529 | — |

| Self-reported illness | BDI score | Spearman's rho | — | — | — | — |
|                      | p-value    | 0.410 | -0.022 | 0.022 | -0.054 | 0.037 | — |

| Self-reported illness | BDI score | Spearman's rho | — | — | — | — |
|                      | p-value    | 0.157 | -0.026 | 0.114 | -0.002 | 0.252 | — |

**Note.** *p < .05, **p < .01, ***p < .001

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Finally, depression among medical students was more explicitly associated with females (\(\text{rho}=0.155, p=0.003\)) and the overall perception of being unhealthy (\(\text{rho}=0.410, p=0.001\)) or sick (\(\text{rho}=0.157, p=0.003\)) (Table 4).

The analysis shows several positive correlations between a few sociodemographic factors. As the age increases, the respondents decrease their chances of identifying themselves as religious (\(\text{rho} = -0.244, p=0.001\)). In addition, age positively impacts the self-estimation of being in a relationship (\(\text{rho}=0.240, p=0.001\)). The overall perception of being sick increases with age (\(\text{rho}=0.114, p=0.03\)) and the overall perception of being unhealthy. On the other hand, self-identifying as religious reduces the perception of being sick (Table 4).

**Conclusion**

The incidence of probable depression among 1st and 2nd-year international medical students was 20.1%. A correlation was found between depression, academic stress, and perceived stress. According to the sociodemographic factors, depression was more explicitly associated with females and the overall perception of being unhealthy or sick. The rate of depression among medical students does not appear to be a result of the medical school experience alone. We find a complex combination of factors that may influence the nature of depression among medical students predisposed to it, such as gender, self-evaluated financial status, and exercise frequency.

**References:**

8. Iorga M, Dondas C, Zugun-Eloae C. Depressed as Freshmen, Stressed as Seniors: The Relationship between Depression, Perceived Stress and Academic Results among Medical Students. *Behavioral Sciences*. 2018; 8(8):70. [Crossref - https://doi.org/10.3390/bs8080070]
Treatment of femoral pseudarthrosis with subtrochanteric localization: a case report

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Abstract
Femur fracture is a relatively common injury and accounts for 15% to 25% of all musculoskeletal (MSK) fractures. Treatment of these fractures accounts for up to 70% of the cost of taping all fractures. Pseudarthrosis, or the so-called "false joint", is a complication resulting after a bone fracture, when treatment is incorrectly performed, not providing sufficient stability of the fracture fragments. There is no consensus definition for the term "pseudarthrosis". Usually, pseudarthrosis is considered to be the absence of bone fusion after a period of 6 months after the fracture, regardless of the type of treatment performed and the presence of the characteristic radiological symptomatology. In subtrochanteric fracture localization, the cause of pseudarthrosis is usually loss of reduction between the fragments resulting from lateralization of the short proximal fragment and a wide femoral groove allowing medialization of the diaphyseal fracture fragment when performing intramedullary osteosynthesis. [2,3] Over the past decades, improvements in orthopaedic fracture grafts have significantly reduced this complication rate in femoral fractures. The treatment of pseudarthroses continues to be a challenge for the surgeon, including the choice of method and type of osteosynthesis implant [1]. We demonstrate a case of successful solution of a problem of pseudarthrosis of the femur with subtrochanteric localization by using a non-standard implant.

Keywords: Pseudarthrosis, osteosynthesis implant, subtrochanteric fracture

Aim and objectives
The aim of this article is to present a case of treatment for pseudarthrosis of the femur, obtained after primary osteosynthesis with an anatomical locking long femoral nail, in a subtrochanteric femoral fracture.

Material and method
Patient aged 83 years underwent surgery in December 2017 for a subtrochanteric fracture resulting from a low-energy trauma to an osteoporotic bone. Blood reposition and metal osteosynthesis was performed using a locking, long anatomic femoral nail. Rehabilitation and loading of the operated limb were performed in standard time frames. On contro examinations with follow-up radiographs, it was found that the fracture showed no tendency to fuse after the ninth postoperative month Fig. (1). At the twelfth postoperative month, a locking nail fracture was noted, most likely caused by metal fatigue resulting in mobility between the bone fragments Fig. (2).

Fig. (1) Ninth postoperative month lacking bone fusion.
The complication - pseudarthrosis of the femur required a second surgical intervention. At one stage, extraction of the broken femoral nail, excision of the pseudarthrosis, and curettage of the bony ends of the femoral fragments were performed, during which the devitalized, marginal bone areas were also removed. This was followed by Bier's cortex boring of the bony ends until bleeding occurred. Fracture stabilization was performed using a locking 5.0 mm LISS distal femoral plate rotated 180 degrees and anatomically contralateral to the corresponding limb. The presence of compound screw holes also allows for compression between the freshened bone fragment surfaces, concomitant with reliable angular stabilization (Fig. 3).

The postoperative inpatient stay was under the protection of antibiotics and anticoagulant. The patient was verticalized on crutches with no weight bearing on the operated limb after removal of drains at 48 hrs. By the end of the hospital stay, gait on crutches was perfected with a gradual increase in distance without straining the operated limb. The patient was dehospitalized with an uneventful surgical wound and recommendations for continuation of antithrombotic prophylaxis 30 days after discharge. The sutures from the surgical intervention were removed on postoperative day 14.

Follow-up examinations were performed at 3, 6, 9, and 12 months postoperatively. The fracture showed a tendency towards fusion as early as the 6th month. Complete consolidation of the bone fragments was observed on the follow-
up radiographs after the 9th month. At the last follow-up examination, the patient was ambulating independently without the use of assistive devices and continued to increase the distance of loading.

Two months later, the patient was admitted to the clinic for treatment of a pertrochanteric fracture of the left femur, which required a new osteosynthesis using the standard method with the DHS system and an additional anti-rotation 7.3 mm. cannulated screw (5) Fig. 4A, 4B.

**Conclusion**

Based on our experience in the treatment of pseudarthrosis in the proximal femur in subtrochanteric fracture, we believe that the rotated anatomically reversed distal femoral plate represents a reliable osteosynthesis device with applications for solving problems of this nature.

**References:**


A case of traumatic upper limb decubitus - optimization of the therapeutic protocol

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Abstract

Traumatic decolman of the skin of the upper extremity, induced by a high-energy reagent, is still encountered despite modern mechanization and automation of production, as well as exceptionally as a result of domestic trauma (1). Such trauma can lead not only to a significant reduction and limitation of function, but also to permanent disability of the affected limb. Damage to the skin and underlying soft tissues has always been and continues to be a challenge for surgeons, both to preserve anatomic structures and to restore full function to the upper extremity. We present a case of traumatic decubitus ulna with a compact wound at the elbow joint and avulsion of skin, subcutaneous tissue, and fascia to the level of the metacarpal joint, without vascular or nerve injury and without fractures of the forearm bones.

Keywords: Traumatic decubitus, decubitus necrosis, free skin flap.

Objective

The aim of the surgical treatment was to preserve the damaged decubitus skin as much as possible, to minimize the risk of complications and last but not least to restore the function of the elbow joint and arm.

Material and methods

A 23-year-old man sustained a traumatic skin decubitus wound in the elbow joint area with the appearance of a circumferential wound (Fig. 1). There was distalization of the traumatized tissue area to the level of the wrist joint, involving skin, subcutaneous tissue, and fascia. On urgent basis, after extensive saline serum lavage of the entire affected area, the devitalized fascial areas were isolated. Debridement was done and necrotic areas were thoroughly removed. With the preparation thus done, we sutured the decellularized circumferential skin-subcutaneous section from distal to proximal direction for the underlying muscle groups. We placed an aspiration drain. We were able to cover the entire decellularized surface (4). A sterile gauze dressing was applied followed by an elastic bandage.

Fig. 1 Diagnostic photograph of the decubitus skin of the upper extremity starting from the elbow joint and reaching to the wrist joint.

The postoperative period continued under the protection of antibiotics and anticoagulants in the hospital. At 36 hours, necrosis began to form around the skin suture along the entire skin circumference, located around the elbow joint. After demarcation, a necrectomy had to be performed, forming a skin defect measuring 7 - 9 cm. Intraoperatively,
material was taken for microbiological examination, which after culture ruled out the presence of bacterial flora and a corresponding risk of wound infection. Treatment continued with epithelizing dressings with colloidal silver. After 10 days, good granulation tissue formed, allowing surgical coverage of the skin defect by free skin flap at the next epap(2). The anterolateral thigh was used as the donor site(3). Two 0.6-mm-thick skin dermatomes were lifted and, after pretreatment, fixed at the site of the formed skin defect around the elbow joint. A postoperative basement membrane and elastic bandage was placed. The sutures were removed on the 15th postoperative day.

**Results**

We reported the results at the end of the first, second and fourth month after the last surgery. At the first month the postoperative traumatic edema involving the forearm and fingers was moderately pronounced. We found a deficit in the range of motion of the elbow joint as follows - extension 25 degrees and flexion 15 degrees, as well as a deficit in the ability to move the fingers. However, we observed complete adaptation of the free skin graft to the soft tissue defect of the elbow joint. At the second month, the swelling of the forearm and hand persisted but showed a tendency to decrease. The elbow joint movements remained within the same parameters, most probably due to the adhesions in the area of the loose skin flap. On this occasion, the patient was referred to a rehabilitation center to address the motor deficit. At the fourth month, we found full range of motion in the elbow joint and in the hand joints, with no residual deficit. The loose skin graft was fully adapted and almost normal in color compared with the rest of the upper extremity skin (Fig. 2).

![Fig. 2 Final result of the treatment of the traumatic upper limb decubitus.](image_url)

**Conclusions**

There is consensus in the literature regarding the treatment protocol for traumatic upper extremity decubitus. The main requirement is to shorten the period from the time of injury to the time of surgical treatment. Operative intervention should be performed as soon as possible after the injury. In this case, the emergency surgery performed within the first hour after the trauma, including extensive lavage, debridement of the devitalized soft tissues and precise suturing of the decannulated skin area, were crucial for therapeutic success. Subsequent elastic dressing prevented the recurrence of hematoma between the detached tissues and the occurrence of septic complications. Rehabilitation performed in several stages after dehospitalization resulted in complete recovery of the elbow joint and arm fusion.

**References:**
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A case report of a suicide with a tropical snake black mamba

Snezha Zlateva, 1Marieta Yovcheva, 1Petko Marinov, 1Georgi Bonchev, 1Ivelina Panayotova, 2Gabriela Kehayova, 2Kristina Stoycheva, 3 Tsonka Dimitrova
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2 Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University, Varna, Bulgaria
3Department of Biology, Faculty of Medicine, Medical University, Varna, Bulgaria

Abstract
We present a clinical case of a 53-year-old man who, with suicidal intent, provoked a bite from a terrarium-reared tropical snake, Black mamba Dendroaspis polylepis. Black mamba venom contains 'dendrotoxins' and 'α-neurotoxins'/curaremimetics'. Clinically, the poisoning begins immediately with a moderately expressed local pain syndrome, 1 hour later the appearance of general toxic neurological, sensory and motor symptoms, 3 hours later a paralytic stage occurs with convulsions, vomiting and hypersecretion from the stomach, respiratory arrest and shock. Treatment was specific and nonspecific within 9 days, after which he was discharged healthy. A specific polyvalent serum against the venom of the Black Mamba is administered 12 hours after the bite. Respiratory paralysis was overcome with intubation and mechanical ventilation for 4 days. Shock and hypotension, pulmonary congestion, and diastolic dysfunction were controlled with daily plasma and dopamine infusions for 5 days. Rhabdomyolysis, toxic encephalopathy, metabolic alkalosis, liver damage, and local lesions are managed with symptomatic treatment.

Keywords: black mamba, alpha neurotoxins, dendrotoxins, suicide

Introduction
Tropical snake bites in Bulgaria are casuistic. We present a case of suicide by inducing a bite from an aquarium-bred tropical snake, Black mamba Dendroaspis polylepis. Black mamba venom contains 'dendrotoxins' and 'α-neurotoxins'/curaremimetics'. Clinically, the poisoning begins immediately with a moderately expressed local pain syndrome, 1 hour later the appearance of general toxic neurological, sensory and motor symptoms, 3 hours later a paralytic stage occurs with convulsions, vomiting and hypersecretion from the stomach, respiratory arrest and shock. Treatment was specific and nonspecific within 9 days, after which he was discharged healthy. A specific polyvalent serum against the venom of the Black Mamba is administered 12 hours after the bite. Respiratory paralysis was overcome with intubation and mechanical ventilation for 4 days. Shock and hypotension, pulmonary congestion, and diastolic dysfunction were controlled with daily plasma and dopamine infusions for 5 days. Rhabdomyolysis, toxic encephalopathy, metabolic alkalosis, liver damage, and local lesions are managed with symptomatic treatment.

Keywords: black mamba, alpha neurotoxins, dendrotoxins, suicide
They bind to nicotinic acetylcholine receptors and thus block the action of acetylcholine on the postsynaptic membrane and cause neuromuscular blockade and hence paralysis. Fasciculins are acetylcholinesterase inhibitors that cause muscle fasciculation. Mambalgins act as inhibitors of acid-sensitive ion channels in the central and peripheral nervous system, causing a pain-inhibiting effect. Without proper treatment, the symptoms of this snake envenomation usually progress to respiratory failure, cardiovascular collapse, and death. Fatality in humans usually occurs after 7 to 15 hours, but can occur up to 15 minutes after bite [4].

Case report

Medical history: Male, GVE 53, with ID no. 2262, admitted on 05/30/2020 at 10:32 p.m. Two hours before hospitalization, with the intention of committing suicide, he himself placed his hand in an aquarium with a Black Mamba and was bitten by the snake. He immediately felt pain at the bite site on the fingers of his left hand. His general condition worsened, he was seen by a relative who sought emergency medical assistance. Urbason 125 mg intravenous was immediately placed in place and referred for hospital treatment. Accompanying diseases are frequent thrombophlebitis of the lower legs, for which he takes Sintrum and Acetylsal. On the occasion of coxarthrosis, an alloplasty of both hip joints was performed.

Objective condition: Male of apparent age corresponding to actual age in preserved general condition. Consciousness-contact, adequate, distime. Hypersthenic habitus, afebrile, takes an active position in bed. Skin and mucous membranes are normal. PLV-unmagnified. Breathing - 16 min, vesicular, without wheezing. CCS - rhythmic, 86 beats/min, clear tones without noise finding, RR 160/90. Liver and spleen - not enlarged, succussio renalis - negative, limbs - on the lower legs of both legs there are skin post-thrombophlebitic changes, without edema. Local status: On the left hand, in the area of the base of the index finger, tenor and second finger, there are several puncture wounds, bite marks that are difficult to notice. The swelling is moderate and covers the whole arm up to the wrist joint, without lymphangitis. Laboratory tests on admission were normal, including serum cholinesterase.

Treatment: oxygen, intubation, IBD, dopamine, plasma, dexamethasone, polyclonal Black Mamba-specific serum, antibiotics, infusions of 3 liters of electrolytes and glucose solutions, fraxiparin, Cormagnesin, Ca gluconate, Allergosan, Quamatel, vitamins B1, B6, B12, Vitamin C, Furantril, RK Mertz and Transmethyl. Local cold Rivanol compresses.

Course of the disease: On the third hour after the bite, profuse sweating and vomiting, convulsions of the face and body, neuromuscular paralysis of the chest muscles and acute respiratory failure begin. He was immediately intubated, put on artificial lung ventilation, sedated and relaxed for 4 days. A nasogastric tube is placed, from which 1200 ml flows out over two days. stomach contents. On the 12th hour of hospitalization, 5 flacons of 50 ml were placed specific polyvalent serum against Black Mamba venom, intravenously. On the fourth day, the patient regained consciousness and was extubated.

Over the next 6 days, toxic encephalopathy was observed, manifesting in a transition from sopor to somnolence and bradypsiclia. He gradually regained clear consciousness until he was discharged on the 9th day. After acute respiratory arrest, the hemodynamics collapses occurred, the arterial pressure within 8 days was 100/70; 90/60, resistant to treatment with continuous infusion of Dopamine 2 g/kg/min and daily transfusion of fresh frozen plasma. Within 5 days, there is interstitial pulmonary congestion, against the background of hypotension and diastolic cardiac dysfunction, established by radiography of the lungs and ultrasound of the myocardium. A rhabdomyolysis syndrome was observed with elevated CPK values to a maximum of 1195 U on day 6, necessitating infusions of electrolyte and glucose solution in a volume of 3 liters, together with furantril, which prevented the onset of renal damage. Clinically, muscle hypotension was observed, slowly recovering by the time of discharge. There was a slight increase in ALAT to 90 U on
day 6, a drop in albumin to 25 mmol/l and total protein to 54 mmol/l, interpreted as liver damage from prolonged hypotension. Discharged on day 9 clinically well.

Discussion
The case observed by us is rare in terms of snakebite being used for suicide, and the Black Mamba species is atypical for Bulgaria and we have no experience in treating this envenomation. There is a report of a Black Mamba suicide involving a woman who kept the snake in an aquarium at her home, and the case ended fatally [6]. Another case of being bitten by a black mamba kept in an aquarium was reported by a professional photographer who took pictures of the snake, including the bite itself, and the photos were published on a website. The photographer did not have any symptoms of poisoning, which was interpreted as a "dry bite". The phenomenon of dry snake bite is known. For the Black Mamba, "dry bites" are about 50% [7]. Regarding the local lesions, in this case report we observed there were several puncture wounds between the first and second toes. The Black Mamba is known to bite multiple times when attacked. In our case, local lesions begin with swelling around the bite site, which spreads in the following days to the axilla. The swelling is without reddening of the skin and without necrosis. It is known in the literature that most tropical snakes possess a protein with enzymatic activity (Phospholipase A), which is responsible for developing necroses, but the venom of the Black Mamba does not possess Phospholipase A. In some cases, only local symptoms of numbness in the area of the bite [5]. Coagulation disorders are characteristic of snake venom, both in the direction of bleeding and the formation of thrombosis. Black mamba venom is known to have little or no hemolytic, hemorrhagic, or procoagulant activity [8]. Our case is typical because we did not observe deviations in the coagulation status. The patient's initial neurological symptoms begin at the scene of the accident, such as blurred vision and paresthesia. Vomiting is also considered a characteristic early symptom. We believe that dendrotoxins, described as acetylcholine stimulators, led to the activation of M-choline receptors in the stomach and from there hypersecretion of gastric juice [8]. The curare-like effect of the neurotoxins was registered 3 hours after the bite with initial myofibrillations and subsequent paralysis of the respiratory muscles with apnea. The APV is decisive in these cases. Cases have been described in the literature where APV brought patients out of a coma for several days, even if no anti-snake serum was administered. Hypotension, shock and myocardial dysfunction are other characteristic manifestations of neurotoxins [8]. In the course of intoxication, we observed vascular collapse with marked hypotension lasting 5 days, as well as diastolic cardiac dysfunction and pulmonary congestion. This required daily plasma transfusions and Dopamine treatment. It is the poor perfusion of the internal organs and the brain that is the reason why we observe transient impairment of liver function and toxic encephalopathy. The treatment carried out is non-specific and specific. The anti-snake serum that was administered to the patient was specific for the Black Mamba, produced in Bulgaria and stored in a refrigerator by the patient himself for many years. According to some authors, without a specific anti-snake serum, patients die, but there are cases when even without its application, thanks to APV and symptomatic agents, people bitten by the Black Mamba survived [5,9].

References:


Detecting changes in EMG after treatment with botulinum toxin in patients after stroke

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Abstract
The purpose of this study will be designed to evaluate the effects of botulinum toxin type A (BoNTA) injection of the rectus femoris (RF) muscle on the electromyographic activity on the knee flexor and extensor and on knee and hip kinematics during gait in patients with hemiparesis exhibiting a stiff knee gait. The methods that will be used are two gait analyses that will be performed on several patients - before and three or four weeks after BoNTA injections. Kinematic and electromyographic parameters will be discussed for the paretic limb. 3-D Gait analyses will be carried out the Motion Analyses System with 4 optoelectronic cameras. About results we should say that It is expected BoNTA treatment to improve gait velocity, to stride length and cadence with an increase of knee angular velocity. In Conclusion, we expect our results to show that BoNTA treatment strongly modified the EMG amplitude and frequency of the injected muscle without changing the EMG activity of synergist or antagonist muscle.

Key words: botulinum toxin type A, EMG, m.rectus femoris, knee flexor, knee extensor, hemiparesis, stroke

Introduction
Human locomotion is a phenomenon of the most extraordinary complexity in which so great are the multitude of individual motions occurring simultaneously in the three planes of space that analysis is difficult without some unifying principle. The adoption of the concept that fundamentally locomotion is the translation of the center of gravity through space along a pathway requiring the least expenditure of energy supplies the necessary unifying principle which permits of qualitative analysis in terms of the essential determinants of gait. The six major determinants are pelvic rotation, pelvic tilt, knee and hip flexion, knee and ankle interaction, and lateral pelvic displacement. Copyright © 1953 by The Journal of Bone and Joint Surgery, Incorporated

The gate after stroke is characterized by a decrease in peak knee flexion during the swing phase of gait and is commonly observed in patients following stroke. One of the principle causes of the gate is spasticity of the rectus femoris muscle (RF). RF spasticity increases the knee extension moment in pre-swing and decreases knee flexion velocity at toe-off, both of which potentially decrease peak knee flexion in swing. Since effective treatments of RF spasticity are available, this mechanism is the most common focus of treatment and Botulinum Toxin type-A injection (BTX-A) is the treatment of choice. The aim is to improve peak knee flexion during swing. BTX-A acts by blocking neurotransmitter release at the neuromuscular junctions, including partial paresis of the injected muscle. It has now been clearly established that BTX-A injection in a spastic RF muscle improves peak knee flexion in swing in stroke patients. Robertson et.al (2009) and Hutin et.al (2010) respectively found increases of 8 and 9 degrees of peak knee flexion during swing one month after BTX-A injection at spontaneous walking speed. In contrast Stoquart et.al (2008) and Caty et.al (2009) found increases of “only” 5 degrees. The reason for this difference remain largely unknown.

Task involves in walking- according to “Rancho Los Amigos” (RLA), California:
1. Weight acceptance
4. Single limb support
5. Swing limb advance
Gait initiations:
  3) A series of events occur from the initiation of body movement to beginning of gait cycle
  6. It is stereotyped activity in both young and old healthy people.
  7. Total duration of this phase is about 0,60 sec.

Types of pathologic gait due to neurological disturbance:

MUSKULAR PARALYSES:
12. SPASTIC- Cirkumduktry gait, Scissoring gait, Dragging or Paralytic gait, Robotic gait( quadriplegic)
   - FLACCID- Lurching gait, Waddaling gait, Gluteus maximus gait, Quadriceps gait, Foot drop or Stapping gait.

Hemiplegic gait: With spastic pattern of hemiplegic leg
10. Hip into extension, adduction and medial rotation
   - Knee in extension
   - Ankle in drop foot with ankle plantar flexion and inversion (equinovarus) which is present during both stance and swing phase.

Electromyography (EMG) is useful tool for the evaluation of abnormal patterns of muscle activation in patients with neurological disorders, helping in clinical decision making. Recently, Phadke et al.(2012) highlighted the problems related with EMG amplitude normalization for the evaluation of the effects of BoNTA in patients with neurological lesions.

2. Methods
2.1. Participants
   About 10 subjects after stroke
2.2. Gait assessment
   Each patient will carry out one gait analysis session on 2 separate days: before BoNTA injections into spastic RF and four weeks after. Gait parameters will be recorded using 4 optoelectronic cameras
2.3. Amplitude. The linear envelope of the EMG signals will be calculated after full wave rectification and low pass filtering at 10Hz (Shiavi et al.,1987)
2.4. Muscle activation time. The TKEO will be applied to the EMG signals in order to calculate the duration of muscle activation throughout the entire gait cycle (Solnik et al.,2010)
2.5. Botulinum toxin injection. An average dose of 164+-50U of BoNTA will be injected into three anatomical points of the spastic RF muscle.

Aim
The aim of this study will be to evaluate changes in the EMG signal of the gate after stroke and knee extensor and flexor muscles after BoNTA injections of the RF.

Results
About results we should say that It is expected BoNTA treatment to improve gait velocity, to stride length and cadence with an increase of knee angular velocity of toe-off and maximal knee flexion in the swing phase. We expect our results to show that BoNTA treatment strongly modified the EMG amplitude and frequency of the injected muscle without changing the EMG activity of synergist or antagonist muscle.

Conclusion
In Conclusion, we expect our results to show that BoNTA treatment strongly modified the EMG amplitude and frequency of the injected muscle without changing the EMG activity of synergist or antagonist muscle. The reduction in RF activation frequency could be the result of increased slow fiber activity. With regard to
these results, it seems that the frequency analysis of EMG signals during gait could be a relevant method to detect the effectiveness of BoNTA in the injected muscle.

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Comparative analysis of the incidence of spinal distortions in adolescents for the period 2009-2021

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Abstract

Purpose: The study objective is to monitor changes in the incidence of spinal distortions in adolescents in the school years 2008/2009 and 2019/2020 in schools within the territory of the town of Kazanlak. The report presents an overview of the manner and organization of preventive screenings of adolescents and options for subsequent treatment.

Materials and methods: The study was carried out by specialists in physical and rehabilitation medicine (PRM) in the school physicians' offices with the consent of the directors, class teachers, parents and the regional education inspectorate. The preventive screenings were carried out completely free of charge for the pupils and the treatment of newly discovered spinal distortions was carried out in outpatient conditions and was taken over by the National Health Insurance Fund.

Results: The study outcomes showed no change in the incidence of spinal distortions of pupils in classes 1 to 4. However, among children from classes 5 to 10 there is a significant increase in the incidence rate over a period of 10 years.

Conclusions: The introduction of modern technologies in pupils' daily lives and their increasingly active lifestyles over the last 10 years has led to an increase in the spinal diseases. PRM specialists are the physicians responsible for restoring preventive screenings for spinal distortions in schools. Our study with subsequent treatment and prevention of these conditions shows a successful approach to dealing with the increased number of scoliosis cases in the town of Kazanlak. Our team believes that this approach can be effectively implemented in each region by the PRM specialists and would have a strong social, economic and health effect in the long term for the population of the Republic of Bulgaria.

Key words: Scoliosis, spinal distortion, preventive screenings, adolescents, rehabilitation

Introduction

The study objective is to monitor the changes in the incidence of spinal distortions in adolescents over a period of 11 years. The study was conducted by means of preventive screenings in the 2008/2009 and 2020/2021 school years within the territory of the town of Kazanlak.

Through this study, we established what impact the introduction of smart phones after 2009 and the growing computerization have had on school age children. The report presents an overview of the manner and organization of preventive screenings of adolescents and the options for subsequent treatment.

Materials and Methods

The screenings were carried out by specialists in physical and rehabilitation medicine (PRM specialists) in the physicians' offices of the respective schools. The study was conducted in 3 schools in the town of Kazanlak. The screenings were carried out with the written consent of the school principals, the class teachers, the parents and the regional education inspectorate. The screenings were carried out free of charge for the pupils. In the school year 2008/2009, they were conducted by Dr. Yordan Gechev, and in 2019/2020 by Dr. Stoimen Gechev and Dr. Gergana Gecheva.

The preventive screenings were carried out through functional evaluation, checkup, palpation and anthropometric measurements. The screened pupils were from classes 1 to 10. In order to quickly systematize the screening outcomes, the pupils were generally distributed in 3 main groups:

- Without change
• Initial changes
• Manifested scoliosis

Changes in the objective status:

**Initial changes** include 1 or more of the following:

- Rigidity of PVM
- Minimum difference in the waist triangles
- Unilateral restriction of lateral flexion
- Functional bent forward posture
- Prominent scapula
- Flat foot (platypodia)

**Manifested scoliosis** include 1 or more of the following:

- Visible curvature of the spine in the frontal plane
- Significant rigidity of the PVM
- Significant difference in the waist triangles
- Unilateral or bilateral restriction of lateral flexion
- Kyphosis
- Higher position of the shoulder joint and scapula
- Flat foot (platypodia)
- Pelvis rotation, lower limb shortening
- Tenderness

**Statistics**

Peaks were noted in the incidence of spinal diseases in every 3 age groups

In 2008/2009, the peaks were in classes 1, 4 and 7
In 2020/2021, the peaks were in classes 2, 5 and 8

Peaks shift by 1 age group over a period of 11 years, but always remain at an equal interval of 3 age groups

The increased incidence of spinal distortions in specific age groups over an equal interval can be explained by the cyclical growth of children in height and body weight

Probably in the distortion rate peaks shown, a large proportion of children in the age group are in a period of increased height growth

In 2008/2009, the percentage of pupils with spinal distortions was higher in the younger classes, **30%** in classes 1 and 4
In 2020/2021, the percentage of pupils with spinal distortions was higher in the older classes: 5 and 8, respectively **41%** and **49%** (nearly half of the children screened)

For the period of study, the high percentage of children with spinal distortions is found to shift from the young age group to the older pupils

This change can be attributed to the greater dependence of older pupils on the smart phones introduced after 2009 and the growing computerization in their daily lives.

The overall incidence rate in screened pupils from classes 1 to 10 in 2008/2009 was **22%**, and in 2020/2021 it increased to **28%**

The percentage of manifested spinal distortion increased significantly, from below 1% in 2008/2009 to 5% in 2020/2021

The increase in the number of severely manifested scoliosis for the studied period can be explained by the lack of preventive screenings in the schools or by the general practitioners.
Discussion
Our proposal to the Association of Physical and Rehabilitation Medicine is to initiate a national campaign for early detection of spinal distortions.

Objectives:
1. Finding children with spinal distortions.
2. Early detection of children predisposed to spinal distortions.
3. Informing pupils and their parents about the importance and consequences of spinal distortions.

Conclusion
138 children received treatment at a medical center. These children were examined in detail with anthropological measurements. Some of them underwent x-ray examinations and plantograms at the discretion of the physicians. Remedial gymnastic groups of 4 children were organized for general strengthening exercises for the children with initial changes. The patients were admitted with referrals for physiotherapy in outpatient conditions, which allowed the implementation of a 10-day treatment course – completely free of charge for the children. The parents and children were informed in detail of the necessary hygiene measures in the daily and school regime as well as of the need to regularly visit the physiotherapy center at least twice a year for followup and treatment of the spinal distortion. The preventive screenings were carried out completely free of charge for pupils and schools. At the end of the preventive screening in the respective school, we prepared an official list of children with initial changes and manifested scoliosis for each individual class. The list was submitted to school principals and the parents of the children with spinal distortions were informed by the class teachers. Contact details of the physicians and the medical center were provided in case the parents wanted to contact us.

Acknowledgments:
Concept development - steps:

**FIRST STEP:** to carry out screening (preventive screening, preliminary inspection) of pupils, completely free of charge, jointly with school authorities and supported locally by public organizations such as:
- Syndicate of Bulgarian Teachers
- Bulgarian Red Cross
- Rotary Club Bulgaria
- Order of Knights Templars
- Inner Wheel Bulgaria etc.

**Second step:** A/ the parents of all children with discovered spinal distortions or predisposed (at increased risk) should be informed through their class teachers. B/ where parents show interest, a detailed examination by a PRM specialist with referral No 3, code 23, may be carried out by the general practitioner and appropriate examinations may be prescribed for diagnosis.

**Third step:** where parents and the school authorities show interest, we can organize treatment sports groups throughout the school year to conduct the remedial gymnastics daily in the sports facilities of the school, for a minimum fee.

**Fourth step:** these preventive screenings could be carried out every year, which would allow us to have direct control over high-risk pupils, and thus we could take into account the effect of the prevention and rehabilitation carried out.

**Fifth step:** on a national scale, a green "school for the back" in different resorts in the country.

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Expression of extracellular matrix and adhesion molecules in mesenchymal stem cells of oral origin - comparison study

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Introduction

In the past decade mesenchymal stem cells (MSC) from oral cavity and dental tissues are broadly investigated. These cells are easily accessible and are capable of self-renewal, multilineage differentiation and plastic adherence (1). Commonly investigated cranio-facial stem cells are derived from alveolar bone, dental pulp of deciduous and permanent teeth, periodontal ligament (PDL), apical papilla and gingiva (2). These cells are known to be able to express wide number of markers, especially MSCs markers (3). The ECM and adhesion proteins production may be useful for determining stemness and regeneration properties of the cells. We strongly believe that cranio-facial stem cells are promising tool for various tissues and structures regeneration and repair. However, detailed characterization and comparison of the ECM and adhesion profile of different mesenchymal stem cell cultures is not yet performed.

The aim of the present research is to isolate primary mesenchymal cell cultures from adult (DPSC) and deciduous tooth pulp (SHED), periodontal ligament (PDLSC), dental apical papilla (SCAP), bone marrow (BMSC) and gingiva (GMSC) and to characterize them for expression of specific extracellular matrix and adhesion molecules using quantitative Real Time RT – PCR analysis.

Materials and methods

Stem cells isolation

The protocols in the current study were approved by the Ethics committee of Medical University-Sofia, Bulgaria. All donors have signed an informed consent. Cells were isolated from dental pulp of permanent (DPSC) or deciduous teeth (SHED), periodontal ligament (PDL), apical papilla (SCAP), bone marrow of alveolar bone (BMSC) and human gingiva (GMSC). The samples were stored in DMEM (Dulbecco’s modified Eagle’s Medium) (Invitrogen, Eugene, OR, USA) supplemented with antibiotics and then transported to the stem cell laboratory. Before further processing the samples were rinsed with phosphate buffered saline (PBS) (Lonza, Verviers, Belgium). The soft tissue samples were obtained as follows: the pulp from permanent teeth was obtained following splitting the teeth at the cementum-enamel junction via sterile fissure burr with high-speed handpiece and copious irrigation; the pulp from deciduous teeth was extracted with sterile barbed broach through the resorbed root canals; the PDL was scraped with sterile scalpel blades from the middle third of the roots; the apical papilla was separated from the root apices of incompletely developed teeth; gingival pieces were obtained during routine periodontal surgery procedures. All soft tissue samples were minced in small pieces and enzymatically digested with 3 mg/mL collagenase type I and 4 mg/mL dispase (Sigma-Aldrich) for 1 h at 37 °C in incubator followed by centrifugation and removal of the supernatant. The cell-pellets were then transferred to plastic cell culture dishes (2 cm) (Greiner Bio – One, Frickenhausen, Germany) containing DMEM supplemented with 1% antibiotic-antimycotic (Sigma-Aldrich, St. Louis, MO, USA) and 20% heat inactivated fetal bovine serum (FBS) (Sigma – Aldrich, St. Louis, USA) and then stored at standard cell culture conditions with 37°C temperature in humidified atmosphere of 5% CO2 and 95% air. The alveolar bone was cut into small pieces and seeded
at the same cell culture conditions. The cell culture media was replaced every 2\textsuperscript{nd} or 3\textsuperscript{rd} day. When reaching 85% confluence the cells were trypsinized (by incubation with 0.5-1 mL 0.025% trypsin/EDTA (Lonza) for 10–15 min at 37°C) and seeded in cell culture flasks (Greiner Bio – One, Frickenhausen, Germany) at a density from $5 \times 10^3$ to $1 \times 10^4$ cells/cm\textsuperscript{2} for further multiplication and passaging. The experiments in the present study were carried out in triplicate with cells from 3\textsuperscript{rd} to 6\textsuperscript{th} passages. The samples were arranged into the following groups: BMSC (ControlGroup), DPSC (Group1), SHED (Group2), SCAP (Group3), PDLSC (Group4) and GMSC (Group5).

**RT2 Profiler PCR Array Test**

Total RNA was extracted with RNeasy Plus Mini kit, cDNA was synthesized with RT2 First Strand Kit. The cultured cells were further processed via FastLane Cell cDNA Kit (Qiagen, Hilden, Germany) according the manufacturer’s instructions. First-strand cDNA was directly prepared and analyzed with RT2 Profiler PRC Array, Human Extracellular Matrix & Adhesion Molecules Array (all QIAGEN, Germantown, MD, USA) following manufacturer’s instructions. Briefly, each sample cDNA was first diluted with RNase-free water and then mixed with RT2 SYBR Green ROX qPCR Mastermix and were transferred onto 96-well plate. The reference gene used in the current experiment is Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). The obtained data was analyzed via the on-line software available at [http://www.qiagen.com/geneglobe](http://www.qiagen.com/geneglobe).

**Statistical analysis**

The data obtained in the present study was presented as the mean ± standard deviation. The statistical analysis is based on Students t-Test and ANOVA via Statistical Package for the Social Sciences (SPSS, IBM Inc., Amronk, USA). A P value <0.05 was used to determine the significant difference between the samples (investigated groups).

**Results**

**RT2 Profiler PCR Arrays Test**

Analysis showed significant differences between the stem cell cultures. Comparison of Collagen isotypes expression showed significant differences in fibrillar collagens and globular collagens expression, particularly Collagen I (fig. 1).

![Fig. 1 Expression of genes for fibrillar and globular collagens](image)

Analysis of integrins showed the most abundant β subunit is ITGB1 followed by ITGB5 and the most abundant α subunit is ITGA8 in most groups except in PDL and BMC, while ITGAV and ITGA5 are mostly
expressed the control group and group 4 (fig. 2). Comparison of matrix-metalloproteinases (MMP) and other proteases also showed differences in the investigated groups (fig. 3, 4).

**Discussion**

The ECM is providing the cell support and is also reservoir for cytokines and growth factors. It consists of wide range molecules including collagens, laminins, integrins, fibronectine, heparin sulfate proteoglycans, etc. (4). Remodelling of ECM by the cells leads to reciprocal interactions leading to specific modulation of cell-cell and cell-ECM behaviour and structure. Analysis of adhesion and ECM markers expression in the present study showed significant differences between the oral mesenchymal stem cell cultures. Collagens are the most abundant proteins in humans, as the superfamily consists of 28 different members (5). We investigated the gene expression of fibrillar and globular collagens in the isolated cells. Comparison of Collagen isotypes expression showed significant differences in fibrillar collagens expression and particularly Collagen I, with highest expression in bone marrow cells and lowest in SCAP. Collagen III and V were significantly less expressed by all studied cells with highest expression again in BMSC and comparatively high expression in PDL and SCAP.

Globular collagens showed bigger variety of expression throughout the cells with all of them expressing Collagen VI. DPSC, PDL and SCAP expressed Collagens XII and XV similar to BMSC and unlike them had higher expression of collagen XVI. SHED and GMSC solely showed significant expression of collagen IV and VII respectively unlike all the other tested cells.

Integrins are transmembrane receptors highly important for cell adhesion. They bind to basement membrane glycoproteins or connective tissue components and also to cell receptors, thus providing stable adhesion and furthermore signaling cascades (6). Integrins have α and β subunits – in mammals eighteen α and eight β subunits are revealed (7). Expression of integrins showed similarity for the most abundant β subunit in the cells is ITGB1 followed by ITGB5. ITGB3 on other hand were mostly expressed by dental mesenchymal cells. Significant differences in the expression of integrin α subunits were discovered. ITGA8 was the most abundant in all groups except in PDL and BMC where ITGAV and ITGA5 prevailed respectively. Stem cell
related ITGA1 and ITGA6 were expressed in DPSC, PDLSC and SCAP and ITGA6 was much lower than ITGA1 in other three cell types.

Proteases play key role in hydrolysis of peptide bonds, thus stimulating the proteins break down. They regulate the fate of many proteins, as well as protein-protein interactions and amplify molecular signals (8). Matrix-metalloproteinases (MMP) are special molecules capable of degrading and modifying the ECM and are known to be able to provide the extracellular homeostasis (9). More than 20 secreted or membrane-associated MMPs are known. Comparison of MMPs gene expression showed highest amounts of MMP2 and MMP14 in all cells with significant difference in other minor expressed types.

All these results suppose differences in cells potential for wound healing, cell migration, modulating the immune response and participation in carcinogenesis of the mesenchymal stem cell subpopulations. We suggest these are different niches of stem cells present in the oral cavity tissues, possessing specific properties and high potential for tissue regeneration.

Expression of the tested markers suggests closeness of phenotype of DPSC, PDLSC and SCAP with BMSC, although SHED and GMSC showed significant differences from all other cells.

**Conclusion**

Oral tissues contain niches of mesenchymal stem cells, having different adhesion and ECM profile, thus possessing different stem cell properties. Considering embryonal development of tooth germ phenotypal closeness of DPSC, PDL and SCAP with BMSC may be expected. Further analysis is needed for better understanding why SHED are so different with DPSC. More comparison experiments between mesenchymal stem cell populations for stem cell niches definition are needed.

**References:**


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Abstract: Complicated metadiaphyseal tibial fractures (CMTF) present a heterogeneous group of injuries for which the surgical approach is determined by the type of fracture personality and the degree of injured surrounding soft tissues. Due to the high variability of these fractures, following the golden operative algorithms is not always appropriate. Standard surgical methods are followed by low, but persistent rate of severe complications. These unsolved problems motivated us to investigate and apply a novel minimally invasive surgical technique, which we named One-Staged Locked Externalized Stabilization (OLES). It is based on Perren’s concept for secondary bone union according to the computational results from our biomechanical experimental Finite Element Model (FEM).

Key words: Tibia, Metadiaphyseal tibial fractures, LCP, External plate fixation.

Introduction: CMTF affect the proximal and the distal third of the tibia and are result from a high-energy trauma - motor vehicle accidents or fall from height. They can have a long-term adverse consequence, associated with significant socio-economic cost. (1-3) Multifragmentary metaphyseal proximal and distal fractures accompanied by severe soft tissue trauma present a significant challenge to the treating orthopaedic trauma surgeon. (4) The soft tissues surrounding the knee and ankle joint are easily affected by the trauma mechanism, and a subsequent fracture fixation further disrupts the blood supply and is a prerequisite for delayed bone union with frequent complications, associated with postoperative wound healing. (5)

Standard method: Contemporary surgical protocols with open reduction and internal fixation (ORIF) require extensive soft tissue exposure, which further impairs the local blood supply, and is the cause for a number of complications, with prolonged recovery time and impaired work capacity (6). For several decades, ORIF with plates has been the gold standard of treatment for these types of unstable tibial fractures, but surgical dissection through the primary injured soft tissues is often followed by risk of early wound disruption, deep infection, septic arthritis, osteomyelitis, joint contractures, nonunion or malunion of the bone fragments, and even amputations of the limb. The evolution in orthopedic instrumentation lead to the development of minimally invasive surgical methods and techniques, such as minimal invasive percutaneous plate osteosynthesis (MIPPO), or intramedullary nailing (IMN), according to the principles of biological fracture fixation. (7-8) MIPPO is a soft tissue sparing surgical approach with respect to the bone biology, fracture healing process, and adjacent blood supply. (9) Despite being anatomically precontoured, and mounted via small skin incisions, LCP plates, may protrude under the skin and can cause substantial surgical wound problems. However, IMN provides an elastic fracture fixation for the meta diaphyseal multifragmentary fractures, allows adequate restoration of limb length equality, the risk of axial malalignment and anterior knee pain is significant. It is determined by the shape and width of the tibial medullary cavity, resembling an hourglass, wide in the metaphyseal zone, and narrowed at the tibial diaphyseal isthmus. In another study of Mahadeva et al., 2008, hybrid single-stage external fixator was found to be the most sparing soft tissue device, with comparable final results to internal plate fixation. (10-11)

Perren’s strain theory and secondary bone healing
The complex interaction between the mechanical environment and cellular repair processes at the fracture site is the basis of Perren's strain concept of the degree of deformation at the fracture region.
Interfragmentary mobility describes the range of micromotions at the fracture site. The strain rate is defined by the change in linear dimensions of the material under the influence of external forces. Longitudinal strain describes the percentage of micromotion at the fracture site and explains the ability of different types of healing tissues to sustain different amounts of interfragmentary mobility. If the degree of longitudinal strain is greater than 100%, it is very likely that the end result will be nonunion of the fracture. With values in the range of 100% to 10%, granulation tissue formation and conditions for fibrous healing are expected. Most interesting is the range between 2% and 10%, where cartilage formation and enchondral ossification underline the indirect or secondary bone healing through the formation of a massive callus. At strain lower than 2%, there are favorable biomechanical conditions for primary bone healing and direct cortical bone formation (12-13-14-15)(fig.1).

![Interfragmentary longitudinal strain](image)

**Figure 1.** Perren’s strain theory.

**Figure 2.** Final FEM results for stiffness, interfragmentary motion (IFM), and longitudinal strain at the fracture site under partial (PWB) and full (FWB) loads of 25 kg and 80 kg, respectively.

**FEM:** A three-dimensional model of the tibia was developed based on two-dimensional CT images using three-dimensional reconstruction. A virtual transverse osteotomy was performed simulating a metadiaphyseal tibial fracture with a defect size of 2 cm, corresponding to a multifragmentary fracture model with a simple, nondisplaced intra-articular component, defined as unstable proximal tibial fracture type AO/OTA 41 C2.2.

The three experimental groups (Gr) with three different plate-bone offsets were designed: Gr-1 with 2 mm plate offset to match an internal plate fixation, the Gr-2 with 22 mm plate offset, simulating an externalized stabilization in patients with thin, soft tissue envelope, and the Gr-3 with 32 mm plate offset, to simulate an externalized plating in patients with thick, soft tissues around the shin bone. The parameters of interest in the simulation model were: construct stiffness, equal to the applied force divided by the resultant displacement measured at the most lateral point of the articular surface, interfragmentary motion, equal to the change in osteotomy width at the most lateral aspect of the fracture and longitudinal deformation (strain) in the fracture zone, which represents the deformation of the bone-implant construct and is calculated, according to Perren's strain concept, by dividing the interfragmentary motion to the width of the fracture defect and expressed as a percentage.(fig.2)

**A novel surgical method - One-Staged Locked Externalized Stabilization with LCP plates**

In the last few years, a method of “Supercutaneous” monolateral external fixation using anatomically precontoured metaphyseal angle-stabled plates, (LISS-DF, LCP), as an external fixator have been reported (16,17). The locking plates are placed over the tibial skin after closed indirect fracture reduction. The plate functions as an external splinting device, and the locking head screws (LHs) provide enough angular stability to the construct (18-19). The CMTF, with their short proximal metaphyseal segment appears to be appropriate, for locking external plate fixation. In the anteromedial aspect of the tibia, the subcutaneous tissue is thin enough, and there are no vital vessels, nerves, or muscles that could be injured after indirect fracture reduction followed by definitive locked external stabilization. When a closed reduction is not possible, stab skin incisions are used. Thus, the plate functions on the principle of an external buttress splinting device, attached to the bone fragments by angularly stable LHs. LHs penetrate into both cortices of the underlying tibia whenever possible.
and are locked in the corresponding holes of the LISS DF plate. That facilitates early mobilization and permits easy patient ambulation, and hygienic care of the skin – screw interface. LES design is aesthetically more acceptable for the patient, compared to the wide range of traditional ExFix systems - monolateral, hybrid, ring, or hexapod designs (20). The One-staged LES with angle-stabled LISS DF plates is a novel surgical method that provides an effective and safe external biological fracture fixation responding to Ganz’s "biological plating" definition for the treatment of CMTF. (fig.3)

![Figure 3. Locked externalized stabilization – our patients with plate and after plate removal.](image)

**Conclusion:** One-staged locked externalized stabilization is an attempt to combine the best of locked internal plate fixation and monolateral external stabilization. The technique is fast, minimally invasive, soft tissue sparing, bloodless, simple, inexpensive surgical alternative with functional and clinical outcomes comparable to the standard surgical methods. It is safe when precise inclusion criteria and presented operative technique is applied. One-staged locked externalized stabilization has the characteristics of flexible biological fracture fixation for natural bone healing.

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Development of interactive software solution “Amira” to optimize and personalize antibiotic prescriptions and fight AMR in Bulgarian hospitals

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Abstract
Irrational use of antimicrobials negatively affects not only the individual patient but leads to severe epidemiological and economic consequences for society. There is a need to develop an optimization approach to improve the therapeutic usability and economic feasibility of antimicrobial agents in Bulgaria. The objective of the on-going Amira project is to develop an interactive software solution to optimize the effectiveness and safety of antibiotics and personalize the antibiotic treatment in clinical settings. A prototype of the software with decision making support regarding appropriate surgical prophylaxis has been developed and is currently being integrated into the clinical practice of two university hospitals in Sofia and Pleven, Bulgaria. The Amira project has won 2 Intel awards recognizing the potential to integrate artificial intelligence and machine learning technologies to allow better observation of the effectiveness and safety of empirical treatment recommendations and their timely adaptation by monitoring antibiotic resistance levels in Europe.

Keywords: AMR, MDR, Antibiotic Stewardship, antibiotics, digital healthcare, AI

Introduction
The appropriate empirical choice of antimicrobial treatment is a key factor for good effectiveness and safety of antibiotics and for reducing the fatality rate of severe infections. However, 20% to 50% of patients are given inappropriate empirical antimicrobial treatment. [1-3]

According to the CDC study published in March 2021, prescribing antibiotics is not appropriate in: 79% of patients with community-acquired pneumonia, 77% of patients with urinary tract infections, 47% of patients prescribing treatment with fluroquinolones, and 27% of patients prescribed an intravenous antibiotic with vancomycin. [4]

Antibiotic Stewardship Programs (ASPs) are organizational and healthcare system-wide approach to support clinicians in their decision making regarding antibiotic treatment, which is designed to improve clinical outcomes and minimize harms done by inappropriate antibiotic prescribing. ASPs also include monitoring and control of the use of antimicrobials to preserve their future effectiveness. [5,6] Hospital antibiotic stewardship programs in Europe and USA have proven to increase infection cure rates while reducing treatment failures, C. difficile infections, adverse drug reactions of antibiotics, the rates of antibiotic resistance, hospital costs and lengths of stay. [6, 7,8]

Objective:
Development of an interactive software application to optimize the therapeutic usability and cost-effectiveness of antimicrobial use in hospital settings.

Materials and methods:
The first step of the AMIRA project, formulated at the beginning of the research and development, was to build a decision support system for antibiotic prophylaxis in patients with upcoming elective surgery which can be easily accessed through any smartphone, tablet and hospital computer with internet connection. The prototype was developed and adapted for the surgical unit of University Hospital "Tsaritsa Yoanna-ISUL". The medical algorithm of the prototype is based on international and national guidelines (where applicable) for surgical prophylaxis for the elective surgeries performed routinely in the unit, the hospitals antibiotic stewardship program and local guidelines for surgical antimicrobial prophylaxis created a few years ago by the Clinical pharmacology and therapeutics sector of University
Hospital "Tsaritsa Yoanna-ISUL" [9], the hospitals data on the local prevalence and resistance of the microorganisms and the summary of product characteristics (SmPCs) of the included antibiotics in the medicinal list of the hospital.

One of the main features of the prototype is the easy-to-use interface. After the diagnosis or indication for antibiotic usage is known, it is to be chosen from the menu (like the shown example in Picture 1 – “Peroperative surgical prophylaxis” in “Small bowel surgery” is selected). Then the specific operation that is to be performed is selected from a drop-down menu (in this case - diverticulectomy), and only the most necessary patient characteristics are requested to be put in to personalise the prophylaxis/treatment (for the different indications the input patient data can be body weight, pregnancy, allergies, kidney and liver function, cardio-vascular diseases, previous antibiotic usage, specific risk factors). In this case the patient is put in to have no allergies, no risk factor for MRSA.

With just a few clicks the application gives personalized recommendations regarding: the pathogens needed to be covered by the prophylaxis (or empirical treatment); the first-line antibiotic for the perioperative antibiotic prophylaxis (or empirical treatment); recommendation about dosage, dosing interval, duration of administration, method of administration, when to repeat the dose; information about very common, common and less common adverse drug reactions and clinically significant drug interactions.

In the shown example (Picture 2) the first line recommendation for antibiotic prophylaxis in this small bowel surgery is Cefazolin 2 g i.v. by 3-minute infusion 30 minutes before the surgery, dissolved in 10 ml of sterile water for injections or 5-10% glucose solution. All the information regarding the antibiotic is presented in an accessible and easy to read format.

When the initial input data is changed, the advice changes accordingly. For example, if instead of diverticulectomy the performed operation is to be small intestinal ileus (obstruction), the patient has allergy to beta-lactams and risk factors for MRSA (Picture 3), the first line recommendation for prophylaxis is now a combination of clindamycin 600 mg i.v. (instead of cephasolin, because of the allergy), metronidazole 500 mg i.v. (because of the obstruction), and vancomyci i.v. (with personalized dose calculated based on the patient’s weight). (Picture 4).

The next version of the application will support the transition from empirical to targeted antimicrobial therapy, alongside with tips for monitoring the effectiveness, safety, and economic feasibility of the selected therapy.

The algorithm is stored on a cloud which allows quick updates and no sensitive patient data is collected, which makes it GDPR safe.

**Results**

A prototype of the Amira software with decision making support regarding appropriate surgical prophylaxis has been developed and is currently being integrated into the clinical practice of two university hospitals in Sofia and Pleven, Bulgaria. The project has received recognition on a European level by receiving 2 consecutive awards from the technological company Intel for the potential of integrating artificial intelligence and machine learning (AI&ML) technologies in the software to optimize further the use of antibiotics by society.

The application has the potential to gather data regarding the prevalence of infections in Europe, the effectiveness and safety of antibiotics, and the antimicrobial resistance rates in different parts of Europe. Gathering and analyzing this data with AI&ML can have many benefits for society like faster adaptation of the guidelines for treating infections to change the prescription behaviors of doctors regarding antibiotics threatened by AMR.
Discussion

To extend the scope of this project and prove the benefits in the clinical practice, Amira is planned to be adapted and implemented in other hospital surgical clinics until the end of 2023 and the impact to be monitored closely. Only then conclusions can be drawn about the actual effectiveness and economic viability of implementing an interactive software solution like Amira for optimization and personalization of the antibiotic treatment.
References:


Reasons for the decision to decline vaccination against COVID-19 in Bulgaria

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Abstract
The study explores the reasons for the COVID-19 vaccine hesitancy in Bulgaria. Between April and May of 2022, a cross-sectional survey on a convenience sample of 1,200 Bulgarian residents was conducted using an online self-administered questionnaire. The results indicated that younger adults and rural residents demonstrated a greater degree of vaccine hesitancy. Higher levels of education and higher income were associated with a greater likelihood of vaccine acceptance. The most important reasons to decline vaccination were fear of side effects, contradictory opinions among physicians, prior COVID-19 sickness, and the misconception that vaccination constituted a violation of the rights. The findings of our study underscores the need for targeted communication and education to increase vaccine uptake and mitigate the impact of misinformation.

Keywords: COVID-19, vaccine, vaccine hesitancy, vaccine refusal, reasons

Introduction
The development and approval of a safe and effective vaccine against COVID-19 provided an opportunity to fight the pandemic, but its availability and distribution were insufficient to curtail the morbidity curve; for the success of the immunization campaign, willingness to receive the vaccine among a greater part of the population became crucial. Several studies found that there were considerable differences in vaccination coverage, both within and between the countries, and the reasons for these differences were the growing anti-vaccination movements and vaccine hesitancy [1, 2].

Even before the pandemic, vaccine hesitancy was identified by the World Health Organization as one of the top 10 threats to global health [3]. It is defined as a "delay in acceptance or refusal of vaccines despite the availability of vaccination services" [4, p. 4163] and represents a substantial obstacle to the success of vaccination efforts. Understanding of the reasons for vaccine hesitancy is essential for the health system preparedness for future outbreaks. Effective public health campaigns have to target hesitant individuals and groups with interventions addressing the reasons for vaccine hesitancy [5].

To fill in the existing knowledge gaps, our study aims to investigate the reasons for the COVID-19 vaccine hesitancy in Bulgaria.
Materials and Methods
A cross-sectional survey of the general Bulgarian population was conducted between April and May 2022 using an online self-administered questionnaire. Participants were recruited via social media and emails using the snowball sampling technique. The final sample included 1,200 adults with residency in Bulgaria. Ethical approval for the survey was granted by the Research Ethics Committee of Medical University-Varna.

The 36-item questionnaire was pretested using a pilot study. The survey collected data on various factors related to COVID-19 vaccination, including vaccination status, beliefs about vaccination, reasons to accept or refuse the COVID-19 vaccine, and sociodemographic characteristics. Respondents were asked to rate the importance of possible reasons to accept or refuse vaccination using a Likert scale, with response options ranging from "very important" to "not important". The responses were subsequently recoded into binary values of "yes" and "no", with "I do not know" responses considered missing.

Descriptive statistics were used to summarize data collected on sociodemographic characteristics and reasons to decline vaccination using jamovi, ver. 2.2.5. A chi-square test was performed to examine the association between sociodemographic variables and individuals' decision to receive the COVID-19 vaccine.

Results
The results indicated that younger adults aged between 18 and 24 years old demonstrated a greater degree of vaccine hesitancy compared to older individuals aged 65 and above ($\chi^2=47.5$, $p<0.001$). In addition, higher levels of education were associated with a greater likelihood of vaccine acceptance, whereas lower education attainment was related to higher levels of vaccine hesitancy ($\chi^2=29.2$, $p<0.001$). Notably, significant differences were observed in vaccination rates between rural and urban areas, with a higher prevalence of vaccine hesitancy found among rural residents ($\chi^2=19.2$, $p<0.001$). Conversely, in larger urban areas, the vaccination rate was found to be significantly higher. Furthermore, our analysis revealed that participants with higher income were more likely to accept the vaccine, whereas those with lower income expressed greater hesitancy towards vaccination ($\chi^2=20.7$, $p=0.02$).

Our study aimed to explore the key reasons underlying individuals' decisions to decline the COVID-19 vaccination. The questionnaire was designed to assess the significance of various factors, including prior COVID-19 sickness, medical conditions thought to be contraindications, incoherent opinions among physicians or contradictory messages in the media, misbeliefs about the vaccine or pandemic, fear of side effects, etc. Out of the 1,200 respondents, 488 were not vaccinated (at the time of the survey, and their responses were included in the analysis about the reasons for their hesitancy or refusal.

The analysis revealed that the most important belief motivating respondents not to get vaccinated was the misconception that vaccination was a form of violation of their rights (Fig. 1), followed by mistrust in vaccine effectiveness. Among the health-related factors, the leading reason was fear of side effects, followed by a previous history of COVID-19 infection. Interestingly, among the factors attributed to the information source affecting negatively vaccination acceptance, contradictory opinions among physicians was the leading one.
Discussion

Our findings suggest that vaccine hesitancy is prevalent among younger adults in Bulgaria compared to older age groups. This may be due to a lack of confidence in vaccine or misinformation circulating on social media platforms [6]. Furthermore, our analysis revealed that individuals with higher educational attainment were more likely to accept the COVID-19 vaccine. Individuals with higher levels of education have access to accurate and reliable health information and have greater trust in science.

The study highlighted significant differences in vaccination rates between rural and urban areas, with a higher prevalence of vaccine hesitancy found in villages. This could be attributed to the restricted access to information and health services in rural areas, as well as the lower levels of education and socioeconomic status [7]. In contrast, better access to health services and health-related information may account for the higher vaccination rates in larger urban areas.

The findings shed light on the reasons underlying COVID-19 vaccine refusal among the Bulgarian population. The results suggest that a significant portion of unvaccinated individuals opt out due to misconceptions about vaccination and a perceived violation of their rights. It is important to address such misinformation in order to increase vaccine acceptance. Fear of side effects has been identified as a factor contributing to vaccine hesitancy, emphasizing the importance of transparent and reliable information and education about the safety and efficacy of COVID-19 vaccines. Additionally, a previous history of COVID-19 infection contributed to vaccine refusal, highlighting the need for targeted communication strategies. Our analysis revealed that contradictory opinions among medical specialists strongly influenced the
vaccination decision, underscoring the importance of consistent and evidence-based messaging from health professionals.

**Conclusion**

Our study provides insights into the key reasons for COVID-19 vaccine hesitancy and refusal in Bulgaria and underscores the need for targeted communication and education to increase vaccine uptake and mitigate the impact of misinformation. Public health communication should emphasize the importance of protecting vulnerable individuals and the community. Efforts should be made to improve access to accurate information and increase awareness. Addressing misinformation regarding the safety and efficacy of vaccines is vital for improving vaccination coverage. Overall, these insights can inform public health strategies and interventions aimed at promoting COVID-19 vaccination uptake in Bulgaria.

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High body mass index: potential health risk for patients with COVID-19 infection and cardiovascular diseases

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Abstract: Aim: Study and analysis of the degree of overweight and obesity in patients hospitalized with COVID-19 infection. Material and methods: The study included 101 patients hospitalized with a diagnosis of COVID-19 in the period January-March 2022. With the help of a medical scale, the anthropometric indicators were measured - height in centimeters and weight in kilograms and the Body Mass Index was calculated using an online calculator. Conclusions: Overweight and obesity are common conditions among hospitalized patients with COVID-19. Other concomitant diseases are arterial hypertension and diabetes mellitus, and their frequency correlates with increasing body weight and high BMI.

Keywords: body weight, body mass index, COVID-19, risk factors

Introduction: Overweight (OW) and obesity have been reported as independent risk factors for severe COVID-19. High body weight increases susceptibility to infections and is a predictor of mortality in patients with COVID-19. They are themselves metabolic diseases and are associated with a number of other chronic diseases (diabetes mellitus (DM), cardiovascular disease (CVD), hypertension (AH), dyslipidemia) that can cause and/or exacerbate, and are associated with increased morbidity, severe course and higher mortality rates among COVID-19 patients. [1, 2, 3]

Aim: The aim of our study was to analyze the incidence and severity of overweight and obesity in patients hospitalized with COVID-19 infection.

Material and methods

The study was conducted among 101 patients hospitalized with a diagnosis of COVID-19 in the period January-March 2022 at a University Hospital in Sofia. Data on age, sex, concomitant diseases, and available vaccination through the structured interview method were also studied in all subjects. With the help of a medical scale, the anthropometric indicators were measured – height in centimeters and weight in kilograms. The BMI was calculated using an online calculator. It is classified according to the World Health Organization (1995) in the following six categories. [4, 5, 6, 7]

Results

The gender distribution shows that 51 (50.50%) of the participants are men and 50 (49.90%) are women. More than half (56.73%) of the respondents have a BMI above the norm. Almost one in three (29.70%) of those hospitalized with COVID-19 was classified as overweight and with a BMI between 25–29.9 kg/m², and one in four (26.73%) with varying degrees of obesity and BMI ≥ 30.0. Only 41.58% of the participants in the study have normal BMI indicators, and the lowest is the share of those with underweight – respectively 1.98%.

Of interest in the study was the classification of BMI levels in obese patients. The highest (40.74%) is the share of patients with grade III obesity and BMI ≥40 kg/m². More than one third (37.04%) of participants with obesity have a BMI between 35-39.9 kg/m², which classifies them as grade II disease, and 22.22% have grade I obesity and BMI 30-34.9 kg/m².

Table 1 presents the concomitant diseases, respectively risk factors for hospital treatment and severe course in patients with COVID-19.
Table 1. Concomitant diseases according to the BMI classification

<table>
<thead>
<tr>
<th>Disease / Risk factor</th>
<th>Normal and underweight</th>
<th>Overweight</th>
<th>Obesity - I, II, III degree</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic lung disease, Asthma</td>
<td>12.90%</td>
<td>3.70%</td>
<td>10.00%</td>
</tr>
<tr>
<td>Stroke</td>
<td>0.00%</td>
<td>3.70%</td>
<td>3.33%</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>12.90%</td>
<td>18.52%</td>
<td>3.33%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19.35%</td>
<td>14.81%</td>
<td>33.33%</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>22.58%</td>
<td>25.93%</td>
<td>33.33%</td>
</tr>
<tr>
<td>CVD - ischemic heart disease, myocardial infarction, heart failure</td>
<td>6.45%</td>
<td>11.11%</td>
<td>3.33%</td>
</tr>
<tr>
<td>Malignant disease</td>
<td>6.45%</td>
<td>7.41%</td>
<td>6.67%</td>
</tr>
<tr>
<td>Hashimoto's thyroiditis and / or other autoimmune disease</td>
<td>6.45%</td>
<td>0.00%</td>
<td>3.33%</td>
</tr>
<tr>
<td>No known comorbidities</td>
<td>12.90%</td>
<td>14.81%</td>
<td>3.33%</td>
</tr>
</tbody>
</table>

The most common concomitant disease is AH. It is found in 22.35% of respondents with underweight and/or normal weight, 25.93% in those with overweight and 33.33% with obesity. The study found that with increasing body weight, the proportion of patients with AH also increases.

Diabetes mellitus is the second most frequently classified concomitant disease, found in the highest relative share of obese patients (33.33%), followed by patients with normal (19.35%) and overweight (14.81%).

Chronic renal failure is most common in overweight patients (18.52%), followed by 12.90% of normal weight patients and only 3.33% in obese patients. The share of respondents with concomitant (chronic lung disease, Asthma) is not small. It is noteworthy that it is found most often (12.90%) in patients classified as normal or underweight. 10.00% of obese and 3.70% of overweight respondents also suffer from concomitant lung disease.

CVD, malignant and autoimmune diseases are also found in the various groups classified according to BMI. Only 3.33% of obese patients, 14.81% of overweight patients and 12.90% of normal weight patients have no known comorbidities.

In three of the patients classified with 2nd and 3rd degree obesity, aged 56 and 35, respectively, and 68 years old, male, the disease is very severe and long. The patients have data for AH, obesity, and two of them with diabetes. Due to the low values of saturation, the hospital treatment with oxygen therapy was necessary for 2, 2 and 1 months, respectively. The 35-year-old patient had severe pulmonary sequelae and post-COVID syndrome.

Discussion

More than half of our respondents have a BMI above the norm. Almost every third (29.70%) of those hospitalized with COVID-19 is classified as overweight, and every fourth (26.73%) with obesity – I, II or III degree.
The study found that the most common comorbidities in the studied patients were AH and diabetes. Their frequency correlates with increasing body weight and high BMI, followed by chronic renal failure, chronic obstructive pulmonary disease, CVD, malignant, autoimmune diseases. Although concomitant diseases are not classified according to BMI in other studies, the same comorbidity has been reported in the literature in patients with COVID infection. AH and diabetes are almost always the leading ones, and their frequency is different in different studies. In a study conducted in the "Homeland of the Epidemic", Wuhan, a study by Jin-jin Zhang et al. found that the most common comorbidities were AH (30.0%) and diabetes (12.1%). However, the authors point out that chronic lung disease and smoking are rare cases. [8]

In a study, cerebrovascular diseases and diabetes have been identified as leading comorbidities responsible for severe COVID-19. Another study ranked them in the following descending order: AH, diabetes, CVD, and cerebrovascular disease. Third – AH and diabetes, and fourth – CVD, including AH, diabetes, chronic obstructive pulmonary disease, chronic renal failure, cancer, obesity. [8, 9, 10]

Our study also found that mortality in men is twice as high as in women. Such are the data in an even larger study conducted by R. Dimova and a team. The same authors also referred to the risk factors for severe course and high mortality in COVID-19 patients, namely CVD, diabetes, chronic lung disease, oncological diseases, chronic renal failure, obesity, liver cirrhosis. [9] The here presented study found that patients with a fatal outcome have a number of comorbidities, the most common of which are CVD, including AH, diabetes mellitus, chronic obstructive pulmonary disease, chronic renal failure, cancer, and obesity. The average age of the deceased was 59.8 years. These data indicate that fatal accidents occur in patients with COVID-19 and a number of predisposing diseases who are also patients of active working age.

Conclusions
In our own study, it was found that the ratio between hospitalized men and women is approximately the same. The mean age of hospitalized patients was 63 years, with a wide age range (20 to 90 years). Approximately 57.0% of hospitalized patients were overweight and obese, which identified them as significant risk factors for inpatient treatment with COVID-19. Over a quarter (27.0%) are classified with varying degrees of obesity, with the highest share of patients with grade III, followed by grade II and last are those with grade I obesity.

The most common concomitant disease is AH. It is found in 22.0% of respondents with underweight and/or normal weight, 26.0% in those with overweight and 33.0% with obesity. The study found that with increasing body weight, the proportion of patients with AH also increases. The next most common concomitant disease in the studied patients is diabetes. Frequency of patients with AH and DM correlates with increasing body weight and high BMI. They are followed by chronic obstructive pulmonary disease, chronic renal failure, CVD, malignant and autoimmune diseases.

Acknowledgements:
This research was supported by the Bulgarian Ministry of Education and Science under the National Program “Young scientists and Postdoctoral Students – 2”

References:


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Analysis of the practical preparation of students nurses to deal with an epileptic seizure

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Abstract
Epilepsy is the most common chronic neurological disease of the brain, characterized by epileptic seizures. The main symptom of epilepsy is epileptic seizures, which are caused by excessive discharges of nerve cells in the brain. The training in the specialty "Nurse" for the educational-qualification degree "Bachelor" includes theoretical and practical training for the disease epilepsy. The aim of the present study is to study and analyze the degree of practical training of the students of the "Nursing" specialty to deal with an epileptic seizure occurring in patients with epilepsy.

Material and methods
documentary, sociological method and graphic analysis of the obtained data were used in the study. The study was conducted among 223 students of the "nursing" specialty

Results: From the analysis of the results, it was found that the percentage of students (53.81%) who consider that they have the necessary practical training to deal with an epileptic seizure is the highest.

Conclusion: The results of the study show that students have a need for additional training, such as a freely chosen discipline

Key words: students, epileptic seizures, practical training

Introduction
Training in the specialty "Nurse" for the educational-qualification degree "Bachelor" is conducted in regular form with a duration of 4 academic years, corresponding to 8 semesters and a horary of 4630 hours. It includes theoretical and practical training. The theoretical preparation is carried out through study disciplines in the form of lectures, seminars and practical exercises, and the practical preparation - through practical training and pre-diploma internship. [1] The acquired theoretical knowledge and practical skills in the four-year training period aim to build professional behavior and attitude, responsibility, security and quality of health care provided in medical practice in the future specialists. [2]

Epilepsy is the most common chronic neurological disease of the brain, which begins in childhood and is of great medical and social significance. [4,8] Reasons for the development of epilepsy can be various factors - genetic and acquired, but more often epilepsy occurs as a result of the simultaneous presence of both genetic and acquired factors. The causes can vary depending on the age of onset of epilepsy.[3,4] Epilepsy is a disease characterized by epileptic seizures. The main symptom of epilepsy is epileptic seizures, which are caused by excessive discharges of nerve cells in the brain.[4,5] Epileptic seizures are divided into convulsive, in which a motor component is present, and non-convulsive, which are not associated with movements - absence, astatic seizures.[6]

Objective
The aim of the present research is to study and analyze the degree of practical training of the students of the "Nursing" specialty to deal with an epileptic seizure that occurred in patients with epilepsy.

Methods and materials
The study was conducted among 223 students from the "nursing" specialty, after completing the fifth semester of study and pre-graduate internship in the period from April 2021 to May 2022. The research used a documentary, sociological method - a questionnaire survey was conducted based on voluntary and anonymous polling based on survey cards developed specifically for the purpose. A statistical
method and a method of graphic analysis of the obtained data were used. The graphs were prepared using the Microsoft Office Excel program.

Results

The age profile of the students from the "nursing" specialty who took part in the survey shows that the group of students from 20 to 30 years old is the largest - 48.43%. In second place are students aged 31 to 40 years - 34.98%. These data show that the students who choose to study nursing are of different age groups, which shows their motivation for choosing this profession. (fig.1)

**Fig. 1. Age distribution of students**

Figure 2 presents the data of the respondents in response to the question "Do you think you have the necessary practical training to deal with an epileptic seizure". The majority of the surveyed students - 53.81% indicated that they have some practical training for dealing with an epileptic seizure in patients with epilepsy. The obtained results show that it is necessary to emphasize practical activity in neurologically ill patients.

**Fig.2. Practical training of students to deal with an epileptic seizure**

The highest relative share (41.25%) of the students asked answered that they were satisfied with the practical training to a certain extent. The data are graphically presented in Fig. 3.
We also studied the opinion of the surveyed students about their knowledge and skills for self-management in case of an epileptic seizure after they had completed the course "Nursing care of the neurologically ill".

Almost half of the surveyed students - 51.57% indicate that they have the necessary knowledge and skills to a certain extent to deal with an epileptic attack in patients with epilepsy. Fig.4.

Discussion
The survey conducted found that the largest group of students (48.43%) aged 20 to 30 years. The percentage of students (53.81%) who believe that they have the necessary practical training to deal with an epileptic seizure is the largest. 41.25% of the respondents were somewhat satisfied with their practical training. There is also a large number of students who believe that they have, to a certain extent, the necessary knowledge and skills to deal with an epileptic seizure in patients with epilepsy.

Conclusion
In conclusion, from the analysis of the obtained results of the conducted study, it was found that the students are familiar with the disease and the epileptic seizures that are characteristic of it, but they are not completely sure that they would handle an epileptic seizure in a patient. The results of the study show that students have a need for additional training, as a freely chosen discipline, because the specifics of neurological diseases make it difficult for them.

Acknowledgements:
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Body composition alterations in individuals with metabolic syndrome

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Abstract

Introduction: Metabolic syndrome is an endocrine condition based on the combination of several inter-related clinical symptoms. It is associated with increased risk for cardiovascular disease and type 2 diabetes mellitus. The aim of the study is to investigate the alterations in body composition in patients with metabolic disorders.

Material/Methods: A sample of 100 subjects at mean age of 54.6 ± 10.5 with metabolic disorders was selected. The indicators for the assessment of body composition were determined by bodyimpedancemetry. Height, waist and hip girths were measured by standardized procedures. Cut-off values for body weight, waist girth, waist-to-hip ratio and central obesity index were also applied. A documentary method for assessment of lipid profile and serum glucose of the subjects was used.

Results: All diagnostic criteria for metabolic syndrome in both sexes are elevated except the HDL-cholesterol. More than 64% of the subjects have deviations from the normal values of the studied anthropometric indicators. Over 90% of men and 74% of women are overweight and obese. The fact, that ¼ of the women with metabolic syndrome have a normal body weight is alarming. According to the waist girth, no significant differences were found between both sexes. In women, 64% are at increased risk for metabolic complications based on the index waist-to-hip ratio, while in men 91.2% are at increased risk.

Conclusion: The results confirm the need for early prevention of overweight and obesity, as well as for a detailed study of the causes of the development of metabolic syndrome in women.

Key words: body composition, metabolic syndrome, anthropometric indicators.

Introduction

Metabolic syndrome (MetS) is a combination of several inter-related clinical symptoms. According to the modern consensus, the diagnosis of MetS is accepted in the presence of visceral obesity (determined by the increased waist circumference ≥ 94 cm for men and ≥ 80 cm for women) in combination with 2 of the following 4 criteria [1]: increased arterial pressure (AP) (systolic ≥130 mmHg, diastolic ≥85 mmHg) or taking antihypertensive medications in a patient with a history of AP; elevated serum levels of triglycerides ≥ 1.7 mmol/L; reduced serum levels of HDL-cholesterol (<1.04 mmol/L for men, <1.29 mmol/L for women) or specific treatment for it; elevated fasting plasma glucose ≥ 5.6 mmol/L or diagnosed type 2 diabetes. In recent years, literature data show relation between body composition, certain anthropometric parameters, and new indicators for assessing cardiovascular risk in individuals with MetS [2, 3]. The aim of the present study is to investigate the alterations in body composition in patients with metabolic disorders.

Methods

A representative sample of 100 subjects (66 % women) at the mean age of 54.6 ± 10.5 with metabolic disorders was selected from practices of general practitioners in Pleven and the region.
The indicators for the assessment of body composition were determined by the method of body impedanceometry (Tanita Body Composition Analyzer TBF-300 M). Height, waist and hip girths were measured by standardized stadiometer and special measuring tape. Body weight categories were assessed based on body mass index (BMI), according to WHO criteria. Cut-off values for waist girth, waist-to-hip ratio (WHR) and central obesity index (waist-to-height ratio) were also applied. A documentary method was used to assess the lipid profile and serum glucose of the subjects. Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of Medical University – Pleven. Statistical package IBM SPSS Statistics v. 26.0 was used for statistical analysis, at a significance level p < 0.05.

**Results and discussion**

All subjects in the study have categorically established MetS. All diagnostic criteria for MetS in both sexes are elevated except for the HDL indicator, which has normal values (Table 1).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sex</th>
<th>Men</th>
<th>Women</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Me</td>
<td>Mean ± SD</td>
<td>Me</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>105.8 ± 9.3</td>
<td>106</td>
<td>94.6 ± 13.8</td>
<td>95.5</td>
</tr>
<tr>
<td>SRR (mm Hg)</td>
<td>136.9 ± 18.4</td>
<td>130</td>
<td>136.6 ± 21.9</td>
<td>135</td>
</tr>
<tr>
<td>DRR (mmHg)</td>
<td>87.1 ± 8.7</td>
<td>85</td>
<td>85.2 ± 12.1</td>
<td>82.5</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.1 ± 1.2</td>
<td>1.9</td>
<td>2.4 ± 1.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.8 ± 1.1</td>
<td>5.5</td>
<td>6.2 ± 1.5</td>
<td>6</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.3 ± 0.5</td>
<td>1.2</td>
<td>1.4 ± 0.4</td>
<td>1.3</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.5 ± 1.0</td>
<td>3.5</td>
<td>3.7 ± 1.3</td>
<td>3.7</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.8 ± 1.2</td>
<td>5.5</td>
<td>5.9 ± 1.5</td>
<td>5.3</td>
</tr>
</tbody>
</table>

More than 64% of the subjects in the study have deviations from the normal values, according to the studied anthropometric indicators and indices (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sex</th>
<th>Men</th>
<th>Women</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Me</td>
<td>Mean ± SD</td>
<td>Me</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.3 ± 10.4</td>
<td>56</td>
<td>54.3 ± 10.6</td>
<td>56.5</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>107.3 ± 7</td>
<td>107.5</td>
<td>108.4 ± 12.6</td>
<td>105</td>
</tr>
<tr>
<td>Waist/Hip Ratio</td>
<td>0.99 ± 0.06</td>
<td>0.98</td>
<td>0.87 ± 0.08</td>
<td>0.88</td>
</tr>
<tr>
<td>Waist/Height Ratio</td>
<td>0.6 ± 0.04</td>
<td>0.61</td>
<td>0.6 ± 0.15</td>
<td>0.59</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.9 ± 7.3</td>
<td>176</td>
<td>158.9 ± 17.5</td>
<td>162</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>92.4 ± 13.4</td>
<td>93.6</td>
<td>79.6 ± 20.9</td>
<td>75.4</td>
</tr>
<tr>
<td>BMI</td>
<td>30.2 ± 3.6</td>
<td>30.5</td>
<td>29.7 ± 6.3</td>
<td>28.8</td>
</tr>
<tr>
<td>Impedance</td>
<td>462.7 ± 71.2</td>
<td>456</td>
<td>505.8 ± 74.7</td>
<td>523</td>
</tr>
<tr>
<td>Body fat %</td>
<td>29.8 ± 5.3</td>
<td>29.9</td>
<td>39.7 ± 7.5</td>
<td>40.7</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>28 ± 7.9</td>
<td>27.8</td>
<td>31.6 ± 12.5</td>
<td>30.7</td>
</tr>
<tr>
<td>Fat Free mass (kg)</td>
<td>64.6 ± 7.9</td>
<td>64.9</td>
<td>46.5 ± 5.9</td>
<td>45.2</td>
</tr>
<tr>
<td>Total Body Water (kg)</td>
<td>47.3 ± 5.8</td>
<td>47.5</td>
<td>34 ± 4.3</td>
<td>33.1</td>
</tr>
</tbody>
</table>

Over 90% of men and 74% of women are overweight and obese. The fact, that ¼ of the women with MetS have a normal body weight, which is 3 times more than men, is alarming (Fig. 1).
Fig. 1. Distribution of the categories body weight by BMI, according to WHO criteria

Although body weight is not a diagnostic indicator of MetS, over ¾ of the women and over 80% of men have a high/very high percentage of body fat and are overweight and obese (Fig. 2).

Fig. 2. Distribution of subjects by the percentage of body fat

According to the waist circumference (WC) indicator, considered as a criterion for increased metabolic risk, no significant differences were found between both sexes (Fig. 3).

Fig. 3. Risk of metabolic complications according to the waist girth
In men, the WHR indicator is more reliable for assessing the metabolic risk in individuals with MetS.
In women with MetS, 63.6% are at increased risk for metabolic complications, assessed by the WHR, while in men 91.2% are at increased risk. Waist-to-height index in both sexes has the same metabolic risk assessment value as the waist girth.

Variations in body composition structure and anthropometric indicators in individuals with MetS allow a more precise metabolic risk assessment to be made. Indicators of visceral obesity in the study have a greater assessment value of MetS risk in the group of men compared to women – WC (p<0.02); waist-to-hip ratio (p < 0.003); visceral obesity index (p < 0.007). In addition, these indicators may predict the risk of insulin resistance to a greater extent than other anthropometric measures in men [2]. Higher variations in BMI, apart from being a predictor of cardiovascular risk, have prognostic value for mortality in various diseases [4]. The relative share of men with overweight and obesity is 91.2% vs. 74.2% for women. The alarming relative share of women with normal weight and MetS found could be related to behavioral risk factors (low physical activity, unbalanced diet, etc.) or sex hormones’ levels. Low physical activity in women with normal weight is associated with a 2.2-fold higher risk of developing insulin resistance [5].

**Conclusion**
The obtained results confirm the need for early prevention of overweight and obesity, as well as for a thorough study of the causes of the development of metabolic syndrome in women.

**References:**

**Conflict of interest:** None Disclosed

**Acknowledgements:** Research relating to this paper was funded by the research program of the Medical University – Pleven, Pleven, Bulgaria (Contract № 3/2016).

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