Publications of papers, presented at:

**Joint Forum:**
11th South-East European Conference of Chemotherapy, Infections and Cancer and 31st Annual Assembly of International Medical Association Bulgaria (IMAB), 28-31 October 2021, Plovdiv, Bulgaria

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**Editor-in-chief of the magazine “Journal of IMAB”:**  
Prof. Dr. Krassimir Metodiev  
Headquarters of International Medical Association Bulgaria (IMAB);  
55, M. Drinov str., 9002 Varna, Bulgaria,  
Tel.: +359/888 712 407  
E-mail: kr.metod@yahoo.com or kr.metod@gmail.com

**Deputy Editor-in-chief:**  
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Tel.: +359/888 628 424  
E-mail: kyuchger@abv.bg

**Journal of IMAB** - 2021; Supplement 31 IMAB & 11 SEEC; Section Medicine.  
ISSN: 1312-773X; DOI: https://dx.doi.org/10.5272/jimab.2021Supplement1
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Editorial Board,
Supplement J.IMAB
Varna, Bulgaria, 2021/2022
Section Medicine:

SARS-CoV-2 distribution in North-East Bulgaria

Tsvetelina Kostadinova 1,2, Zhivka Stoykova 1,3, Tatina Todorova 1,2, Denis Niyazi 3,4, Milena Bozhkova 3,4, Svetomira Bizheva 4, Temenuga Stoeva 3,4

1) Laboratory of Virology, University Hospital “St. Marina”, Varna, Bulgaria
2) Medical College, Medical University Varna, Varna, Bulgaria
3) Department of Microbiology and Virology, Medical University Varna, Varna, Bulgaria
4) Laboratory of Microbiology, University Hospital “St. Marina”, Varna, Bulgaria

Abstract

The purpose of the present work was to analyze the frequency of SARS-CoV-2 positive samples in North-East Bulgaria and the spatiotemporal distribution of COVID-19 during the first two years of the pandemic (2020-2021). We found that the distribution of SARS-CoV-2 was not related to seasons and there were significant regional differences in the timing and the strength of the waves’ peaks.

Keywords: SARS-CoV2, epidemiology, seasonality

Introduction

In December 2019, unknown viral pneumonia emerged in Wuhan, China. A month later, a novel coronavirus was isolated and subsequently named SARS-CoV-2. The first positive cases in Bulgaria were detected on 08.03.2020, and a week later, the Virology Laboratory of the University Hospital “St. Marina” in Varna reported the first positive case in North-East Bulgaria. The laboratory of the University Hospital “St. Marina” has been routinely testing samples from all regions of North-East Bulgaria since the very beginning of the COVID-19 pandemic. During March and April 2020, positive samples were sporadic, but since May 2020, the detection of SARS-CoV-2 has become regular. The laboratory has remained the principal COVID-19 diagnostic unit for the whole territory of North-East Bulgaria till the end of 2020 and has tested samples from both hospitalized and ambulatory individuals. Then, with the extensive increase in the amount of infected and contact people, the regions in the area had developed their diagnostic units. Thus, in 2021, most of the tested samples in the laboratory were only from Varna Region.

The purpose of the present work was to analyze the frequency of SARS-CoV-2 positive samples in North-East Bulgaria and the spatiotemporal distribution of COVID-19 during the first two years of the pandemic (2020-2021). We also tried to identify seasonal, age and gender trends in the epidemiology of the infection.

Materials and Methods

Between 01.05.2020 and 30.09.2021, we tested a total of 36781 naso-oropharyngeal swabs and bronchoalveolar lavages via RT-PCR analysis (28027 in 2020 and 8754 in 2021). RNA extraction was performed with SaMag Viral Nucleic Acid Extraction Kit using a SaMag-12 instrument (Sacace Biotechnologies, Italy) or Maccura Mag-Bind RNA Extraction Kit using an Allsheng’ Auto-Pure 32A System (Maccura Biotechnology, China). RNAs were amplified with a SARS-CoV-2 Real-TM kit (Sacace Biotechnologies, Italy). Both extraction and amplification were according to the standard manufacturer’s instructions.

Results and Discussion

As of 30th September 2021, the Bulgarian authorities reported a total of 500112 COVID-19 infected individuals in the country (37752 of which in Varna Region, 9440 – in Dobrich Region, 16801 – in Ruse Region, 7141 – in Silistra Region, 5835 – in Targoviste Region, and 11413 – in Shumen
From May 2020 to September 2021, the positive samples in the Virology Laboratory of the University Hospital “St. Marina” were 33.6% of all tested (31.0% for 2020 and 41.7% for 2021). Our data confirmed the presence of four waves in the northeast part of the country (Figure 1). The number of positive samples was significantly higher than the average for the country which could be explained by the prevalence of hospitalized patients, especially after December 2020.

Interestingly, in January and February, when usually in Bulgaria there is a seasonal peak in the incidence of influenza and other respiratory diseases, the new coronavirus was less dominant. The seasonal distribution of the known seasonal coronaviruses is well documented in 21 countries and showed a model similar to those of influenza virus and RSV distribution – in the North hemisphere, infections are predominantly from December to March (2). In addition, during the second year of the pandemic, in 2021, more cases in August and September were registered compared to the same months of 2020. Therefore, the appearance of new variants, the prolonged circulation of the virus in the population and the population’s behavior were more important for the epidemiological pattern of the infection than the seasonality.

It is of great interest to compare also the regional differences in the epidemiology of COVID-19. During the first pandemic year, we tested samples from six of the seven North-East regions from both hospitalized and ambulatory patients (including those with epidemiological indications but excluding individuals without clinical and epidemiological indications). Regions Shumen and Ruse were the first to enter the first wave of the pandemic (Figure 2) in June 2020, while in the other regions the first wave started in July 2020. In Targoviste Region, the first cases appeared relatively late and the peak of the first wave was registered in September 2020. In Shumen Region, the peak was in August. This discordance in the timing of the waves’ peaks could be explained with disproportional sampling – in Varna and Ruse Regions we tested mainly hospital’s samples, while in Shumen, Targoviste and Dobrich Regions we tested samples from both hospitalized patients and contact persons. The samples from...
Silistra Region were also from individuals with epidemiological indications mainly from nursing homes and after local outbreaks. An alternative explanation could be found in the demographic particularities, migration and life dynamic in these regions.

Figure 2. Regional distribution by months of the positive SARS-CoV-2 samples (outpatients and hospitalized patients) from May to December 2020.

All age groups showed an increased proportion of positive results during 2021 compared to 2020. Especially, the active population aged 20-69 years was infected to a greater extent during the second year of the pandemic. Only individuals > 70 years were more affected in 2020. Differences between the years also existed in the gender distribution of the positive samples. During 2020, males were significantly more often diagnosed as positive for SARS-CoV-2 (36.1% of all tested males were positive versus 27.8% of females). The trend was completely different in 2021 – 42% of the tested females were positive versus 41.3% of males. COVID-19 cases among children and young people were least frequent among all age groups. Possible reasons for the milder infection in children are the different reactions of the immune system, the lack of chronic diseases and comorbidities and the decreased expression of ACE2 receptor in the nasal epithelium (3).

Conclusions
- There is a different seasonal and regional distribution of COVID-19 during the two years of the pandemic (2020-2021).
- Younger age groups are less affected by COVID-19 and less hospitalized (or at least they are less registered because of asymptomatic and mild infections).
- Positive cases are more frequent during the second year of the pandemic (2021) for both sexes and most of the age groups except people aged >70 years. This difference could be due to the more advanced immunization and/or social distance.

References:
Prevalence of COVID-19 and vaccination status among patients with pemphigus: A retrospective study

Tsvetana Abadjieva ¹², Zhaneta Zheliazkova ¹²

¹) Department of Dermatology and Venereology, Medical Faculty, Medical University of Plovdiv
²) Clinic of Dermatology and Venereology, St. George’s University Hospital of Plovdiv

Summary
Patients with pemphigus are managed with immunosuppressants and are at higher risk of infections.
Purpose: To assess the prevalence of COVID-19 and vaccination status among patients with pemphigus.
Material and Methods: Forty patients with pemphigus were followed. They were contacted by telephone or in-person.
Results: Ten (25%) patients reported to have been diagnosed with COVID-19 infection. Two (5%) patients were vaccinated for COVID-19, ten (25%) patients refused vaccination, and 28 (70%) patients were undecided about vaccination.
Conclusions: There is no evidence that patients with pemphigus are more likely to contract COVID-19 than healthy individuals. Communication with pemphigus patients and information on the risk-benefit ratio of vaccination is recommended.

Key words: pemphigus, COVID-19, SARS-CoV-2, coronavirus, vaccination, complications

Introduction
Pemphigus consists of a group of severe, life-threatening bullous diseases mediated by autoantibodies. ¹¹ Patients with pemphigus are managed with immunosuppressants and are at higher risk of infections.

Material and Methods
Forty patients with pemphigus were contacted by telephone or in-person. The following information was collected: whether they had had COVID-19; the severity of the course of coronavirus disease; complications of COVID-19; influence of COVID-19 on the course of pemphigus; vaccination for COVID-19; adherence to measures against COVID-19.
The variables were described by means and standard deviations (±SD), and percentages. Statistical analyses were performed with data analysis software IBM – SPSS Statistics v.23.

Results
The mean age of the pemphigus patients was 57.50±14.097 (30-87) years. Female patients were 27 (67.5%), aged 30 to 87 years, male patients were 13 (32.5%), aged 40 to 76 years. Ten (25%) patients reported to have been diagnosed with COVID-19 infection confirmed by PCR or antigen test for SARS-CoV-2. The mean age of the patients who had had COVID-19 was 57.90 ±15.242 (40-87) years. The course of coronavirus infection was mild to moderate. The patients recovered without complications and without exacerbation of pemphigus. Two (5%) patients were vaccinated for COVID-19, ten (25%) patients refused vaccination, and 28 (70%) patients were undecided about vaccination (Table 1).
Vaccinated patients were men aged 52 and 64 years. They had no exacerbation of pemphigus. All patients reported strict adherence to measures against COVID-19.
Table 1. COVID-19 and Vaccination Status among Patients with Pemphigus

<table>
<thead>
<tr>
<th></th>
<th>Patients (N=40)</th>
<th>Females (n=27)</th>
<th>Males (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COVID-19</td>
<td>10 (25%)</td>
<td>5 (18.5%)</td>
<td>5 (38.5%)</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>2 (5%)</td>
<td>0</td>
<td>2 (15.4%)</td>
</tr>
<tr>
<td>Refused vaccination</td>
<td>10 (25%)</td>
<td>8 (29.6%)</td>
<td>2 (15.4%)</td>
</tr>
<tr>
<td>Undecided</td>
<td>28 (70%)</td>
<td>19 (70.4%)</td>
<td>9 (69.2%)</td>
</tr>
</tbody>
</table>

Discussion

One pemphigus patient at the Dermatologic Clinic at Alexandrovska Hospital, Sofia, Bulgaria, was suspected for COVID-19 [2]. In patients with pemphigus in Italy, some authors have reported no incidence or isolated cases of COVID-19. [3, 4, 5, 6] Similar data were published by other authors from Iran and Turkey. [7, 8, 9]

A large population-based cohort study in Israel established that the mortality from COVID-19 among pemphigus patients was similar to the control group. [10] There are no published data that patients with pemphigus are more susceptible to COVID-19 than healthy individuals.

A cross-sectional study on the COVID-19 vaccine acceptance and hesitancy in patients with immunobullous diseases established that 90.5% of 707 patients received vaccination against COVID-19, and 12.3% (79) of the patients experienced a flare of their bullous disease. [11] Two patients with pemphigus vulgaris in remission experienced a flare of the disease after administration of the first dose of Pfizer and Moderna vaccines. The pemphigus patients received the second vaccine dose without further exacerbation of the disease. The patients developed IgG antibodies against the SARS-CoV-2 one month after the second dose. [12] Isolated cases of development of pemphigus following SARS-CoV-2 vaccination have been reported. [13, 14] It is recommended that every patient with AIBD be vaccinated with one of the approved vaccines against COVID-19. [15]

Conclusions

There is no evidence that patients with pemphigus are more likely to contract COVID-19 than healthy individuals. Communication with pemphigus patients and information on the risk-benefit ratio of vaccination is recommended.

References:


Challenges in a private hospital management during the Covid-19 pandemic

Krassimira Chachova-Rancheva¹, Nevena Tsacheva¹, Lidia Hristova²

¹Uni Hospital OOD General Medical Facility, ²Medical University - Sofia, Faculty of Public Health

Abstract
The COVID 19 pandemic has been wide spreading with a strong mutability of its causative agent, SARS CoV – 2, for the recent two years. This epidemic situation puts to trial our healthcare system, including the management of a highly specialized hospital. An exceptionally elevated morbidity with temporary loss of work capacity of totally 141 employees diagnosed with U 07.1, U 07.2 was identified in the general hospital where totally 670 were employed in 2020. Forty-eight people out of them were frequent and long-term sick employees. They form a frequency of 178.0 cases per 100 employees and the labor loss frequency is 3,013.4 per 100 employees. The above indices represent 21% of the staff, 39% of all patients and 33.8% of the frequent and long-term sick employees. Considering these very high indicators of morbidity with temporary loss of work capacity, the preventive focus expressed in health care and secondary prophylactics for all patients, sustainable work of the Occupational Health and Safety Committee with targeted analysis of high-risk employees in a changing work environment and labor process in a complicated COVID situation poses a challenge to the hospital management.

Introduction
The pandemic caused by SARS CoV - 2 as an infectious disease is spreading rapidly with strong mutability of the causative agent. The pandemic affects an increasing number of people in a number of countries and on different continents provoking a cumbersome public health situation and a genuine challenge to healthcare facilities and their management. The situation around the disease is changing with rapid trends. Several "waves" of high morbidity and constantly increasing mortality rates have been reported according to the official statistic only until March 2021. This data is applicable to medical and other specialists and the proven cases by this date were 13,180 as 3,180 out of them were doctors, 4,462 nurses, 2,182 house keepers, 282 paramedics and 2, 434 other staff. Governments and governmental authorities are forced to proceed in a prompt manner in order to restrict the infection spread and hospital management faces issues to ensure the safety of both its staff and patients. Numerous measures which ensure the sustainable operation of the hospitals were required to be taken despite the situation.

Material and methods
The objective study of the incidence of morbidity with temporary loss of work capacity emphasizing on absences from work caused by this infectious agent, including the levels of frequent and long-term sick employees in order to ensure reliable occupational health and safety conditions is among the top challenges of the private hospital management in the COVID pandemic situation. We applied an individual approach of studying the morbidity with temporary loss of work capacity based on a methodology which was developed by us and circulated to all occupational medicine offices at a national level by the General Labor Inspectorate at the Ministry of Labor and Social Policy. The applied data collecting methods from the original sick notes, data processing and sorting according to indices which are important both for the employee and the employer and analysis of the indicators obtained through specifically developed software provide both individual and summarized characteristics of absences from work split by diseases and diagnoses and the damage caused to the workforce what is crucial for the hospital management and the associated labor losses.
Results and discussion

An exceptionally elevated morbidity with temporary loss of work capacity of totally 141 employees diagnosed with U 07.1, U 07.2 was identified in the general hospital. Forty-eight people out of them were frequent and long-term sick employees. They result into issuing 251 primary sick notes and 4,249 labor losses. Respectively, the frequency of their cases 178.0 per 100 employees and the labor loss frequency is 3,013.4 per 100 employees. The above indices represent 21% of the staff, 39% of all patients and 33.8% of the frequent and long-term sick employees which presents a top priority challenge to the hospital management. The latter is attributed to the fact that the share of primary sick notes was 69.3% of all registered cases in 2020. The work losses caused by SARS Cov - 2 are 35.1% of all registered work losses. These features of morbidity with temporary loss of work capacity outline COVID 19 as the leading determining factor for the employees’ morbidity and at the same time it determines other indicators, including permanent disability. The latter shows an increased level with 15% in 2021 which ensues from other comorbidities of medical professionals or complications of this infectious agent affecting many other systems, virtually the whole body with moderate to severe clinical forms of the disease course [1, 2, 3].

If we refer to the description of the clinical picture made by other authors, many patients experience a loss of smell (anosmia) and taste (dysgeusia), arthralgias and myalgias. Common symptoms are fever (58.66%), cough (54.52%), dyspnea (30.82%), general malaise (29.75%) and fatigue (28.16%). In addition, if we consider other also common symptoms such as neurological (20.82%), dermatological (20.45%), anorexia (20.26%), myalgia (16.9%), sore throat (14.41%), runny nose (14.29%), headache (12.17%), chest pain (11.49%) and diarrhea (9.59%), the number of difficulties and issues to be solved by the management get multiplied. [4, 5, 6]. All this occurs on the background of an age structure of the working healthcare specialists tending towards to the senior age groups.

Conclusions

The healthcare professionals who take care for COVID-19 patients in hospital facilities should be registered and monitored in accordance with national occupational risk procedures and occupational health principles. Based on the identified high risk of infection associated with the health care provision and considering the recommendations made by other institutions, the following specific recommendations are suggested: Managing the hospital facility with the objective to overcome these morbidity rates is a challenge with a pronounced preventive focus on the health care and follow-up prevention for all sick employees, sustainable work of the Occupational Health and Safety Committee when outlining relevant measures to define the occupational health and safety policy at the hospital and the data base for maintaining the personal health and professional characteristics and all topical events in the period 2021-2022, including regular examinations, tests and targeted analyses of people working in a highly hazardous and variable environment and work process under a complex COVID situation.

The European Center for Disease Prevention and Control attaches importance to the following other also common symptoms such as neurological (20.82%), dermatological (20.45%), anorexia (20.26%), myalgia (16.9%), sore throat (14.41%), runny nose (14.29%), headache (12.17%), chest pain (11.49%) and diarrhea (9.59%), the number of difficulties and issues to be solved by the management get multiplied. [4, 5, 6]. All this occurs on the background of an age structure of the working healthcare specialists tending towards to the senior age groups.

Conclusions

The healthcare professionals who take care for COVID-19 patients in hospital facilities should be registered and monitored in accordance with national occupational risk procedures and occupational health principles. Based on the identified high risk of infection associated with the health care provision and considering the recommendations made by other institutions, the following specific recommendations are suggested: Managing the hospital facility with the objective to overcome these morbidity rates is a challenge with a pronounced preventive focus on the health care and follow-up prevention for all sick employees, sustainable work of the Occupational Health and Safety Committee when outlining relevant measures to define the occupational health and safety policy at the hospital and the data base for maintaining the personal health and professional characteristics and all topical events in the period 2021-2022, including regular examinations, tests and targeted analyses of people working in a highly hazardous and variable environment and work process under a complex COVID situation.

The European Center for Disease Prevention and Control attaches importance to the following characteristics for the risk assessment for the population, including the working healthcare specialists:

- The risk from a COVID-19 infection associated severe disease for people from the European Union/European Economic Area and the United Kingdom is currently considered moderate for the general population and high for the elderly people and people suffering from chronic diseases.

In addition, the risk from a minor illness and the subsequent impact on social and work-related activities is considered high.

- The risk from intercommunity COVID-19 transmission in the European Union/European Economic Area and the United Kingdom is currently considered very high.
- The risk from widespread COVID-19 transmission from a national community in in the European Union/European Economic Area and the United Kingdom in the next weeks is high.
- The risk associated with the COVID-19 transmission to healthcare and social care institutions with large vulnerable populations is considered high.
• The risk for people from the European Union/European Economic Area who travel or reside in geographical areas with a presumed distribution in the community is very high. Considering the above, the risk from becoming infected with COVID-19 for the hospital staff and the challenge to the management of coping with its spreading is identified as very high and requiring immediate action to be taken.

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Healthcare-associated infections in the shadow of COVID – 19

Tsonko Paunov¹, Deyana Ilieva¹, Mariya Marinova², Kalina Stoyanova¹, Miglena Kolarova¹, Eliyana Ivanova¹

¹) Medical University – Varna, Bulgaria; 2) UMHAT "St. Marina"-Varna, Bulgaria

Abstract
The purpose of the study is to present data on the impact of the COVID-19 pandemic on Healthcare-associated infections (HAIs) at University Hospital "St. Marina"-Varna, Bulgaria.

Material and methods: The study covers the first three-quarters of 2019, 2020, and 2021. The data was collected from the Hospital's epidemiological surveillance system.

Results and discussion: The distribution of HAIs showed a decrease in the registered cases in 2020 compared to 2019 and almost doubled in 2021 (0.60%). The leading pathogen is A. baumannii. E.coli and C.albicans increased their impact in 2021. Ventilator-associated events and surgical-site infections predominate in the HAIs-profile.

Conclusion: The COVID-19 pandemic affected both HAIs-prevalence and pathogen profiles.

Introduction
Healthcare systems were not globally prepared for the coronavirus disease 2019 (COVID-19) pandemic. Hospitals came under pressure, with a limited number of health professionals, constrained resources, and inefficient methods for infection control. In new and stressful work conditions, increased use of antibiotics, insufficient personal protective equipment (PPE) and disinfectants, the risk of HAIs expanded because all hospital efforts were redirected and focused on reducing SARS-CoV-2 transmission [1,2,3].

The purpose of the study is to determine the impact of the COVID-19 pandemic on the incidence and structure of HAIs at the biggest Hospital in Northeastern Bulgaria - University Multiprofile Hospital for Active Treatment (UMHAT) "St. Marina"-Varna.

Material and methods
The retrospective descriptive study covers the first three-quarters of 2020 and 2021 compared with the data from 2019, a period without COVID-19. The data was collected from the Hospital's official information system, including - monthly epidemiological surveillance reports to the Regional Health Inspectorate (RHI) and microbiological laboratory data. The etiological profile, clinical structure, morbidity, and distribution of HAIs in the different hospital wards were analyzed. Particular attention was adverted to indicative infections - central line-associated bloodstream infections (CLABSIs), catheter-associated urinary tract infections (CAUTIs), ventilator-related infections (VAEs), surgical site infections (SSIs). The profile of the specific for the hospital potential multidrug-resistant organisms (MDROs) was also analyzed.

Results
The analysis of the overall number of HAIs for each year's three-quarterly interval showed an unusual decrease in the number of registered infections during the first year - the cases declined from 306 in 2019 to 259 in 2020. On the opposite, a significant increase (n=439) was observed in 2021. The relative ratio of HAIs (based on the number of all admitted patients for the same period) is 0.60% for 2019 and 0.57% for 2020. Likewise, an observed increase of 0.97% was established in 2021. The trend was significantly expressed in intensive care units and corresponded to the more severe prognoses of the patients there.

The structure of HAIs in different hospital wards of UMHAT "St.Marina", Varna is presented in Figure1. The largest number of HAIs for each of the three periods was registered in the Clinic of Anesthesiology and Intensive Care (CAIC). Similar to the overall data in CAIC, the registered HAIs
decreased for 2020, compared to 2019. In the first three-quarters of 2021, HAIs significantly increased, with a resultant prevalence of 29.61%. In the first quarter of 2021, a new specialized intensive care unit only for COVID-19 patients (COVID-19 ICU) was opened, and approximately 1/3 of the HAIs (n=135) for that year were registered there. In 2021 a spike of HAIs was observed also in the Second surgery clinic, where an influx of COVID-19 patients was admitted from all over Eastern Bulgaria. The staff had to be trained for the new conditions, especially those that had never been engaged previously in intensive care units. The Infection Control Team (ICT) worked around the clock at an overwhelming pace in multilayered levels of their official duties: provided, distributed, and trained staff to wear PPE; managed the reserve and supply of disinfectants; distributed donations from various organizations; supervised patients and visitors to wear masks properly; participated in laboratory testing of patients and hospital staff; planned the vaccination schedule, communicated with the Ministry of Health, RHI and other organizations and gave recommendations for proper implementation of constantly developing pandemic measures and protocols. The instructions varied and changed overnight, often adapting to deficiencies of a different nature or responding to healthcare personnel’s fears. In several periods of extreme shortage, it was necessary to reuse PPE, and in others, 2-3 layers of personal costumes or masks were used. This did not improve infection control and probably facilitated the transmission of nosocomial strains to some extent. In this situation, ICT and the whole hospital staff were unsuccessful in adhering to established infection control practices, resulting in underreporting HAIs for 2020. Similar shortcomings were observed in other studies in different countries [2,3,5].

Analyzing these weaknesses at the end of 2020, the Hospital's management, the HAI Control Committee, and ICT have redoubled the efforts to improve surveillance and control of HAIs. The results in 2021 are visible and correspond with the actual and expected increase in the registered cases, which lead to the continuous and adequate improvement of the measures to reduce HAIs in the UMHAT "St. Marina", Varna.

Figure 2. demonstrates the etiological agents diagnosed in the Microbiology Laboratory of UMHAT "St. Marina".

The leading pathogen causing HAIs since 2012 is A. baumannii [4] and remained with the highest prevalence for the investigated period - 18.63% (2019); 21.23% (2020); 23.23% (2021). K. pneumoniae and P. aeruginosa, typical of previous years [4], shift ranking to E. coli in 2020 and C. albicans in 2021.
E. coli prevalence rose from 10.42% (2020) to 12.30% (2021) and was even more noticeable for C. albicans - 5.41% (2020) and 11.39% (2021).

Antibiotic pressure led to the increased detection of fungi and highly resistant bacteria in HAIs clinical samples, as noted by other authors [2, 3].

Regarding the clinical characteristics, VAEs and SSIs predominate in the HAIs-profile. VAEs recorded a twofold rise from 23.94% (2020) to 42.82% (2021), or an overall increase of 55.91%. Other researchers noted a similar trend at the beginning of the pandemic, associated with a larger number of patients in need of a respirator and various other factors that can influence the growth of VAEs [5, 6]. Notable also is the constant increase of the SSIs - 13.39% (2019); 20.84% (2020); 26.42% (2021). Other authors note a decline probably due to the limited planned operations [5]. Analyzing the clinical structure in 2021, compared to 2020, we find a slight increase of 1.23% in Bloodstream Infections (BSIs). There is a decrease in the relative share of lower respiratory tract infections (LRTI) by 1.09% and CAUTIs by 6.38%. Following the global trends, CLABSIs rise by 3.54% in 2020, reaching 12.36% of all HAIs. A similar increase (up to 47%) is observed in different geographical areas during the same period [5, 7]. In 2021, the relative share of CLABSIs in UMHAT "St.Marina" decreased by 7.6%.

**Conclusion**

Our analysis shows that despite the incomplete registration of HAIs at the beginning of the COVID-19 pandemic, in 2021, the expected number of HAIs exceeds the usual morbidity. HAIs are registered mainly in ICU. Due to the antibiotic pressure, C. albicans and highly resistant strains of A. baumannii, E. coli, dominated in the last period. VAEs increased by 55.91%. SSIs also have a constant trend of expansion. Joint efforts of the Hospital's management, ICT, HAI Control Committee, and all staff are needed to improve the surveillance and control of HAIs.

**References:**


The Covid-19 Pandemic - Challenges Facing Training at the Medical University - Plovdiv

Anna Mihaylova¹, Petya Kasnakova², Dobromira Shopova³, Yolena Gesheva⁴, Desislava Bakova⁵, Maria Semerdzhieva⁵

1) Specialty „Assistant Pharmacist”, Medical College, Medical University - Plovdiv, Bulgaria
2) Specialty „Rehabilitator”, Medical College, Medical University - Plovdiv, Bulgaria
3) Department of Prosthetic Dentistry, Faculty of Dental Medicine, Medical University - Plovdiv, Bulgaria
4) Faculty of Medicine, Student 5th year, Medical University – Plovdiv, Bulgaria
5) Department of Health Care Management, Faculty of Public Health, Medical University – Plovdiv, Bulgaria

Abstract
With the introduction of the state of emergency the training process passed into a remote electronic environment for training medical professionals.
The purpose of this article is to present some major challenges for training at the Medical University - Plovdiv in the context of the COVID-19 pandemic.
Material and Methods: An anonymous survey was conducted involving 340 students from the MU of Plovdiv.
Results: The respondents are convinced that professional skills are absolutely necessary for their formation as medical specialists. Students indicate their relationships with their teachers as “excellent” (26.47%). Participation in additional practical training in a real work environment will contribute to successful professional realization (69.41%)
Conclusion: Despite the difficult situation for students and teachers, it managed to maintain one of the main priorities of the Medical University - Plovdiv related to the continuous improvement of the quality of education in all forms, including in the electronic environment.

Keywords: COVID-19, challenges, e-learning

Introduction
With the introduction of the state of emergency to limit the spread of the coronavirus, physical distancing was required, in which the training process passed into a remote electronic environment for training medical professionals. All participants in the learning process were placed in a new situation [1, 2]. Urgent measures were taken to continue the learning process were aimed at developing and using human and material resources with the support and widespread dissemination of innovation and digitalization [3]. The introduced absent form of education at the Medical University - Plovdiv necessitated remote conducting of lectures, educational-practical classes and exams, causing a digital leap in medical education. The interactive educational environment replaces traditional learning, providing conditions for the realization of effective group dynamics between the subjects in the learning process, guided by the teacher, as a manager of the learning process. The transition to distance learning caused by COVID-19 was made possible by the dynamics of technological modernisation and the active transfer of scientific knowledge and achievements on a global scale [4, 5].
The purpose of this article is to present some major challenges for training at the Medical University - Plovdiv in the context of the COVID-19 pandemic.

Material and Methods
An anonymous survey was conducted involving 340 students from the Medical University of Plovdiv, majoring in Medicine, Dentistry and Pharmacy and Health Care. The questions in the survey addressed
different aspects of learning process during COVID-19 pandemic. The questionnaire was created in Google platform and was distributed only online.

**Results and Discussion**

During the distance learning students most often used information tools through synchronous communication applications - Microsoft Teams, edo.mup, Moodle, Zoom, Google meet, sites with scientific information and video sharing sites. Most often, students communicate with teachers through the Microsoft Teams platform (89.2%) or by email (25.9%). The respondents are convinced that professional communication skills with your teachers, with the administration staff, with IT-help are absolutely necessary for their formation as medical specialists. Students indicate their relationships with their teachers as “excellent” in (26.47%), "very good" in (35.88%), and with the administrative staff as “good” in (30.58%). Students self-assess their preparation as insufficient in terms of communication skills with patients and their relatives in (26.47%) to carry out their professional activities. According to the results of the study, the application of distance learning complicates practical knowledge for the future professional activity of the medical specialists.

In the higher medical school there are various organizational forms of education: lectures, practical classes, seminars, individual work, clinical practice, summer internships, undergraduate internships, etc. Theoretical training is related to the lecture, as the main classical form of training. The modern requirements to the classical forms of education are related to the change of the content, the methods and the means for conducting in accordance with the new goals. According to the students, the thematic program of the lectures is well structured (76.47%). The way of teaching encourages the search for up-to-date scientific literature (69.99%). The lecturer discusses various theories and methods aimed at practical problems related to the future profession. In this way, the student during the lecture is placed closer to the practice, to the patient, to the real conditions in the future.

The aim of theoretical training is to create professional competence. The survey confirms the high satisfaction of students in the lecture course (76.46%).

![Fig.1. Assessment of students for theoretical training](image)

The training of students is aimed at acquiring basic professional knowledge and skills. In the process of training they acquire theoretical and practical knowledge in special disciplines, which subsequently form the future specialists.
The respondents indicate that they prefer to acquire knowledge, skills and abilities by exercising in a real environment in the presence of a patient/client and like to learn the material by conducting training sessions. According to the students, theoretical training is sufficient for successful practice/internship (52.35%). The practice/internship is extremely useful in a real environment (71.76%) and independent work. The independent work of students in the educational process is a prerequisite for increasing the professional practical training, for successful entry and exercise of the profession. Participation in additional practical training in a real work environment will contribute to successful professional realization (69.41%). The problem of whether distance learning by medical professionals is more effective than conducting it in a real environment remains debatable, especially for practical training.

Conclusions
The situation with the COVID-19 pandemic has necessitated a rethinking of the way of education and training are provided in all medical specialties in order to meet the demands of a rapidly changing world. It could be seen as a unique opportunity to radically solve a number of problems of medical education, as it gives an indisputable chance, based on the synthesized experience of goal setting and pandemic management, to transform the educational profile in our country. Despite the difficult situation for students and teachers, it managed to maintain one of the main priorities of the Medical University - Plovdiv related to the continuous improvement of the quality of education in all forms, including in the electronic environment.

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Address for correspondence: Anna Mihaylova, PhD
15A Vasil Aprilov Blv. Plovdiv, Bulgaria
e-mail: annamihaylova@abv.bg
The impact of Covid-19 pandemic on the structure of of the temporary disability of health workers in a state hospital

Lidiya Hristova¹, Rouja Nikolova¹, Galya Stankova¹, Alisa Rumenovery², Nevena Tzacheva³

¹) Department of Occupational Medicine, Faculty of Public Health, Medical University - Sofia, 2) Faculty of Medicine, Sofia University 3) Occupational Medicine Service, Unihsipital Hospital

Abstract
In response to the COVID-19 pandemic, working medical and non-medical care professionals are at extremely high risk, leading the way in the fight against SARS-CoV-2. The statistics in the Republic of Bulgaria as of 21.11.2021 show over 17053 cases of medical specialists infected with COVID-19, of which 4577 doctors and 5663 nurses. The pandemic has imposed increased demands on health professionals related to timely information about health aspects, risks and possible situations in the workplace that may lead to exposure. To maintain the safety of these high-risk workplaces, it is necessary to ensure a clear dialogue between health professionals, sharing the latest information from clinical protocols, guidelines, measures and solutions to ensure effective implementation. The data unequivocally show that by providing adequate measures, instructions and training in health and safety, the exposure and risks of medical professionals are limited and reduced.

Introduction
Worldwide, the COVID-19 pandemic has claimed the lives of thousands of doctors and nurses. A report by the human rights organization Amnesty International from the end of 2020 mentions at least 7,000 medics who fell victim to COVID-19. Among the most endangered are workers who sanitize hospitals. According to the WHO, by June 2021 Between 80,000 and 180,000 health workers have died from COVID-19, and in response to the COVID-19 pandemic, working medical and non-medical care professionals are at extremely high risk, leading the way in the fight against SARS-CoV. 2. According to the Bulgarian Medical Union, more than 2,200 medics from COVID-19 were infected and more than 50 doctors died in 2020. The data show that most of them worked in hospitals, the rest of them were part of According to the statistics in the Republic of Bulgaria as of November 21, 2021, the number of cases of medical specialists infected with COVID-19 is 17053, of which 3026 are nurses and 350 are paramedics. For the same period 15.4% of infected doctors are registered out of the total number of registered doctors in Bulgaria and 20% are infected by the total number of health care specialists (nurses, orderlies, paramedics). Those working in the health sector are among the most vulnerable and affected occupational groups in the COVID-19 pandemic. Therefore, their protection must be a priority for any national policy on health and safety at work.

Materials and methods
In 2020, we studied the health status of 1,495 employees of a multidisciplinary hospital for active treatment, who served more than 23,000 patients in 2020 alone. The incidence of temporary disability is the broadest and most objective basis for assessing negative health. The study of the health status of the working medical and non-medical specialists in a multidisciplinary hospital for active treatment shows the state and trends in the health of the medical workers under specific working conditions. In our study, the age group of working medical professionals over 45 years is 61% of all workers, followed by the age groups from 26 to 35 years and from 36 to 45 years, the specific working conditions and the organization of the work process.

Result and discussion
Interpreting the results obtained for the absolute number of issued and posted sick leaves (with relatively small variation in the number of employees) by nosological units and classes, according to the
International Classification of Diseases ICD-10, we can focus on the search for causal relationship between the impact of specific risk factors and the negative changes in the health condition of the employees in the studied medical institution [1]. We identified and assessed the hazards to which the employees of the medical institution were exposed, which included not only the exposure to biological agents (SARS-CoV-2), but also unregulated working hours, night shifts and prolonged use of personal protective equipment [2, 3, 4, 5, 6, 7, 8]. We also witnessed stigma, discrimination, burnout, psychological violence and harassment [9, 10, 11].

The processing of 699 primary sick leaves with 14,007 job losses issued in 2020 shows the following: in terms of number of cases and days, "infectious diseases" are leading in the structure of morbidity with temporary incapacity for work. With the diagnosis of COVID-19 and viral infection, 229 cases or 33% of the total number of cases and 2609 or 19% of the total loss of days were registered unspecified. The average duration in days is 13.30 days, above the national average. For the studied period there are no data on the lethal outcome of sick health professionals.

Table 1. Distribution by indicators of morbidity with temporary incapacity for work for the class of diseases “Some infectious diseases according to ICD-10 for 2020.

<table>
<thead>
<tr>
<th>Prof. group of diseases on ICD 10</th>
<th>Frequency / Case</th>
<th>Frequency factor</th>
<th>Frequency / severity</th>
<th>Weight factor</th>
<th>Average duration in days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Some infectious and parasitic diseases</td>
<td>237</td>
<td>17,36</td>
<td>3151</td>
<td>230,76</td>
<td>13,30</td>
</tr>
<tr>
<td>Covid - 19, virus identified</td>
<td>161</td>
<td>11,79</td>
<td>2609</td>
<td>191,07</td>
<td>16,2</td>
</tr>
<tr>
<td>Covid - 19, unidentified virus</td>
<td>14</td>
<td>1,03</td>
<td>141</td>
<td>10,33</td>
<td>10,1</td>
</tr>
<tr>
<td>Viral infection, unspecified</td>
<td>50</td>
<td>3,66</td>
<td>292</td>
<td>21,38</td>
<td>5,8</td>
</tr>
<tr>
<td>Coronavirus unspecified infection, unspecified</td>
<td>4</td>
<td>0,29</td>
<td>63</td>
<td>4,61</td>
<td>15,8</td>
</tr>
<tr>
<td>Other specified viral diseases</td>
<td>2</td>
<td>0,15</td>
<td>10</td>
<td>0,73</td>
<td>5</td>
</tr>
<tr>
<td>Enterovirus unspecified infection, unspecified</td>
<td>3</td>
<td>0,22</td>
<td>16</td>
<td>1,17</td>
<td>5,3</td>
</tr>
</tbody>
</table>

Conclusion
The high morbidity and mortality among health professionals requires the development and implementation of programs for health and safety at work, which should include: regulations of the health institution on health and safety at work, contract with occupational health service, responsible person and committee on health and safety. safe working conditions. Employer's commitment is training and instruction on health and safety of health workers, risk assessment and communication and prevention of occupational risks. Mandatory stages in the program should be related to early detection, diagnosis, treatment, care for occupational diseases and accidents, reporting, investigation and reporting of incidents, information system and monitoring. In the multidisciplinary hospital for active treatment the established procedures for safety and health at work are observed. The employer has provided the
medics with protective equipment and provided training for their proper use. Workers self-monitor for signs of disease and isolate themselves and, if necessary, get vaccinated. The data clearly show that when safety measures are at the right level, there are fewer victims.

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Correspondence to: Assoc. Prof. Lidiya Hristova, MD, PhD,
Department of Occupational Medicine, Faculty of Public Health, Medical University- Sofia; 8, Byalo more Str., 4th floor, 48, Sofia, Bulgaria; e-mail: lidiahristoff@gmail.com
Role of 18F-FDG PET/CT for unsuspected recurrence of head and neck carcinoma in patient restaged for the colorectal carcinoma

Tsvetelina Yordanova¹,², Borislav Chaushev²

¹)Department of Imaging Diagnostics, Interventional Radiology and Radiotherapy, Faculty of Medicine, Medical University Prof. dr. P Stoyanov- Varna, Bulgaria
²)Department of nuclear medicine, UMHAT „St. Marina“ Varna, Bulgaria

Abstract
We detected early recurrence of head and neck cancer (HNC) with 18F-FDG positron emission tomography/computed tomography (PET/CT) in a patient with known primary tumor in abdomen. Diagnosis of a HNC is usually achieved by a combination of patient history, physical examination and either nasopharyngoscopy and/or laryngoscopy with directed biopsy. 18F-FDG PET/CT permits the evaluation of both metabolic and anatomic characteristics of disease, which has proven to be a major advance for evaluation of recurrence. We report a patient with primary abdominal tumor. 67-years-old male with colorectal carcinoma. He was referred to our hospital for 18F-FDG PET/CT examination with biochemical suspicion of progression. The 18F-FDG PET/CT exam found only one hypermetabolic zone above the clavicle, on the left side of the larynx. We studied the medical records and found out that the patient was operated for laryngeal carcinoma. The patient did not report any symptoms. 18F-FDG PET/CT correct identified unsuspected local recurrence of the HNC. Our clinical case shows that 18F-FDG PET/CT can be used for detection of unsuspected recurrence of HNC in patient referred for restaging another primary carcinoma (colorectal cancer) and helped timely treatment of newly diagnosed recurrence of laryngeal cancer.

Key words: early recurrence, Head and neck cancer, 18F-FDG PET/CT, unsuspected laryngeal recurrence, follow-up, asymptomatic patient.

Introduction
Head and neck cancers are heterogeneous diseases and represent 6-10% of all malignant neoplasms. Malignant head and neck cancers include those of the mouth and oral cavity, nasal cavity and sinuses, nasopharynx, oropharynx, salivary glands, hypopharynx and larynx. Diagnosis of a HNC is usually achieved by a combination of patient history, physical examination and either nasopharyngoscopy and/or laryngoscopy with directed biopsy. The anatomic changes resulting from surgery or radiation significantly limit the use of CT and MRT for the detection of the recurrence, whereas FDG-PET/CT maintains 83-100% sensitivity, 78-98% specificity and 81-98% accuracy for the detection of recurrent disease [1]. PET/CT permits the evaluation of both metabolic and anatomic characteristics of disease, which has proven to be a major advance for evaluation of recurrence. The detection of early recurrence of head and neck cancer (HNC) is challenge.

Case description
We report a patient with primary abdominal tumor. Sixty-seven years old male patient with colorectal carcinoma, T2N0M0G2. He was referred to our hospital for 18F-FDG PET/CT examination in April 2017, with biochemical suspension of progression. He had increased level of tumor marker: Ca 19,9- 74. The ultrasound of abdomen and pelvis did not show any abnormality. He was treated with hemicolecotomy one year ago. The whole body 18F-FDG PET/CT exam found only one hypermetabolic zone above the clavicle, on the left side of the larynx (figure №1 and №2).
**Fig №1.** Patient restaged for the colorectal carcinoma. The maximum intensity projection (MIP) 18F-FDG PET scan show pathological uptake on the left side in larynx, SUVmax28. The histology result diagnosed a recurrence of laryngeal carcinoma.

The uptake in head and neck area had pathological SUVmax-28. The patient underwent consult with ear-nose-throat specialist and the endoscopy did not show macroscopic tumor, but the biopsy and histology result diagnosed a recurrence of laryngeal carcinoma. We studied the medical records and found out that the patient was operated for laryngeal carcinoma T2N0M0G1 nine years ago (frontolateral resection of larynx). The patient did not report any symptoms.

**Fig №2.** Patient restaged for the colorectal carcinoma. The fused 18F-FDG PET/CT show pathological uptake in larynx. The histology result diagnosed a recurrence of laryngeal carcinoma.

**Discussion**
A relevant example is the case of colorectal cancer, in which early detection of local recurrence or liver metastases, at a stage when they are still resectable, can provide up to 40% long-term survival with treatment [2]. 18F-FDG PET/CT surveillance detected recurrences more sensitively than computed tomography (CT) and magnetic resonance imaging or chest CT [3]. The current treatment of choice for squamous cell head and neck cancers involves surgery or radiotherapy for early stage cancer and a multimodal approach including surgery, chemotherapy and radiotherapy for advance staged cancer. 18F-FDG PET/CT has been recognized as a valuable imaging tool for the detection of *tumor recurrence and second* primary cancer both during posttreatment surveillance and at initial staging [4].
[5]. However, routine posttreatment imaging of asymptomatic patient is not recommended in current National Comprehensive Cancer Network clinical practice guidelines [6]. The correct and timely identification of local–regional recurrence or a single metastasis will affect the choice of definitive, often curative therapy, such as surgery, innovative ablation procedures, or 3-dimensional intensity-modulated radiotherapy, preceded by aggressive systemic chemotherapy regimens when indicated. Both patients with suspected recurrence and those without clinical symptoms have demonstrated that PET/CT imaging is superior to physical examination and conventional imaging to detect recurrent loco-regional disease (7). Currently, the detection rate of recurrence in patients who attend regular clinical follow-up is poor, less than 1% in asymptomatic patients. FDG PET-CT may enable survivors to be stratified into groups based on likelihood of having recurrent disease. Optimal surveillance pathway can be developed, reserving most intense imaging regimes and most frequent follow-up survivors at high risk of recurrence (8).

**Conclusion**

Our clinical case shows that 18F-FDG PET/CT can be used for detection of unsuspected recurrence of head and neck cancer referred for restaging another primary carcinoma (colorectal cancer) and helped timely treatment of newly diagnosed recurrence of laryngeal cancer.

**References:**

Address for correspondence: Tsvetelina Yordanova, Department of nuclear medicine, UMHAT „St. Marina“ Varna, Bulgaria, bul „Hristo Smirnenski“ 1, Email: cvetijordanova@gmail.com
Abstract

Introduction/ Purpose: $^{68}$Ga-prostate-specific membrane antigen (PSMA) has been found in tumor-associated neovasculature endothelial cells of some non-prostatic malignancies. The aim is to present an unusual clinical case of $^{68}$Ga-PSMA-avid incidental synchronous malignancy in primary staging for PC.

Materials and Methods: We present a case of a 73-year-old male, in whom a second primary metastatic non-small cell lung cancer (NSCLC) was discovered with a $^{68}$Ga-PSMA PET/CT done for staging of PC.

Results: PSMA-PET was performed and fusion PET/CT images revealed a soft-tissue lesion in the apex of the left lung and one ipsilateral hilar lymph node with increased tracer uptake, which were subsequently histologically confirmed as primary squamous cell lung cancer with associated lymph node metastasis.

Conclusion: Incidental lung lesions on $^{68}$Ga-PSMA PET/CT in PC patients should provoke a differential diagnosis of primary lung cancer. The variable PSMA avidity can potentially put forward to develop the PSMA theranostic concept outside of PC.

Keywords: $^{68}$Ga-PSMA PET/CT, synchronous primary lung cancer

Introduction

Prostate-specific membrane antigen (PSMA) is a 100 kDa type II transmembrane cell surface glycoprotein with influence on the folate hydrolase and neurocarboxypeptidase activity [1]. PSMA expression in cell membranes of prostate cancer (PC) is significantly increased (100- to 1000-fold) that in benign cells and other PSMA-expressing tissues such as kidney, proximal small intestine, and salivary glands [2]. $^{68}$Ga-PSMA PET/CT has shown excellent results in imaging of PC: in primary staging and the evaluation of biochemical recurrence however, recurrent PC is main indication for the novel imaging modality [3]. PSMA-PET/CT hybrid imaging provides superior specificity and sensitivity in comparison with conventional techniques [4]. Nevertheless, it has been shown that various normal nonprostatic tissues and benign lesions also express PSMA and consequently demonstrate tracer avidness. Infection and inflammation may show increased tracer uptake, therefore imitating malignant disease [5]. PSMA has also been found in tumor-associated neovasculature endothelial cells of some nonprostatic malignancies, including NSCLC, possibly due to tumor-associated angiogenic factors and endothelial cell proliferation [6]. However, synchronous $^{68}$Ga-PSMA-avid malignancies are rare (0.7%) in PC patients [7]. As PSMA expression is not confined to PC, knowledge of increased PSMA uptake in non-prostatic pathologies is essential to optimize interpretation. The underlying case report represents PSMA expression in squamous cell lung cancer. Targeting PSMA-expressing neovessels might represent an encouraging treatment option in aggressive solid tumors. The revelation of patients, who might benefit from PSMA ligand-based radionuclide therapy, is of considerable clinical importance. PURPOSE: The aim is to present an unusual clinical case of $^{68}$Ga-PSMA-avid incidental synchronous malignancy in primary staging for PC.
Materials and Methods

Case report

We present a case of a 73-year-old male, in whom a second primary metastatic NSCLC was discovered with a \(^{68}\)Ga-PSMA PET/CT done for staging of high-risk PC. The patient was recently diagnosed with prostate adenocarcinoma, Gleason score 7 (4 + 3) with high initial serum prostate-specific antigen levels (sPSA)- 33.2 ng/ml. Actual sPSA level was 34.13 ng/ml. He was referred for a \(^{68}\)Ga-PSMA PET/CT scan prior to treatment with curative-intent: surgery or radiotherapy. The whole-body \(^{68}\)Ga-PSMA PET/CT images were performed according to existing EANM procedure Guidelines [8] using a PET/CT scanner (Philips Gemini TF) with 16 slice CT. Standard males preparation included well-hydration prior to the study and during the uptake period, furosemide administration (20 mg i.v, quickly before \(^{68}\)Ga-PSMA tracer injection) and voiding promptly before PET/CT imaging acquisition. PET/CT imaging was performed 65 minute after intravenous application of 185 MBq (5 mCi) of \(^{68}\)Ga-PSMA, produced at the nuclear pharmacy of the Nuclear Medicine Department in UMHAT “St. Marina” - Varna, Bulgaria.

Results and discussion

PSMA-PET was performed and in addition to the PSMA expression in the known primary PC (fig. 1 A-D), fusion PET/CT images revealed a soft-tissue lesion in the apex of the left lung and one ipsilateral hilar lymph node with increased tracer uptake, which were subsequently histologically (TRU CUT biopsy) confirmed as primary squamous cell lung cancer (G2) with associated lymph node metastasis (fig. 2 A-D).

Figure 1 A-D, Figure 2 A-D. The patient had a cT2a, Gleason score 4+3=7 tumor and actual sPSA value of 34.13 ng/ml. Anamnestic data presented chest pain and cough. The PSMA PET/CT revealed increased PSMA activity in the biopsy-proven prostate cancer, pT2acN0cM0 (fig. 1/ images, arrows: A- fused axial PET/CT, B- axial Low dose CT, C- fused coronal PET/CT, D- fused sagittal PET/CT) and intense uptake in a 34/30 mm soft-tissue peripheral tumor in the apex (upper lobe / S2 segment) of the left lung (fig. 2/ images, arrows): A-maximum-intensity projection (MIP), B- fused axial PET/CT, D- axial PET and one ipsilateral hilar lymph node (fig. 2 C- axial PET) with increased tracer uptake (with SUVmax-values up to 5.6). The PSMA expressing lung tumor as well hilar lymph node were considered highly suspicious for synchronous primary malignancy- lung carcinoma with ipsilateral hilar lymph node metastasis. No sign of bone or other metastases was found (cT2a cN1 cM0). The patient was referred for further examinations with subsequent histological confirmation of primary squamous cell lung cancer with associated lymph node metastasis. The male was also referred for \(^{18}\)F-FDG PET/CT and
radiotherapy for lung cancer. The planned examination and treatment were not realized due to worsening of the concomitant psychiatric disease: with data for psycho-organic syndrome, with frequent episodes of psychomotor agitation. Several nonprostatic neoplastic diseases express PSMA on their cell membrane or in the endothelial cells of tumor neovasculature and therefore demonstrate increased PSMA uptake [5]. Recent studies have reported the PSMA expression in a subset of NSCLCs, especially in tumor-associated neovascularization [6, 9]. Neovascular expression of the PSMA ligand was found in 49% of NSCLC, furthermore high PSMA expression was associated with higher tumor grading (G3/G4) [9]. According to data from another published study, the percentages of NSCLC patients with PSMA positive tumor cells and PSMA positive tumor neovasculature endothelial cells were 54.02% and 85.06%, respectively. Incidental lung lesions on 68Ga-PSMA in PC patients are not uncommon and should provoke a differential diagnosis of primary lung cancer, particularly if clinical and morphological suspicions are present [10]. Increased PSMA expression in lung adenocarcinoma has been recently reported [11, 12]. Although, no such evidence are existing for small cell lung cancer [13]. Quantitative analysis of PSMA PET/CT is not able to differentiate certainly between lung lesions of various genesis in males with PC. Primary lung cancers demonstrate high PSMA expression comparable to PC metastases [14]. Case with unusual pattern of uptake in 18F-FDG and 68Ga-PSMA PET/CT in patient with primary lung adenocarcinoma was previously reported: a spiculated nodule in the apex of the left lung with intense 68Ga-PSMA uptake, but no pathological 18F-FDG uptake [15]. Differential diagnosis is of considerable importance, since misinterpretation and subsequently misdiagnosis may cause to unnecessary or false therapy approach. In the present case, the PSMA PET/CT detected a synchronous malignancy (primary squamous cell lung cancer) with associated lymph node metastasis. According to our clinical experience in this context, the best approach is the assessment of PET/CT findings, the morphological characteristics of the lung lesions and histopathological evaluation in high suspicious cases.

Conclusion

Incidental lung lesions on 68Ga-PSMA PET/CT in PC patients are not uncommon and should provoke a differential diagnosis of primary lung cancer. The degree of tracer uptake is not reliable for differentiating between pulmonary lesions of different origin in PC patients, which is of considerable clinical importance. Whereas, the variable PSMA avidity can potentially put forward to develop the PSMA theranostic concept outside of PC and 68Ga-PSMA may become a promising target antigen in lung cancer for radioligand therapy of suitable candidates.

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Role of the G-ALLELE of the RS1800896 promoter polymorphism in the interleukin-10 gene in the brain tumors susceptibility: a case-control study

Ivan Mindov¹, Stefan Valkanov¹, Bozhidar Tzvetanov¹, Ridvan Yusuf¹, Spaska Stanilova², Lyuba Miteva²*

¹Department of Neurosurgery, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
²Department of Molecular biology, Immunology and Medical genetics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

Abstract
The study aimed to analyze the significance of a functional genetic variant in the IL10 gene promoter (rs1800896) for brain cancer susceptibility. The genotyping was performed by allele-specific PCR in 160 participants. Serum IL-10 was quantified by the ELISA assay. Our results showed that the carrying of the variant G-allele of rs1800896 polymorphism (AG+GG genotypes), could be associated with a significantly higher risk for both primary and metastatic brain tumors development (OR=2.11, 95% CI: 1.075-4.173; p=0.029). In addition, in cases with primary brain tumors, carriers of G-allele showed higher IL-10 serum levels compared to the AA-genotype (5.5±1.78 vs. 1.94±0.84 pg/ml) in contrast to cases with metastatic brain tumors.

Our preliminary study suggested that the IL10 rs1800896*G allele might be associated with the genetic susceptibility of the primary brain tumor development and sustain tumor metastasis due to its functional effect to enhance IL-10 production.

Keywords: glioblastoma, cytokine, cancer

Introduction
The relationship between inflammation and cancer is well defined. The inflammation is known as the seventh hallmark of cancer that considerably influences cancer development and progression. Brain cancers show cellular and molecular heterogeneity, part of which reflects the diversity of cytokines, other signaling molecules involved in cancer cell clonal selection. Primary brain cancer is one of the rare types of cancers associated with a low survival rate around the world. In addition, brain metastases often arise from primary lung (39–56%), breast (13–30%), as well as gastrointestinal (6–9%) cancers [1]. IL-10 maintains the balance between pro- and anti-inflammatory cytokine levels in the periphery as well as within the central nervous system (CNS) [2]. IL-10 has the potential to promote the proliferation of U87MG glioma cells in a dose-dependent manner [3], and was pointed as a therapeutic target in the curing of glioma [4]. IL-10 may also increase the metastatic potential of lung cancer cells [5], breast cancer [6], and colorectal cancer [7]. A functional genetic variant -1082 A>G (rs1800869) in a promoter region of the IL10 gene was confirmed. The G-allele was associated with high levels of IL-10 production [8] and has been widely investigated in cancer predisposition including in our previous studies [9]. However, the associative studies about the IL-10-1082A/G and brain cancer development and progression are limited.

Based on the above, the aim of the study was to investigate the distribution of rs1800896 variant in IL10 among cases with brain tumors in an attempt to analyze its significance for brain cancer susceptibility.

Methods
DNA samples were isolated from a total of 160 participants, 77 cases and 83 controls. The genotyping of rs1800896 was performed by allele-specific PCR assay, described previously [9]. The patients’ group was composed of 40 (52%) male and 37 (48%) female with a mean age of 62.53±11.17 yrs. The
diagnosis was confirmed histologically at University Hospital “Prof. St. Kir kovich”, Stara Zagora, Bulgaria. According to the diagnosis, 32 (42%) cases have primary tumors, including 19 cases with the high grade and 13 cases with low-grade glial tumors, and 45 (58%) cases were with metastatic brain tumors. The control group consist of 45 (54%) males and 38 (46%) females with a mean age of 58.6±9.2 yrs. Serum levels of IL-10 were quantified by the ELISA test. The statistical analysis was performed using the Statistica (StatSoft, Inc., USA) v. 12. The t p-value less than 0.05 was considered statistically significant.

**Results and Discussion**

The observed genotypic and allelic frequencies rs1800896 polymorphism in *IL10* among cases and control are presented in **Table 1**.

**Table 1.** Genotypic and allelic frequencies rs1800896 polymorphism in the *IL10* among cases and controls

<table>
<thead>
<tr>
<th>rs1800896</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA-genotype</td>
<td>19 (25%)</td>
<td>34 (41%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>AG-genotype</td>
<td>47 (61%)</td>
<td>40 (48%)</td>
<td>2.1 (1.042-4.243)</td>
<td>0.037</td>
</tr>
<tr>
<td>GG-genotype</td>
<td>11 (14%)</td>
<td>9 (11%)</td>
<td>2.19 (0.769-6.216)</td>
<td>0.138</td>
</tr>
<tr>
<td>AG+GG genotypes</td>
<td>58 (75%)</td>
<td>49 (59%)</td>
<td>2.11 (1.075-4.173)</td>
<td>0.029</td>
</tr>
<tr>
<td>A-allele</td>
<td>85 (55%)</td>
<td>108 (65%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>G-allele</td>
<td>69 (45%)</td>
<td>58 (35%)</td>
<td>1.51 (0.964-2.371)</td>
<td>0.071</td>
</tr>
</tbody>
</table>

*OR* - odds ratio; *CI* - confidence interval

The genotypic distribution of the selected polymorphism of *IL10* in the control group was in Hardy–Weinberg equilibrium (χ²=0.234; p=0.889). In a comparison of AA vs. AG+GG, the carrying of the variant G-allele of rs1800896 polymorphism was associated with a significantly higher risk of brain tumors (OR = 2.11, 95% CI = 1.075-4.173; p=0.029). The same tendency was observed for the subgroup analysis among cases with primary brain tumors (OR = 2.478, 95% CI = 0.963-6.378; p=0.055) and metastatic brain tumors OR = 1.908, 95% CI = 0.864-4.215; p=0.107) without reaching statistical significance, probably due to the small sample size.

These data are in accordance with other studies exploring the significance of rs1800896 polymorphism for carcinogenesis. Resent meta-analyses have reported the association between GG-genotype and increased risk of breast cancer [10], as well as increased lung cancer risk associated with G-allele [11]. It should be noted that some other studies have reported increased cancer risk for A-allele, or were not able to confirm any association of this genetic variant. Such discrepancy is not surprising, taking into consideration well-known ethnic heterogeneity or specificity and heterogeneity of cancers. Amirian et al [12] explored several genetic variants, including rs1800896 among 373 Caucasian, adult glioma patients and 365 cancer-free controls. They have found a significantly increased risk of the GG-genotype of *IL10* rs1800896 compared with the AA/GA genotypes (OR: 1.57, 95% CI: 1.11 – 2.23; p=0.012). Also, the increased risk with GG-genotype in the adjusted analysis according to the grade of
glioma (for grade IV glioma OR:1.61, 95%CI: 1.07-2.44; p=0.016) was reported. Respectively, we may assume that the carrying of rs1800896*G-allele is associated with a significantly higher risk of brain tumors.

Bearing in mind the functional role of IL10 rs1800896 polymorphism, we quantified serum IL-10. A significantly higher IL-10 in cases with metastatic brain tumors than in primary tumors (15.8±5.7 pg/ml vs. 4.2±1.3 pg/ml; p=0.011) was detected. Respectively, the subgroups analysis of serum IL-10 according to the origin of brain cancer and rs1800896 was performed (Figure 1).

**Figure 1.** IL-10 serum level in the primary and in the metastatic brain tumors according to the rs1800896 IL10 genotype. The data are presented as mean ± s.e.m.

Cases with primary brain tumors carriers of AG+GG genotypes showed a tendency of higher IL-10 compared to AA-genotype (mean 5.5 ± 1.78 vs. 1.94 ± 0.84 pg/ml, respectively). Contrary, in cases with metastatic brain tumors, higher IL-10 serum levels were detected in AA-genotype than in AG+GG genotypes without reaching statistical significance. Although our data are preliminary, we may hypothesis that IL-10 and the functional IL10 rs1800896 polymorphism have an impact on both susceptibilities to primary and metastatic brain tumorigenesis.

**Conclusion**

Our preliminary study suggested that the rs1800896 G allele in IL10 might be associated with genetic susceptibility of primary brain tumor development and sustain tumor metastasis due to its functional effect to enhance IL-10 production.

**References:**


**Acknowledgment:** This work was supported by Grants NIP2/2019 and NIP9/2021 from the Fund for Scientific projects from Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria.

**Corresponding author:** Galina Gavazova phone number: +359899800636; Bulgaria, 4000 Plovdiv, Hr. Botev 3; e-mail: gavazovagalina@gmail.com
18F-FDG PET/CT in finding cutaneous, muscular and pancreatic involvement in IgA multiple myeloma – case report with literature review

Tanya Stoeva 1,2, Aneliya Klisarova, 1,2

1 Nuclear Medicine Department - UMHAT “Sveta Marina” Varna, Bulgaria
2 Department of Imaging Diagnostics, Interventional Radiology and Radiotherapy, Faculty of
Medicine, Medical University of Varna, Bulgaria

Abstract
Introduction: Multiple myeloma (MM) is a malignant disease which affects the plasma cells in the
bone marrow, leading to bone destruction. There’s subtype of patients with extramedullary MM in
which monoclonal plasma cells can be found in distant sites of the body.
Materials and methods: We present a case of 45-years-old male patient with IgA MM who had been
referred for 18F-FDG PET/CT for monitoring the treatment response.
Results: The 18F-FDG PET/CT showed numerous osteolytic lesions in the bones of the axial and
appendicular skeleton, in places with a pronounced soft tissue component. Multiple FDG-avid lesions
in muscles, skin and subcutaneous tissue, retroperitoneal and pancreatic involvement were found as
well.
Conclusion: EMD of skin, muscles and pancreas is uncommonly seen. In this case report, we
displayed the important role of 18F-FDG for detecting diffuse EMD, which can be used as prognostic
factor and in favor for monitoring therapy response and progression.
Key words: multiple myeloma, 18F-FDG PET/CT, extramedullary multiple myeloma

Introduction
Multiple myeloma (MM) is a malignant disease which affects plasma cells as it makes them multiply
uncontrollably in the bone marrow, leading to overproduction of monoclonal paraprotein (M protein),
bone destruction by predominantly osteolytic lesions and displacement of other hematopoietic cell lines.
Multiple myeloma, although rare, is the second most common malignant hematological disease after
Non-Hodgkin's lymphoma [1]. It occupies 1% in terms of morbidity among all types of cancer and 13% of
all hematological neoplasms [2]. In 2015, about 40,000 people in Europe were diagnosed with MM
and this number is expected to increase up to 46,000 by 2025 [3]. It is reported that the average age of
diagnosis of the disease is between 66-70 years, with 37% under 65 years. MM is extremely rare under
the age of 30 - the frequency varies between 0.02% -0.3%, prevailing among the male population [4].
On the flip side, there’s a subtype of patients with extramedullary multiple myeloma in which
monoclonal plasma cells can be found in distant sites of the body. It is reported with a frequency of 7%
to 20% in disease presentation and 6% to 20% in progression [5]. Cutaneous and muscular involvement
from multiple myeloma are rare clinical findings that usually occurs in end-stage of the disease and
predicts a poor prognosis. There’s only a few case reports and retrospective analyses in the literature
that exhibit this rare phenomenon. Purpose: In this case report, we aim to present a rare case of middle-
age male patient with IgA multiple myeloma and the role of 18F-FDG PET/CT for finding out the
diffuse cutaneous, muscular and pancreatic involvement from the primary hematological neoplasm.

Materials and methods
We present a case of 45-years-old male patient with IgA multiple myeloma diagnosed in December
2017. Anamnestic data reported pain in his lower back and bones, radiating to the lower extremities with
a progressive character with duration of 6-8 months. Pathological fracture of the right femoral neck and
immunodeficiency syndrome were found as well. Laboratory data showed anemia, monoclonal
paraprotein type IgA, lambda 19.3% = 14.3 g / l, high urea levels up to 43 mmol/L and creatinine to 993
μmol/L. Biopsy of tumor formation on his back was performed. The histopathological analysis revealed infiltration of the skin from multiple myeloma. The patient received 6 cycles of chemotherapy with CyBorDex and maintenance therapy with Bortezomib. In June 2018, the patient was referred for 18F-FDG PET/CT for monitoring the treatment response. The scan was performed on PET/CT (Philips Gemini TF) using 6.8 mCi 18F-FDG after proper preparation of the patient.

**Results and discussion**

The 18F-FDG PET/CT showed numerous osteolytic lesions in the bones of the axial and appendicular skeleton, in places with a pronounced soft tissue component (at the level of Th10 with propagation to the spinal canal). Along with the described changes, many metabolically inactive lytic bone lesions were scanned as well. Multiple FDG-avid lesions in muscles, skin and subcutaneous tissue, retroperitoneal and malignant involvement of the pancreas were found (Figure 1) (Figure 2).

![Figure 1](image1.jpg)

**Figure 1**- fused axial PET/CT image of FDG-avid muscle lesion in the left lower leg

![Figure 2](image2.jpg)

**Figure 2**- 18F-FDG PET/CT fused axial image- malignant involvement of pancreas with a retroperitoneal lesion.

Extramedullary multiple myeloma is a rare manifestation of multiple myeloma (MM) [6] and often associated with poor prognosis because of the anaplastic up to undifferentiated character of the plasma
cells. The estimated median survival is approximately eight months [7]. PET/CT imaging is playing an important and integral role in diagnosis and monitoring the response to treatment in various extramedullary localizations [8]. The number of visible-FDG focal lesions, the presence of extramedullary disease (EMD) and the maximum standardized values of uptake (SUVmax) are reliable prognostic 18F-FDG PET/CT parameters at diagnosis, before stem cell transplantation, in recurrence or progression as sensitivity and specificity are reported in the range of 80–100% [9]. In a meta-analysis by Lu et al (2012) [10] for the assessment of PET/CT in intramedullary and extramedullary lesions in MM, data from 14 studies (395 patients) were summarized. They reported that 18F-FDG-PET/CT at detection of extramedullary lesions had 96% sensitivity and 77.8% specificity. These data indicate that PET/CT has good diagnostic accuracy for the detection of active MM, in particular for extramedullary spread. In a literature review of 400 articles it is found that 82.2% of EMD is most commonly seen in upper respiratory and gastrointestinal tracts followed by urinary tract, skin, lungs and breasts [11]. Multiple myeloma involvement of pancreas in combination with skin, muscles, suprarenal glands, kidneys or lungs are extremely rare [12]. Most cases of plasma cell infiltration in the pancreas are microscopic. Well-formed masses are unusual and can be represented as a focal mass or as a diffuse enlargement of pancreas with or without bile obstruction [13]. For the increased FDG uptake in muscles and skin, there is a wide spectrum of causes such as exercising, postprandial acquisition, compensatory hypertrophy, inflammatory granulomatous disease, primary or metastatic tumors [14].

**Conclusion**

Extramedullary involvement of skin, muscles and pancreas in MM is uncommonly seen. In this case, we displayed the important role of 18F-FDG for detecting diffuse EMD in a middle-age male patient with IgA multiple myeloma which can be excellent prognostic factor and in favor for monitoring therapy response and progression of the malignant formations.

**References:**


Comparison of (18F)FDG PET/CT, Computer Tomography and (68Ga)DOTATATE PET/CT in the imaging of neuroendocrine tumors of the small intestine and their metastases - case report and review of literature

Chausheva S.¹,², Valchev G.¹,³

Medical University, Department Diagnostic Imaging, Interventional Radiology and Radiotherapy
Clinic Nuclear medicine, UMHAT “St. Marina” – Varna
Clinic Imaging Diagnostics, UMHAT “St. Marina” – Varna

Abstract
Neuroendocrine tumors arise from neuroendocrine cells scattered through the body and demonstrate an overexpression of somatostatin receptors (SSTRs). Two thirds of all NETs originate from the gastrointestinal tract.

Purpose: The purpose of this case report is to provide a review of the difference in the use of (18F)FDG PET/CT, CT and 68Ga (DOTATATE) PET/CT in the assessment of neuroendocrine tumors of the small intestine and their metastases.

Material and methods: We present a case of a 41-year-old male patient with a neuroendocrine tumor of the small intestine and peritoneal metastasis who underwent two PET/CT scans with (18F)FDG and Ga-68 DOTATATE and a CT with contrast.

Results: The results from Ga-68 DOTATATE PET/CT are crucial in the detection of suspicious lesions.

Conclusion: Ga-68 DOTATATE PET/CT should be the preferred imaging modality for diagnostic and detection of metastatic lesions from neuroendocrine tumors.

Keywords: Neuroendocrine tumors, (68Ga)DOTATATE PET/CT, (18F)FDG PET/CT, CT.

Introduction
Neuroendocrine tumors (NETs) are a heterogenous group of rare tumors [1]. These types of neoplasms arise from neuroendocrine cells scattered through the body and demonstrate an overexpression of somatostatin receptors (SSTRs). Two thirds of all NETs originate from the gastrointestinal tract[2]. The World Health Organization is classifying NETs into low grade (G1), intermediate (G2) tumors (NETs) and high grade carcinomas (G3). The classification is based on on Ki67 proliferation index and mitotic count [3]. The groups are as follows: Grade 1: Ki67 1-2% and/or up to 1 mitosis/10 HPF, Grade 2: Ki67 3-20% and/or 2-20 mitoses/10 HPF, and Grade 3: Neuroendocrine carcinomas (NECs): Ki67 >20% and/or >20 mitoses/10 HPF. It is incontrovertible that these tumours can be difficult to identify, especially in their early stages, because of there prosaic symptoms such as diarrhea, abdominal cramps, headaches and rashes [4]. Imaging is valuable not only for diagnosing and staging, but also in the follow-up of recurrent or metastatic disease. In neuroendocrine tumours metastases are a common finding. In recent study it was discovered that 29% of patients with a neuroendocrine tumour were found to have metastatic disease at initial diagnosis. Only 14% of the metatatic lesions where in the peritoneum [5]. Current diagnostic modalities include computed tomography (CT), magnetic resonance imaging (MRI), transabdominal ultrasound (US), endoscopic US (EUS). Imaging of such tumors is often difficult, they are often limited in detecting such tumors and their metastases, because of the small sizes and variable anatomic locations. The development of hybrid scanners as PET/CT and new radiopharmaceuticals as 68Ga-labeled somatostatin analogs are the perfect combination where functional imaging findings may be directly correlated to morphology and reveal additional metastases compared to or other conventional imaging modalities like CT [6]. Different studies have shown the high diagnostic exactness of 68Ga-DOTATATE PET/CT in diagnosing primary NETs (G1 and G2) and their metastases. In
comparessement with (18F) FDG PET/CT which has low accumulation regarding these histological types of tumors [7].

**Purpose**
The purpose of this case report is to provide a review of the difference in the use of (18F) FDG PET/CT, CT and 68Ga (DOTATATE) PET/CT in the assessment of neuroendocrine tumors of the small intestine and their metastases.

**Materials and methods**
A 41-year-old male patient was admitted at the University Hospital “St. Marina” in Varna, Bulgaria with atypical, crampy abdominal pain, nausea and vomiting. A midline laparotomy was performed. Intraoperative a tumor formation of the small intestine was found and packages of lymph nodes in the mesentery. Resection of the tumor formation and latero-lateral small intestinal anastomosis was performed with a histological result of neuroendocrine tumor G1. Whole body F18-FDG PET/CT was performed which showed discrete zones of low active FDG uptake in the peritoneum. Peritoneal lesions were suspected but because of the low accumulation of the radiopharmaceutical in G1 NET, the findings where not conclusive [Fig. 1].

Figure 1. - Advantages of 68Ga-DOTATATE PET/CT over F18-FDG PET/CT. (A) Axial F18-FDG PET/CT fused images showed low metabolic activity in peritoneum. (B) Axial 68Ga-DOTATATE PET/CT fused images demonstrating increased DOTATATE uptake in the multiple peritoneal lesions with better delineation in comparison to the F18-FDG PET/CT scan.

After 32-month-long therapy with Sandostatin lar a contrast-enhanced CT of thorax, abdomen and pelvis was performed, but no lesions were found. The patient continued with the therapy and 3.5 months later a whole body Ga68 DOTATATE PET/CT was conducted. Axial PET/CT fused images shows peritoneal lesions with high somatostatin receptor expression [Fig. 2].

Figure 2. - Advantages of 68Ga-DOTATATE PET/CT over conventional imaging. (A) Maximum intensity projection PET image revealed presence of multiple focal areas of increased radiotracer uptake in the peritoneum. (B) Axial 68Ga-DOTATATE PET/CT fused images showed multiple peritoneal lesions were seen with better delineation on 68Ga-DOTATATE PET/CT maximum-intensity-projection.(C) Axial contrast-enhanced CT image. The peritone lesions were too small to be seen on original reading of axial contrast-enhanced CT images and only became apparent with knowledge of 68Ga-DOTATATE PET/CT findings.

**Discussion**
Regarding the gastrointestinal tract NETs are the second most common group of tumors. There clinical symptomatology can be vague and often they can be very difficult to detect. Due to these factors the diagnosis is often made when the disease is at an advanced stage and metastases are present. The most common locations of metastatic lesions from gastrointestinal tract NETs are the liver, peritoneum, lung,
and bone [9]. Accurate patient management and appropriate treatment of neuroendocrine tumors depends on the correct staging of NET, especially if distant metastatic lesions are suspected. In different studies comparisons between somatostatin receptor PET/CT and conventional imaging modalities were conducted and the higher diagnostic performance of somatostatin receptor PET/CT for the detection of metastatic disease was demonstrated. The effectiveness of Gallium-68 DOTATATE PET/CT correlate with the NET differentiation; in fact as NET differentiation heightened lowered, radiolabelled somatostatin analogues uptake increased in metastatic NET lesions [8]. The opposite is true with the 18F-FDG uptake which is reduced or absent at all. Based on these facts we can conclude that Gallium-68 DOTATATE PET/CT is more sensitive to G1 and G2 NETs, while (18F) FDG is sensitive to G3. With the additional information oncologists and surgeons are able to achieve a better treatment plan. Follow-up scans can be performed to establish each patient’s response to treatment.

**Conclusion**

Somatostatin receptor imaging with 68Ga-DOTA-somatostatin analog-PET/CT provide high sensitivity for imaging of G1 and G2 types of NET lesions and should always be a part of the tumor staging, preoperative imaging and re-staging. Visualization of small peritoneal lesions and primary small-intestinal NETs is facilitated by 68Ga-DOTA-somatostatin analog-PET/CT.

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Clinical significance of Hepatitis D virus genotype I infection

Denitsa Tsaneva-Damyanova

Department of Microbiology and Virology-Medical University, Varna, Bulgaria.

Abstract
Background: Hepatitis D virus (HDV) causes the most aggressive of all viral hepatitis leading to fibrosis, cirrhosis and hepatocellular carcinoma and liver failure. There are eight different HDV genotypes worldwide, with genotype I being widely spread in Europe.

Purpose: To evaluate the clinical significance of hepatitis D virus genotype I infection in patients with chronic HDV infection.

Materials and methods: The investigation was conducted among 12 chronic HDV carriers. HDV sequencing and clinical assessment of the severity of the liver dysfunction was made.

Results: All of the HDV strains belonged to genotype I.

Conclusions: Genotype I is the most widespread HDV genotype in Bulgaria. The severity of liver disease caused by HDV is thought to be associated with the HDV.

Keywords: HDV, genotype I, HDV cirrhosis

Introduction
About 15 million people worldwide have been diagnosed with HDV. Hepatitis D virus causes the most aggressive of all viral hepatitis leading to fibrosis, cirrhosis and hepatocellular carcinoma and liver failure. HDV infection remains one of the leading causes of death from viral hepatitis and an indication for liver transplantation. There are eight different HDV genotypes worldwide (I to VIII) with genotype I being widely spread in Europe, Middle East and America [1].

Materials and methods
The investigation was conducted among 12 chronic HDV patients - 66.7% males (95% CI: 34.9%-90.1%, n=8) and 33.3% females (95% CI: 9.2%-65.1%, n=4), patients of “St.Marina” University Hospital, Varna, from 28 to 62 years (mean age 45.3 ± SD 12.17), in the period 2013-2019.

HDV serological status of the target group was defined via commercially available ELISA tests. Nucleic acid was extracted from 200 µl serum samples (GeneAll Exgene Viral DNA/RNA kit. Synthesis of cDNA was performed using Minotech Biotechnology kit in a final volume of 10 µl. HDV-specific nested PCRs were performed with reverse transcribed cDNA samples as template, strictly following the protocol of the manufacturer. The products were analyzed by electrophoresis on 1 % agarose gel and the expected length of 359 bases was confirmed in all of them. Sequences analysis of the received data was performed via BLAST in NCBI (National Center for Biothechnology and Information) database. Statistical analyses were performed by SPSS ver. 23 software package. Results were expressed as mean (±) SD or median (range) as appropriate. Data were analysed by Pearson’s χ2test. Two-sided p-values < 0.05 were considered as statistically significant. Confidence intervals (95%CI) were determined.

Result
Of all patients tested for HDV (n = 12) with proven liver disease were anti-HDV (+) in ELISA. A statistically significant difference was found between the anti-HDV Ab seropositivity of individuals in the 28-39 age group and that in the other age groups (Pearson’s χ2=4.48, p=0.03 <0.5). HDV RNA positive results in PCR were found in all of the twelve patients.

All patients were genetically analyzed via direct sequencing of the HDV RNA amplicons. The genotype assignment was based on the analysis of the sequences that corresponded to nucleotides.
between 906 and 1256. When comparing the obtained HDV sequences with sequences correlating to HDV from the database, they are all closely related to genotype I of HDV and circulating among Bulgaria’s neighboring countries.

Nucleotide similarity among the 12 isolated sequences ranged from 84 % to 99% (mean 89.9%) (Table 1).

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>% of query cover</th>
<th>% of identity</th>
<th>NCBI description</th>
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<tr>
<td>1</td>
<td>Female</td>
<td>61</td>
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</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>51</td>
<td>96%</td>
<td>81.20%</td>
<td>Human hepatitis delta virus encoding delta-antigen RNA, complete cds</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>28</td>
<td>89%</td>
<td>94.48%</td>
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<tr>
<td>4</td>
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<td>50</td>
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<td>93.20%</td>
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<td>5</td>
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<td>39</td>
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<td>92.99%</td>
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<td>6</td>
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<td>93%</td>
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<td>82.54%</td>
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<td>62</td>
<td>99%</td>
<td>93.31%</td>
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</table>
Table 1. General information for HDV tested patients, % of query cover and identity of the obtained results, compared with the NCBI database

We have encountered variable clinical manifestations among these 12 patients target group (95%CI: 42.8% - 94.5%, n=9). Chronic HDV was found in 16.7% (95%CI: 2.1% - 48.4%, n=2). Liver cirrhosis was found in 75% of the neighboring countries. target group (95%CI: 42.8% - 94.5%, n=9). Five of the patients with cirrhosis-55.6% (95% CI: 21.2% - 86.3%, n=5) died due to complications. Hepatocellular carcinoma (HCC) was found in 8.3% (95% CI: 0.2% - 38.4%, n=1) of them.

Discussion
Isolates of HDV genotype 1 were found in all parts of the world and lead from asymptomatic liver disease to fulminant hepatitis [3]. We haven’t found other reports about HDV genotype prevalence in Bulgaria. In our research 75 % of the tested patients were with cirrhosis at different clinical stage. As per the literature 60% -70% of patients with chronic HDV would develop liver cirrhosis. HDV is the cause of almost half of the cases of liver cirrhosis and HCC in Turkey [4]. Since genotypes may influence the clinical outcome of the disease and shows geographic dissimilarities, it seems that is important to determine the occurrence of diverse genotypes in population, which may contribute to the clinical settings. Patients with HDV cirrhosis show negative trend to fast progression and decompensation of liver disease. Patients with HBV and HDV chronic infection have a twofold higher risk of developing cirrhosis and die and threefold higher risk of developing HCC compared with HBV monoinfected individuals [5].

Conclusion
HDV genotype I was found prevalent in Bulgaria. The severity of liver disease caused by chronic HDV is thought to be associated with the HDV genotype and viral loads. HDV accelerates the development of liver dysfunction with its complications. Analysis of more HDV sequences, in particular from areas not yet studied will continue to improve our knowledge of the virological and epidemiological properties of HDV.

References:
The Role of Dynamic Renal Scintigraphy with $^{99m}$Tc-DTPA in the diagnoses of Congenital Hydronephrosis. Clinical case:

Borislav Chaushev

Clinic of Nuclear Medicine, UMBAL `St. Marina`- Varna, Medical University – Varna, Bulgaria.

Abstract

Purpose: Congenital anomalies of the urinary tract are approximately a third of all prenatally diagnosed abnormalities. They are the most common renal as well as urological disorders of childhood. The aim of our study was to evaluate the role of Dynamic Renal Scintigraphy with $^{99m}$Tc-DTPA in children with congenital hydronephrosis.

Material and Method: Eleven children aged between 2 and 17 years were admitted to the department of Nuclear Medicine with Urinary Tract Infection and were investigated with Dynamic Renal Scintigraphy with $^{99m}$Tc-DTPA.

Results: Hypofunctional to afunctional left kidney. Normal scintigraphy of the right kidney.

Conclusion: The Dynamic Renal Scintigraphy allows to establish the degree of urodynamic dysfunction. It can be used not only to diagnose hydronephrosis but can be one of the leading methods for post correctional surgery follow up functional assessment.

Key words: Dynamic Renal Scintigraphy (DRS), 99mTc-DTPA (Diethylen triamine penta acetic acid calcium trisodium hydrate), Urinary Tract Infection (UTI)

Introduction

Congenital anomalies of the urinary tract are approximately a third of all prenatally diagnosed abnormalities. They are the most common renal as well as urological disorders of childhood. Their frequency is between 3-6% per 1000 new born babies. Congenital nephrological anomalies are responsible for the development of chronic renal disease in 55% of the cases.

One of the most common congenital renal abnormalities are the anomalies of the pyelo-urethral segment which often leads to hydronephrosis. Hydronephrosis irrespective of its aetiology is quantified in 3 grades. (1,4,5)

Clinical presentation of Hydronephrosis includes frequent Urinary Tract Infection’s (UTIs), restlessness as abdominal pain equivalent in infancy and significant leukocyturia and bacteriuria. Hydronephrosis can be diagnosed antenatally between 20 and 30 gestational weeks.

All new born diagnosed prenatally with hydronephrosis should have Ultrasonography (US) examination between the 4 – 6 day from birth (3,4,6)

The aim of our study was evaluated the role of Dynamic Renal Scintigraphy with 99mTc-DTPA in the children with congenital hydronephrosis (7)

Materials and Methods

Eleven children aged between 2 and 17 years were admitted to the department of Nuclear Medicine with UTI and were investigated with Dynamic Renal Scintigraphy (DRS) with $^{99m}$Tc-DTPA (Diethylene triamine penta acetic acid calcium trisodium hydrate). The study had confirmed the established by US diagnosis of hydronephrosis in all.

Eight of the children had corrective surgery and DRS follow up elevation.

One of the children, the 7-year old A.R.R. had corrective surgery at age of 3 for left sided hydronephrosis due to pyeloureteral transition stenosis. A.R.R. had not been reviewed after the operation. A week prior to admission child developed abdominal pains and dysuria.
On admission there was elevated CRP of 200 and significant bacteriuria. US of the kidneys revealed hydroureteronephrosis of the left kidney. Grade II increased parenchymal echogenicity and poor visualization of the pyramids. The right kidney had normal appearance.

After antibiotic treatment the patient was booked for DRS with $^{99m}$Tc-DTPA. Dynamic Renal Scintigraphy with $^{99m}$Tc-DTPA is part of the imaging algorithm of assessment of hydronephrosis. It is done with SPECT/CT scanner. (6,7)

A typical renogram has 3 phases. The first phase is known as the vascular transit phase which represents radiotracer entering the kidneys. It usually lasts about 30 to 60 seconds. The second phase is known as the tubular concentration phase or parenchymal transit phase normally lasts 1 to 5 minutes when radiotracer appears in the tubules. It is represented by a peak in the renogram. The third phase is noted by a downslope in the renogram indicating excretion of the radiotracer from the kidneys and clearance from the collecting system.

It usually starts at 4-8 minutes after radiotracer injection.

The DRS with $^{99m}$Tc – DTPA can also be used as a follow up study after corrective surgery at 6 months and 1 year.

The patient received 111 MBq (3mCi) bolus injection of $^{99m}$Tc – DTPA while in the supine position. Renal dynamic scintigraphy was acquired on a SPECT/CT scanner (MEDISO) with a low-energy collimator, a 128 × 128 matrix, and 20% energy window after dynamic scanning for sixty minutes.

**Results**
The Dynamic Renal Scintigraphy with 3.1 mCi $^{99m}$Tc – DTPA indicated:

Right kidney - preserved perfusion and activity during the parenchymal phase without zones of retention during the excretory phase.
The functional curve was normal with $T_{max}$ – 6min, $T_{1/2}$ – 15min.

Differential function (2-3min) – 75.7%.

Left kidney – results indicated reduced perfusion and activity in the parenchymal phase without zones of retention during the excretory phase.

Functional curve is significantly reduced with isosthenuria course. $T_{max}$ – 12.11min, $T_{1/2}$ – 17min.

Differential function (2-3min) – 24.3%.

Results consistent with hypofunctional to afunctional left kidney. Normal scintigraphy of the right kidney.

Patient was referred to Urology and had left nephrostomy tube inserted.

**Discussion**
The congenital hydronephrosis is a common anomaly of the urinary tract. It can be diagnosed antenatally. The diagnosis requires postnatal verification with other imaging modalities.

**Conclusion**
The Dynamic Renal Scintigraphy allows to establish the degree of urodynamic dysfunction. It can be used not only to diagnose hydronephrosis but can be one of the leading methods for post correctional surgery follow up functional assessment.

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Etiological and clinical structure of the healthcare-associated infections in the University Hospital "St. Marina" – Varna for a period (2016-2020)

Ilieva Deyana¹, Tsonko Paunov¹, Mariya Marinova², Desislava Vankova¹, Miglena Kolarova¹, Eliyana Ivanova¹

1. Medical University-Varna
2. University Hospital "St. Marina"-Varna

Abstract
Healthcare-associated infections (HAIs) are a major and increasing cause of morbidity and mortality around the world. There is a need for adequate evidence-based guidelines in order to control the HAIs [1,2,3]. Aim: To analyze the HAIs-profile for a period 2016-2020 and to track the accuracy of registration. Methods: A retrospective descriptive study was conducted based on the registered HAIs in the monitoring system of the hospital. Results: Within the period 2016-2020, 342158 patients were hospitalized. An average of 33.71% received an antimicrobial therapy. The registered prevalence of HAIs is 0.56%. The leading etiological agent causing HAIs is A.baumannii (18.59%). The surgical site infections (SSI) predominate (17.87%). Conclusion: Despite the lower number of patient admissions and the growing consumption of antibiotics, the hospital rates of HAIs did not exceed the national rates. A lot has to be done in order to improve the hospital surveillance and the antibiotic stewardship policies.

Introduction
Healthcare-associated infections (HAIs) are a major public health challenge. Globally, the morbidity and mortality analyses show that HAIs are an important and growing threat for patients’ safety. HAIs are a significant financial burden on patients, healthcare workers and healthcare systems. There is a great effort by specialist to develop surveillance systems and infection control methods. Without surveillance, there are no effective methods of infection control. [4,5]

The aim of the report is to study the etiological and clinical profile of the HAIs at the University Hospital "St. Marina" Varna for a five-year period (2016-2020), as well as the percentage of unrecognized and untested cases for the same period, which will contribute to the improvement of the registration and control of these infections.

Methods
Retrospective descriptive study was carried out (in 2021) using the hospital register of HRIs for the five-year period (2016-2020). We used data from: the official documents/registration forms of the patients' medical histories; and from the microbiological examination documents. The data was structured and analyzed with Microsoft Excel v. 16.0.

Results
The descriptive data analyses cover the five-year period - 2016-2020 (Fig. 1). Compared to 2016 in 2017 the patient admission decreased to 71.45% and remained similar in the following years.
The analyses show that the antibiotic consumption had been increasing from 24.64% in 2016 to 39.76% in 2020 (share of all the hospitalised patients that had an antibiotic prescription). Inevitably, the increased antibiotics use is associated with increased resistance to antibiotics. [6] Initially in Bulgaria, this indicator was adopted in the national surveillance system as an indirect indicator of the presence of infections. [7] The practice shows that the escalation of HAIs is caused by increased and unnecessary consumption of broad-spectrum antibiotics, well studied in CR-Acinetobacter spp. [8] The current results correlate with ECDC data which shows that at a country level, a majority of EU countries reported a substantial decrease of antibiotics consumption, between 2019 and 2020, for both the community and the hospital sector. Seven countries (Estonia, Greece, Hungary, Italy, Latvia, Malta and Portugal) reported a decrease in the community, but an increase in the hospital sector. Bulgaria reported an increase of antibiotics consumption in both the community and the hospital sector. [9] The reported data of the current study confirms the need for better antibiotic stewardship for the improvement of the trend of HAIs in the hospital.

Despite the increase in antibiotic consumption, a decrease in the relative share of HAIs from 0.74% (2017) to 0.50% (2020) is registered. This is less than the average level for Bulgaria 1.0-1.4 % [10], Europa 3.5-10% [11,12], China 2.45%. [13]. In 2020, with the onset of the COVID-19 pandemic, in UH
“St. Marina” incomplete registration of HAIs was observed and therefore strains with high antibiotic resistance predominate.

Comparing the etiological structure (Table 1.) there is a tendency for a leading place and maintaining the relative share of *A. baumannii* as causative agent by HAIs 18.59% (15.40%-21.74%). On the second and on the third place alternately during the period are infections caused by *K. pneumoniae* (11.71%) and *P. aeruginosa* (12.17%). In the last year *E. coli* is in second place on the list with 12.54 %. In contrast to our results in the EU the most frequently isolated microorganism was *P. aeruginosa* in ICU-acquired pneumonia episodes, CNS in ICU-acquired SIs, and *E. coli* in ICU-acquired UTIs.[11] It is important to note that for the studied period the share of etiologically unrecognized and unexamined infections is average 2.62%.

The investigation of clinical structure shows some specifics of HAIs categories in the hospital in focus. Despite the high absolute values of the registered lower-respiratory tract infections (LRTIs) n=129 (2017) and ventilator-associated events (VAEs) n=119 (2018), the total share of SSI (surgical-site infections) is the largest for the studied period (17.87 %, n=348). In this order for the period following are 16.90% (n=329) VAEs, 14.69% (n=286) LRTI, 11.92% (n=232) catheter-associated urinary tract infections (CAUTIs), 11.50% (n=224) bloodstream infections (BSIs), 9.45% (n=184) central line-associated bloodstream infections (CLABSI). Globally, the data on the relative incidence of SSI (17%) are comparable to ours. Another study for EU reported a lower frequency of SSI varied from 0.5% to 10.1%.[14] Our study shows the same trend with decreasing frequency in 2019 (12.50%) and increase in 2020 (23.10%).

Conclusions
The presented study shows that despite the lower number of patient admissions and the growing consumption of antibiotics, the annual actual hospital rate of HAIs was 0.5 % and did not exceed the national rates from 1 to 1.4%. At a hospital level, these facts are similar also for several key clinical HAIs categories - catheter-associated urinary tract infections (CAUTI), central line-related bloodstream infections (CLABSI), ventilator-related events (VAEs) [10,11]

The values below nationally infection rate are results of improved infection control but not only. Unfortunately, after a period of a stable state-related to the HAIs, followed a negligence and incomplete registration. These facts most clearly were expressed at the start of the COVID-19 pandemic in 2020, when healthcare systems were collapsing. The dominant nosocomial strain in all years has been *A. baumannii* with an average relative share of 18.59% and a constant increasing trend. The clinical structure is dominated by SSI with a relative share of 17.87% and a trend maintained for the period. Institutionally, relevant national guidelines, better surveillance system, adequate registration and an antibiotic stewardship is recommended in order to turn down the rising HAIs trends.

References:


Miglena Kolarova¹, Albena Toneva¹,², Kichka Dimitrova², Zhaneta Stoyanova², Teodora Dimitrova¹, Tsonko Paunov¹

¹. Medical University Varna, Bulgaria
². Multiprofile hospital for active treatment – Varna – Military medical academy

Abstract
The goal of healthcare is to create a safe environment in medical institutions for patients and staff. According to the retrospective analysis of the structure of healthcare associated infections in MHAT) - Varna at the MMA - Sofia for the period from 2010 to 2020. for the period 2010 to 2020, S. aureus, Pseudomonas spp., Escherichia coli are most often isolated in the intensive care units for infections defined as "other" infections. at the site of surgical treatment and associated with a urinary catheter.

Introduction
Healthcare-associated infections (HAIs/NI) are a global medical problem due to their widespread prevalence, the burden on the patient's health, staff, and the state's economy by prolonging hospital stays and increasing the cost of their treatment. Patients subject to invasive treatments and procedures have a higher risk of HAIs [5]. The mass character of medical care received, the widespread use of antibiotics, changes in the demographic structure of the population, the nature of pathogens, and the organism's immune reactivity are leading factors for the occurrence, development, and spread of HAIs. According to Ribarova (Nosocomial Infections, Sofia 2000, Simel Press), HAIs influence patients, their family members, staff, and healthcare facilities themselves. HAIs are registered in both developed and developing countries and affect approximately 1.4 million patients each day. The Center for Disease Control and Prevention (CDC) estimates that 1.7 million HAIs contribute to 99,000 deaths each year and are among the top ten leading causes of death. The number of death cases associated with HAI is highest in pneumonia and blood infections (bacteremia/sepsis). The highest morbidity is among ICU patients, followed by high-risk neonatal wards. [4, 5, 6, 7].

Objective: To study the frequency and structure of the HAIs in MHAT - Varna at the Military Medical Academy - Sofia for 2010 - 2020.

Material and methods: To achieve this goal, a retrospective analysis of the data on registered HAIs for a period of ten years (2010 - 2020) was applied, the procedures for registration of HAIs on the territory of MHAT - Varna at the Military Medical Academy were studied, as well as articles and publications linked to the topic of the report. The documentary method and the comparative analysis are supplemented by statistical data processing for HAIs from MHAT - Varna to MMA - Sofia. The results of the study are presented in graphical and tabular form.

Results and discussion
The analysis of the data from the register in MHAT - Varna to the MMA shows that for the period 2010-2020, were hospitalized 83602 patients and 7127 cases of HAIs were reported. The established average morbidity is 85.25 per 1,000 patients. There are no significant differences in the registered indicators concerning the number of hospitalized patients and the number of reported HAIs during the years for the observed period. The risk factors for the infections acquired in MHAT - Varna to
the Military Medical Academy (MMA) are elderly patients, immunosuppression, extended hospital stay, many major chronic diseases, invasive procedures, mechanical ventilation support. Two pathophysiological factors are required for the development of HAIs: decreased host protection and colonization by infectious agents such as Gram-positive coci (Staphylococci, Streptococci) and Gram-negative bacteria (Acinetobacter, Pseudomonas, Enterobacter, Klebsiella) from the environment, medical staff, other infected patients or attendants [2]. The etiological structure of HAI is variable, determined not only by the ward's profile but also related to the mass consumption of antibiotics, sometimes incorrect. Multidrug-resistant organisms (MDROs) become part of the patient's microbiome and can subsequently cause infections that are difficult or even impossible to treat. Most nosocomial infections arise from endogenous bacterial flora, although many critically ill patients eventually become colonized with resistant bacterial hospital strains. The pathogens caused by HAIs are characterized by significant environmental resistance, high virulence, invasiveness, and polydrug resistance. The analysis of etiologic diagnosis in MHAT - Varna for 2010-2020 indicates a wide variety of microorganisms with Staphylococcus spp. domination. Staphylococcus aureus led throughout the whole monitored period and was isolated in 10.50% of respondents. Staphylococcus epidermidis was diagnosed in 9.27 % of the cases. Most cases of HAIs caused by S.aureus were registered in 2015 and 2020, respectively 62 and 69. Pseudomonas aeruginosa was isolated from 69 cases (9.57%) in 2011. Escherichia coli was isolated in 8.52% of the studied cases (n = 56) in 2016, followed by other members of the family Enterobacteriaceae, which have relative shares between 2 - 4%. The relative share of "other", unconfirmed or unspecified pathogens is high - 41.83%, isolated in 1 904 cases, respectively. According to the nosological structure, HAIs are divided into 11 groups according to their localization in organs and systems. [3]. Leading place in the structure of the registered HAIs during the whole monitored period in MHAT - Varna - MMA are occupied by infections defined as "others" – with a relative share 36.49% (n = 2173 cases), followed by SSIs with a relative share 30.71% (n = 1829 cases). Urinary tract infections were registered in 729 cases with a relative share of 12.24%. (Fig.1).

**Fig.1 Registered healthcare associated infections by systems for 2010-2020**
The frequency of occurrence and spread of HAIs is directly dependent on the profile of the hospital ward due to differences in hospital structures and in the volume and severity of cases in MHAT - Varna at the MMA. Differences in the morbidity and structure of NI in the different departments of the medical institution are established. The wards with high risk for HAIs in the hospital are the ICU, followed by the wards with surgical profiles, where large in volume and duration surgical interventions of high-risk patients are performed. The Clinic of Thermal Trauma and Plastic Surgery is located on the territory of the hospital. Prolonged hospitalization in this critical care unit increases the risk of nosocomial infections. Medium-risk wards are surgical wards that perform clean, low-volume, short-stay surgeries in low-risk patients. Therapeutic profile wards are low risk (Fig.2).

Conclusions
For the period 2010-2020, the leading position among the isolated causative agents of HAIs is occupied by \textit{S.aureus} (10.50\%), \textit{P.aeruginosa} (9.57\%), \textit{S.epidermidis} 9.27\%), and \textit{E.coli} (8.52%). The microbiologically unproven infections have the highest relative share al of HAIs - 41.83\%. The relative share of HAI is highest in intensive care units (39.5\%), and leading in the nosological HAI-profile are infections defined as "others" (36.5\%), followed by SSIs (30, 71\%) and urinary tract infections (12.24\%).

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Monitoring the effect of a combined methodology in chronic pain syndrome in lumbosacral region

Georgi Stoyanov¹, Stamenka Mitova¹, Margarita Avramova¹

¹ Department of Kinesitherapy, Faculty of Public Health, Healthcare and Sports, South-West University “Neofit Rilski”, Blagoevgrad, Bulgaria

Abstract
Chronic lumbosacral pain affects 84% of people. Aim: The aim of the present study is to determine the effect of specialized tangentor combined with magnet and myofascial technique in patients with chronic pain syndrome in lumbosacral region. Methodology: The study included 62 persons with a proven chronic lumbosacral pain. Patients were randomly divided in two groups. Visual Analog Pain Scale and a Modified Merle d’Aubigne scale is used to assess pain and manual muscle testing to evaluate muscle weakness. Results: The results show improvement of the pain level, muscle weakness and quality of life for the EG patients. Conclusions: The study demonstrates the effectiveness and positive impact of myofascial techniques for patients with chronic lumbosacral pain.

Key words: chronic pain, myofascial, low back, spine

Introduction
Underwater jet massage stimulates blood circulation and lymphatic circulation, thus stimulate metabolism and contribute to the detoxification of the body. Common effects on tissues are muscle relaxation, tension reduction, improvement of peripheral blood circulation and microcirculation. Magnetotherapy is a non-invasive method in alternative medicine. Low back pain (LBP) is one of the most frequent disabilities reported in Western countries (Coulombre, et.al. 2017). Therapeutic exercise is a common conservative intervention used by clinicians to decrease pain, improve disability, and restore muscular function (Brian et.al, 2017). Lower back pain is the most common example of chronic musculoskeletal pain (IASP, 2017; Stoyanov et al., 2020). The aim of the present study is to determine the effect of this combined therapy in patients with chronic non-specific pain syndrome in lumbosacral region.

Material and Methods
Participants: A sample of 75 participants were evaluated. 62 of them met the inclusion criteria and were randomly divided in two experimental group (EG) and control group (CG). All participants (n=62) have proven chronic syndrome in lumbosacral region. The EG consists 34 participants mean age (X±SD) 46.36±5.61years. The CG was formed of 28 participants, mean age 46.29±6.81 years.

Test protocol and Instruments: VAS and Modified Merl d’Aubigne Scale were used to assess the pain threshold and manual muscle testing to assess muscle weakness, so the Roland-Morris Questionnaire to assess the quality of life of the patients. Procedure: The duration of kinesitherapy procedures for patients in both groups was 45 minutes, four times a week, for a period of 8 weeks. Patients in the EG were given a specialized tangent combined with a magnet every other day for the entire treatment period with a duration of the procedure of 15-20 minutes. Myofascial techniques were applied for 15 minutes 4 times a week. We used Muscle - energy techniques (MET), Position-release techniques (PRT) and Isometric contact of antagonists. Manual-soft tissue mobilization - was conducted 4 times a week. Specialized exercise – a combination with analytical exercise for back
and abdominal muscles. The CG received all the same treatment except the specialized tangenter therapy. **Statistical analysis:** We used the primary statistics variables like: Median (Mdn), arithmetic mean (±), standard deviation (Sd). In term to calculate statistically, significant differences we use Mann-Whitney test to compare independent quantitative variables (Glushkova et.al, 2014).

**Results**
Mean values obtained of VAS for the EG before and 8 weeks after specialized therapy were as follows: 7.18±0.69mm, and 3.96±0.53mm (Mann Whitney, p<0.001). For the CG mean values obtained for the CG before and after the administered complex therapy were as follows: 7.57±0.74mm, and 5.75±0.65mm after therapy, without statistically significant differences before and after treatment (Fig. 1. A.).

![Graphs showing results](image)

**Figure 1.** Dynamic of the results of VAS – A; Merl d’Aubigne Scale – B; MMT –muscles of the back – C Roland and Morris Quiessonnaire– D for the Experimental and Control groups

*** Statistically significant differences, Mann-Witney test p < 0.001 after complex therapy

** Statistically significant differences, Mann-Witney test p < 0.03 after complex therapy
Mean values of Merl d’Aubigne Scale for the EG are represented on the Fig. 1. B. The results of MMT for the back muscles before treatment for EG were 3.25±0.57 and 4.40±0.5 at the end. For the CG data shows 3.28±0.46 before and 3.78±0.42 8 weeks after therapy (Fig. 1 C.). MMT of m. rectus abdominis for the EG before and after therapy was respectively 3.44±0.5 and 4.5±0.5. For the CG before and after therapy was respectively 3.36±0.5 and 3.75±0.52. Mean values of the Morris and Roland questionnaire before and 3 months after therapy shows for the EG 14.94±1.92 before and 8.44±9.37 after therapy. For the CG were respectively 14.86±1.82 before and 11.0±1.41 after kinesitherapy (Mann Whitney, p<0.05) (Fig. 1. D.)

**Discussion**

The results show fast positive effect on the pain symptoms for patients of EG. Pain significantly reduce, and this in turn leads to improvement in range of movement and functionality of the spine (Mitova et al.,2020). We believe that this is due to the analgesic and anti-inflammatory effect of tangent therapy in combination with the magnet therapy and myofascial techniques (Bordoni. et.al. 2017). The use of a tangentor in combination with a magnet has a positive effect on the symptoms. The tangent allows painless, but at the same time deep massage of the tissues, which are directly affected. This in turn makes it much more effective as a recovery procedure. At the same time, the especially built-in magnet has been shown to have a positive effect on the musculoskeletal system. Manual-soft tissue mobilization with its profound impact increases hyperemia and trophic treated structures, which helps anesthesia by removing metabolic products. On the other hand, causes reflex and reduce regional nociception (Bourne, Talkad, Varacallo, 2020). Included in the experimental protocol exercise for stretching and relaxation of the shortened muscles in conjunction with analytical exercise post-isometric relaxation techniques, specific exercise increase mobility of the spine and contribute to the reduction of muscle imbalance. The myofascial approach not only helps to overcome the painful symptoms, but is also a valuable tool for rehabilitation (Andreev et.al. 2020). All this in turn lead to the improvement of functionality of the spine and better the quality of life of the patients with the studied pathology (Mitova, et.al, 2020).

**Conclusion**

The data presented demonstrates the effectiveness and positive impact of specialized tangenter with magnet and myofascial techniques for patients with chronic lumbosacral pain. The pain reduction leads to improvement of the range of movement and function of the spine. Finally yet importantly, patients in EG showed an improvement in their quality of life, their psycho-emotional state and the fear of re-suffering.

**References:**


Effective communication as a factor affecting adherence to pharmacotherapy

Silviya Mihaylova¹, Desislava Aleksandrova¹, Antoaneta Tsvetkova¹, Sylvia Stamova²

¹ Medical College, Medical University - Varna, Bulgaria
² Pharmaceutical Chemistry, Faculty of pharmacy, Medical University - Varna, Bulgaria

Abstract
Communication is a key element of health and pharmaceutical care which is directly related to the treatment outcome. This research aimed to assess the pharmacists’ perception of effective communication as a factor affecting adherence to the prescribed treatment. Anonymous individual questionnaires were distributed to community pharmacist’s. The questionnaire proposed 6 statements with five response options (also called the five-point Likert scale). Respondents unanimously agreed with the statements that good communication and trust in the doctor (96%), and the provision of pharmaceutical care (91.1%) had a positive influence and could lead to better adherence outcomes. Communication with patients, which reveals their preferences, needs and values, is a precondition for improving the degree of adherence to the treatment plan.

Keywords: effective communication, adherence, pharmacotherapy, pharmaceutical care

Introduction
The strategy for effective communication aims to improve the compliance and adherence to the treatment plan and to ensure control over the therapeutic effect of the treatment. By receiving professional information the patient gains competency, takes responsibility and gets the opportunity for self-management of the disease [1]. According to Melnikow and Kiefe patients do not follow the prescribed treatment strictly which is an indicator of poor communication and presents a significant problem in the provision of healthcare. The lack of good doctor-patient communication leads to serious costs for healthcare services and an increased number of hospital admissions [2]. Usually, patients do not understand the complicated professional language of doctors so in the pharmacy they can get answers to unasked questions about their medication regimen. Pharmacists are intermediaries between the doctor and the patient in providing timely prevention and treatment [3]. Through effective communication the pharmacist can acquire information about the patient’s condition, complaints and problems. The proper communication approach promotes an active patient involvement in the therapeutic process [4]. According to Col et al, pharmacists, with their knowledge of medicines, can identify and solve non-adherence problems and positively influence the outcome of different treatment plans through high quality pharmaceutical care [5]. The aim of this research is to study pharmacists’ perception of effective communication as a factor affecting adherence to the prescribed treatment.

Materials and methods
The survey was conducted online in May 2020 through a sociological method, using a Google form. Anonymous individual questionnaires were distributed to community pharmacist’s. The questionnaire proposed 6 statements with five response options (also called the five-point Likert scale, ranked from “Strongly disagree” to “Strongly agree”). Data was processed through software included in the Google forms.

Results
The results of the questionnaires are presented in Table 1.
Pharmacists’ perception of factors influencing adherence

<table>
<thead>
<tr>
<th>Statements</th>
<th>Strongly agree</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
<th>Strongly disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>The more medicines are prescribed to patients, less likely they are to take them.</td>
<td>31.5</td>
<td>47.6</td>
<td>18.5</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>The more frequent the dosing, less likely it is all doses to be taken.</td>
<td>35.5</td>
<td>40.3</td>
<td>16.1</td>
<td>7.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Good communication and trust in the doctor can result in improved adherence.</td>
<td>82.3</td>
<td>13.7</td>
<td>2.4</td>
<td>1.6</td>
<td>0.0</td>
</tr>
<tr>
<td>The pharmacist can positively influence adherence by providing pharmaceutical care.</td>
<td>75.0</td>
<td>16.1</td>
<td>8.1</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Adherence to pharmacotherapy in children is stricter because parents supervise it.</td>
<td>69.4</td>
<td>25.0</td>
<td>3.2</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>There are specific factors affecting adherence in patients over 65 years – polypharmacy, impaired vision, forgetfulness and higher sensitivity to ADR.</td>
<td>64.5</td>
<td>25.8</td>
<td>8.1</td>
<td>0.0</td>
<td>1.6</td>
</tr>
</tbody>
</table>

A total of 124 respondents participated in the survey of whom 74 (59.7%) were pharmacy technicians and 50 were pharmacists (40.3%). Regarding the first statement pharmacists either strongly agreed (31.5%) or agreed (47.6%) that polypharmacy reduced the likelihood of adherence to the prescribed regimen. Less than 3% of respondents (2.4%) expressed disagreement. Respondents strongly agreed (35.5%) or agreed (40.3%) with the statement that the more frequent the dosing, the less likely it was that all doses would be taken, whereas 8% disagreed with that. Respondents unanimously agreed with the statements that good communication and trust in the doctor (96%) and the provision of pharmaceutical care (91.1%) influenced adherence positively and could result in an improved adherence. Survey results regarding adherence in children and in patients over 65 were similar. Around 65% strongly agreed and 25% agreed with the suggested statements.

**Discussion**

Pharmacotherapy adherence is a challenge in contemporary healthcare systems. It is important for patients that communication also includes explanations about the causes of the symptoms, duration of the treatment, possible side effects and contraindications, the price of the medicines, the dosing frequency, the appropriate dosage form and the need for monitoring [6]. It is well known that adherence to the prescribed pharmacotherapy is affected by negative attitudes towards the medicines, inadequate knowledge, high costs or fear of possible side effects [7]. According to the World Health Organization there are 5 main factors influencing treatment adherence: the social and economic, health-care team and system-related, condition-related, therapy-related, and patient-related [8]. The six statements used in our survey are associated with two of these factors – health-care team and system-related, and therapy-related. The results of our study show that discussing the therapy-related factors will contribute for a higher adherence rate. As reported by Fernandez-Lazaro et al. when patients know their therapeutic plan a higher level of adherence can be predicted [9]. According to the results from this study, the pharmacist can positively influence the degree of treatment adherence by applying the concept of pharmaceutical care. In paediatrics, parents are an additional factor that impacts child adherence to treatment. In their communication with parents, it is essential for medical professionals to establish how much parents know about the disease, its seriousness and the need for treatment. Thus an adequate child adherence to
the therapeutic regimen will be guaranteed [10]. Communication with patients over 65 has its own specific features. Discussing the risk of drug-related problems as well as the fear of possible drug interactions can be a key to achieving patient adherence to the treatment. Other factors that should be considered in counselling about the pharmacotherapy are: patients’ reduced vision and hearing; strength and motor coordination; cognitive limitations; depression and social isolation; comorbidities; polypharmacy and other.

**Conclusion**

The results of this study correspond with other studies on the subject and confirm that good communication with patients can lead to better adherence outcomes. The introduction of a functioning digital healthcare in Bulgaria will provide new opportunities for an enhanced collaboration among the different healthcare professionals and the patient.

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Abstract
A quarter of the global cases of cancer are in Europe although its population is less than 10 % of the world population. The overall economic impact of cancer in Europe amounts to €100 billion annually and 40% of cancer cases are preventable. “Gynaecological transvaginal ultrasound examination for early detection of gynaecological conditions was conducted among 1800 women between 2017 and 2020 located in Varna in the North East Region of Bulgaria. Among the examined 1800 women, 527 pathological findings were detected where the largest share was that of women with uterine fibroids, followed by endometrial polyps, pelvic and ovarian tumours. The most affected group were women between 35 and 55, the peak age being 45-55.

Introduction
Cervical cancer continues to be a significant health issue that affects middle-aged women in less developed countries. According to data from the WHO it was the fourth most common cancer in women [1]. Cervical cancer is the second most common form of cancer in women between 15 and 44 in Europe. Annually, about 325.3 million women who have turned 15 are at risk of developing cervical cancer [2]. In 2020, an estimated 604 000 women were diagnosed with cervical cancer worldwide and about 342 000 women died from the disease [3]. Within the member states of the European Union there is a trend for reduction in mortality in the countries in Western and Central Europe and an increase of mortality in the countries from Eastern Europe. Data analysis of the situation in the Republic of Bulgaria for 2020 [4] shows that the incidence rate of cervical cancer is 28.2 per 100 000 women, compared to a European average of 15.0 per 100 000 women. The mortality rate of cervical cancer in Bulgaria - 14.1, is more than twice the European average of 6.71 per 100 000 women. While the average mortality rate of cervical cancer in the EU has been falling, the trend in Bulgaria is increasing. The five-year relative survival rate in Bulgaria, 55%, is also below the average of Europe which is 63%. Across Eastern Europe, Bulgaria ranks third in terms of cervical cancer mortality rates after Romania and Moldova.

The registered cases of malignant neoplasms in 2016 in the province of Varna were 18 631 in total. The relative number was 3940.7 against a country average of 4036.0 (per 100 000 people). The highest number was that of female breast cancer (1411.6) and gynaecological cancers (1026.8). The rate of new cases of cancer in 2016 in the province of Varna was 454.2 compared to a county average of 435.5 (per 100 000 people). The highest rate belonged to gastrointestinal cancer (113.6) and female breast cancer (108.7) [5]. These data indicate the need for an intense focus on evidence-based preventive approaches in healthcare. Numerous studies have shown that preventive health screenings and primary care consultations significantly increase the life expectancy, especially in the age group from 30 to 49 [6]. Cancer prevention and promotion of healthy lifestyles also help fight obesity and other non-communicable diseases such as cardiovascular diseases and diabetes since they have common risk factors. About 3 % of healthcare budgets are used for health promotion and disease prevention [7]. Without taking additional action, the annual number of new cases of cervical cancer is expected to increase from 570 000 to 700 000 between 2018 and 2030, while the annual number of deaths is projected to rise from 311 000 to 400 000, mostly in the developing countries [8]. In pursuance of an early detection and screening of reproductive diseases in women over 18, a municipal programme was
launched in 2017 in HSOGN - Varna. The programme ‘Preventive healthcare for women: transvaginal ultrasound examination’ ran between 2017 and 2020 covering 1800 female residents of the Municipality of Varna.

**Results and discussion**

**Target group:** Female residents of the Municipality of Varna with gynaecological complaints and symptoms.

**Implementers:** Obstetrician-gynaecologists from HSOGN–Varna OOD, certified for the relevant diagnostic methods.

The majority of the pathological findings described below were identified for the first time and the patients were not aware of their existence. Comparability of the results of the pilot study is presented in Table 1.

**Table 1: Summary of the detected pathological findings**

<table>
<thead>
<tr>
<th>Pathological findings</th>
<th>15-20</th>
<th>21-25</th>
<th>26-30</th>
<th>31-35</th>
<th>36-40</th>
<th>41-45</th>
<th>46-50</th>
<th>51-55</th>
<th>56-60</th>
<th>60+</th>
<th>Total number of findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine fibroid</td>
<td>2</td>
<td>9</td>
<td>16</td>
<td>21</td>
<td>49</td>
<td>83</td>
<td>49</td>
<td>30</td>
<td>12</td>
<td>271</td>
<td>527</td>
</tr>
<tr>
<td>Endometrial polyp</td>
<td>2</td>
<td>4</td>
<td>11</td>
<td>18</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>59</td>
</tr>
<tr>
<td>Endometrial hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>8</td>
<td>7</td>
<td>5</td>
<td>6</td>
<td>14</td>
<td>3</td>
<td>55</td>
</tr>
<tr>
<td>PCOS</td>
<td>3</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>11</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Descensus/Prolapse</td>
<td></td>
<td>1</td>
<td>2</td>
<td>5</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Tumours in the true pelvis</td>
<td></td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>3</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Infertility</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td></td>
</tr>
<tr>
<td><strong>Total number of findings</strong></td>
<td>1</td>
<td>14</td>
<td>33</td>
<td>46</td>
<td>60</td>
<td>91</td>
<td>114</td>
<td>78</td>
<td>58</td>
<td>32</td>
<td>527</td>
</tr>
</tbody>
</table>

The largest share of identified pathological findings was in the age when hormonal changes occurred and early signs of perimenopause were observed. The main symptoms of uterine fibroids noted in the case histories were heavy menstrual bleeding or bleeding between periods. The study subjects reported abdominal and pelvic pain, problems with urination and bowel movements and increased vaginal discharge. However, they did not attach significance to those symptoms despite their duration. Due to a low level of awareness among patients about the importance of annual gynaecological exams pathological findings in the study sample detected during the first two years of the programme were disturbing. The incidence rate of pathological findings in the sample group during the programme period was 29.28%. The proportion of women below the age of 40 diagnosed with endometrial polyp is worrying since it is more likely in women between 40-50 years old. The pathological changes found in the study group indicate an increased incidence rate of PCOS and ovarian cancer in younger age groups.

Ovarian cysts are the most common ovarian condition and it is considered that almost every woman in childbearing age has been diagnosed with an ovarian cyst. A cause of concern is that ovarian cancer has been diagnosed among the younger age groups. The risk of developing ovarian cancer increases by 5% with every year of regular hormone use.

According to information from the patients’ medical records, a large part of the study subjects with ovarian tumours reported previous hormonal regulation of their ovulation (stimulation/suppression). The
growth in the number of detected uterine fibroids and endometrial polyps in the younger age groups is determined by the fact that these conditions are often asymptomatic and are found incidentally. In such cases the fibroid can be detected during a visit to an internal medicine specialist for another health issue. In women of reproductive age who are planning a pregnancy this might cause a problem both in terms of the fibroid growth and in terms of pregnancy complications. Screening examinations are needed to prevent reproductive health problems in women and to reduce or remove pathological findings. The lower incidence of diagnosed ovarian cancer and PCOS is subject to the early signs experienced by patients which urges them to look for a solution to the problem or provokes a consultation with a specialist. With a proper diagnosis and behaviour the reproductive function is restored, the survival rates and quality of life are improved. Regarding the pathological findings in other organs in the true pelvis, an upward trend is observed due to an increase in the incidence of diseases of the urinary system, the digestive system and the surrounding tissues. These diseases have different origins (diet errors; various infection agents; genetic predisposition and other factors) but they also affect the reproductive function and quality of life, and might lead to the development of other disorders.

Conclusion
The success of the programme is significant and women have shown interest in the modern diagnostic tools. The benefits of the initiative are tangible and lead to improving the attitudes towards reproductive health and an increased personal health responsibility. The programme has helped women of different ages, from the Municipality of Varna to pay attention to reproductive health issues. Some of them have received relevant treatment and have become more responsible and informed about the health problems associated with each of the life stages.

References:
1. World Health Organisation https://www.who.int/health-topics/cervical-cancer#tab=tab_1
Effects of CB1 antagonist on mechanical nociception in olfactory bulbectomized rats

Dobrinka Doncheva¹, Mariya Ivanova¹, Roman Tashev², Margarita Velikova¹

1) Department of Physiology and pathophysiology, MU-Varna, Bulgaria
2) Department of Physiology and pathophysiology, MU-Sofia, Bulgaria

Abstract

The endocannabinoid (ECS) system is one of the endogenous systems involved in the control of pain. It consists of the endocannabinoids (EC), the two main types of cannabinoid receptors (CB1 and CB2) and the enzymes for the synthesis and degradation of EC. CB1 receptors are primarily expressed in the CNS; their activation mediates most of the physiologic effects of the EC. Abnormal activity of the ECS has been implicated in the development of depressive disorders [1]. Studies suggest that depression is associated with altered pain perception [2]. Previously we have demonstrated modulatory effect of CB receptor ligands on pain withdrawal thresholds in rats with a model of depression – olfactory bulbectomy [3]. The aim of our study was to examine the effect of selective CB1 receptor antagonist SR141716A on nociception of rats.

Introduction

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Materials and methods

Surgical procedure. Bilateral olfactory bulbectomy (OBX) was performed according to the method described by Kelly et al. [4]. SR141716A (Rimonabant,RIM), (Sanofi, France) was administered intragastrally at a dose of 3 mg/kg.

Animals. The experimental animals (male Wistar rats), were divided into 3 groups where RIM was administered for 7, 14 and 21 days after bulbectomy. The control groups, treated with saline included: sham-operated and OBX rats, tested 7,14 and 21 days after OBX. The sham surgery was performed in the same way as in the case of olfactory bulbectomy without removal of the olfactory bulbs.

Assessment of mechanical nociception (paw-pressure test). The changes in the nociceptive response were determined by the paw-pressure test, using analgesimeter. A gradually increasing pressure was applied to the dorsal surface of 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 (paw withdrawal) threshold.

Statistical analysis. Data were analysed by one-way ANOVA and further analysed by post hoc Student-Newman-Keuls (SNK) test.

Result and discussion

ANOVA demonstrated a significant effect for factor drug ($F_{5,47} = 8.951; P \leq 0.001$) on pain threshold. Rimonabnt significantly decreased the pain threshold after 7 ($P \leq 0.04^*$), 14 ($P \leq 0.04^*$) and 21 days ($P \leq 0.05^*$) of administration, as compared to the respective sham-saline treated controls (Fig. 1).
ANOVA showed a significance for factor bulbectomy (F_{1,16} = 41.213; P \leq 0.002). Post-hoc SNK test demonstrated that the olfactory bulbectomy increased of the pain threshold (P \leq 0.001^{***}) as compared to the sham operated controls. ANOVA demonstrated a significant effect for factor day (F_{6,55} = 3.315; P \leq 0.005). Rimonabant, given 14 days to OBX rats produced a significant antinociceptive effect by increasing the pain threshold as compared to both saline- treated OBX rats (P \leq 0.002^{**}) and to the sham-operated rats (P \leq 0.004^{***})(Fig.2). OBX rats, treated with RIM for 7 or 21 days after bulbectomy, did not show any significant difference in the pain threshold as compared to the respective saline-treated OBX-controls; the threshold was higher (P \leq 0.004^{***} and P \leq 0.04^{*} respectively) as compared to the sham-operated controls (Fig.2).

It is well known that cannabinoids act on multiple nociceptive pathways and elicit antinociceptive effects [5]. Numerous studies provided evidence that the cannabinoid agonists inhibit pain behavior in different experimental models [6]. The nociceptive effect of SR141716A observed by us in the sham-operated rats supports the data for the functional role of CB1 receptor in pain control [7]. The olfactory bulbectomy as a model of depression induces biochemical, neurotransmitter, physiological, behavioral, etc. changes in rodents. The nociceptive response in the paw-pressure test, where the threshold to mechanical pressure stimulation is assessed, was higher in OBX rats. Interestingly, the CB1 antagonist showed antinociceptive effect in OBX rats when administered intragastrically 14 days after OBX. This finding was unexpected, as far as the ability of SR141716A to attenuate the antinociceptive effects of cannabinoid agonists have been demonstrated in several experiments [8]. Our previous studies revealed that the 7 day intracerebroventricular injection of Rimonabant increased pain sensitivity to mechanical pressure i.e., produced hyperalgesia [3].

**Conclusion**

The blockade of the CB1 receptors by 14 day intragastral administration of SR 141716A increased the mechanical response thresholds in OBX rats. The results suggest that abnormal functioning of the ECS may contribute to the altered mechanical nociception in rats with an OBX model of depression.

**Fig. 1.** Effects of Rimonabant administered per os for 7, 14, 21 days in sham-operated rats on nociception. Asterisks depict comparisons of pain threshold (AU) in sham RIM-treated rats vs. respectively sham saline-treated rats. n = 8. *P ≤ 0.05. Means (± S.E.M.) are presented.

**Fig. 2.** Effects of Rimonabant (SR141716A), administered per os for 7, 14 and 21 days on nociception in OBX rats. Asterisks depict comparisons of pain threshold (AU) in RIM-treated OBX rats vs. OBX
saline-treated rats. Circles depict comparisons after 7, 14 and 21 days RIM treated OBX rats vs. sham operated saline-treated rats. n = 8. *P ≤ 0.04, **P ≤ 0.02, ***P ≤ 0.004 Means (± S.E.M.) are presented.

References:

Lipid emulsion reduces complications and mortality in patients with severe drug intoxications

Gabriela Kehayova¹, Snezha Zlatevaⁱ, Petko Marinovⁱ, S. Dimitrova¹

1) Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University, Varna, Bulgaria

Abstract

Purpose: The incidence of acute drug intoxications with antidepressants, neuroleptics and cardiovascular drugs is constantly increasing worldwide. In recent years, intravenous lipid emulsions (LEs) have been successfully used as part of complex therapy. Aim: The aim of the study was to compare the incidence of complications and mortality in patients with acute drug intoxications, some of whom have received LE to standard therapy. Material/methods: The object of the study was 265 patients with severe drug intoxications that have passed through the Clinic of Toxicology at the Naval Hospital in Varna during the period 2010-2018. Statistical and documentary methods were used. Results: Mortality in the LE group was 2.29 times lower than in patients, treated with standard resuscitation therapy alone. Conclusion: treatment with LE is clinically more effective than treatment with standard therapy alone in acute intoxications with cardiotoxic drugs.

Key words: drug overdose, lipid emulsion, mortality

Introduction

Acute drug intoxication is a serious global problem. The most commonly overdosed drugs are antidepressants, neuroleptics and cardiovascular drugs. In case of overdose, these drugs cause severe and sometimes fatal cardiovascular complications despite timely treatment. In recent years, intravenous lipid emulsions (LEs) have been successfully used as part of complex therapy of severe intoxications with lipophilic drugs and local anesthetic systemic toxicity. According to the American Society of Regional Anesthesia guidelines, the use of LE includes bolus intravenous dose LE 1.5 ml/kg for one minute followed by continuous intravenous infusion of 0.25 ml/kg/min for 20 - 60 min (15 ml/kg for 60 min) until circulation is restored [1]. The aim of the study was to compare the incidence of complications and mortality in patients with acute drug intoxication, some of whom have received LE to standard therapy.

Materials and methods

The object of study was 265 patients with severe drug intoxications that have passed through the Clinic of Toxicology at the Naval Hospital in Varna for the period 2010-2018. Patients were divided into two groups: a control group without LE, treated only with standard therapy (216) and a study group receiving LE to standard therapy (49). The study is retrospective and covers an 8- year period. Access to medical records is provided with the permission of the Ethics Committee of the Naval Hospital in Varna. The history of the disease, the conducted treatment, the performed laboratory and instrumental examinations and the outcome of the conducted therapy in the Clinic of Toxicology at the Naval Hospital in Varna were studied. The statistical analysis was performed using the statistical functions of "Microsoft 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 shows that both groups of patients- control without LE and study group with LE are comparable in demographics and comorbidity (P<0.05). Acute drug intoxications are more common among women up to the age of 45 (P<0.05). Depression is the most common in both groups (P<0.05). Intoxications with antidepressants, neuroleptics and cardiovascular drugs are the most common in both
groups (P<0.05). In drug poisoning with both direct and indirect cardiotoxic effects such as antidepressants and neuroleptics, LE reduces bradycardia, normalizes high heart rate in atrial fibrillation, normalizes prolonged QT interval, overcomes arterial hypotension and shock. In acute intoxications with drugs with direct cardiotoxic effect (verapamil), LE manages prolonged QT-interval, AV-block, sinus bradycardia, arterial hypotension and shock. LE significantly reduces metabolic acidosis, hyperglycemia and hypoxemia (P<0.05). No acute renal impairment, cerebral edema and multi-organ failure were observed in the study group with LE (P<0.01).

Mortality in patients, treated with LE is 2.29 times lower than in those treated without LE and this statement is true with 95% probability (P<0.05).

No side effects in hemodynamics, pulmonary, renal, hepatic function and fat metabolism were observed in any of the patients treated with LE. LE does not lead to: increased transaminases, pulmonary edema, thrombocytopenia, does not impair renal function and does not increase cholesterol and triglycerides (P<0.05).

Discussion

The results show the highest incidence of suicide attempts with cardiotoxic drugs such as benzodiazepines, antidepressants, calcium channel blockers, beta blockers and cardiac glycosides. Similar data are available from other studies [2]. The analysis shows that acute drug intoxications are most common among the working population and are more common among women and in people with depression. Our data on suicide demographics are similar to other studies [3]. Comparable to our results are the studies of other researchers regarding the effect of cardiac conduct by LE. According to these studies LE stabilizes hemodynamics and overcomes shock in the first hours of infusion [4]. Successful therapy of verapamil and diltiazem intoxication with LE has been confirmed by other researchers [5,6]. Our study confirms the benefit of LE because in the group who ingested twice the dose of verapamil, but treated with LE, there were no deaths. The study coincides with the opinion of other authors, who also confirm the safety of LE [7].

Conclusion

The treatment with LE is clinically more effective than treatment with standard therapy alone in acute intoxications with cardiotoxic drugs. LE reduces mortality 2.29 times than in the control group as well as the frequency and severity of complications in the course of intoxication. LE is safe because no pulmonary edema, liver, kidney damage or thrombocytopenia have been reported in any patient.

Abbreviation list:
LE- lipid emulsion

Acknowledgement

Special thanks to prof. Snezha Zlateva and prof. Petko Marinov for the support and valuable advice in conducting the study.

References:


Address for correspondence:
Gabriela Kehayova, MD, PhD
Chief Assistant Professor, Department of Pharmacology, Toxicology and Pharmacotherapy, Faculty of Pharmacy, Medical University, Varna, Bulgaria
E- mail: gabi_stier@yahoo.com
**Physical assessment of patients with anorexia nervosa and depression**

Rosica Popova¹; Maria Nikolova²

¹Sofia University ”St.Kl.Ohridski”; University Hospital “Lozenetz”
²Department of Epidemiology and Hygiene; Medical University-Sofia

**Abstract**

Anorexia nervosa (AN) is an eating disorder and often is combined with depressive symptoms. PURPOSE: To find the correlation between the degree of malnutrition and the degree of depression among patients with AN.

MATERIAL and METHODS: We analyzed 38 patients with AN by anthropometric measurements, biomarkers, Beck’s depression inventory (BDI) and statistical methods.

RESULTS: We measured the body composition of patients - 50% are with mild, 31.5% with moderate, 5.3% - with severe malnutrition, 13.2% - no malnutrition. From biomarkers - Lymphocytes, Transferrin, K⁺, BUN and 25-OH vit D were under the reference values. According the results of Beck’s test we found that all the patients with severe and extreme depression showed impaired biomarkers and anthropometric indicators.

CONCLUSION: There is a significant relationship between the severity of depression and severity of malnutrition among patients with AN.

**Key words:** Anorexia nervosa, Depression, Malnutrition

**Introduction**

AN is a mental disturbance of dietary behavior with a constant confusion of dietary habits and weight control, and that leads to significant impairment of physical health and psychosocial functions. [1] AN is usually related with malnutrition which is associated with macro- and micronutrient deficits. Depression is a mental disturbance which is associated with constant sadness and lack of interest to usual activities that makes us happy, together with inability to do every day obligations for a period minimum of 2 months. [2] Many studies confirm that the progression of AN is associated with the presence of depression. [3]; [4] Usually, AN is associated with less food and energy intake which leads to insufficiency of some nutrients, strictly related to our mood and their deficiency leads to deterioration of depression. The severity of depression with its consequences also impact on the model of food intake and the appearance of nutrient deficiencies.

**Objective**

The aim of our study was to find the correlation between the degree of malnutrition and the degree of depression among patients with AN, to predict the outcome of the underlying disease.

**Materials and methods**

We analyzed 38 patients with AN according to the 3 basic criteria of Diagnostic and Statistical Manual of Mental Disorders (DSM-5). [5] We used the following methods: 1) Anthropometric methods – by Bio-impedance analysis (BIA) we measured the body composition of patients – Fat mass (FM), Fat-free mass (FFM), Total Body Water (TBW), Body Mass Index (BMI) (kg/m²) = Body weight (kg) / height (m²) ; 2) Biomarkers – Complete Blood Count, Serum iron, Transferrin, Albumin, Na, K, Cl, Ca, P, BUN, TSH, 25-OH Vit D; 3) BDI for depression – 21 questions with four expected answers; 4) Statistical methods for calculation of average value, median and coefficient of correlation - a p-value <0.001 had been considered statistically significant for all tests.
Results
The group of AN patients had average age of 22.8 years. Ten of the patients were adolescents and we used the anthropometric indices BMI-for-age which are applied according the discriminative criteria of WHO for assessing growth and development of children. [6] The referent value of Fat Mass (FM%) for men was 13-29.3% and for women – 26.1 - 39.6% [7] According to the standards of BMI and FM (%), 86.8% (33 patients) had low BMI and all of them (100%) - low FM (%). The average BMI was 17.8 kg/m² and the average FM (%) was 15.2%. BMI usually shows the functional capacity and the survival rate. According to BMI the categorization of malnutrition among our patients was as following:

- 17-18.49 kg/m² – mild malnutrition – 19 patients (50%)
- 16-16.99 kg/m² – moderate malnutrition – 12 patients (31.5%)
- <16 kg/m² – severe malnutrition – 2 patients (5.3%)

Only 13.2% (5 patients) had normal body weight. Systematic analyses of randomized trials show that the level of malnutrition determines the higher risk for co-morbidity and mortality. [8] Those patients (36.9 % - moderate and severe) need strict monitoring of the health and nutritional status. Besides, we measured the FFM and calculated the FFMI according the formula: FFMI = FFM / H (m²) (FFMI – fat free mass index; H – height), as for men FFMI>17 kg/m² and for women FFMI>15kg/m². All the patients with AN had lower FFMI which is related with high risk of osteoporosis, heart failure and other metabolic disorders. [9]

From biomarkers we found abnormal values of Transferrin, K⁺, Lymphocytes, BUN (blood urea nitrogen), 25-OH Vit D. Patients with AN usually have low-protein diet which influence adversely on Transferrin`s values and we had 5 patients with values under the reference level. The low concentration of potassium was found in 8 patients which was associated with high risk of cardiac arrest, cardiomyopathy, tetany and encephalopathy [10]. Patients with AN showed mainly low BUN or close to the normal range. The reason was the low protein intake and the exhausted muscle mass. All the AN patient had low Vit D. It is well known its antidepressant effect. [11] The low lymphocytes are associated with malnutrition, especially T lymphocytes are affected, and more specific the ratio CD4:CD8. It is established that adequate nutritional support increases the ratio and the number of the T lymphocytes. More than half of our patients had lymphocytopenia (less than 1.9·10³ cells/ml).

Beck’s Depression Inventory (BDI) is easy to implement and according to the total points we assessed the severity of depression. To simplify the analysis, we divided the patients into 3 groups – the first group is with no depression (3;8%) and mild disturbance (4;11%), the second – with borderline (5;13%) and moderate depression (15;39%) and the third – with severe (8; 21%) and extreme (3;8%) depression.
Tabl. 1 Average values of the abnormal anthropometric and biochemical results in the 3 groups distributed by BDI

The abnormal biomarkers and anthropometric measurements that characterized malnutrition are shown on table 1 and we compared them with the severity of depression - results of BDI (average points of the groups). The correlation coefficient \( r \) is between -0.993 (vit D) to -0.927 (BMI) which means there is a strong inverse linear relationship between the anthropometric values and biomarkers to the level of depression, or as severe is the malnutrition as more depressive are the patients. We posed the null hypothesis that there was no relationship between above parameters and the points of depression, we rejected it because \( p<0.001 \) which proved that there is a significant correlation between malnutrition and depression.

**Conclusion**

The anthropometric measurements and the biomarkers of the AN patients show inversely proportion to the degree of depression. Our study suggests the need of detailed assessment of depression among patients with AN because the symptoms of depression aggravate the malnutrition of AN and the outcome of the disease. The severity of depression influence on the evolution of AN as the extreme depression suggest bad prognosis and the moderate depression is a good predictor of gaining weight and improving the psychosomatic status of the patients.

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**Address for correspondence:**
Rosica Popova
Department of Neurology, Psychiatry, Physical Therapy and Rehabilitation, Preventive Medicine and Public Health, Medical Faculty, Sofia University “St.Kl.Ohridski”; University Hospital “Lozenetz” Kozjak Str №1, Sofia, Bulgaria; e-mail: rosical702@yahoo.com
Bioenergetic Metabolism of Patients with Parkinson’s Disease – a Pilot Study

Danail Minchev¹,², Valentina Mihaylova¹, Valcho Naydenov³,⁴, Maria Kazakova¹,², Anastasiya Trenova³,⁴, and Victoria Sarafian¹,²

¹Department of Medical Biology, Medical University – Plovdiv, Bulgaria
²Research Institute at MU-Plovdiv; Plovdiv, Bulgaria
³Department of Neurology, Medical University – Plovdiv, Bulgaria
⁴Department of Neurology, University Hospital “Kaspela”, Plovdiv, Bulgaria

Abstract

Impaired bioenergetic functions have been previously demonstrated as significant pathogenetic factors of Parkinson’s disease (PD). Reactive oxygen species, mitochondrial mutations, and mitochondrial dysfunction have been proved to play a crucial role in PD pathogenesis. This pilot study includes eight PD patients undergoing a conservative (L-DOPA or Dopamine-agonists) three-month therapy. The Mito Stress protocol was applied on isolated PBMCs to obtain the bioenergetic profiles of the PD patients. All metabolic experiments were performed using a Seahorse XFp machine. Our results show alterations in several parameters of mitochondrial respiration. After therapy, the Spare Respiratory Capacity decreases by approximately 20%, while the Coupling Efficiency parameter marks a significant increase by almost 40%. In conclusion, mitochondrial function is altered in PD patients. Further research efforts on a larger group of patients are still needed for the complete elucidation of the antiparkinsonian therapy on mitochondrial functions.

Introduction

Parkinson’s disease (PD) is the second most common neurodegenerative disorder worldwide. A growing body of evidence has suggested that mitochondrial dysfunction plays a crucial role as a pathogenetic mechanism in PD (Błaszczyk, 2016) [1]. Numerous studies have described elevated oxidative stress, decreased levels of antioxidants, and frequent mitochondrial mutations in post mortem brain samples from PD individuals (Malpartida et al., 2021) [2]. Moreover, impaired bioenergetic function in isolated peripheral blood mononuclear cells (PBMCs) and fibroblasts from PD patients were also observed (Teves et al., 2018) [3]. In this context, the present study aims to investigate the metabolic profiles of isolated PBMCs from PD patients and to evaluate the possible association between cellular energy metabolism and disease progression.

Materials and Methods

The experimental design of the present study was approved by the Ethics Committee of the Medical University – Plovdiv (Protocol № 6/2021). Eight PD patients undergoing antiparkinsonian therapy (four with L-DOPA, four with pramipexole) for three months were included in the study. The diagnosis was confirmed in all patients by a team of certified professionals from the Department of Neurology according to the internationally accepted criteria [MDS-PD (Movement Disorder Society – Parkinson’s Disease) and UPDRS (Unified Parkinson's Disease Rating Scale)]. Blood samples were collected from the patients before treatment initiation and after three months on a stable-dose therapy by a standard venipuncture. Peripheral blood mononuclear cells (PBMCs) were isolated from the PD patients via gradient centrifugation (with Pancol). Prior to analysis, the isolated PBMCs were stored overnight in a complete RPMI medium (supplemented with 10% fetal bovine serum) in a 5% CO₂ incubator at 37°C. Bioenergetic experiments on isolated PBMCs are performed with a Seahorse XFp flux analyzer using a Mito Stress protocol according to the manufacturer’s procedure. The Seahorse XFp analyzer measures two basic metabolic rates simultaneously: extracellular acidification rate (ECAR) and oxygen consumption rate (OCR), indicative of glycolysis and mitochondrial respiration, respectively (Legmann et al., 2011) [4].
Results and Discussion

The sequential injection of oligomycin, FCCP, and rotenone allows the calculation of a set of parameters reflecting mitochondrial function, such as Basal Respiration, Maximal Respiration, Proton Leak, Spare Respiratory Capacity, and Coupling Efficiency. From a mathematical point of view, the Spare Respiratory Capacity is the difference between the basal level of OCR and the Maximal Respiration, reached by the mitochondria following the addition of FCCP. In turn, the Coupling Efficiency parameter represents the percentage ratio of the ATP Production and the Basal Respiration. It represents the actual proportion of basal respiration OCR used by the cells to sustain the ATP production to meet their energetic needs. By measuring the previously mentioned respiratory parameters, we discovered that the complex treatment approach reduces the FCCP-dependent OCR and Spare Respiratory Capacity in PD PBMCs. Moreover, our pilot metabolic experiments indicate that the Coupling Efficiency parameters change significantly in PBMCs of PD patients following a three-month treatment course. While the Spare Capacity shows a decrease of about 20% on average, the Coupling Efficiency increases by almost 40% (Figure 1). Our findings are in accordance with previous studies describing alterations in the OCR parameters in different neurological pathologies. Since the energy requirements of neurons are high, mitochondrial dysfunction may play a crucial role in neurodegenerative diseases, as several emerging studies suggest. For instance, Bell et al. (2020) [5] have assumed that the Spare Respiratory Capacity in fibroblast correlates with early neuropsychiatric symptoms of Alzheimer’s Disease (AD). This correlation together with our experimental results suggests that moderate changes in the activity of the electron transport chain in mitochondria can significantly affect the disease phenotype. A reduced mitochondrial ATP production was also found to correlate with the downregulation of the PD-related gene PINK1 (Ghandi et al., 2009) [6] in human neurons. Another key protein factor involved in PD development, α-syn, was proven to modulate the activity of ATP synthase by interacting with its α subunit (Ludtmann et al., 2016) [7]. Since the number of patients examined in the present study is relatively low, our results are inconclusive in terms of the possible effects of the therapy on mitochondrial function.

Figure 1. A) Difference in spare respiratory capacity in PD patients before and after therapy B) Difference in coupling efficiency in PD patients before and after therapy.

Conclusions

This is the first study to present a functional analysis of cellular bioenergetic metabolism in a group of Bulgarian patients with PD. Our results suggest moderate alterations in several parameters of mitochondrial respiration.
Acknowledgements
This study was supported by MU-Plovdiv – Grant № DPDP 01/2021

References
Potential antimicrobial activity of new metronidazole derivatives against pathogenic clinical isolates

Sylvia Stamova¹, Neli Ermenlieva², Emilia Georgieva³, Svetlana Georgieva¹

1) Department of Pharmaceutical chemistry, Faculty of Pharmacy, Medical University “Prof. Paraskev Stoyanov” - Varna, Bulgaria
2) Department of microbiology and virusology, Faculty of Medicine, Medical University “Prof. Paraskev Stoyanov” - Varna, Bulgaria
3) Training sector ”Medical Laboratory Assistant”, Medical college, Medical University “Prof. Paraskev Stoyanov” - Varna, Bulgaria

Abstract
The present study aims to investigate potential antimicrobial activity of new metronidazole derivatives against clinical isolates of Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Candida albicans. A total of six different concentrations (6.25-200 µg/ml) of two newly synthesized metronidazole derivatives (MTD1 and MTD2) were tested by disc diffusion method of Kirby-Bauer on Mueller-Hinton agar. The selection of solvents was very precisely according to solubility of new compounds. Therefore, we have used the following solvents: NaCl 0.9%, Phosphate buffer pH 7.2, methanol and ethanol.

The methanol and ethanol solutions of metronidazole derivatives showed antimicrobial activity against S. aureus and C. albicans. Derivatives MTD1 and MTD2 (concentrations 100 µg / ml and more) demonstrated inhibition zones of 12-14 mm, both against S. aur derivatives-Ethyl 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide) benzoate (MTD1) and Butyl 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide) benzoate (MTD2)eus and against Candida. For each sample in NaCl 0.9% and Phosphate buffer there was not detected antimicrobial activity more than the controls. The tested derivatives did not show antibacterial activity against E.coli and B. subtilis strains.

Keywords: new amide derivatives, metronidazole, antimicrobial activity, clinical isolates

Introduction
Antibacterial resistance is a global problem, and it affects millions of 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]. 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 [2]. New different antimicrobial amides [3,4] and peptides (AMPs) have received highlighted attention as a possible alternative approach to fighting infections caused by antibiotic-resistant bacterial strains [1,5].

The amide bond is the critical bond that forms the backbone of peptides, proteins, and a wealth of other biomolecules [6,7]. Medicinally, this group is significant for amide-forming reactions. It is worth noting that it is evaluated to be the most common reaction carried out in the pharmaceutical industry [7,8] and approximately a quarter of all marketed drugs (and two-thirds of all drug candidates) contain at least one amide bond [7,9]. A consideration of how amides interact with biological targets is also a key aspect of drug discovery [7,10]. In addition, therapeutic amides and peptides have high selectivity, efficacy, and low toxicity [11].

The presented literary data pointed our research on synthesis and evaluation of amide derivatives of metronidazole. The present study aimed to evaluate the potential antimicrobial activity of new metronidazole derivatives against clinical isolates of Staphylococcus aureus, Bacillus subtilis, Escherichia coli and Candida albicans.
**Materials and methods**

Two new amide metronidazole derivatives—Ethyl 4-((2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide)benzoate (MTD1) and Butyl 4-((2-(2-methyl-5-nitro-1H-imidazol-1-yl)acetamide)benzoate (MTD2), non-described in literature, were synthesized and structurally characterized by IR, NMR spectroscopy and TLC, HPLC chromatography methods [12].

Antimicrobial activity of MTD1 and MTD2 against *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans* were tested with two diffusion susceptibility tests—disk diffusion method. The pathogenic strains of *E. coli*, *S. aureus*, *B. subtilis* and the fungal strain *C. albicans* are clinical isolates of urine, nasal secretions, faeces and wound secretions, respectively.

**Kirby-Bauer disk diffusion susceptibility test**

The technique includes 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 (MTD1, MTD2 and metronidazole as a control sample). Each sterile filter disk (HiMedia®, provided by Ridacom, Bulgaria) was placed for five seconds in the appropriate test solution, sufficient for complete irrigation. Controls were set for the four diluents, which were used for the preparation of suspensions of the active substances—NaCl 0.9%, Phosphate buffer pH 7.2, methanol and ethanol. The samples were incubated aerobically for 24 hours at 37°C. All samples were made in triplicate.

**Results and discussion**

Antimicrobial activities of metronidazole derivatives are presented in Table 1.

**Table 1.** Reporting of antimicrobial activity of metronidazole and two metronidazole derivatives (MTD1 and MTD2) against *Staphylococcus aureus* and *Candida albicans* (inhibition zones in mm).

<table>
<thead>
<tr>
<th>S. aureus</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>µg/ml</strong></td>
<td><strong>Solutions with CH₃OH</strong></td>
</tr>
<tr>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>-</td>
</tr>
<tr>
<td>MTD1</td>
<td>-</td>
</tr>
<tr>
<td>MTD2</td>
<td>-</td>
</tr>
</tbody>
</table>

Legend: (-) - no zone of inhibition

Disc-diffusion method is preferred for rapid identification of bioactive metabolites and is usually used for initial screening of antimicrobial activity of different compounds. Testing antibacterial activity of metronidazole derivatives revealed that ethanol and methanol solutions showed the highest activity against *Staphylococcus aureus* (13 mm), methanol solution against *Candida albicans* (14 mm) and no activity against *Escherichia coli* and *Bacillus subtilis*. Moreover, two of the tested solutions—with saline and phosphate buffer did not show any inhibition effect. No antimicrobial effect was observed with the established control samples with metronidazole.
Conclusion
The results presented in our study showed that the metronidazole derivatives-Ethyl 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide) benzoate (MTD1) and Butyl 4-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) acetamide) benzoate (MTD2) could be a helpful source of antibacterial compounds. The methanol solution of metronidazole derivatives had antimicrobial activities against *Staphylococcus aureus* and *Candida albicans* and the ethanol solution – against *S. aureus*. This basic research can help to develop antimicrobial drugs from metronidazole derivatives.

References:
Vitamin D status among patients with sarcopenic obesity

Maria Nikolova1; Rosica Popova2

1Department of Epidemiology and Hygiene; Medical University-Sofia
2Sofia University “St.Kl.Ohridski”; University Hospital “Lozenetz”

Abstract
Vitamin D insufficiency and deficiency are more common among patients with sarcopenic obesity (SO) compared to obesity-only or sarcopenia or healthy subjects.
Purpose: To assess the correlations of plasma 25(OH) vitamin D levels with indices of body composition examined by DXA.
Material and Methods: 62 adult subjects consented to participate – 27 men (43.5 %) and 35 women (56.5 %). Fan-beam dual-energy X-ray (DXA) body composition analysis was performed on a Lunar Prodigy Pro bone densitometer. Vitamin D was measured by electro-hemi-luminescent detection as 25(OH)D Total.
Results: The serum 25(OH) D level was correlated significantly only to the whole body bone mineral content and indicators of the risk of sarcopenia - the appendicular lean mass index (ALMI) and the ALM-to-BMI index.
Conclusion: In our study it was established that the indicators that predetermine the risk of SO significantly correlate with vitamin D deficiency.
Key words: Vitamin D, Body composition, Sarcopenic obesity

Introduction
The negative correlations of serum vitamin D levels with different indices of obesity, such as body weight, BMI, WC and waist-to-hip ratio (WHR), were extensively studied [1]. Vitamin D is stored in the liver and adipose tissue [2]. On the other hand, the contribution of lean mass has been questioned in a few studies based on dual X-ray absorptiometry (DXA) and was generally found to be less important than that of fat mass [1-2]. The results were rather inconclusive showing association with the appendicular lean mass in one of those studies [3] and no association with lean mass in another one [4]. A number of DEXA-based studies have been focused on ALM and various fat and lean mass indices, however, have been designed to assess the prevalence and characteristics of sarcopenia without examining correlations with vitamin D status [5]. There have been isolated studies examining the relationship between plasma vitamin D levels and obese sarcopenia. Three studies found that vitamin D deficiency and insufficiency are more common in people with obese sarcopenia than in those who are obese or have only sarcopenia or are healthy [6-7].

Objective
The aim of our study was to assess the correlations of plasma 25(OH) vitamin D levels with indices of body composition examined by DXA with an emphasis on lean and bone mass as well as on indices such as android/gynoid fat, appendicular lean mass (ALM) and appendicular lean mass index (ALMI), fat-mass indexes (FMI), fat-free mass indexes (FFMI) and the ALM-to-BMI index.
Materials and methods: This is a cross-sectional observational study. The inclusion criteria were age between 19 and 60 years and willingness to participate. The exclusion criteria were severe or chronic diseases or medications known to affect body weight, immobilization, and others known to induce morbid obesity.
We analyzed 62 patients– 27 men (43.5 %) and 35 women (56.5 %). Their mean age was 45.3 ± 9.5 years. Body weight was measured by a calibrated digital scale (Tanita BC 420 MA, Tanita Inc., Japan). Fan-beam dual-energy X-ray (DXA) body composition analysis was performed on a Lunar Prodigy Pro
bone densitometer (GE Lunar, Chicago, IL, USA). Additionally a number of ratios were calculated - fat mass ratios (Trunk/Total, Legs/Total, Arms + Legs / Trunk, Android/Gynoid), as well as appendicular lean and fat mass (ALM and AFM, in kg). Height corrected parameters were calculated according to recent publications – fat mass index (FMI, in kg/m2), fat-free mass index (FFMI, in kg/m2), ALM index (ALM/height2, in kg/m2) and LM/Height2 [8]. Plasma 25(OH) Vitamin D was measured by electro-hemi-luminescent detection as 25(OH)D Total (ECLIA on an Elecsys 2010 analyzer, Roche Diagnostics, Switzerland). Statistical analyses were done using the SPSS 23.0 statistical package for Windows (SPSS Inc., Chicago, IL, USA).

**Results** - Serum levels of 25(OH) D of the whole group were 33.0 ± 17.3 nmol/l (median – 29.1 nmol/l). In men they were 36.5 ± 18.1 nmol/l; and in women 30.3 ± 16.3 nmol/l. The difference between both sexes was not significant. 48.4 % of the study population had vitamin D insufficiency, 37.1 % had deficiency, and only 3.2 % had values above 30 ng/dl (75 nmol/l).

Table 1. Summarizes the data related to the various indices characterizing the lean mass (LBMI, FFMI, ALM, ALMI and the ALM-to-BMI ratio), as well as the indicators and indices characterizing the fat distribution and the class of obesity (AFM, FMI, A/G ratio and ratios: trunk fat to total body fat ratio, leg fat to total body fat ratio, limb fat to trunk fat ratio).

<table>
<thead>
<tr>
<th>INDICATOR</th>
<th>Total (n=62)</th>
<th>Men (n=27)</th>
<th>Women (n=35)</th>
<th>Reference values (RV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value</td>
<td>SD</td>
<td>Median</td>
<td>Mean value</td>
</tr>
<tr>
<td>LBMI (kg/m²)*</td>
<td>18.18</td>
<td>2.73</td>
<td>18.37</td>
<td>20.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFMI (kg/m²)*</td>
<td>19.19</td>
<td>2.76</td>
<td>19.36</td>
<td>21.60</td>
</tr>
<tr>
<td>ALM (kg)*</td>
<td>23.26</td>
<td>5.91</td>
<td>21.43</td>
<td>29.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALMI (kg/m²)*</td>
<td>7.92</td>
<td>1.35</td>
<td>7.52</td>
<td>9.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALM-to-BMI ratio*</td>
<td>0.70</td>
<td>0.17</td>
<td>0.66</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appendicular Fat Mass (kg)**</td>
<td>17.78</td>
<td>4.47</td>
<td>18.63</td>
<td>16.00</td>
</tr>
<tr>
<td>FMI (kg/m²)**</td>
<td>14.52</td>
<td>3.67</td>
<td>14.30</td>
<td>13.00</td>
</tr>
<tr>
<td>A/G ratio*</td>
<td>1.11</td>
<td>0.15</td>
<td>1.11</td>
<td>1.24</td>
</tr>
<tr>
<td>FMR - Trunk/Total fat *</td>
<td>0.54</td>
<td>0.06</td>
<td>0.54</td>
<td>0.58</td>
</tr>
<tr>
<td>FMR- Legs/Total fat</td>
<td>0.34</td>
<td>0.05</td>
<td>0.34</td>
<td>0.30</td>
</tr>
<tr>
<td>FMR- Arms+Legs fat/Trunk fat</td>
<td>0.81</td>
<td>0.18</td>
<td>0.81</td>
<td>0.67</td>
</tr>
</tbody>
</table>
* statistically reliable sex-based differences (p< 0.001); ** statistically reliable sex-based differences (p<0.005); *** RV for FMI (kg/m²) men: normal levels – 3.0–6.0 kg/m²; with class I obesity – between 9.0 and 12.0 kg/m²; with class II obesity – between 12.0 and 15.0 kg/m²; with class III obesity – more than 15.0 kg/m². women: normal levels – 5.0–9.0 kg/m²; with class I obesity between 13.0 and 17.0 kg/m²; with class II obesity between 17.0 and 21.0 kg/m²; with class III obesity more than 21.0 kg/m²; **** RV for FFMI (kg/m²): overweight: men – normal levels 21.8–24.4 kg/m²; and women respectively – normal levels of 17.1–18.4 kg/m²; obesity: men – normal levels > 24.5 kg/m²; and women respectively – normal levels > 18.5 kg/m²

The mean values of the ALM-to-BMI index in all overweight people are lower than the recommended levels, which requires an assessment of the risk of obese sarcopenia development in this group. About ¾ (75.8%) of all subjects who underwent DXA measurement had increased values of the A/G fat ratio (> 1). All studied men and 57% of studied women had a raised A/G index characterizing the risk of visceral obesity.

**Conclusion**

In the present survey it was established that the indicators that predetermine the risk of sarcopenia (low levels of the ALM-to-BMI index, lean mass in arms (Reg. Arms-Lean)) significantly correlate with vitamin D deficiency. Reliable associations were found only with the whole body bone mineral content, the index of lean mass in arms and legs (ALMI) and the ALM-to-BMI, indicating the importance of the lean mass. Vitamin D showed a very low dependence on fat mass percentage and fat mass index (FMI) only in men. In addition, the multiple regression model, including related parameters, could explain only 7% of the change in serum 25(OH)D levels.

**References:**

Effects of Rimonabant on Locomotor and Exploratory Activities in Olfactory Bulbectomized Rats

D. Doncheva¹, R. Tashev², M. Velikova¹

1. Dept. Physiology and Pathophysiology, MU Varna, Bulgaria
2. Physiology and Pathophysiology, MU, Sofia, Bulgaria

Abstract
The bilateral olfactory bulbectomy (OBX) is a validated model of depression. The removal of bulbi olfactorii produces a syndrome of behavioural, neurochemical, neuroendocrine, immune, etc. alterations, that resembles human depressive disorder. OBX rat is not only a relevant animal model of (major) depression but also is an adequate model for exploring the links that exist between the neurotransmitter, behavioral, endocrine and immune systems. Behavioural abnormalities, developed by the OBX rodents, include hyperlocomotion, memory disturbances, reduced sexual activity, hyperemotionality. Endocannabinoid system (ECS) plays role in many physiological processes, including mood, learning and memory. It is also involved in the pathogenesis of anxiety and mood disorders. The involvement of the endocannabinoid system in the control of mood and locomotor activity is poorly understood. The behavioral effects of endocannabinoids are mediated through the CB1 receptors, which are widely expressed in the brain, including the olfactory bulb, cortical regions (neocortex, pyriform cortex), hippocampus, amygdala, etc.). Cannabinoid type 1 receptor antagonist SR-141716 (Rimonabant, RIM) is a selective CB1-receptor antagonist which also has properties of inverse agonist. Rimonabant antagonizes the effects of Δ9-THC, the main psychoactive compound in marijuana.
**Aim** of the present study is to investigate the effects of cannabinoid CB1-receptor antagonist SR-141716A on locomotor and exploratory activities of olfactory bulbectomized (OBX) rats and to evaluate the significance of the time interval for the administration of the drug.

**Material and methods**

Bilateral olfactory bulbectomy (OBX): The surgical procedure involved drilling two burr holes 2 mm in dm at the points 7.5 mm anterior to bregma and 2 mm from the mid-line on its both sides. The bulbs were aspirated with a needle attached to a water pump.

Drugs: Rimonabant (RIM), SR-141716A (Sanofi, Fr) was administered intragastrally for 14 days at a dose of 3 mg/kg.

Experimental animals: Male Wistar rats, treated with RIM, were divided into 4 groups:
1/ Sham-operated - RIM;
2/ RIM, administered prior to OBX (14d RIM, OBX)
3/ RIM, given immediately after OBX (OBX, 1-14d RIM);
4/ RIM administered after development of depressive-like state (OBX, 14-28d RIM).

The control groups were Sham-saline, OBX-saline, tested on 14 and 28 day after OBX.

**Results**

- RIM administered 14 days before bulbectomy (14d RIM, OBX) and on the background of a developed depressive-like state (OBX, 14-28d RIM) increased the total number of horizontal and vertical movements for the 5-minute period of observation, compared to both Sham-controls and OBX-controls (Fig 2 A,B).

- RIM administered 14 days immediately after bulbectomy (OBX, 1-14d RIM,) showed no significant changes in the number of movements as compared to the OBX rats (Fig 2). This indicates that during this period, CB1-receptor blockade did not interfere with the pathological processes that underlie OBX-induced hyperlocomotion.

- OBX-rats typically show disturbed habituation: the number of movements does not decrease gradually for the period of observation, instead, activity increases after the 2nd min., i.e. the animal cannot orient itself in the new environment (Fig 1 A,B). After RIM-administration, exploratory activity in the new environment (habituation) remained impaired in all groups, similarly to the OBX-controls (Fig A).
**FIG. 1.** A,B. Effect of Rimonabant (SR-141716A), on exploratory activity - horizontal (A) and vertical (B) movements in OBX rats (n = 8)

**FIG. 2.** A,B. Effect of Rimonabant (SR-141716A), on locomotor activity - total number of horizontal (A) and vertical (B) movements in OBX rats (14-day intragastral administration, (n = 8)

*** p<0.001 comparison with sham-operated controls;  p<0.001 comparison with OBX-controls

**In conclusion,** our data showed a modulatory influence of CB1R blockade on hypermotility of OBX-rats. The time interval for administration of RIM is of significance for the locomotor, but not for the exploratory disturbances induced by the bullectomy.

**References:**


Investigation of antimicrobial activity of commercial essential oils of Thymus vulgaris

Neli Ermenlieva¹, Emilia Georgieva², Sylvia Stamova³, Gabriela Tsankova¹, Silviya Mihaylova², Antoaneta Tsvetkova²

¹Department of Microbiology and Virology, Faculty of Medicine, Medical University of Varna
²Medical college of Varna, Medical University of Varna, Bulgaria
³Department of Pharmaceutical chemistry, Faculty of Pharmacy, Medical University of Varna, Varna, Bulgaria

Abstract
Essential oils are volatile, natural, complex compounds known for their strong odor and different medicative properties. They are used as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic, and locally anesthetic agents. The aim of this study is to evaluate the potential antimicrobial activity of two (for external and internal use) commercial essential oils of Thymus vulgaris against Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922. The study was conducted in Medical College – Varna, Bulgaria. The antimicrobial activity of thyme essential oils was determined by the use of the Kirby-Bauer disk diffusion susceptibility test. This inhibitory effect was observed by the diameters above 20 mm of the zones of inhibition. The results demonstrate that the Thymus vulgaris essential oils tested possess strong antimicrobial properties, and may in the future represent a new source of natural antiseptics with applications in the pharmaceutical industry.

Key words: essential oils, Thymus vulgaris, antimicrobial activity, E. coli, S. aureus

Introduction
Essential oils are volatile, natural, complex compounds known for their strong odor and different medicative properties. They are used as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic, and local anesthetic agents [1]. These properties are currently actual but the knowledge about their antimicrobial activities is more extended now than in the past. The chemical composition of thymus essential oils shows variations, the mainly reported compounds are geraniol, linalool, gamma-terpineol, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol [2, 3]. Carvacrol and thymol are cyclic organic compounds containing a methyl group, hydroxyl group, and an isopropyl group. The two structures differ from each other according to the position of the hydroxyl group. The key difference between carvacrol and thymol is that carvacrol contains a hydroxyl group at the ortho position of the benzene ring whereas thymol contains a hydroxyl group at the meta position of the benzene ring [4]. In addition, the thyme essential oils exhibit significant antimicrobial activity [5,6 ] as well as strong antioxidant properties [7, 8, 9].

Aim
The aim of this study is to evaluate the potential antimicrobial activity of two (for external and internal use) commercial essential oils of Thymus vulgaris against Staphylococcus aureus ATCC 29213 and Escherichia coli ATCC 25922.

Materials and methods
The study was conducted in Medical Collage – Varna, Bulgaria. The antimicrobial activity of oregano essential oils was determined by the use of Kirby-Bauer disk diffusion susceptibility test against Escherichia coli and Staphylococcus aureus. We made dense seed of 0.5 MF standardized bacterial culture on the surfaces of Mueller–Hinton agar media (HiMedia®, provided by Ridacom, Bulgaria). A stock solution of 10% (v/v) thyme oil in DMSO (1%) was prepared. Sterile filter discs (HiMedia®, provided by Ridacom, Bulgaria) were soaked with the appropriate volume (3, 5, 10 µL) of the test
compounds (thyme essential oil-for external use and thyme essential oil-for internal use) and after the surfaces of the culture media have dried, the discs were placed on it. Controls were set for the solvent, which was used for the preparation of suspensions of the active substances-DMSO. All samples were performed in triplicate.

Results
The results of the reported antimicrobial activity of *Thymus vulgaris* essential oils (for external and internal use) against *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922 via disk-diffusion method are shown in Table 1 and Figure 1. The two essential oils showed high antimicrobial activity against these bacterial strains.

Table 1. Antimicrobial activity of thyme essential oils against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 determined by disk-diffusion method (zones of inhibition in mm).

<table>
<thead>
<tr>
<th>Essential oil</th>
<th><em>S. aureus ATCC 29213</em></th>
<th><em>E. coli ATCC 25922</em></th>
<th>DMSO 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10% stock solutions, μL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td><em>Thymus vulgaris essential oil</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(internal use)</td>
<td>23 mm</td>
<td>30mm</td>
<td>40mm</td>
</tr>
<tr>
<td><em>Thymus vulgaris</em> (external use)</td>
<td>26mm</td>
<td>30mm</td>
<td>32mm</td>
</tr>
</tbody>
</table>

Legend: - no zones of inhibition

Figure 1. Antimicrobial activity of *Thymus vulgaris* essential oils (external and internal use) against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 determined by disk-diffusion method.
Conclusion

The antimicrobial activity of thyme essential oils (external and internal form) was efficient in the control of *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. The results demonstrate that the *Thymus vulgaris* essential oils tested possess strong antimicrobial properties, and may in the future represent a new source of natural antiseptics with applications in the pharmaceutical industry.

References:

Todorka Sokrateva¹, Deyana VankoVA¹, Diana Ivanova¹, Sławomir Wilczyński², Vanya Koleva³, Yana Koleva⁴, Dobromir Enchev⁴, Yoana Kiselova-Kaneva¹

1) Department of Biochemistry, molecular medicine and nutrigenomics, Medical University – Varna, (Bulgaria);
2) Department of Basic Biomedical Science, Medical University of Silesia in Katowice, Sosnowiec (Poland);
3) Department of Biology, University „K. Preslavsky“, Shumen, (Bulgaria);
4) Department of Chemistry, University "Prof. D-r Asen Zlatarov"- Burgas, (Bulgaria);
5) Department of Chemistry, University „K. Preslavsky“, Shumen, (Bulgaria)

Abstract

Our goal was to examine physicochemical characterization of a synthesized compound 5-Ethyl-5-methyl-4-bromo-2-N-buthylamido-2,5-dihydro-1,2-oxaphosphol-2-oxide (Br-oxph-1), to perform in silico metabolism prognosis and to test its cytotoxicity and gene expression effects. In silico modelling revealed 37 probable metabolites some of them with DNA and protein binding properties. At low concentrations, Br-oxph-1 potentiates cell proliferation and its IC50 is 0.442 mg/mL. Concentration of 0.25 mg/mL caused most prominent effect on GCLc and GPx1 expression causing 8 (p<0.01) and 3 (p<0.001) fold change, respectively.

Key words: organophosphorus, (Q)SAR, gene expression

Introduction

Oxaphosphols belong to five membered heterocyclic compounds, containing oxygen and phosphorus atoms. Due to their high biological activity, they have been used in the pharmaceutical, agricultural and chemical industries. Br-oxph-1 are structurally analogous with drugs that are used in treatment of viral infections and cancer [1, 2]. Data from in vivo studies with different plant and animal test–systems as well as in vitro studies with human carcinoma cells reveal mitodepressive, clastogenic and turbagenic effects of analogue Br-oxph and its inhibitory effects of growth of human lung and liver carcinoma cells [3-5]. A recent study on cytotoxic activity of Br-oxph-1 has showed stronger in vitro inhibitory effect on proliferation/viability of human hepatoma cell line SK-HEP-1 in comparison to Br-oxph [3]. There is no data about biological activity Br-oxph-1 on normal cells. The aim of present study was to perform physicochemical characterization of Br-oxph-1 and to assess its biological activity in silico and in vitro on normal cell culture model.

Materials and methods

The compound was synthesized according procedures described earlier [4] and is a structural analog to 4-bromo-N,N-diethyl-5,5-dimethyl-2,5-dihydro-1,2-oxaphosphol-2-amine-2-oxide (Br-oxph). Quantum-chemical and physicochemical molecular indexes were calculated using the Quantum-chemical package HyperChem with the semi-empirical AM1 method after geometric optimization. Predicting of liver metabolism, DNA and protein binding of Br-oxph-1 was performed using the Organization for Economic Co-operation and Development (OECD) (Q)SAR Toolbox software (ver.1.0). J774A.1 cell culture were subcultivated in standard conditions in complete growth medium containing as described by supplier (American Type Culture Collection) Dulbecco’s Modified Eagle’s medium with phenol red, 4.5 g/L glucose and L-glutamine (DMEM, Lonza), 10% fetal bovine serum (FBS, Sigma-Aldrich) and 100 U/mL penicillin/streptomycin mixture (Sigma-Aldrich, Germany). Viability of J774A.1 cells treated with increasing concentration of Br-oxph-1 (0.0625 – 10 mg/mL) for
20 hours was evaluated using the MTT test as described earlier [6]. Gene expression analysis in J774A.1 cells treated with 0.125, 0.25 and 0.5 mg/mL of Br-oxph-1 was measured with Quantitative real-time PCR as described earlier [7]. Results are presented as mean ± standard deviation of mean (Mean±SD) for MTT test and as mean ± standard error of means (Mean±SEM) for gene expression analysis. The comparison of two independent groups was done with Student's t-test.

Result and discussion

Results of calculated electronic molecular indexes of the studied compound are: Molecular mass M=296,14 a.e.m; $\text{E}_{\text{homo}}=-10.59397$ eV; $\text{E}_{\text{lumo}}=-0.5569724$ eV; Electronegativity $\text{E}_N=-5.5754712$; Molecular dipole moment $\mu=5.505$; Total Energy $\text{E}_{\text{tot}}=-662792.7$ kcal/mol; Van der Waals surface $S=505.09$ A; Van der Waals volume $V=769.88$ A; Hydration energy=-2.02 kcal/mol; Hydrophobicity log $P=1.62$; Molar refractivity $\text{MR}=66.36$ A; Polarization $\alpha=23.73$; Molecular reactivity $\text{HARD}=5.0184979$; Number of proton acceptors (nO, nN)=3; Number of proton acceptors (nO, nN)=1. Considering molecular indexes Br-oxph-1 appears to be an electronegative stable molecule (Etot=-66279.7 kmol/mol) capable to donate an electron couple to a donor acceptor bond (E$_{\text{homo}}$=-10.59397 eV). It passes through membranes (logP=1.62) which can be related to its intramolecular effects on cell metabolism, including proliferation and gene expression. Among the molecular parameters and their recommended values according to Lipinski's 5th Rule [8] are: log $P<5$; $M<500$; number of proton acceptors (nO, nN)<10; number of proton donors (nOH, nNH)<5. These parameters are satisfactory for the Br-oxph-1.

Considering molecular effects of potential drugs in vitro there is a need to know about their metabolism and to identify possible intermolecular interactions of the compound and its metabolites. Liver metabolites as predicted with (Q)SAR Toolbox (v.1.0) software are present on Fig. 1. In silico modelling did not reveal any DNA and/or protein binding activity of parental structure (Br-oxph-1). Total of thirty-seven probable metabolites were suggested by the OECD (Q)SAR Toolbox software. Fourteen of them were indicated to bind to DNA (№ 1, 15, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37); twelve to proteins (№ 1, 15, 6, 9, 12, 18, 20, 23, 25, 30, 33, 36); five – both DNA and proteins (№ 1, 15, 30, 33, 36) and sixteen bind neither (№ 2, 3, 4, 5, 7, 8, 10, 11, 13, 14, 16, 17, 19, 21, 22, 24). Data from in silico prediction are consistent with the results on the genotoxic and cytotoxic effects of the similar compound Br-oxph on various experimental in vitro and in vivo test subjects [1-5].

**Fig. 1. Liver predicted metabolites of Br-oxph-1.**

A: 1) -CH$_2$-CH$_3$, 15) -CH(OH)-CH$_3$; B: 2) R1: -Br; R2: -OH; R3: -CH$_2$-CH$_3$, 3) R1: =O; R2: - R3: -CH$_2$-CH$_3$, 4) R1: -H; R2: -OH; R3: -CH$_2$-CH$_3$, 16) R1: -Br; R2:-OH; R3:-CH(OH)-CH$_3$; C: 12) R: -CH$_2$-CH$_3$, 23) R: -CH(OH)-CH$_3$; D: 5) R1: -CH$_3$; R2: -CH$_2$-CH$_3$; R3: -CH$_2$-OH, 6) R1: -CH$_3$; R2: -CH$_2$-CH$_3$; R3: -CHO, 7) R1: -CH$_3$; R2: -CH$_2$-CH$_3$; R3: -COOH, 8) R1: -CH$_3$; R2: -CH$_2$-OH; R3: -CH$_3$, 9) R1: -CH$_3$; R2: -CH$_2$-OH; R3: -CH$_3$, 10) R1: -CH$_3$; R2: -CH$_2$-COOH; R3: -CH$_3$, 11) R1: -CH$_2$-CH$_3$; R2: -CH$_2$-OH; R3: -CH$_3$, 13) R1: -COOH; R2: -CH$_2$-OH; R3: -CH$_3$, 14) R1: -CH$_3$; R2: -CH$_2$-CH$_3$; R3: -
CH(OH)-CH₃, 17) R₁: -CH₃; R₂: -CH₂-CH₃; R₃: -CH(OH)-CHO, 19) R₁: -CH₃; R₂: -CH₂-CH₂-OH; R₃: -CH(OH)-CH₃, 20) R₁: -CH₃; R₂: -CH₂-CHO; R₃: -CH(OH)-CH₃, 21) R₁: -CH₃; R₂: -CH₂-COOH; R₃: -CH(OH)-CH₃, 22) R₁: -CH₂-CH₃; R₂: -CH₂-OH; R₃: -CH(OH)-CH₃, 24) R₁: -COOH; R₂: -CH₂-CH₃; R₃: -CH(OH)-CH₃, 25) R₁: -CH₃; R₂: -CH₂-CH₃; R₃: -COO-CH₃; E: 27) R₁: -OH; R₂: -CH₃; R₃: -CH₂-OH; R₄: CH(OH)-CH₃, 28) R₁: -OH; R₂: -CH₃; R₃: -CH₂-CH₃; R₄: -CH(OH)-CH₂-OH, 29) R₁: -OH; R₂: -CH₂-OH; R₃: -CH₂-CH₃; R₄: -CH(OH)(CH₂)₂-OH, 30) R₁: -OH; R₂: -CH₃; R₃: -CH₂-CH₃; R₄: -CHO-CH₃, 31) R₁: -OH; R₂: -CH₃; R₃: -CH₂-CH₃; R₄: -CH₂-CH₃, 32) R₁: -OH; R₂: -CH₃; R₃: -CH₂-CH₂-OH; R₄: -CH₂-CH₃, 33) R₁: -OH; R₂: -CH₃; R₃: -CH₂-COOH; R₄: -CH₂-COOH, 34) R₁: -OH; R₂: -CH₃; R₃: -CH₂-CH₃, 35) R₁: -OH; R₂: -CH₃; R₃: -CH₂-CH₃; R₄: -CH₂-CH₂-OH, 36) R₁: -OH; R₂: -CH₃; R₃: -CH₂-CH₃; R₄: -CH₂-COOH.

MTT test may reveal possible mitogenic or inhibitory activity on cell proliferation and/or cytotoxic effects. Cytotoxic activity of Br-oxph-1 to macrophages was poor – IC₅₀ = 0.442 mg/mL (Fig. 2). In comparison, the IC₅₀ to SK-HEP-1 adenocarcinoma cell line is 0.19 mg/mL [3], which makes it about 2.3 times less toxic to macrophages. Furthermore – the proliferation/viability of macrophages after treatment with lower concentrations (0.0625-0.25 mg/mL) of Br-oxph-1 increases and is maximum (165%) is at 0.25 mg/mL (about ½IC₅₀). Such proliferative effect has not been observed in cancerous SK-HEP-1 cells and when treated with the same concentration of the compound the vitality of cells was only 3.38% [3]. These data demonstrate a selective Br-oxph-1 cytotoxic activity in vitro against to the cells of the lines studied and lower susceptibility of normal J774A.1 cells. Concentration of 0.5 mg/mL causes rapid decline in cell viability to 25.03% and further increase in concentration to 10 mg/mL (20 fold) did not contribute to a significant change in this parameter. In contrast, treatment with 0.3 mg/mL and higher concentrations of Br-oxph-1 leaded to 100% cell death in SK-HEP-1 line [3].

**Fig. 2.** J774A.1 macrophage cells treated with different concentrations of Br-oxph-1. A) Cell viability [%]; B) mRNA expression levels of GCLc; C) mRNA expression levels of GPx1.

We measured changes in expression of antioxidant defense related proteins – glutamate cysteine ligase (GCLc) and glutathione peroxidase 1 (GPx1). Br-oxph-1 at concentration of 0.125 mg/mL decreased GCLc mRNA levels by a half (p<0.05) and increased GPx1 levels by 2.18 (p<0.05). Concentration of 0.25 mg/mL most potently stimulated transcription of GCLc and GPx1 causing 8 (p<0.01) and 3 (p<0.001) fold change, respectively. Regarding the observation of the effect of Br-oxph-1 on studied genes, we may conclude it and/or its intracellular metabolites provoke glutathione related antioxidant system. Established rapid decline in expression levels of both enzymes coincide with the same effect in cell viability at 0.5 mg/mL Br-oxph-1. Apparently, this concentration overcomes protective capacity of the cells, leading to pronounced cell death, accompanied by decay in cell functions, including protein expression.

**Conclusions**

Data from in silico prediction, cytotoxicity and gene gene expression analysis indicate that Br-oxph-1 exerts prominent biological effect in J774A.1 macrophages and exerts appropriate physicochemical parameters, which make it a suitable candidate for drug development.
References:


Address for correspondence:
Todorka Sokrateva
Department of Biochemistry, molecular medicine and nutrigenomics, Faculty of Pharmacy, Medical University – Varna
Address: 84 Tzar Osvoboditel Str. 9000 Varna (Bulgaria)
E-mail: sokrateva@mu-varna.bg
Evaluation of academic stress among international students

Gabriela Panayotova¹, Sylvia Pavlova, Marya Ivanova¹, Dobrinka Doncheva¹, Margarita Velikova¹

1) Department of Physiology and pathophysiology, MU-Varna, Bulgaria
2) Department of Department of Social medicine and healthcare organization, MU-Varna

Abstract
Medical students experience high levels of stress during their course of academic studies. The aim of our survey was to examine the academic factors affecting the levels of stress of international medical students and to evaluate the differences in the stress levels related to the academic demands. Twice in the academic year (November and May), the participants - 1st-year international medical students, filled University Stress Scale (USS) and Academic Factors Stress Scale (AFSS). The results from the analysis of the USS scores indicate that the items, causing frequently stress were “Academic/coursework demands” and “Procrastination”. Most of the factors evaluated as ones producing moderate stress, according to the ASDS, were related to the study. “Worrying about examination” and “Difficulty in remembering all that is studied” showed a decrease from November to May, still being factors for a moderate level of stress experienced by the students.

Introduction
Studies have shown that university students experience high level of stress during their course of education [1, 2]. There are reports that medical students are more likely to experience psychological distress, anxiety and depression than other university students [3]. International students trained in the English course in Medical university of Varna, Bulgaria have to adapt to the cultural, linguistic and social differences in the foreign country, as well as to the specifics of the educational system, which may cause higher levels of stress. The aim of our study was to evaluate the levels of academic stress among international medical students and to identify the factors that make student’s life more stressful. Our survey included 1st year international students at the Medical University of Varna, Bulgaria, enrolled in the 6-years medical studies in English. The recruitment of the participants commenced after receiving approval from the University research ethics committee. In November, 2018 at the end of the 1st semester, 212 volunteers signed an informed consent form, and completed a sociodemographic questionnaire. We used two scales to assess the stress related to the study in the university: University Stress Scale (USS) and Academic Stress Factors Scale (ASFS). In May, 2019 at the end of the 2nd semester, 110 students once again participated in the survey. The purpose of our study was to investigate the academic factors that affect international students' stress levels at the Medical University of Varna, Bulgaria.

Materials and methods
The USS includes 21 items and measures the stress experienced by university students [4]. The overall intensity of the stress can be evaluated - a score ≥ 13 is predictive of psychological distress. Our team developed an Academic Stress Factors Scale (ASFS), which includes 26 items, concerning different domains such as study stress, self-inflicted stress, relationship stress, stress related to adaptation difficulties, teacher-student interactions. The level of stress, which is related to each factor, was graded from 0 to 3 (factor producing no stress (0), slight (1), moderate (2), high (3) stress.

Analyses used IBM SPSS v. 26 (Statistical Product and Service Solutions). The complete sample received descriptive data on the result of interests. Mean standard deviation (M) and standard errors (±) were reported for all items. Frequencies and percentages were reported for all self-evaluation, behavior-related and demographic variables. Internal consistency reliability of the scale measurement was estimated using Cronbach’s Alpha. Finally, Paired t-test was used to identify the differences in the
means of the items of each scale measured in November and May of data collection. The significance of the tests was based on a two-sided α at 0.05.

**Result and discussion**

95% of participants were aged 18-23 years. Most of them were female (56%), single (71%). 49% identified themselves as non-alcohol consumers, 15.6% as smokers; 55.5% spent 2-4 h studying/day; sleeping patterns varied between 4-8 hours/night. Less than 6% of the students reported having some chronic illnesses. Most students reported being financially stable (95%) and supported by parents (85.7%). The results of the reliability tests of the USS and ASFS do not reveal significant differences. Cronbach Alpha values ranged between 0.7 and 0.8, indicating high consistency between the items included in the scales. Specifically, Cronbach Alpha of USS data collected in November was 0.779, whereas Cronbach Alpha of ASFS data was 0.861. Interestingly, there was a slight increase in the internal consistency of the reported outcomes in May, as Cronbach Alpha for USS was 0.819, and for the ASFS it was 0.869. All scale items demonstrated great consistency, and no item was removed off the scale. 37.73% of the students scored ≥ 13 on the USS scale in November, and the percentage increased to 51.81% in May. That shows students' psychological stress levels have grown by the end of their first year of medical school. The mean score of the items included in the USS scale varied between 0 and 2 (i.e. causing “not at all” stress and causing "frequently" stress in the past month). The items causing frequently stress (1.5 and above scores) were “Academic/coursework demands” (M=1.72±0.05) and “Procrastination” (M=1.55±0.06). The items causing sometimes stress (score 1.0) were “University/college environment” (M=1.0±0.05) and “Parental expectations” (M=1.04±0.06). The item comparison of the USS scale in the two periods of evaluation, November and May, indicated significant changes in four items (Fig.1).

![Fig.1. The item comparison of the USS in the two periods of evaluation.](image)

While the burden of the “Academic/course demands” (p=0.011) and the “Language/cultural/ issues” (p=0.007) decreased at the end of the academic year, the “Physical health problems” (p=0.05) and stress from “Work” (p=0.05) increased with the approaching examination session. Except for “Academic/course work demands” the other three items were not related to stress, having mean values around 0.5, respectively, for “Language/cultural/ issues” (M=0.66±0.06); “Physical health problems” (M=0.53±0.04); “Work” (M=0.6±0.05).

The scores of all items included in the ASFS scale varied between 0 and 2, indicating no stress or moderate level of stress related to certain academic life factors. Items inducing moderate levels of stress (1.5 and above scores) were “Exam” (M=2.15±0.06); “Worrying about examination” (M=1.91±0.06) “Difficulty in remembering all that is studied” (M=1.67±0.06); “Lack of concentration during study hours” (M=1.56±0.06), Examination syllabus too heavy (M=1.5±0.05). Among the factors least related to stress (0.5 and below scores) were “ Unable to discuss academic failures with parents” (M=0.5±0.05); “Inadequate space or room for study” (M=0.53±0.06); “Lack of fluency while speaking the English language” (M=0.51±0.05); “Unable to find friends” (M=0.33±0.05); “Inability to adapt to the new social & cultural environment” (M=0.44±0.05) “Lack of support from parents” (M=0.24±0.04).
The results of the comparison of the ASFS items did not indicate significant changes for the two observed periods, except the item “Worrying about examination”, which has decreased from 1.9 to 1.5 as a mean score ($t=3.08, p=0.003$), still being factors for a moderate level of stress experienced by the students. Furthermore, the influence of "Ten-twelve hours of study for the tests" as a stress factor decreased from 1.33 to 1.06 ($p=0.05$) (Fig.2).

**Fig. 2.** The item comparison of the ASFS in the two periods of evaluation

**Conclusion**
This study provided evidence that during the first year of the medical study the percentage of international students in the Medical University of Varna, who report higher than average levels of stress increases. The factors that cause stress were the high-academic demands and the students’ ability to respond to them. However, the adaptation difficulties, cultural problems, teacher-student interactions, relationships and family problems appear not to contribute to the academic stress experienced by the students.

**References:**
Schizophrenic disorders in forensic psychiatric expertises in the civil process

Sonya Maneva, Adelina Dzhordzhanova

First psychiatric clinic, UMHAT “St. Marina” – Varna
Department of psychiatry and medical psychology, Medical University – Varna

Abstract
The aim of this research is to analyze the disorders of the schizophrenic spectrum in forensic psychiatric expertises in the civil process. Object of this study are a total of 327 persons, certified by experts in civil cases with subsequent court decisions by the District Court – Varna over a period of 5 years. The results are statistically processed with SPSS v. 20.0. Variational and comparative analysis have been conducted.

Key words: Schizophrenic disorders, forensic psychiatric expertise, civil process

Introduction
Schizophrenia is a serious mental disorder with notable social significance. The disturbances of perception, the affective variability in mood, the pathological symptoms of thought and cognition, all lead to a disturbance in the functioning of the individual to such a degree that treatment is a necessity for the safety of the patient, as well as that of those around them [1]. Despite the obvious need for psychiatric help, the provision of the necessary treatment of persons with schizophrenia is often problematic [2]. Whenever a patient does not wish to be treated, a forensic psychiatric expertise is appointed by the judicial authorities. In those cases, the patients believe that the purpose of the psychiatric evaluation is for them to be punished additionally and an emphasis is placed on the opposing points of view of the patient (i.e., achieving of freedom) in contrast to that of the expert (i.e., ensuring an adequate and effective treatment) [3, 4]. Some researches analyze the structure of forensic psychiatric expertises and the omissions perceived in them [5, 6, 7]. Unlike other countries, the Republic of Bulgaria has no established standard for the preparation of forensic psychiatric expertises, according to which the expertises should be made, which would raise the quality of the expert activity.

The aim of this research is to analyze the disorders of the schizophrenic spectrum in forensic psychiatric expertises in the civil process.

Materials and methods
Objects of this study are a total of 327 persons, certified by experts in civil cases with subsequent court decisions by the District Court – Varna over a period of 5 years. The results are statistically processed with SPSS v. 20.0. Variational and comparative analysis have been conducted.

Results
Of all examined persons, 99 (30.3%) present with disorders of the schizophrenic spectrum with an average age of 47.6 years (17-80 y.). Amongst the forementioned, males are predominant (62.6 %) and 54.5 % are genetically burdened with psychiatric disorder. Of all 99 certified persons, 92 are diagnosed with paranoid schizophrenia, 4 with delusional disorder, 1 with hebephrenic schizophrenia, 1 with simple schizophrenia and 1 with schizoaffective disorder.

In the certified persons with paranoid schizophrenia the following pathological symptoms have been registered – disorders of the thought process, hallucinations and delusional interpretations of reality, affective disorders with predominance of pathologically modified emotions, severe disorders of the self, lack of insight and parathymia and parabulia.

In the certified with delusional disorder the following psychopathological symptoms have been established - disorders of the thought process, affective disorders with predominance of pathologically modified emotions, disorders of orientation for the self and situations, disorders of attention and memory and lack of insight.
A significant difference is established in the number of psychopathological symptoms and the determined diagnoses \((p < 0.01)\), with an established simultaneous occurrence of mild mental retardation and paranoid schizophrenia whenever the intellectual quotient – IQ is determined to be in the range of 50-69 (tabl.1).

Tabl. 1. Psychopathological symptoms in the psychiatric diagnoses noted in the expertises regarding incapacity (count)

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Legend of the psychopathological symptoms:
1. IQ 50 – 69
2. IQ 40 – 49
3. IQ 30 – 39
4. IQ below 30
5. Disorder in speech and language
6. Hallucinations or delusions of persecution
7. Disorganization and disorientation
8. Affective disorder with a predominance of pathological changes in emotions
9. Disorders of orientation for personality and situation
10. Disorders of attention and memory, which disturb the reproduction of information, relevant to decision-making
11. Somatoform disorders
12. Quantitative and qualitative disorder in self-assessment
13. Alcoholism
14. Negative symptoms
15. Paranoid psychosis

The analysis proves that in 11 of the issued by the court settlements the laws on which they are based are not cited. According to those decisions, 10 of the certified persons are diagnosed with paranoid schizophrenia and one - with schizoaffective disorder.

The number of certified persons by the Law on persons and families is highest – 75. Of them 73 present with paranoid schizophrenia, one - with hebephrenic schizophrenia and one - with simple schizophrenia. Following in numbers are the persons certified by the Law on obligations and contracts - 10, of which 7 present with paranoid schizophrenia, and 3 - with delusional disorder. On cases based on the Family codex two persons are certified, of which one presenting with paranoid schizophrenia and one – with delusional disorder. By the Law on inheritance a single subject has been an object of expert evaluation \((p=0.001)\).

With regards to placement under full guardianship, the greatest percentage of the examined suffer from paranoid schizophrenia – 31.30 \%. Similarly, in the cases where the court decides for limited guardianship, prevailing are the certified persons suffering from paranoid schizophrenia – 45.80 \%.

In 88.9 \% of the cases the forensic psychiatric expertise is accepted by the court, in 9.1 \% - rejected, and in 2.0 \% the certified persons have passed away during the conduction of the civil case.

**Discussion**

Schizophrenia is a severe disabling chronic mental disorder, which significantly impairs the quality of life of the patients and leads to disability, higher death rates, social isolation and unemployment. The lack of insight of the individual regarding the current reality and the occurring changes in their behavior, may create conditions for the occurrence of dangerous for the patient and those around them situations [8, 9]. In the occurrence of such dangerous situations, in some cases court proceedings are initiated. Whenever the patient presents with a psychiatric disorder, or with a possibility of such a disorder, a forensic psychiatric expertise is appointed.

As opposed to other studies, which describe forensic psychiatric expertises and their application to the ruling of a court decision [6,7,10], this research presents empiric proof for the process of assessment of
persons with disorders of the schizophrenic spectrum. This completes the existing literature which focuses mostly on the validity of the instruments for evaluation [11,12] The main drawback of the preparation of a forensic psychiatric expertise in the Republic of Bulgaria is that, up to this moment, there is no issued and accepted standard on the structure of the expertise, which experts could use for their work [13].

**Conclusion**

The present research proves that persons with disorders of the schizophrenic spectrum are often subjects of forensic psychiatric expertises in civil proceedings. For an expert evaluation to be objective, the experts need a legally accepted identical methodology.

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Study in dynamics of postvaccine and postexposure humoral immune response to SARS-CoV-2

Martina Bozhkova 1,2,3, Yordan Kalchev 1,2,4, Gergana Lengerova 1,2,4, Tanya Veliyanova5, Teodora Kalfova1, Steliyan Petrov1, Danyal Pasha6, Hristo Taskov1,2,3, Marianna Mudjeva1,2,3,4

1. Department of Microbiology and Immunology, MU-Plovdiv, Bulgaria
2. Research Institute at MU-Plovdiv, Bulgaria
3. Laboratory of Clinical Immunology, St. George University Hospital – Plovdiv, Bulgaria
4. Laboratory of Microbiology, St. George University Hospital – Plovdiv, Bulgaria
5. Department of Infectious Diseases, Parasitology and Tropical Medicine, MU-Plovdiv, Bulgaria
6. Medical Student, Faculty of Medicine, MU-Plovdiv

Abstract
The nature and duration of specific postvaccine and postexposure immunity against SARS-CoV-2 are important parameters for the development of herd immunity and management of the epidemic. We performed a prospective, longitudinal study to evaluate the humoral immune response induced by mRNA vaccines against SARS-CoV-2 compared to COVID-19 reconvalescent patients. A total number of 113 fully vaccinated individuals with two doses of mRNA vaccine and 40 reconvalescent patients were tested for RBD IgG antibodies and virus-neutralizing antibodies, over six months after vaccination or infection. Our data demonstrated that levels for specific humoral immunity decreased significantly 6 months postvaccination or infection with SARS-CoV-2 but probably are still protective.

Key words: COVID-19, humoral immunity, neutralizing antibodies, RBD IgG antibodies, SARS-CoV-2 vaccines

Introduction
A novel beta coronavirus Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) was described as the causative agent of COVID-19, which spread rapidly and caused health, economic and social crisis (1). In the absence of specific and effective antiviral treatment for COVID-19, long-lasting adaptive immune protection (individual and herd), induced by vaccines or postexposure to the virus, is currently the major tool to deal with the ongoing pandemic (2). Several studies described that specific T-cellular and humoral immune responses to SARS-CoV-2 in reconvalescent individuals last about 8 months (3-6). Similar findings were published post-vaccination (7, 8). However, it is still unclear whether the humoral response to SARS-CoV-2 after vaccinations and infection is the same or differs by nature, duration, and kinetics. The aim of this study was to characterize the antibody persistence and to compare the kinetics of humoral response after infection and vaccination against SARS-CoV-2.

Material and Methods
The group of recovered COVID-19 patients (RP) included 40 individuals (13 males and 27 females) with a median age of 44.5± 22.5 years. Blood samples were collected about 2 months (65.5 ± 37.5 days) and 6 months (mean 198 ± 36 days) post symptoms onset. The group of vaccinated individuals (VI) included 113 donors (36 males and 77 females) vaccinated with mRNA vaccines - Pfizer-BioNTech (Comirnaty) and Moderna (Spikevax). The median age of VI was 46.5± 27.5 years. Blood samples were collected about 2 months (mean 52 ± 23 days) and 6 months (mean 191.5± 33.5 days) after the second dose of the vaccine. Humoral immune response to SARS-CoV-2 was evaluated by detection of receptor-binding-domain (RBD) IgG antibodies (bioMerieux IgG II, VIDAS® PC) and neutralizing antibodies (nAb) by the % of inhibition (cPass Neutralization Antibody Detection kit GenScript, ELISA). T-test was used to compare variables. A p-value below 0.05 was considered statistically significant.

Results
The data about the RBD binding antibodies are presented in Figure 1. On the 2\textsuperscript{nd} month, the levels of IgG BAU/ml were significantly higher in VI compared to RP (mean BAU/ml: 508.6 vs 319.4, \(p<0.001\)). On the 6\textsuperscript{th} month, the levels of antibodies decrease in both groups (for RP from mean 319.4 BAU/ml to mean 151.4 BAU/ml; \(p<0.01\) and for VI – from mean 508.6 BAU/ml to mean 152.9 BAU/ml; \(p<0.001\)). This decline was observed in all individuals (Fig. 1B) and was much faster in VI compared to RP (% of reduction: 69.9 \% vs. 52.6 \%). On 6\textsuperscript{th} month there was no significant difference in levels of binding IgG in RP and VI (mean BAU/ml: 151.4 vs. 152.9; \(p>0.05\)). Figure 2 presents the results for \% of inhibition. On the 2\textsuperscript{nd} month, the values for \% of inhibition are higher in the group of VI compared to RP (mean 84.7 \% vs 69.8 \%, \(p<0.01\)). Between 2\textsuperscript{nd} and 6\textsuperscript{th} month in the VI group, the values of \% of inhibitions decreased from mean 84.7 \% to mean 60.1 \%; \(p<0.01\). In contrast in the group of RP, no significant decline was observed (mean 69.8 \% vs mean 66.6; \(p=0.99\)). It should be noted, that on the 6\textsuperscript{th} month the values of inhibition below 50 \% were recorded in 37.8\% of VI and in 19\% of RP, which indicated that the neutralizing antibodies were exhausted more rapidly in the VI group. We observed a moderate correlation between IgG BAU/ml and \% of inhibition between both tested groups on 2\textsuperscript{nd} month (VI - \(R^2 = 0.41\); \(p<0.01\); RP - \(R^2 = 0.64\); \(p<0.01\)) and 6\textsuperscript{th} month (VI - \(R^2 = 0.48\); \(p<0.01\); RP - \(R^2 = 0.42\); \(p<0.01\)).

Figure 1. (A) Levels of RBD IgG BAU/ml on 2\textsuperscript{nd} and 6\textsuperscript{th} month after vaccination and post symptoms onset (\(* = p<0.01\), \(* *= p<0.001\)). (B) RBD IgG BAU/ml data for each VI plotted on 2\textsuperscript{nd} and 6\textsuperscript{th} month (each line represents 1 participant).

Figure 2. (A) Levels of neutralizing antibodies by \% of inhibition on 2\textsuperscript{nd} and 6\textsuperscript{th} month after vaccination and post symptoms onset (\(* * * = p<0.001\)), (B) \% of inhibition data for each VI plotted on 2\textsuperscript{nd} and 6\textsuperscript{th} month (each line represents 1 participant).

Discussion

By comparing the results of antibody levels in both tested groups, we found that there were different kinetics of the humoral immune response. Despite the higher values of RBD IgG on the 2\textsuperscript{nd} month in the group of VI, there was no significant difference on the 6\textsuperscript{th} month between the two tested groups. We observed a much faster decrease of the antibody levels in the VI group. This tendency is in accordance with other studies (7). The higher antibody levels found in VI may be due to a stronger stimulus to the immune system after the administration of two doses of vaccine compared with a single encounter with the virus. It is known that the level of antibodies in reconvalescent is related to the disease severity and the viral load (9). Since we tested reconvalescent patients with mild symptoms, one could expect also a weaker immune response. Khoury D. et al. proved that values above 50\% for neutralizing-antibodies can be protective (10). Our study demonstrated that on the 6\textsuperscript{th} month in 62.2\% of VI and in 81\% of RP values of \% inhibition were above 50 \%. This suggests that despite decreased levels of specific antibodies, the majority of tested individuals still have protective immunity against the virus on the 6\textsuperscript{th} month.

There are contradictory data about the correlation between RBD IgG and neutralizing antibodies to SARS-CoV-2. Cassaniti I. et al. reported no significant correlation (8). In opposite, we found a moderate correlation between the values RBD IgG and neutralizing antibodies, which is in agreement with the results of Johnson M. et al and Peterhoff D. et al. (11).
Conclusion
Our study demonstrated that the specific humoral immunity to SARS-CoV-2 decreased over six months in both vaccinated and convalescent individuals. However, the levels of IgG/RBD and neutralizing antibodies in both groups were still protective in the majority of individuals. These results would serve to clarify the time interval and frequency of revaccination, as well as to develop scientific-based policies and programs for the administration of vaccines against SARS-CoV-2.

Acknowledgments
This study was supported by Project "National University Complex for Biomedical and Applied Research, related to BBMRI-ERIC" (NUCBАR-BBMRI.BG) – a site of the National Roadmap of Research Infrastructure of Bulgaria 2021-2027, agreement D01-395 / 18.12.2020.

References
Multiplex PCR for rapid identification of pathogens directly from positive blood cultures

Gergana Lengerova 1,2,3, Yordan Kalchev 1,2,3, Ralitsa Raycheva 4, Michael Petrov 1,3

1. Department of Microbiology and Immunology, Faculty of Pharmacy, MU-Plovdiv, Bulgaria
2. Laboratory of Microbiology, University Hospital St. George, Plovdiv, Bulgaria
3. Research Institute at Medical University - Plovdiv, Bulgaria
4. Department of Social Medicine and Public Health, Faculty of Public Health, MU-Plovdiv

Abstract: The present study aims to determine the diagnostic significance of multiplex PCR for a more accurate and rapid microbiological diagnosis of positive blood cultures compared to routine microbiological testing. This method makes it possible to detect associated bloodstream infections and genes encoding antibiotic resistance (blaKPC, mecA, vanA/B) directly from the positive blood culture. Multiplex PCR has many advantages over conventional microbiology methods still used for the diagnosis of bacteremia/fungemia. Prompt and reliable simultaneous detection of the most common pathogens associated with bloodstream infections would guide physicians to manage and optimize treatment in a timely manner.

Introduction: Bloodstream infections (BSI) are characterized by high morbidity and mortality [1]. Rapid diagnosis of positive blood cultures is essential for the patients, but routine microbiological methods take a relatively long time and can lead to inappropriate initial therapy, development of antimicrobial resistance, prolonged hospital stays, increased medical costs, and reduced survival rate of patients [1-3]. Unfortunately, the detection of microorganisms directly from blood encounters many difficulties, which explains why diagnostic techniques that can identify pathogens directly from positive blood cultures on the market are scarce.

Aim: To study the application of multiplex PCR (mPCR) for the rapid detection (in 70 minutes) of bacterial pathogens and resistance genes directly from positive blood cultures.

Material and methods: 72 positive blood cultures were analysed from patients admitted at the University Hospital St. George, Plovdiv. Blood cultures from patients with clinical and laboratory abnormalities were collected by venipuncture in compliance with the antiseptics requirements and transported as soon as possible. The study was conducted in the Laboratory of Microbiology at the University Hospital St. George, Plovdiv, and in the Department of Microbiology and Immunology of the Medical University - Plovdiv. The blood cultures were incubated in BacT/ALERT 3D-60 (bioMérieux, France). Molecular genetic analysis was performed on the positive blood cultures by a multiplex polymerase chain reaction (FilmArray, bioMérieux, France). It is a closed system that contains all the reagents needed for the stages of isolation, amplification, and detection of specific genes. The blood culture identification (BCID) panel of the mPCR is capable of simultaneous detection of 25 pathogens and 3 genes encoding antibiotic resistance, directly from the positive blood culture, skipping the steps of isolation and cultivation of the microorganisms. The applied software package performs the interpretation of the obtained results. The results were compared with the routine biochemical methods, semi-automated and automated microbial identification systems, including Vitek-2 Compact, Vitek MS Plus (bioMérieux, France). Antibiotic susceptibility was determined by the Bauer-Kirby disk-diffusion method and MIC by the VITEK-2 Compact. Processing and analysis of raw data were performed with statistical package software for social sciences SPSS Statistics v.22 (IBM, USA). A less than p<0.05 was considered statistically significant.

Results and discussion: The new method - mPCR, has several advantages over the conventional microbiological techniques – direct identification from the positive blood culture, higher speed, simplified workflow, and minimized risk of contamination. FilmArray BCID allows for the simultaneous detection of certain bacteria and fungi within 70 minutes [1]. The present study included 40 men (55.6%) and 32 women (44.4%) with a mean age of 46.7±3.2 years. The patients were mainly from the clinics of Pediatrics, Anesthesiology and Intensive Care Unit (ICU), Cardiac Surgery, Infectious diseases. In 65 (90.3%) of the positive blood cultures, the mPCR detected microorganisms, and in 7 (9.7%) no microorganisms were found by the applied
method. These results were confirmed by classical identification methods in 63 (87.5%) cases. In 7 (9.72%) cases, the classical methods identified microorganisms, which were not included in the test spectrum of mPCR - *Corynebacterium spp.*, *Kocuria spp.*, aerobic and anaerobic spore-forming bacteria. In the remaining 2 (2.77%) cases mPCR proved the presence of *S. pneumoniae*, which was not confirmed by the routine methods, due to the lack of bacterial growth. Clinically, the patients had symptoms of lobar pneumonia and had already started antimicrobial therapy. These cases show the potentiality of the method for the detection of microorganisms in cases when the empirical antimicrobial therapy has already been initiated. We found a statistically significant difference between the relative proportions of PCR results compared to routine microbiological methods (*z*=5.10; *p*=0.0001). The BCID panel accelerates the identification of the most common bacteria and fungi that cause BSIs. It can also be used as a helpful tool in addition to routine microbiological analysis for rapid and prompt testing. These observations were confirmed by a study in the University of South Carolina on the potential of FilmArray BCID on 783 patients. According to the team of MacVane *et al.*, the panel complements but does not replace conventional microbiological methods of identification and antimicrobial susceptibility testing, which we also confirm with our study [2].

The method allows the detection of more than one pathogen in one sample. Associated bloodstream infections were found in 9 (12.5%) of the studied patients, including combinations of only Gram-negative bacteria, as well as Gram-negative and Gram-positive microorganisms. Polymicrobial bloodstream infections were also confirmed by routine diagnostic tests. Among the mPCR-detected pathogens Gram-positive isolates (n=29; 37.2%) dominated over the Gram-negative (n=25; 32.1%), followed by *Candida spp.* (n=15; 19.2%) and non-fermentative bacteria (n=9; 11.5%). Figure 1 depicts the distribution (n/%) of the detected microorganisms. The included genes for antibiotic resistance in the panel were detected in 8 (11.2%) of the samples – *blaKPC* (n=3), *mecA* (n=3) and *vanA/B* (n=2). They were also confirmed by routine tests for antibiotic susceptibility. Using the mPCR, we can identify infections caused by antibiotic-resistant strains, that have not been adequately treated. This may allow clinicians to start targeted antibiotic treatment or de-escalate the given therapy, thus reducing the costs from the empirical application of broad-spectrum antibiotics. Early and appropriate antimicrobial treatment of BSIs has been proven to increase survival rates in several studies [3,4]. In the present study, we calculated a total sensitivity of the method of 90.1% and 100% sensitivity for the detection of resistance genes. Although the number of pathogens is limited, several previous BCID panel studies reported excellent sensitivity and specificity [1,6,7]. Similar to our results, the studies of Altun *et al.*, Blaschke *et al.* showed that FilmArray is a reliable system for rapid identification of bacteria and fungi from positive blood cultures with high sensitivity and specificity [8,9].

Fig 1. Relative distribution (%/n) of microorganisms from blood cultures detected by mPCR.

**Conclusion:** The FilmArray BCID panel is an accurate test for the rapid identification of pathogens and resistance genes directly from positive blood cultures. Furthermore, it is a reliable method for the simultaneous detection of several pathogens. These advantages and the rapid identification time would promote the
initiation of adequate antimicrobial therapy and improve the medical care and management of patients with BSIs.

**Acknowledgments:** This work was funded by project NUCBAS-BBMRI.BG, Contract D01-395/18.12.2020 (Ministry of Education and Science) within the frame of the Bulgarian National Roadmap for Research Infrastructure.

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