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Supplement J.IMAB

Varna, Bulgaria, 2021/2022

Section Medicine:

SARS-CoV-2 distribution in North-East Bulgaria

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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

Region) (<https://data.egov.bg/data/resourceView/cb5d7df0-3066-4d7a-b4a1-ac26525e0f0c>). From 1st May 2020 to 30th September 2021, a total of four waves of COVID-19 were registered with more significant peaks during the second (November-December 2020) and the third wave (March-April 2021). The relatively low severity of the first wave could be explained with the strict containment measures at the beginning of the pandemic. Similarly, the infection in many European countries showed a higher intensity during the second wave because of the increased infectiousness of the new mutations and the partial abandonment of the measures (1).

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.

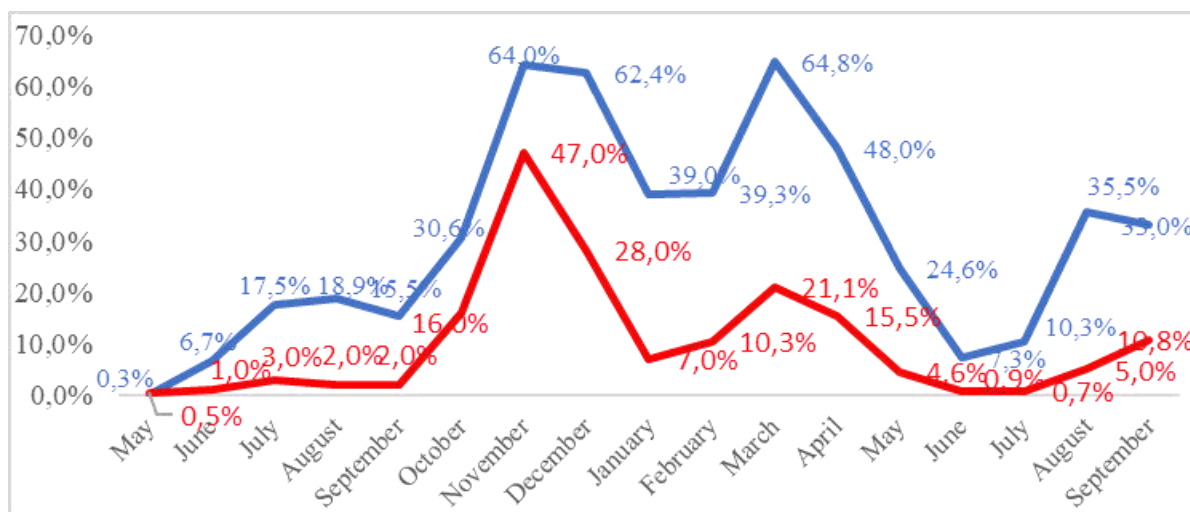


Figure 1. Proportion of positives samples in Bulgaria (in red) and in University Hospital “St. Marina”, Varna (in blue) (01.05.2020 – 30.09.2021).

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.

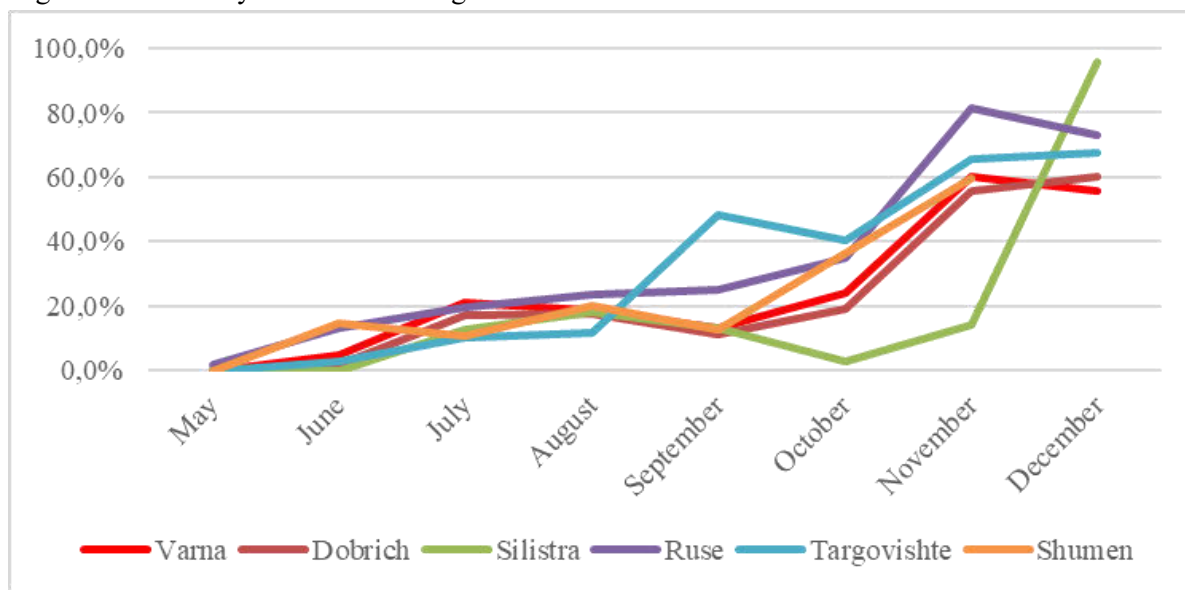


Figure 2. Regional distribution by months of the positive SARS-CoV-2 samples (outpatients and hospitalized patients) from May to December 2020.

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:

1. Fokas AS, Kastis GA. SARS-CoV-2: The Second Wave in Europe. J Med Internet Res. 2021 May 1;23(5).
2. Nichols GL, Gillingham EL, Macintyre HL, et al. Coronavirus seasonality, respiratory infections and weather. BMC Infect Dis. 2021 Dec 1;21(1):1–15.
3. Zimmermann P, Curtis N. Why is COVID-19 less severe in children? A review of the proposed mechanisms underlying the age-related difference in severity of SARS-CoV-2 infections. Arch Dis Child. 2020 May 1;106(5):429–39.

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

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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.

[1] 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 (\pm 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

	Patients (N=40)	Females (n=27)	Males (n=13)
COVID-19	10 (25%)	5 (18.5%)	5 (38.5%)
Vaccinated	2 (5%)	0	2 (15.4%)
Refused vaccination	10 (25%)	8 (29.6%)	2 (15.4%)
Undecided	28 (70%)	19 (70.4%)	9 (69.2%)

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:

- Schmidt E, Kasperkiewicz M, Joly P. Pemphigus. *Lancet*. 2019 Sep; 394 (10201): 882-894. doi: 10.1016/S0140-6736(19)31778-7 [PubMed]
- Drenovska K, Vassileva S, Tanev I, Joly P. Impact of COVID-19 on autoimmune blistering diseases. *Clin Dermatol* 2021 May-Jun; 39(3): 359-368. doi: 10.1016/j.clindermatol.2021.01.007 [PubMed]
- Balestri R, Rech G, Girardelli CR. Occurrence of SARS-Co-2 during mycophenolate mofetil treatment for pemphigus. *J Eur Acad Dermatol Venereol* 2020 Sep; 34(9): e435-436. doi: 10.1111/jdv.16578 [PubMed]
- Carugno A, Sena P, Raponi F, Test ER, Vezzoli P. Patients with bullous skin diseases in a high-epidemic COVID-19 area, Bergamo, Italy. *Br J Dermatol* 2020 Sep; 183(3): 589-591. doi: 10.1111/bjd.19266 [PubMed]
- Di Altobrando A, Patrizia A, Abbenante D, Bardazzi F. Rituximab: a safe therapeutic option during the COVID-19 pandemic. *J Dermatol Treat* 2020 Jul 29; 1. 10.1080/09546634.2020.1800565 [PubMed]
- Sinagra JL, Vedovelli C, Binazzi R, Salemme A, Moro F, Mazzanti C, et al. Case report: Complete and fast recovery from severe COVID-19 in a pemphigus patient treated with rituximab. *Front Immunol* 2021 April 16; 12: 665522. doi: 10.3389/fimmu.2021.665522 [PubMed]
- Ghalamkarpour F, Pourani MR. Aggressive course of pemphigus vulgaris following COVID-19 infection. *Dermatol Ther* 2020 Nov 8: e14398 doi: 10.1111/dth.14398 [PubMed]

8. Shahidi-Dadras M, Abdollahimajd F, Ohadi L, Tabary M, Agahri F, Mozafari N, et al. COVID-19 in pemphigus vulgaris patients with previous rituximab therapy: a tele-medicine-experience. *J Dermatol Treat* 2020 Jul 9; 1-2. doi:10.1080/09546634.2020.1789041 [PubMed]
9. Uzuncakmak TK, Özkoca D, Askin O, Kutlubay Z. Can rituximab be used in the treatment of pemphigus vulgaris during the COVID-19 pandemic? *Dermatol Ther* 2021 Jan; 34(1): e146476. doi:10.1111/dth.14647 [PubMed]
10. Kridin K, Schonmann Y, Weinstein O, Schmidt E, Ludwig RJ, Cohen AD. The risk of COVID-19 in patients with bullous pemphigoid and pemphigus: A population based cohort study. *J Amer Acad Dermatol* 2021 Jul; 85:79-87. doi.org/10.1016/j.jaad.2021.02.087 [PubMed]
11. Kasperkiewicz M, Strong R, Mead K, Yale M, Zillikens D, Woodley DT, et al. COVID-19 vaccine acceptance and hesitancy in patients with immunobullous diseases: a cross-sectional study of the International Pemphigus and Pemphigoid Foundation. *Br J Dermatol* 2021 Nov 29. doi: 10.1111/bjd.20906 [PubMed]
12. Damiani G, Pacifico A, Pelloni F, Iorizzo M. The first dose of COVID-19 vaccine may trigger pemphigus and bullous pemphigoid flares: is the second dose therefore contraindicated? *J Eur Acad Dermatol Venereol* 2021 Oct; 35(10): e645-e647 13 doi: 10.1111/jdv.174772 [PubMed]
13. Thongprasom K, Penagis N, Phattarataratip E, Samaranayake L. Oral pemphigus after COVID-19 vaccination. *Oral Dis* 2021 Sep; 00: 1-2. doi: 10.1111/odi.14034 [PubMed]
14. Solimani F, Mansour Y, Didona D, Dilling A, Ghoreschi K, Meier K. Development of severe pemphigus vulgaris following SARS-CoV-2 vaccination with BNT162b2. *J Acad Dermatol Venereol* 2021 Oct; 35(10): e649-e651 doi: 10.1111/jdv.17480 [PubMed]
15. Guidance from the EADV Task Force Autoimmune Blistering Diseases during the COVID-19 pandemic. Publication date: 2020 Apr 17 Update: 2021 Jan 14 Available from: www.eadv.org/covid-19/task-force [Internet]Section Dental Medicine:

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

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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 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 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.

References:

1. Yang J. Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: a systematic review and meta- analysis. *Int J Infect Dis.* 2020; 94:91-5; doi:10.1016/j.ijid.2020.03.017
2. Tzacheva N., *Occupational Medicine in Republic of Bulgaria: Theory, Methodology, Practice, Abstract on Doctor of Science, Sofia, 2014, 170.* [in Bulgarian];
3. Tzacheva, N, *Risk Assessment by Clinics and Wards in the COVID 19 Context, UNI HOSPITAL, 2021 (under print)* [in Bulgarian];
4. da Rosa Mesquita R, Francelino Silva Junior LC, Santos Santana FM, et al. Clinical manifestations of COVID-19 in the general population: systematic review. *Wien Klin Wochenschr.* 2020;1-6. doi:10.1007/s00508-020-01760-4.
5. Centers for Disease Control and Prevention (CDC). Interim U.S. Guidance for Risk Assessment and Public Health Management of Healthcare Personnel with Potential Exposure in a Healthcare Setting to Patients with 2019 Novel Coronavirus (2019-nCoV) 2020 [cited 2020 20 February]. Available from: <https://www.cdc.gov/coronavirus/2019-ncov/hcp/guidance-risk-assesment-hcp.html>
6. <https://www.ecdc.europa.eu/en/current-risk-assessment-novel-coronavirus-situation>

Healthcare-associated infections in the shadow of COVID – 19

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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 a significant portion of accepted emergency cases also tested positive for COVID 19. Several studies in the comparable time frame [1,2,3] describe two alternatives to the situation of HAIs. During the first period of the pandemic, in the hospitals/wards where the intensity of care for COVID-19 patients has increased moderately, the introduced emergency epidemic measures have led to the correct registration and reduction of HAIs. On the contrary, in overloaded hospitals/wards, HAIs were not reported at optimum, especially at the beginning in the initial moment of fear of the new infectious threat and a worldwide shortage of respirators, PPE, disinfectants, and staff [1]. The second case situation was observed in UMHAT "St. Marina", Varna. Due to the large capacity and high-tech equipment, 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.

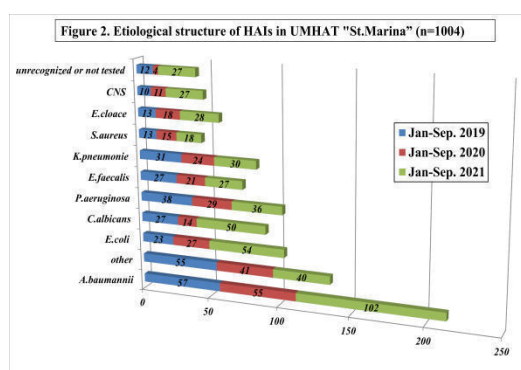
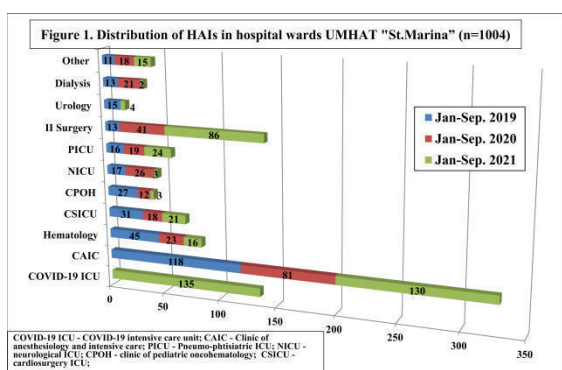


Figure 2. demonstrates the etiological agents diagnosed in the Microbiology Laboratory of UMHAT "St. Marina".

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:

1. Palmore TN, Henderson DK, Healthcare-associated infections during the coronavirus disease 2019 (COVID-19) pandemic, *Infect Control Hosp Epidemiol.* 2021, 1372-1373. doi:10.1017/ice.2021.377
2. Perez S, Innes GK, et al. Increase in Hospital-Acquired Carbapenem-Resistant *Acinetobacter baumannii* Infection and Colonization in an Acute Care Hospital During a Surge in COVID-19 Admissions — New Jersey, February–July 2020. *MMWR Morb Mortal Wkly Rep.* 2020; 69:1827–1831. DOI: <http://dx.doi.org/10.15585/mmwr.mm6948e1>
3. Prestel C, Anderson E, et al. *Candida auris* outbreak in a COVID-19 specialty care unit—Florida, July–August 2020. *Morb Mortal Wkly Rep.* 2021;70:56–57.
4. Monov D, Kolarova M, et al. Infections related to medical care and their outbreak in the conditions of the Varna district for the period 2014-2016, *Varna Medical Forum*, 6: 117-123.
5. Weiner-Lastinger LM, Pattabiraman V, Konnor R, Patel P, Wong E, Xu SY et al. The impact of coronavirus disease 2019 (COVID-19) on healthcare-associated infections in 2020: A summary of data reported to the National Healthcare Safety Network. *Infect Control Hosp Epidemiol*, (2021). 1-14. doi:10.1017/ice.2021.362
6. 2019 National and State Healthcare-Associated Infections Progress Report. Centers for Disease Control and Prevention (CDC) website. Published December 2020. Accessed April 30, 2021; <https://www.cdc.gov/hai/data/portal/progress-report.html>.
7. Patel PR, Weiner-Lastinger LM, Dudeck MA, et al. Impact of COVID-19 pandemic on central-line-associated bloodstream infections during the early months of 2020, National Healthcare Safety Network. *Infect Control Hosp Epidemiol.* 2021 Mar :1-4. doi: 10.1017/ice.2021.108. PMID: 33719981; PMCID: PMC8047389

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

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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%).

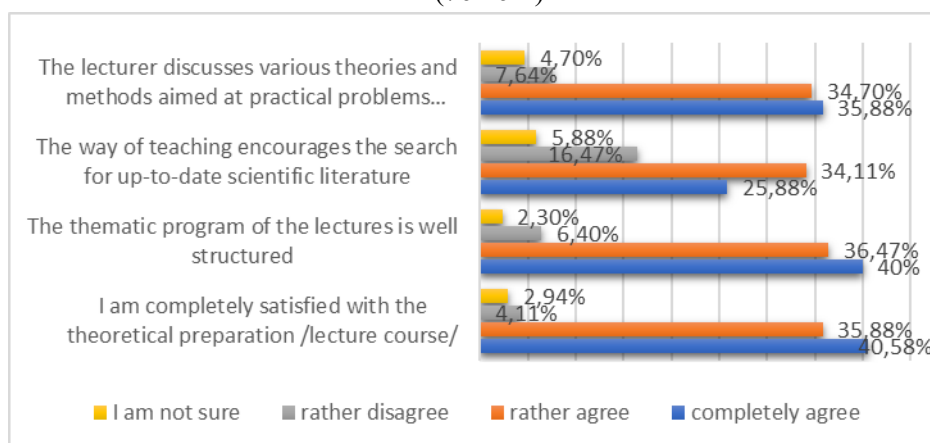


Fig.1. Assessment of students for theoretical training

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.

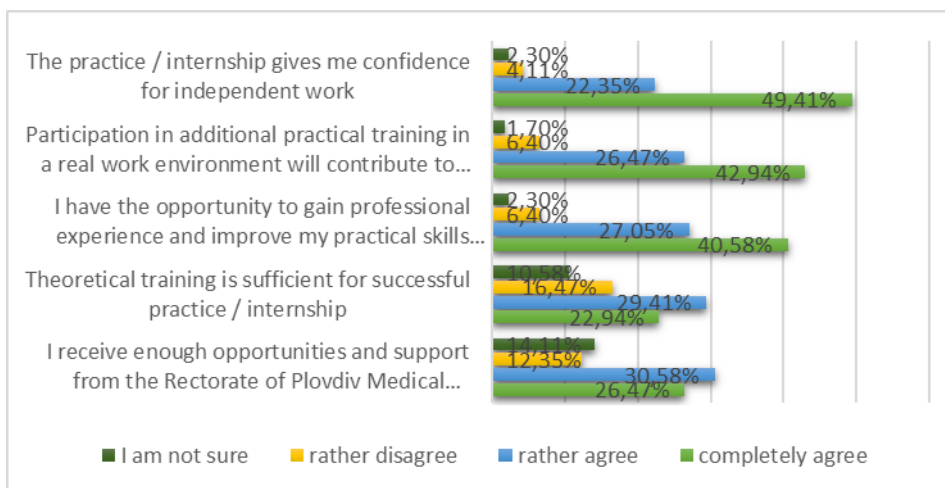


Fig. 2. Evaluation of practical training during hybrid training

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.

References:

1. Biebeler H, Schreiber D. [Training in corona time: results of an empirical study on the effects of the corona pandemic on training companies]. Scientific discussion papers, 2020 [in German] [[Internet](#)]
2. Garrote A, Neuenschwander M, Hofmann J, Mayland C, Niederbacher E, Prieth V et al. Distance learning during the coronavirus pandemic: analysis of challenges and conditions for success, 2020 [in German] [[Internet](#)]
3. Yaneva A, Mateva N, Kilova K. [Serious games application in modern education]. Health policy and management (supplements), 2020; 20: 113-115. ISSN 1313-4981 [in Bulgarian]
4. Kilova K, Kitova T. Student opinion on the quality of distance medical learning in pandemic conditions. Journal of Environmental Protection and Ecology, 2021; 22 (4): 1706–1714; ISSN 1311-5065 [[CrossRef http://www.jepe-journal.info/](http://www.jepe-journal.info/)]

5. Kilova K. [Distance learning in the conditions of a pandemic through the view of the students from Medical University – Plovdiv]. Scientific works of the Union of Scientists in Bulgaria - Plovdiv, Series G – Medicine, Pharmacy and Dental Medicine. 2020, XXV:210-214 [in Bulgarian] [[Internet](#)]

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The impact of Covid-19 pandemic on the structure of of the temporary disability of health workers in a state hospital

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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 peri 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.

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

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.

References:

1. Tzacheva, N Unified methodology for analysis and assessment of the health status of insured workers, National Center for Hygiene, Medical Ecology and Nutrition, Sofia, 2001. [in Bulgarian];
2. ILO Monitor: COVID-19 and the world of work. Second edition. Geneva: International Labour Organization; 2020 (https://www.ilo.org/global/topics/coronavirus/impacts-and-responses/WCMS_740877/lang-en/index.htm, accessed 5 September 2020).
3. Working for health and growth: investing in the health workforce. Report of the High-Level Commission on Health Employment and Economic Growth. Geneva: WHO 2016 (<https://apps.who.int/iris/bitstream/handle/10665/250047/9789241511308-eng.pdf>, accessed 5 September 2020).
4. Hristova L., N. Tzacheva, R. Nikolova, V. Grigorov, Retrospective dynamic study of the current morbidity and nonmedical specialists in pre – hospital health care, *Journal of IMAB*, ISSN: 1312-773X, 2021 Apr-Jun; 27(2); DOI: 10.5272/jimab.2021272.3741
5. Hristova L., N. Tzacheva, R. Nikolova, A five-year retrospective period study of period prevalence with view to working environment and work process in two diagnostic and consulting centers in Varna, *J of imab*, ISSN: 1312-773X (Online), 2021 jan-mar; 26(1):2981-2984; DOI: 10.5272/jimab.2020261.2981
6. Hristova L., N. Tzacheva, Working conditions and morbidity in Bulgarian healthcare professionals, targeted study on leading disabilities, Lap Lambert Academic Publishing, 2020, ISSN: 978-620-3-20213-7: 46; www.lap-publishing.com/catalog/details/store/gb/book/978-620-3-20213-7/working-conditions-and-morbidity-in-bulgarian-healthcare-professionals
7. Hristova L, Filipidou Hr, Chernaeva M, Tsacheva N. Diseases with temporary disability of the respiratory system, working in a multidisciplinary hospital in Varna, 21 International Pulmonary Congress, Thessaloniki. 2016. 11 – report. [in Greece]
8. Occupational health and safety risks in the healthcare sector - Guide to prevention and good practice. European Agency for Safety and Health at Work. www.ilo.org/dyn/travail/docs/1965/osh.pdf
9. Pappa S, Ntella V, Giannakas T, Giannakoulis VG, Papoutsis E, Katsaounou P. Prevalence of depression, anxiety, and insomnia among healthcare workers during the COVID-19 pandemic: a systematic review and meta-analysis. *Brain Behav Immun.* 2020;88:901–7 (<https://doi.org/10.1016/j.bbi.2020.05.026>, accessed 5 September 2020).
10. Samuneva-Zhelyabova M, Lyubomirova K, Kundurzhiev T. Sleep Disorders and Fatigue among Emergency Healthcare Workers. *J of IMAB.* 2020 Apr-Jun;26(2):3163-3167. DOI: 10.5272/jimab.2020262.3163
11. Samuneva-Zhelyabova M., K Lyubomirova, T Kundurzhiev, Ergonomic risk factors and physical fatigue among ambulance workers in Bulgaria, *European Journal of Public Health*, Volume 30, Issue Supplement_5, September 2020, ckaa166.524, <https://doi.org/10.1093/eurpub/kaa166.524>

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Role of 18F-FDG PET/CT for unsuspected recurrence of head and neck carcinoma in patient restaged for the colorectal carcinoma

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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 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

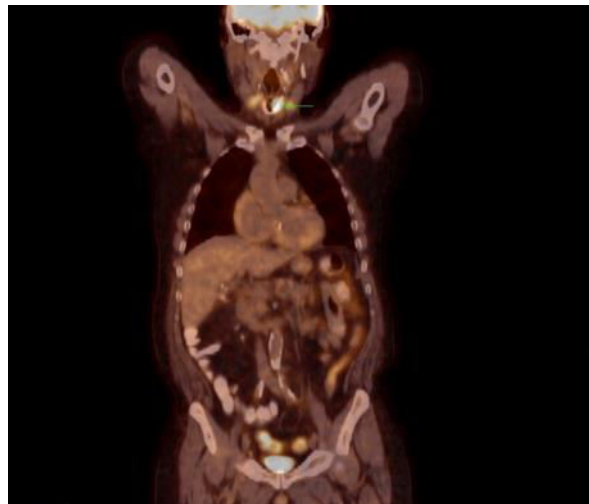
We report a patient with primary abdominal tumor. Sixty-seven years old male patient with colorectal carcinoma, T2N0M0G2. He was referred to our hospital for 18F-FDG PET/CT examination in April 2017, with biochemical suspicion of progression. He had increased level of tumor marker: Ca 19,9- 74. The ultrasound of abdomen and pelvis did not show any abnormality. He was treated with hemicolectomy 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 in patient referred for restaging another primary carcinoma (colorectal cancer) and helped timely treatment of newly diagnosed recurrence of laryngeal cancer.

References:

1. Al-Ibraheem A, Buck A, Krause BJ, Scheidhauer K, Schwider M. Clinical applications of FDG PET and PET/CT in head and neck cancer. *J Oncol* 2009; 2209:208725 Crossref Medline Google Scholar
2. Adam R, Vinet E. Regional treatment of metastasis: surgery of colorectal liver metastases. *Ann Oncol*. 2004;15(suppl 4):iv103–iv106. CrossRefPubMedGoogle Scholar
3. Kim SA, Roh JL, Kim JS, Lee JH, Lee SH, Choi SH, Nam SY, Kim SY. 18F-FDG PET/CT surveillance for the detection of recurrence in patients with head and neck cancer. *Eur J Cancer*. 2017 Feb;72:62-70. doi: 10.1016/j.ejca.2016.11.009. Epub 2016 Dec 24. PMID: 28027517. [PubMed]
4. Moon, S. H., Choi, J. Y., Lee, H. J., Son, Y. I., Baek, C. H., Ahn, Y. C., Ahn, M. J., Park, K., & Kim, B. T. (2015). Prognostic value of volume-based positron emission tomography/computed tomography in patients with nasopharyngeal carcinoma treated with concurrent chemoradiotherapy. *Clinical and experimental otorhinolaryngology*, 8(2), 142–148. <https://doi.org/10.3342/ceo.2015.8.2.142> [PubMed]
5. Krabbe, C. A., Pruijm, J., Dijkstra, P. U., Balink, H., van der Laan, B. F., de Visscher, J. G., & Roodenburg, J. L. (2009). 18F-FDG PET as a routine posttreatment surveillance tool in oral and oropharyngeal squamous cell carcinoma: a prospective study. *Journal of nuclear medicine : official publication, Society of Nuclear Medicine*, 50(12), 1940–1947. <https://doi.org/10.2967/jnumed.109.065300> [PubMed]
6. Pfister, D. G., Spencer, S., Adelstein, D., Adkins, D., Anzai, Y., Brizel, D. M., Bruce, J. Y., Busse, P. M., Caudell, J. J., Cmelak, A. J., Colevas, A. D., Eisele, D. W., Fenton, M., Foote, R. L., Galloway, T., Gillison, M. L., Haddad, R. I., Hicks, W. L., Hitchcock, Y. J., Jimeno, A., ... Darlow, S. D. (2020). Head and Neck Cancers, Version 2.2020, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network : JNCCN*, 18(7), 873–898. <https://doi.org/10.6004/jnccn.2020.0031> [PubMed]
7. Isles, M. G., McConkey, C., & Mehanna, H. M. (2008). A systematic review and meta-analysis of the role of positron emission tomography in the follow up of head and neck squamous cell carcinoma following radiotherapy or chemoradiotherapy. *Clinical otolaryngology : official journal of ENT-UK ; official journal of Netherlands Society for Oto-Rhino-Laryngology & Cervico-Facial Surgery*, 33(3), 210–222. <https://doi.org/10.1111/j.1749-4486.2008.01688.x> [PubMed]
8. Wong W. L. (2021). PET-CT for Staging and Detection of Recurrence of Head and Neck Cancer. *Seminars in nuclear medicine*, 51(1), 13–25. <https://doi.org/10.1053/j.semnuclmed.2020.09.004> [PubMed]

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⁶⁸Ga-PSMA-avid Primary Squamous Cell Lung Cancer on PET/CT: Incidental synchronous malignancy in primary staging for Prostate Cancer (PC): a Case report and literature review

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Abstract

Introduction/ Purpose: ⁶⁸Ga-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 ⁶⁸Ga-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 ⁶⁸Ga-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 ⁶⁸Ga-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: ⁶⁸Ga-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]. ⁶⁸Ga-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 ⁶⁸Ga-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 ⁶⁸Ga-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).

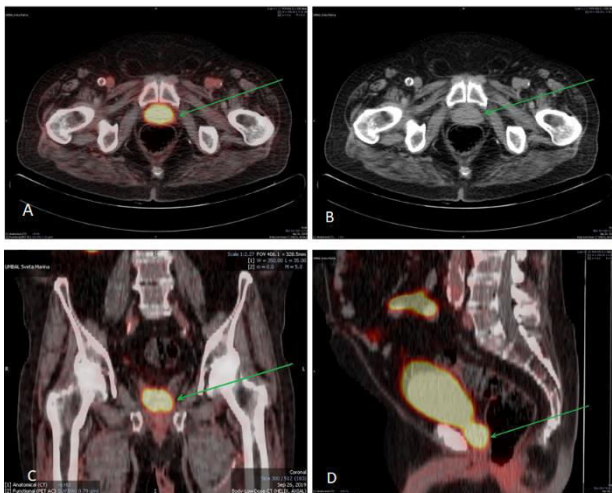


Figure 1 A-D

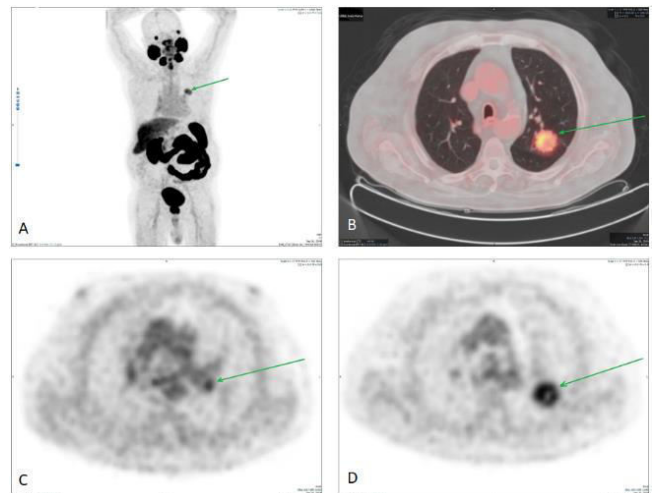


Figure 2 A-D.

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.

References:

1. Gordon I, Tretiakova M, Noffsinger A, Hart J, Reuter V, Al-Ahmadie H. Prostate-specific membrane antigen expression in regeneration and repair. *Mod Pathol.* 2008; 21:1421–1427. <https://doi.org/10.1038/modpathol.2008.143>
2. Bostwick DG, Pacelli A, Blute M, Roche P, Murphy GP. Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: a study of 184 cases. *Cancer.* 1998; 82:2256–61. [https://doi.org/10.1002/\(SICI\)1097-0142\(19980601\)82:11<2256::AID-CNCR22>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1097-0142(19980601)82:11<2256::AID-CNCR22>3.0.CO;2-S)
3. Corfield J, Perera M, Bolton D, & Lawrentschuk N. ⁶⁸Ga-prostate specific membrane antigen (PSMA) positron emission tomography (PET) for primary staging of high-risk prostate cancer: a systematic review. *World Journal of Urology.* 2018; 36(4): 519–527. [doi:10.1007/s00345-018-2182-1](https://doi.org/10.1007/s00345-018-2182-1)
4. Perera M, Papa N, Christidis D, Wetherell D, Hofman MS, Murphy DG et al. Sensitivity, specificity, and predictors of positive ⁶⁸Ga-prostate-specific membrane antigen positron emission tomography in advanced prostate cancer: a systematic review and meta-analysis. *Eur Urol.* 2016; 70(6):926–937 DOI: [10.1016/j.eururo.2016.06.021](https://doi.org/10.1016/j.eururo.2016.06.021)
5. Shetty D, Patel D, Le K, Bui C, Mansberg R. Pitfalls in Gallium-68 PSMA PET/CT Interpretation-A

- Pictorial Review. *Tomography*. 2018; 4(4):182-193. doi: [10.18383/j.tom.2018.00021](https://doi.org/10.18383/j.tom.2018.00021). PMID: [30588504](https://pubmed.ncbi.nlm.nih.gov/30588504/); PMCID: [PMC6299744](https://pubmed.ncbi.nlm.nih.gov/PMC6299744/).
6. Wang H, Wang S, Song W, Pan Y, Yu H, Si T, et al. Expression of Prostate Specific Membrane Antigen in Lung Cancer Cells and Tumor Neovasculature Endothelial Cells and Its Clinical Significance. *PLoS ONE*. 2015;10(5):e0125924. doi: [10.1371/journal.pone.0125924](https://doi.org/10.1371/journal.pone.0125924)
7. Osman MM, Irvani A., Hicks RJ and Hofman MS. Detection of Synchronous Primary Malignancies with 68Ga-Labeled Prostate-Specific Membrane Antigen PET/CT in Patients with Prostate Cancer: Frequency in 764 Patients. *Journal of Nuclear Medicine*. 2017 Dec; 58 (12):1938-1942. DOI: <https://doi.org/10.2967/jnumed.117.190215>
8. Fendler WP, Eiber M, Beheshti M, Bomanji J, Ceci F, Cho S et al. ⁶⁸Ga-PSMA PET/CT: Joint EANM and SNMMI procedure guideline for prostate cancer imaging: version 1.0. *Eur J Nucl Med Mol Imaging*. 2017; 44:1014–1024. <https://doi.org/10.1007/s00259-017-3670-z>
9. Schmidt LH, Heitkötter B, Schulze AB, Schliemann C, Steinestel K, Trautmann M et al. Prostate specific membrane antigen (PSMA) expression in non-small cell lung cancer. *PLoS One*. 2017;12(10):e0186280. doi: [10.1371/journal.pone.0186280](https://doi.org/10.1371/journal.pone.0186280)
10. Usmani S, Sadeq A, Marafi F, Esmail A, al Kandari F, & Ahmed, N. 68Ga-PSMA Uptake in the Lung. Metastatic Versus Primary Lung Tumor. *Clinical Nuclear Medicine*. 2019 Feb; 45(2):80-82. doi: [10.1097/rlu.0000000000002870](https://doi.org/10.1097/rlu.0000000000002870)
11. Jochumsen MR, Gormsen LC, Nielsen GL. 68Ga-PSMA avid primary adenocarcinoma of the lung with complementary low 18F-FDG uptake. *Clin Nucl Med*. 2018;43:117–119. DOI: [10.1097/rlu.0000000000001935](https://doi.org/10.1097/rlu.0000000000001935)
12. Chia JY, Loi HY, Khor LK, Lee KS, Seow YH. Primary lung adenocarcinoma with 68Galium prostate-specific membrane antigen-PET/CT scan avidity in a patient on surveillance after prostatectomy. *Clin Genitourin Cancer*. 2018;16: 525–527. DOI: [10.1016/j.clgc.2018.03.009](https://doi.org/10.1016/j.clgc.2018.03.009)
13. Sharma P. 68Ga-PSMA–Avid Small Cell Lung Cancer on PET/CT. *Clinical Nuclear Medicine*. 2020 Dec;45(12):1016-1017. doi: [10.1097/RLU.0000000000003270](https://doi.org/10.1097/RLU.0000000000003270)
14. Pyka T, Weirich G, Einspieler I, Maurer T, Theisen J, Hatzichristodoulou G et al. 68Ga-PSMA-HBED-CC PET for Differential Diagnosis of Suggestive Lung Lesions in Patients with Prostate Cancer. *J Nucl Med*. 2016; 57:367–371 DOI: [10.2967/jnumed.115.164442](https://doi.org/10.2967/jnumed.115.164442)
15. Jochumsen MR; Gormsen LC, Nielsen GL. 68Ga-PSMA Avid Primary Adenocarcinoma of the Lung with complementary low 18F-FDG Uptake, *Clinical Nuclear Medicine*: 2018. 43 (2):117-119 doi: [10.1097/RLU.0000000000001935](https://doi.org/10.1097/RLU.0000000000001935)

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

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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. Kirkovich”, 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

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

OR - odds ratio; CI - confidence interval

The genotypic distribution of the selected polymorphism of *IL10* in the control group was in Hardy–Weinberg equilibrium ($\chi^2=0.234$; $p=0.889$). 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**).

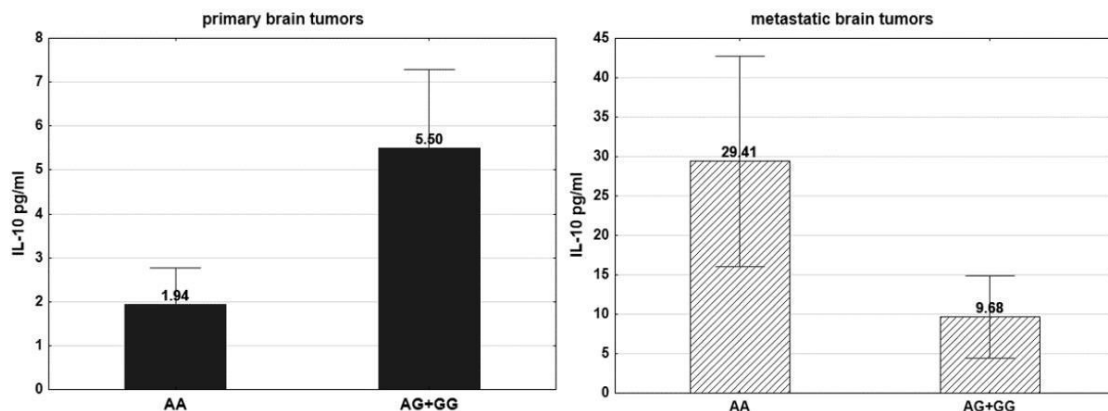


Figure 1. IL-10 serum level in the primary and in the metastatic brain tumors according to the rs1800896 *IL10* genotype. The data are presented as mean \pm s.e.m.

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:

- Schroeder T, Bittrich P, Kuhne JF, Noebel C, Leischner H, Fiehler J, et al. Mapping distribution of brain metastases: does the primary tumor matter? *J Neurooncol.* 2020 Mar; 147(1):229-35.
- Burmeister AR, Marriott I. The interleukin-10 family of cytokines and their role in the CNS. *Front Cell Neurosci.* 2018 Nov 27;12:458.
- Zhang Z, Huang X, Li J, Fan H, Yang F, Zhang R, et al. Interleukin 10 promotes growth and invasion of glioma cells by up-regulating KPNA 2 in vitro. *J Cancer Res Ther.* 2019;15(4):927–32.
- Qi L, Yu H, Zhang Y, Zhao D, Lv P, Zhong Y, et al. IL-10 secreted by M2 macrophage promoted tumorigenesis through interaction with JAK2 in glioma. *Oncotarget.* 2016 Nov;7(44):71673-85.
- Zeng L, O'Connor C, Zhang J, Kaplan AM, Cohen DA. IL-10 promotes resistance to apoptosis and metastatic potential in lung tumor cell lines. *Cytokine.* 2010 Mar;49(3):294-302.
- Méndez-García LA, Nava-Castro KE, Ochoa-Mercado TL, Palacios-Arreola MI, Ruiz-Manzano RA, Segovia-Mendoza M, et al. Breast cancer metastasis: Are cytokines important players during its development and progression? *Interferon Cytokine Res.* 2019 Jan;39(1):39-55.

7. Stanilov N, Miteva L, Deliysky T, Jovchev J, Stanilova S. Advanced colorectal cancer is associated with enhanced IL-23 and IL-10 serum levels. *Lab Med*. 2010 March; 41(3):159–63.
8. Larsson L, Rymo L, Berglundh T. Sp1 binds to the G allele of the -1087 polymorphism in the IL-10 promoter and promotes IL-10 mRNA transcription and protein production. *Genes Immun*. 2010 Mar;11(2):181-7.
9. Miteva LD, Stanilov NS, Deliysky TS, Stanilova SA. Significance of -1082A/G polymorphism of IL10 gene for progression of colorectal cancer and IL-10 expression. *Tumour Biol*. 2014 Dec;35(12):12655-64.
10. Zhu Z, Liu JB, Liu X, Qian L. Association of interleukin 10 rs1800896 polymorphism with susceptibility to breast cancer: a meta-analysis. *J Int Med Res*. 2020 Apr;48(4):300060520904863.
11. Ding K, Yi M, Li L, Zhang Y. Interleukin polymorphisms and protein levels associated with lung cancer susceptibility and phenotypes. *Expert Rev Clin Immunol* 2021;17(9):1029-40.
12. Amirian E, Liu Y, Scheurer ME, El-Zein R, Gilbert MR, Bondy ML. Genetic variants in inflammation pathway genes and asthma in glioma susceptibility. *Neuro Oncol*. 2010 May;12(5):444-52.

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18F-FDG PET/CT in finding cutaneous, muscular and pancreatic involvement in IgA multiple myeloma – case report with literature review

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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

$\mu\text{mol/L}$. 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 ^{18}F -FDG PET/CT for monitoring the treatment response. The scan was performed on PET/CT (Philips Gemini TF) using 6.8 mCi ^{18}F -FDG after proper preparation of the patient.

Results and discussion

The ^{18}F -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**).

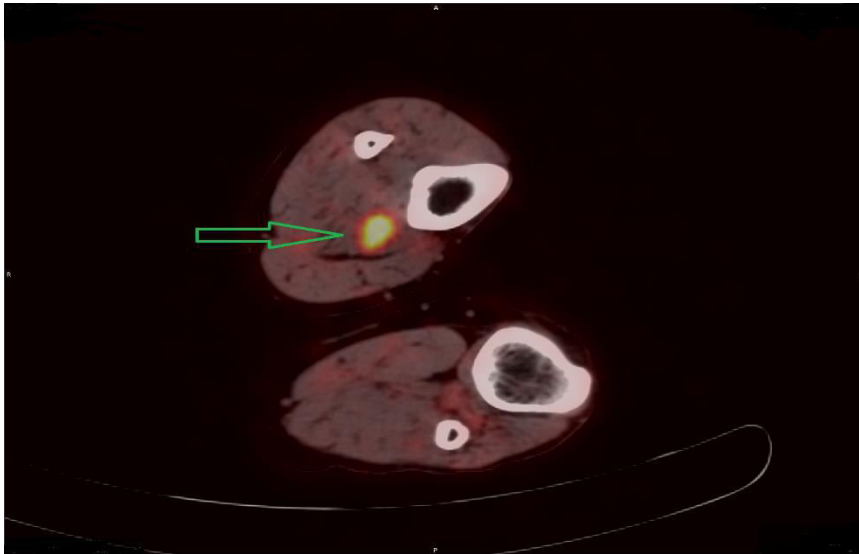


Figure 1- fused axial PET/CT image of FDG-avid muscle lesion in the left lower leg

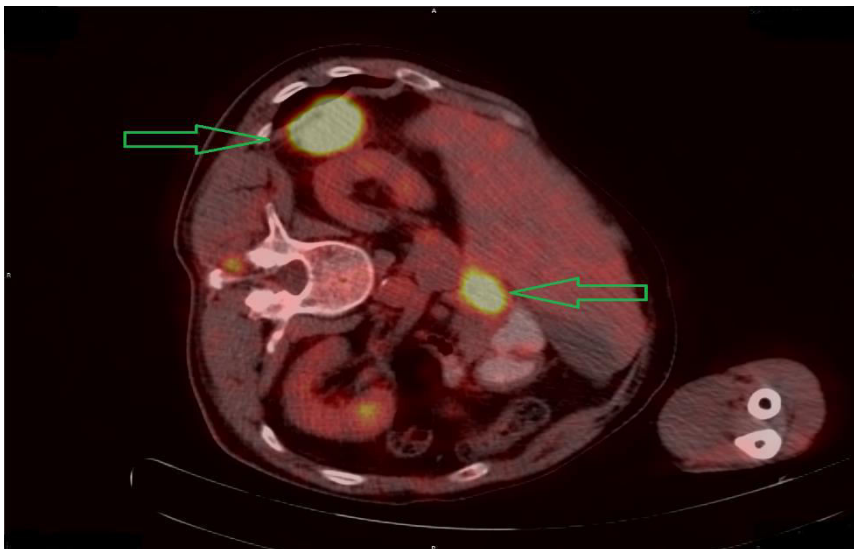


Figure 2- ^{18}F -FDG PET/CT fused axial image- malignant involvement of pancreas with a retroperitoneal lesion.

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 (SUV_{max}) 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:

- [1] Kazandjian, D., *Multiple myeloma epidemiology and survival: A unique malignancy. Semin Oncol.* 2016 Dec; 43(6): 676–681. Published online 2016 Nov 10. doi: 10.1053/j.seminoncol.2016.11.004
- [2] Raab, M. S., Podar, K., Breitkreutz, I., Richardson, P. G., & Anderson, K. C., *Multiple myeloma. The Lancet* (2009)., 374(9686), 324–339. [https://doi.org/10.1016/s0140-6736\(09\)60221-x](https://doi.org/10.1016/s0140-6736(09)60221-x)
- [3] *Incidence of myeloma. (n.d.). Retrieved March 27, 2021, from <https://www.mpeurope.org/about-myeloma/incidence-of-myeloma/>*
- [4] Blade J, Kyle RA, Greipp PR. Multiple myeloma in patients younger than 30 years. Report of 10 cases and review of the literature. *Archives of internal medicine.* 1996;156:1463–1468. DOI: [10.1001/archinte.1996.00440120125014](https://doi.org/10.1001/archinte.1996.00440120125014)
- [5] Sevcikova, S., Minarik, J., Stork, M., Jelinek, T., Pour, L., & Hajek, R. *Extramedullary disease in multiple myeloma – controversies and future directions. Blood Reviews* (2019), 36, 32–39. <https://doi.org/10.1016/j.blre.2019.04.002>
- [6] A. Alegre, A. Granda, C. Martínez-Chamorro et al., “Different patterns of relapse after autologous peripheral blood stem cell transplantation in multiple myeloma: clinical results of 280 cases from the Spanish Registry,” *Haematologica* 2002, vol. 87, no. 6, pp. 609–614..
- [7] Holliday, A. C., Khan, M. I., Mazloom, S. E., Chavan, R. N., & Grider, D. J. *Metastatic Multiple Myeloma to the Skin. Case Reports in Dermatological Medicine*, 2019, 1–3. <https://doi.org/10.1155/2019/7930123>
- [8] Bhutani, M., Foureau, D. M., Atrash, S., Voorhees, P. M., & Usmani, S. Z.. Extramedullary multiple myeloma. In *Leukemia* (2019) (Vol. 34, Issue 1, pp. 1–20). Springer Science and Business Media LLC. <https://doi.org/10.1038/s41375-019-0660-0>
- [9] Nanni, C., Zamagni, E., Farsad, M., Castellucci, P., Tosi, P., Cangini, D., et al.. *Role of 18F-FDG PET/CT in the assessment of bone involvement in newly diagnosed multiple myeloma: preliminary*

- results. *European Journal of Nuclear Medicine and Molecular Imaging* (2006), 33(5), 525–531. <https://doi.org/10.1007/s00259-005-0004-3> <https://doi.org/10.1007/s00259-005-0004-3>
- [10] Lu, Y.-Y., Chen, J.-H., Lin, W.-Y., Liang, J.-A., Wang, H.-Y., Tsai, S.-C. et al.. *FDG PET or PET/CT for Detecting Intramedullary and Extramedullary Lesions in Multiple Myeloma. Clinical Nuclear Medicine* (2012), 37(9), 833–837 <https://doi.org/10.1097/RLU.0b013e31825b2071>
- [11] Alexiou C, Kau RJ, Dietzfelbinger H, et al. *Extramedullary plasmacytoma: tumor occurrence and therapeutic concepts. Cancer* 1999; 85:2305-2314
- [12] Köse, M., Buraniqi, E., Akpınar, T. S., Kayacan, S. M., & Tükek, T.. *Relapse of Multiple Myeloma Presenting as Extramedullary Plasmacytomas in Multiple Organs. Case Reports in Hematology*, 2015, 1–6. <https://doi.org/10.1155/2015/452305>
- [13] Gupta, P., Rice, G. D., Abraham, K., Ghole, V., & Ketkar, M.. *Extramedullary plasmacytoma of the pancreas and jejunum. Clinical Imaging*, (2009), 33(3), 240–243. doi: [10.5946/ce.2014.47.1.115](https://doi.org/10.5946/ce.2014.47.1.115)
- [14] Karunanithi, S., Soundararajan, R., Sharma, P., Naswa, N., Bal, C., & Kumar, R. *Spectrum of Physiologic and Pathologic Skeletal Muscle¹⁸F-FDG Uptake on PET/CT. American Journal of Roentgenology* . (2015), 205(2), W141–W149. <https://doi.org/10.2214/AJR.14.13457>

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

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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

comparissement with (18F) FDG PET/CT which has low accumulation regarding these histological types of tumors [7].

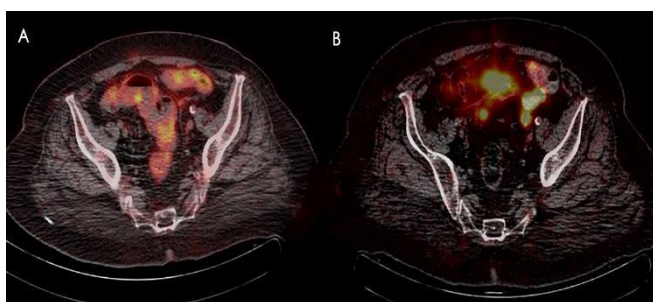
Purpose

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 were not conclusive [Fig. 1].

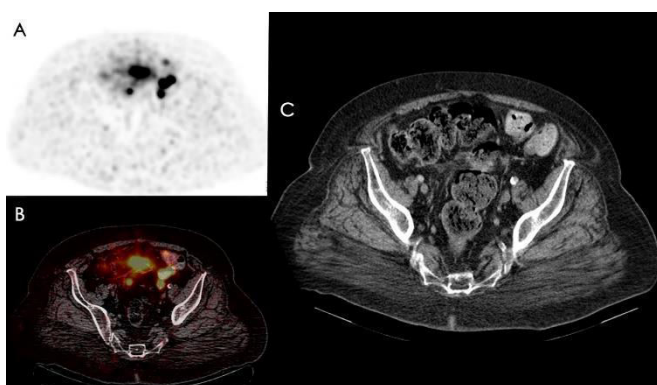
Figure 1. - Advantages of 68Ga-DOTATATE PET/CT over F18-FDG PET/CT. (A) Axial F18-FDG PET/CT fused images showed low metabolic activity in peritoneum. (B) Axial 68Ga-DOTATATE PET/CT fused images demonstrating increased DOTATATE uptake in the multiple peritoneal lesions with better delineation in comparison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

References:

1. Kocha W, Maroun J, Kennecke H, Law C, Metrakos P, Ouellet JF, Reid R, Rowsell C, Shah A, Singh S, Van Uum S, Wong R. Consensus recommendations for the diagnosis and management of well-differentiated gastroenterohepatic neuroendocrine tumours: a revised statement from a Canadian National Expert Group. *Curr Oncol*. 2010 Jun;17(3):49-64. doi: 10.3747/co.v17i3.484. PMID: 20567626; PMCID: PMC2880904.
2. Modlin IM, Latich I, Zikusoka M, Kidd M, Eick G, Chan AK. Gastrointestinal carcinoids: the evolution of diagnostic strategies. *J Clin Gastroenterol*. 2006 Aug;40(7):572-82. doi: 10.1097/00004836-200608000-00003. PMID: 16917396.
3. Hauso O, Gustafsson B, Kidd M, Waldum H, Drozdov I, Chan A, Modlin I. Neuroendocrine tumour epidemiology. *Cancer*. 2008;113:2655-2664. doi: 10.1002/cncr.23883. [[PubMed](#)] [[CrossRef](#)] [[Google Scholar](#)]
4. Modlin IM, Latich I, Zikusoka M, Kidd M, Eick G, Chan AK. Gastrointestinal carcinoids: the evolution of diagnostic strategies. *J Clin Gastroenterol*. 2006 Aug;40(7):572-82. doi: 10.1097/00004836-200608000-00003. PMID: 16917396.
5. Bodei L, Sundin A, Kidd M, Prasad V, Modlin IM. The status of neuroendocrine tumor imaging: from darkness to light? *Neuroendocrinology*. 2015;101(1):1-17. doi: 10.1159/000367850. Epub 2014 Sep 10. PMID: 25228173
6. ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Radiological, Nuclear Medicine & Hybrid- Anders Sundin¹, Rudolf Arnold², Eric Baudin³, Jaroslaw B Cwikla⁴, Barbro Eriksson⁵, Stefano Fanti⁶, Nicola Fazio⁷, Francesco Giammarile⁸, Rodney J. Hicks⁹, Andreas Kjaer¹⁰, Eric Krenning¹¹, Dik Kwekkeboom¹², Catherine Lombard-Bohas¹³, Juan M O'Connor¹⁴, Dermot O'Toole¹⁵, Andrea Rockall¹⁶, Bertram Wiedenmann¹⁷, Juan W Valle¹⁸, Marie-Pierre Vullierme¹⁹,
7. Liu X, Li N, Jiang T, Xu H, Ran Q, Shu Z, Wu J, Li Y, Zhou S, Zhang B. Comparison of gallium-68 somatostatin receptor and ¹⁸F-fluorodeoxyglucose positron emission tomography in the diagnosis of neuroendocrine tumours: A systematic review and meta-analysis. *Hell J Nucl Med*. 2020 May-Aug;23(2):188-200. doi: 10.1967/s002449912108. Epub 2020 Jul 27. PMID: 32716410.
8. De Dosso S, Treglia G, Pascale M, Tamburello A, Santhanam P, Kroiss AS, Pereira Mestre R, Saletti P, Giovannella L. Detection rate of unknown primary tumour by using somatostatin receptor PET/CT in

patients with metastatic neuroendocrine tumours: a meta-analysis. *Endocrine*. 2019 Jun;64(3):456-468. doi: 10.1007/s12020-019-01934-9. Epub 2019 Apr 19. PMID: 31004334

9.Sanli Y, Garg I, Kandathil A, Kendi T, Zanetti MJB, Kuyumcu S, Subramaniam RM. Neuroendocrine Tumor Diagnosis and Management: ⁶⁸Ga-DOTATATE PET/CT. *AJR Am J Roentgenol*. 2018 Aug;211(2):267-277. doi: 10.2214/AJR.18.19881. Epub 2018 Jul 5. PMID: 29975116.

Clinical significance of Hepatitis D virus genotype I infection

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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 assesment 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 $45.3 \pm SD 12.17$), 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 μ l serum samples (GeneAll Exgene Viral DNA/RNA kit. Synthesis of cDNA was performed using Minotech Biotechnology kit in a final volume of 10 μ l. 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 (\pm) SD or median (range) as appropriate. Data were analysed by Pearson's χ^2 test. 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 $\chi^2=4.48$, $p=0.03 < 0.5$). 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).

No.	Sex M/F	Age	% of query cover	% of identity	NCBI description
1	Female	61	94%	87.43%	Hepatitis delta virus dTk27 LHD gene for large HD antigen, genomic RNA, strain dTk27
2	Male	51	96%	81.20%	Human hepatitis delta virus encoding delta-antigen RNA, complete cds
3	Male	28	89%	94.48%	Hepatitis delta virus isolate Patient_13 large delta antigen and small delta antigen genes, complete cds
4	Female	50	93%	93.20%	Hepatitis D virus Greek isolate 33 hepatitis delta antigen gene, partial cds
5	Female	39	91%	92.99%	Hepatitis D virus Turkish isolate 08 hepatitis delta antigen gene, partial cds
6	Male	62	99%	93%	Hepatitis delta virus viral cRNA for HD-Ag (HD gene), strain dFr5143
7	Female	56	93%	92.60%	Hepatitis D virus Greek isolate 22 hepatitis delta antigen gene, partial cds
8	Male	29	93%	92.26%	Hepatitis delta virus isolate p3534 delta antigen gene, partial cds
9	Male	55	84%	82.54%	Hepatitis D virus United States isolate 18 hepatitis delta antigen gene, partial cds
10	Male	46	84%	82.54%	Hepatitis D virus Greek isolate 32 hepatitis delta antigen gene, partial cds
11	Male	35	95%	94.28%	Hepatitis delta virus cRNA for LHD (HD gene), strain dFr4586
12	Male	62	99%	93.31%	Hepatitis D virus Russian isolate hepatitis delta antigen gene, partial cds

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:

1. WHO. [Internet] Hepatitis D. Available from: <https://www.who.int/news-room/fact-sheets/detail/hepatitis-d>. Accessed 5 December 2021.
2. Niro GA, Smedile A, Andriulli A, Rizzetto M, Gerin JL, Casey JL. The predominance of hepatitis delta virus genotype I among chronically infected Italian patients. *Hepatology*. 1997 Mar;25(3):728-34. doi: 10.1002/hep.510250339. PMID: 9049226.
3. Fattovich G, Giustina G, Christensen E, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. *Gut*. 2000.
4. Degertekin H, Yalcin K, Yakut M, et al. Seropositivity for delta hepatitis in patients with chronic hepatitis B and liver cirrhosis in Turkey: a meta-analysis. *Liver Int*. 2008.
5. Shirazi R, Ram D, Rakovsky A, Bucris E, Gozlan Y, Lustig Y, et al. Characterization of hepatitis B and delta coinfection in Israel. *BMC Infect Dis*. 2018;18(1):97. doi: 10.1186/s12879-018-3008-x.

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

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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), ^{99m}Tc-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 ^{99m}Tc-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 hydronephrosis 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 Tmax – 6min, T1/2 – 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. Tmax – 12.11min, T1/2 – 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.

Reference:

1. Анадолийска А., М. Гайдарова, Детска нефрология 1 и 2 Част 2008.
2. Близнакова Д., Ръководство по ултразвукова диагностика на отделителната система в детска възраст, Варна 1995.
3. Буева А., А. Анадолийска, Св. Маринова, С. Томова, Постнатално верифициране на пренатално доказаните аномалии на отделителната система, Педиатрия, 1998, 1, 34-36.
4. Минков М. Детска урология, София, изд. Лик, 2004.
5. Чакърски В., Атлас по ултразвукова диагностика, 2004, София, Медицина и физкултура.

6. Asku N et al. Postnatal management of infants with antenatal detected hydronephrosis, *Pediatr. Nephrol.*, 2005, 20, 1252-1259.
7. O'Reilly PH., Consensus Committee of the Society of Radionuclides in Nephrourology. Standardization of the renogram technique for investigating the dilated upper urinary tract and assessing the results of surgery. *BJU Int.* 2003 Feb;91(3):239-43. [[PubMed](#)]

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

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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.

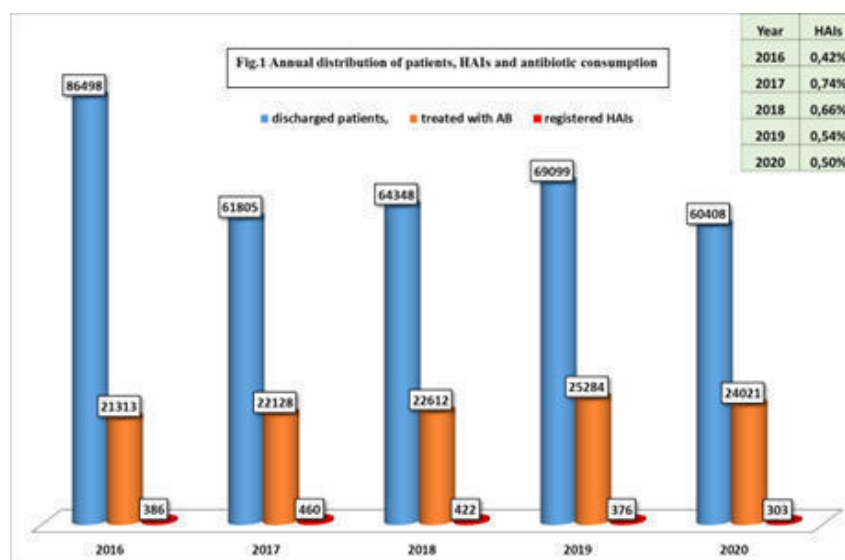


Table1 Etiological structure of HAI in University Hospital "St.Marina in period 2016-2020

	2016	2017	2018	2019	2020
<i>A.baumannii</i>	65	100	65	71	61
<i>P.aeruginosa</i>	59	43	57	45	33
<i>K.pneumonie</i>	47	52	64	39	26
<i>E.coli</i>	30	33	35	27	38
<i>E.doace</i>	30	30	20	15	22
<i>CNS</i>	32	26	14	14	16
<i>E.faecalis</i>	17	31	20	35	23
<i>C.albicans</i>	12	11	18	35	17
<i>S.aureus</i>	13	28	15	15	18
<i>Unrecognized or not tested</i>	11	11	11	14	4
<i>other</i>	70	95	103	66	45
	386	460	422	376	303

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 **etiologic 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 BSIs, 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 SSIs (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 (CLABSIs). Globally, the data on the relative incidence of SSIs (17%) are comparable to ours. Another study for EU reported a lower frequency of SSIs 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 SSIs 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:

1. N. Joel Ehrenkranz MD et al, Control of Health Care-associated Infections (HAI): Winning Both the Battles and the War, Journal of General Internal Medicine volume 26, pages340–342 (2011)
2. S. Mayor, “Hospital-acquired infections kill 5000 patients a year in England,”*BMJ*, vol. 321, no. 7273, p. 1370, 2000.
3. Monov D, E. Zheleva et al. Infections related to medical care and their outbreak in the conditions of the Varna district for the period 2014-2016, Varna Medical Forum, , 6, 117-123
4. Jaffar A. Al-Tawfiq, Paul A. Tambyah, Healthcare associated infections (HAI) perspectives, Journal of Infection and Public Health, Volume 7, Issue 4, 2014, Pages 339-344
5. V.D. Rosenthal, S. Guzman et al, The attributable cost, length of hospital stay, and mortality of central line-associated bloodstream infection in intensive care departments in Argentina: a prospective, matched analysis, Am J Infect Control, 31 (2003), pp. 475-480

6. Patrick DM, Marra F et al. Per capita antibiotic consumption: how does a North American jurisdiction compare with Europe?, *Clin infect Dis*, 2004, vol. 38 (pg. 11-7)
7. Mihaylova L., S. Iliev, N. Gatcheva, Incidence rates and microbial etiology of nosocomial infections in University hospital "Dr G. Stranski" – Pleven: Epidemiological analysis 2003-2005, *Official Journal of Bulgarian Association for Prevention and Infection Control – BulNoso*, 2012, Volume 9, No 1–2,101-109
8. Chen IL et al.. Antibiotic consumption and healthcare-associated infections caused by multidrug-resistant gram-negative bacilli at a large medical center in Taiwan from 2002 to 2009: implicating the importance of antibiotic stewardship. *PLoS One*. 2013 May 30;8(5):e65621.
9. European Centre for Disease Prevention and Control. Antimicrobial consumption in the EU/EEA (ESAC-Net) - Annual Epidemiological Report 2020. Stockholm: ECDC; 2021.
10. Vacheva R., M. Todorova, N. Gacheva, T. Kamenova. Nosocomial infections in the Republic of Bulgaria in the period 2007-2009. *Information Journal of NCIPD* 2010; 6: 4-46
11. European Centre for Disease Prevention and Control, Annual Epidemiological Report for 2017, Healthcare-associated infections acquired in intensive care units
12. WHO Guidelines on Hand Hygiene in Health Care, First Global Patient Safety Challenge "Clean Care is Safer Care", Geneva, World Health Organization, 2009
13. Jian Sun, Wen Qin et al., "Analysis of Continuous Prevalence Survey of Healthcare-Associated Infections Based on the Real-Time Monitoring System in 2018 in Shandong in China", *BioMed Research International*, vol. 2021, Article ID 6693889, 7 pages, 2021.
14. European Centre for Disease Prevention and Control. Healthcare-associated infections: surgical site infections. ECDC. Annual epidemiological report for 2017. Stockholm: ECDC; 2019.

Epidemiological structure of healthcare-associated infections in Multiprofile hospital for active treatment - Varna – Military medical academy for the period 2010 - 2020.

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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 cocci (*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

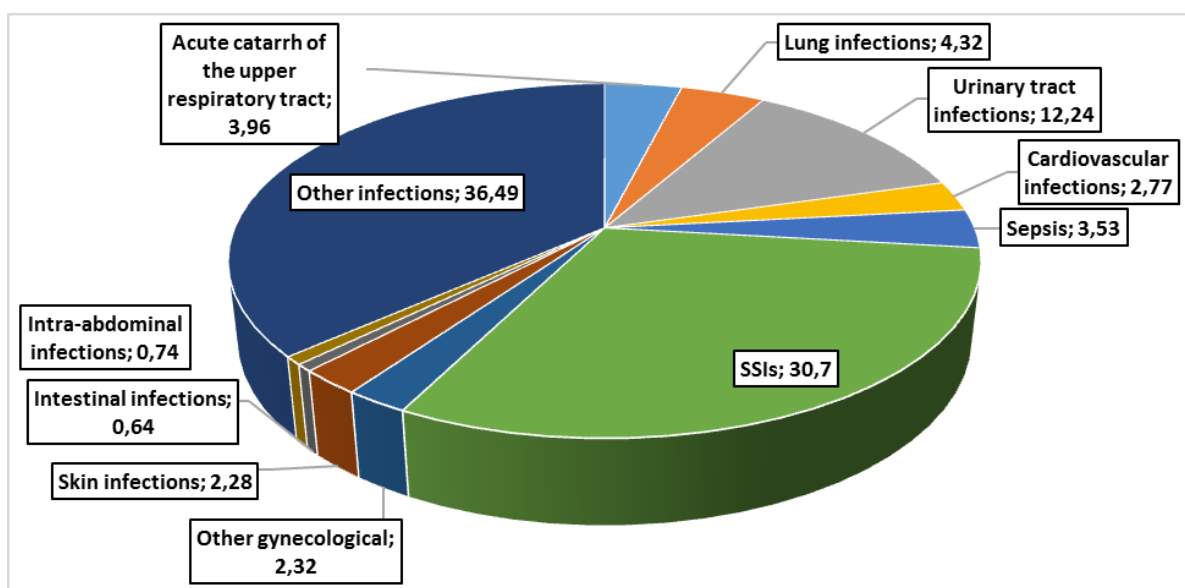
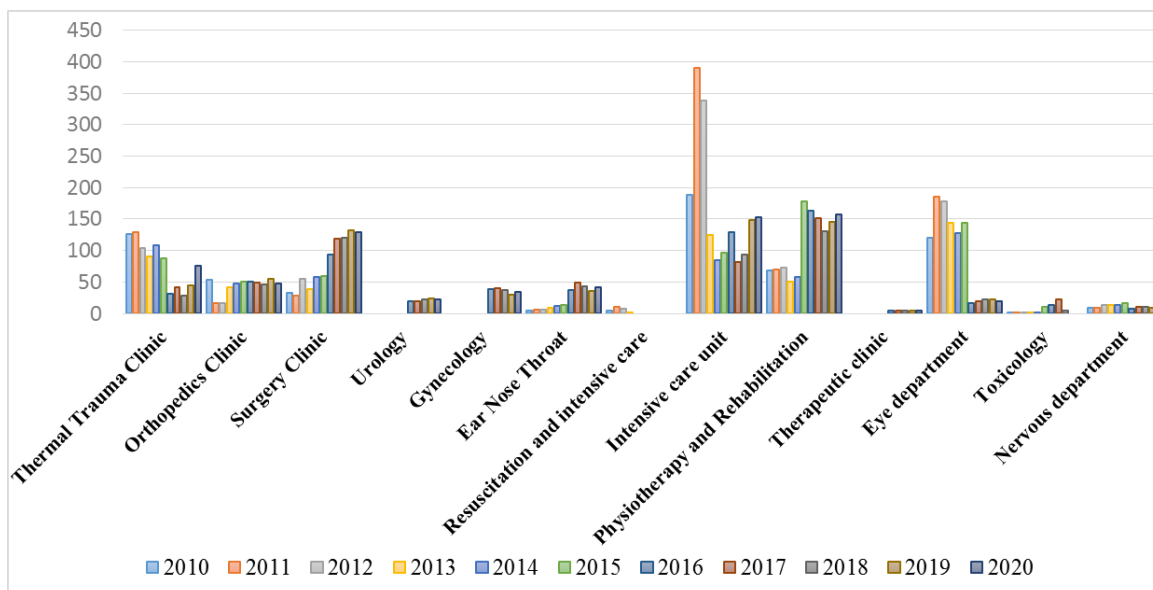


Fig.2 Distribution of healthcare associated infections by clinics and departments



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 *S.aureus* (10.50%), *P.aeruginosa* (9.57%), *S.epidermidis* 9.27%), and *E.coli* (8.52%).) The microbiologically unproven infections have the highest relative share of HAIs - 41.83%. The relative share of HAI is highest in intensive care units (39.5%), and leading in the nosological HAIs-profile are infections defined as "others" (36.5%), followed by SSIs (30, 71%) and urinary tract infections (12.24%).

References:

1. Ribarova Nelly, Nosocomial Infections, Sofia 2000, Simel Press
2. Атакишизаде С.А., З.О.Караев. Этиологические факторы внутрибольничных инфекций Азербайджанский Медицинский Университет, г.Баку Биомедицина бр.4 2015
3. **Anderson, D. J., et al.** 2008. Strategies to prevent surgical site infections in acute care hospitals. *Infect. Control Hosp. Epidemiol.* 29 (Suppl. 1):S51-S61. [[PubMed](#)] [[Google Scholar](#)]
4. Doshi RK, Patel G, MacKay R, Wallach F. Health care-Associated Infections: Epidemiology, Prevention, and Therapy. *Mount Sinai J Med* 2009; 76: 84– 94.

5. [Emily R. M. Sydnor](#), [Trish M. Perl](#) Hospital Epidemiology and Infection Control in Acute-Care Settings [Clin Microbiol Rev.](#) 2011 Jan; 24(1): 141–173. doi: [10.1128/CMR.00027-10](https://doi.org/10.1128/CMR.00027-10) PMID: [21233510](https://pubmed.ncbi.nlm.nih.gov/21233510/)
6. Ostrowsky B. Epidemiology of Health care-Associated Infections. In: Bennett & Brachman's Hospital Infections. 5th edition. Wolters Kluwer Lippincott Williams & Wilkins, Philadelphia. 2007; 3-23.
7. Scott RD II. The Direct Medical Costs of Health care-Associated Infections in U.S. Hospitals and the Benefits of Prevention. Atlanta: Centers for Disease Control and Prevention, 2009. [http://www.cdc.gov/ncidod/dhqp/pdf/ Scott_CostPaper.pdf](http://www.cdc.gov/ncidod/dhqp/pdf/Scott_CostPaper.pdf) *Accessed July 8, 2011

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

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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 ($\bar{X} \pm SD$) 46.36 \pm 5.61years. The CG was formed of 28 participants, mean age 46.29 \pm 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 tangentor therapy. *Statistical analysis:* We used the primary statistics variables like: Median (Mdn), arithmetic mean (\pm), 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 ± 0.69 mm, and 3.96 ± 0.53 mm (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 ± 0.74 mm, and 5.75 ± 0.65 mm after therapy, without statistically significant differences before and after treatment (Fig. 1. A.).

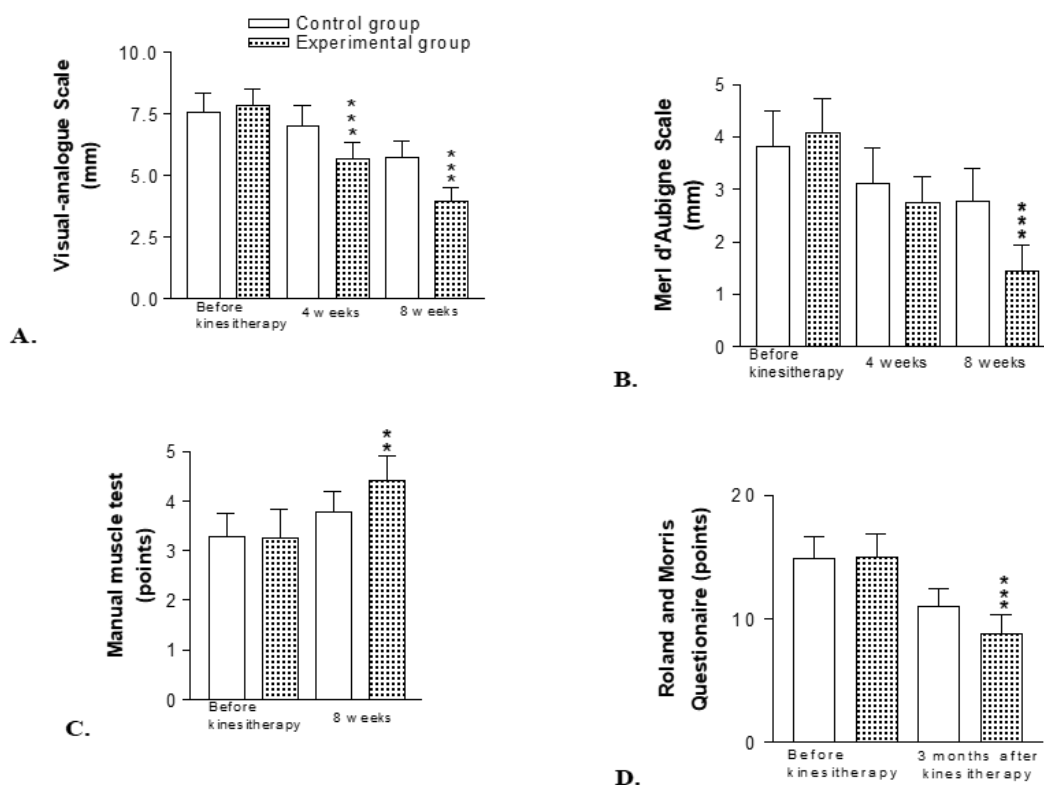


Figure 1. Dynamic of the results of VAS – **A**; Merl d’Aubigne Scale – **B**; MMT –muscles of the back – **C** Roland and Morris Questionnaire– **D** for the Experimental and Control groups

*** 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 tangentor 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:

1. Andreev D, Avramova M, Mitova S. (2020) Manual therapy and kinesiotaping in chronic low back pain. *Knowledge International Journal* 41 (3), 561-566 [Online Issue: <https://ikm.mk/ojs/index.php/KIJ/article/view/4312>]
3. Bordoni B, Marelli F, Morabito B, Sacconi B. (2017). The indeterminable resilience of the fascial system. *J Integr Med.* Sep;15(5):337-343 [Doi: 10.1016/S2095-4964(17)60351-0]
4. Bourne M, Talkad A, Varacallo M. (2020) Anatomy, Bony Pelvis and Lower Limb, Foot Fascia, StatPearls Publishing; *Treasure Island (FL)*: May 20 [Online Issue: <https://www.ncbi.nlm.nih.gov/books/NBK526043/>]
5. Bruan J. et.al. (2017) Core stability exercise versus general exercise for chronic low back pain. *Journal of Athletic Training* 52(1):71-72; [Doi: 10.4085/1062-6050-51.11.16]
6. Coulombre B, et.al. (2017). Core stability exercise versus general exercise for chronic low back pain. *Journal of Athletic Training* 2017;52(1):71-72; [Doi: 10.4085/1062-6050-51.11.16]
7. IASP (2017) Myofascial pain. <http://www.iasp.ain.org/files/> / Musculo skeletal Pain Fact Sheets 14 / *Myofascial Pain_Final* [Online Issue: <https://www.iasp.pain.org/GlobalYear/MusculoskeletalPain>]
8. Glushkova M, Nikolova E, Glushkov Iv, Pacheva P. (2014) Application of the method of

- mahalanobis in identifying early and limited motor development of children, Research in Kinesiology Vol.42, No.1, pp. 60- 65 [Online Issue: <https://fsprm.mk/wp-content/uploads/2014/06/Pages-from-RIK12014-za-Sajtot-12.pdf>]
10. Mitova St, Gramatikova M, Avramova M, Stoyanov G. (2020). A complex approach to Musculoskeletal dysfunction in the spine. *Journal of Physical Education and Sport (JPES)*, 20 (6) 3316-3322 [Doi:10.7752/jpes.2020.s6449]
 11. Mitova S, Gramatikova M, Chongov B, Avramova M. (2020) Research of the possibilities of laser acupuncture in musculoskeletal dysfunctions in the area of the spine, *Journal of IMAB Annual Proceeding (Scientific Papers)*, Peytchinski Publishing Ltd., ISSN: 1312-773X (Online), Issue: 2020, vol. 26, issue3 [Doi:10.5272/jimab.2020263.3298]
 12. Stoyanov G, Avramova M, Mitova St, Gramatikova M. (2020). Frequency and Prevalence of Chronic Pain syndrome the spine. *Knowledge International Journal*, vol.42, No 4, 777-783; [Online Issue: <https://ikm.mk/ojs/index.php/KIJ/article/view/4614>]

Effective communication as a factor affecting adherence to pharmacotherapy

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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.

Table 1: Pharmacists' perception of factors influencing adherence

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

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.

References

1. Karaivanova M. et al. Pharmacotherapy and problems of the clinical pharmacy. First edition. *Softtrade*. Sofia; 2014; 22-23 [in Bulgarian].
2. Melnikow J. & Kiefe C. (1994). Patient compliance and medical research: issues in methodology. *Journal of general internal medicine*, 9(2), 96–105. <https://doi.org/10.1007/BF02600211>
3. Neuvonen PJ, Backman JT, & Niemi M (2008). Pharmacokinetic comparison of the potential over-the-counter statins simvastatin, lovastatin, fluvastatin and pravastatin. *Clinical pharmacokinetics*, 47(7), 463–474. <https://doi.org/10.2165/00003088-200847070-00003>
4. Kamusheva M, Petkova V. Chapter 3 Communication in Pharmacy Settings. *Textbook on Pharmaceutical Care for Students in Pharmacy*. Sofia; 2018:72–119. Bulgarian.
5. Col N, Fanale JE, & Kronholm P (1990). The role of medication noncompliance and adverse drug reactions in hospitalizations of the elderly. *Archives of internal medicine*, 150(4), 841–845.
6. Jackson JL, Chamberlin J, & Kroenke K (2001). Predictors of patient satisfaction. *Social science & medicine* (1982), 52(4), 609–620. [https://doi.org/10.1016/s0277-9536\(00\)00164-7](https://doi.org/10.1016/s0277-9536(00)00164-7)
7. Sabaté E. *Adherence to long-term therapies: evidence for action*. Switzerland: World Health Organization; 2003.
8. Turcu-Stiolica A, Doica IP, Ungureanu BS, Rogoveanu I, Florescu DN, Subtirelu MS et al., (2021). Development and Validation of a Questionnaire to Measure Medication Adherence to Direct-Acting Agents in Patients with Hepatitis C. *Pharmaceutics*, 13(10), 1683. <https://doi.org/10.3390/pharmaceutics13101683>
9. Fernandez-Lazaro CI, García-González JM, Adams DP, Fernandez-Lazaro D, Mielgo-Ayuso J, Caballero-Garcia A et al. (2019). Adherence to treatment and related factors among patients with chronic conditions in primary care: a cross-sectional study. *BMC family practice*, 20(1), 132. <https://doi.org/10.1186/s12875-019-1019-3>
10. Petkova E, Popov D. Compliance and adherence to bronchial asthma treatment. *InSpiro 2015*; (5), 33 [in Bulgarian].

Preventive healthcare for women – transvaginal ultrasonography – a pilot study

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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

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

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
2. National program for primary prevention of cervical cancer 2021 – 2024 <https://www.strategy.bg/FileHandler.ashx?fileId=25614> [in Bulgarian]
3. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71:209–49. doi:10.3322/caac.21660.
4. Bruni L, Albero G, Serrano B, Mena M, Collado JJ, Gómez D, et al., ICO/IARC Information Centre on HPV and Cancer (HPV Information Centre). Human Papillomavirus and Related Diseases in Europe. Summary Report 22 October 2021. [<https://hpvcentre.net/statistics/reports/XEX.pdf>]
5. National Statistical Institute Republic of Bulgaria, 2018 http://www.nsi.bg/sites/default/files/files/publications/Zdraveopazvane_2017.pdf
6. Preventive Care, 2021 <https://www.publichealth.org/public-awareness/preventive-care-schedule/>
7. https://ec.europa.eu/health/non_communicable_diseases/cancer_bg
8. World Health Organisation <https://www.who.int/news/item/17-11-2020-a-cervical-cancer-free-future-first-ever-global-commitment-to-eliminate-a-cancer>

Effects of CB1 antagonist on mechanical nociception in olfactory bulbectomized rats

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Abstract

The endocannabinoid system modulates the activity of neurotransmitter systems in CNS and regulates many physiological functions. Its role in the control of pain and emotional states is well established. Disturbances in the ECS are associated with psychiatric disorders, including depression. Aim of the study: to examine the effect of the selective CB1 receptor antagonist SR141716A on nociception of rats with a model of depression - olfactory bulbectomy (OBX). SR141716A (Rimonabant) was administered intragastrally for 7, 14 and 21 days after bulbectomy. OBX rats showed higher pain threshold in the paw-pressure test. Rimonabant additionally increased pain threshold upon 14 days administration, as compared to the OBX controls; when given 7 or 21 days after bulbectomy, the CB1 antagonist did not produce significant changes. The blockade of the CB1 receptors in the two-week interval, when the depressive-like state developed, increased the mechanical response thresholds in OBX rats.

Introduction

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 the nociception of olfactory bulbectomized rats.

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. Rimonabant 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 ($P \leq 0.001^{000}$) 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 ($P \leq 0.004^{000}$) (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 ($P \leq 0.004^{000}$ and $P \leq 0.04^0$ 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. $n = 8$. * $P \leq 0.05$. Means (\pm S.E.M.) are presented.

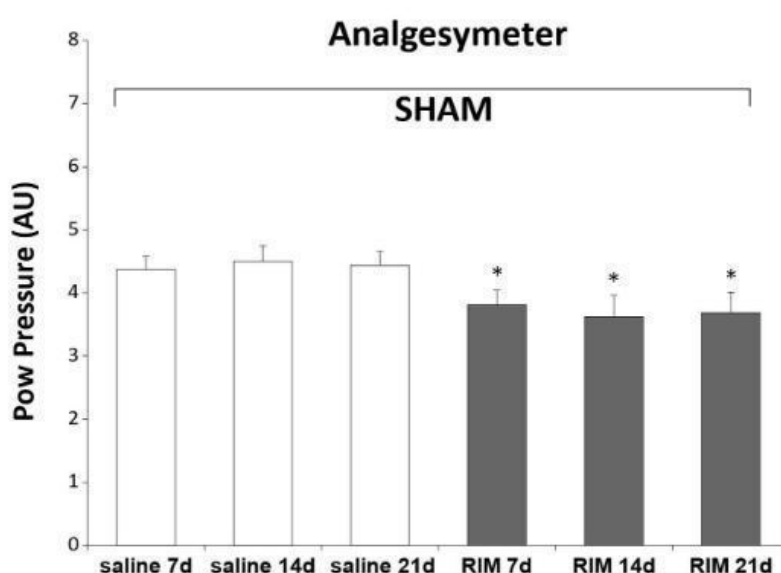
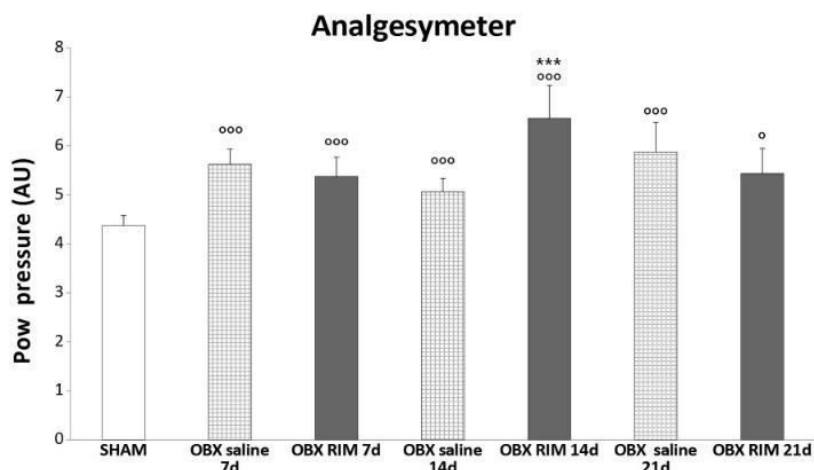


Fig.2. Effects of Rimonabant (SR141716A), administered per os for 7, 14 and 21 days on nociception in OBX rats. Asterisks depict comparisons of pain threshold (AU) in RIM-treated OBX rats vs. OBX

saline-treated rats. Circles depict comparisons after 7, 14 and 21 days RIM treated OBX rats vs. sham operated saline-treated rats. n = 8. ^oP ≤ 0.04, ^{oo}P ≤ 0.02, ^{ooo}P ≤ 0.004 Means (± S.E.M.) are presented.



References:

1. Gallego-Landin I, García-Baos A, Castro-Zavala A et al. Reviewing the Role of the Endocannabinoid System in the Pathophysiology of Depression. *Front Pharmacol.* 2021;6(12):762738 [PubMed <https://pubmed.ncbi.nlm.nih.gov/34938182/>]
 2. Thompson T, Correll CU, Gallop K, et al. Is Pain Perception Altered in People With Depression? A Systematic Review and Meta-Analysis of Experimental Pain Research. *The Journal of Pain.* 2016;17(12):257-1272 [PubMed <https://pubmed.ncbi.nlm.nih.gov/27589910/>]
 3. Tashev RE, Stavreva GT, Velikova MS. Subchronic Central Administration of Cannabinoid Ligands Modulates Nociception in Bulbectomized Rats. *Folia Med.* 2019 31;61(4):540-544 [PubMed <https://pubmed.ncbi.nlm.nih.gov/32337866/>]
 4. Kelly JP, Wrynn AS, Leonard BE. The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther.* 1997;74(3):299-316. [PubMed <https://pubmed.ncbi.nlm.nih.gov/9352586/>]
 5. Vučković S, Srebro D, Vujović KS, Vučetić Č, Prostran M. Cannabinoids and Pain: New Insights From Old Molecules. *Front Pharmacol.* 2018;9:1259. [PubMed <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6277878/>]
 6. Walker JM, Hohmann AG, Martin WJ, Strangman NM, Huang SM, Tsou K. The neurobiology of cannabinoid analgesia. *Life Sci.* 1999;65(6-7):665-73 [PubMed <https://pubmed.ncbi.nlm.nih.gov/10462067/>]
 7. Martin WJ, Tsou K, Walker JM. Cannabinoid receptor-mediated inhibition of the rat tail-flick reflex after microinjection into the rostral ventromedial medulla. *Neurosci Lett* 1998, 242:33–36 [PubMed <https://pubmed.ncbi.nlm.nih.gov/9509998/>]
- Starowicz K, Malek N, Przewlocka B. Cannabinoid receptors and pain. *Wiley Interdisciplinary Reviews: Membrane Transport and Signaling* 2013;3(2):121-132. [Crossref-
<https://onlinelibrary.wiley.com/doi/10.1002/wmts.83>]

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

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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 conduction 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

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References:

1. Weinberg GL. Treatment of local anesthetic systemic toxicity (LAST). *Reg Anesth Pain Med.* 2010 Mar-Apr;35(2):188-93. doi: 10.1097/AAP.0b013e3181d246c3. PMID: 20216036.
2. Lindberg G, Bingeors K, Ranstam J, Råstam L, Melander A. Use of calcium channel blockers and risk of suicide: ecological findings confirmed in population based cohort study. *BMJ.* 1998 Mar 7;316(7133):741-5. doi: 10.1136/bmj.316.7133.741. PMID: 9529409; PMCID: PMC28478.
3. Hawton K, Bergen H, Simkin S, Cooper J, Waters K, Gunnell D, Kapur N. Toxicity of antidepressants: rates of suicide relative to prescribing and non-fatal overdose. *Br J Psychiatry.* 2010 May;196(5):354-8. doi: 10.1192/bjp.bp.109.070219. PMID: 20435959; PMCID: PMC2862059.
4. Meaney CJ, Sareh H, Hayes BD, Gonzales JP. Intravenous lipid emulsion in the management of amlodipine overdose. *Hosp Pharm.* 2013 Nov;48(10):848-54. doi: 10.1310/hpj4810-848. PMID: 24421438; PMCID: PMC3859284.

5. Montiel V, Gougnard T, Hantson P. Diltiazem poisoning treated with hyperinsulinemic euglycemia therapy and intravenous lipid emulsion. *Eur J Emerg Med.* 2011 Apr;18(2):121-3. doi: 10.1097/MEJ.0b013e32834130ab. PMID: 21088599.
6. Vuković- Ercegović G, Perković-Vukčević N, Đorđević S, Šegrt Z, Potrebić O, Janković S, Jović-Stošić J, Marinković N. Successful usage of intravenous lipid emulsion in treatment of acute verapamil poisoning: A case report. *Vojnosanitetski Pregled.* Directory of Open Access Journals. 2017; 74 (3): 278-281. doi: <https://doi.org/10.2298/VSP150901010V>
7. Sebe A, Dişel NR, Açıkalın Akpınar A, Karakoç E. Role of intravenous lipid emulsions in the management of calcium channel blocker and β -blocker overdose: 3 years experience of a university hospital. *Postgrad Med.* 2015 Mar;127(2):119-24. doi: 10.1080/00325481.2015.1012480. Epub 2015 Feb 14. PMID: 25684131.

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Physical assessment of patients with anorexia nervosa and depression

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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/m^2) = Body weight (kg) / height (m^2); 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: $FFMI = FFM / H (m^2)$ (FFMI – fat free mass index; H – height), as for men $FFMI > 17 \text{ kg/m}^2$ and for women $FFMI > 15 \text{ kg/m}^2$. 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 $1.9 \cdot 10^3$ sells/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.

Characteristic of patients Lab Exams/anthropometry	Patients with severe and extreme depression (36)	Patients with moderate and borderline clinical depression (23,9)	Patients with mild mood disturbance and normal status (10,7)
BMI kg/m ²	16,2	17,6	17,9
FM%	19,2	20,8	24,1
FFMI kg/m ²	12,8	14,3	14,8
Transferrin (µmol/l)	44,6	58,1	62,3
K ⁺ (mmol/l)	3,4	3,8	4,9
BUN (mmol/l)	1,8	3,7	5,1
25-OH Vit D (nmol/l)	28,5	32,6	39,3
Ly (cells/ml)	1,7	1,9	2,3

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.

References:

1. Attia E, Walsh T. Anorexia Nervosa. *Am. J. Psychiatry*, 2007, 164(12): 1805-10
2. de Zwart PL, Jeronimus BF, de Jonge P. Empirical evidence for definitions of episode, remission, recovery, relapse and recurrence in depression: A systematic review. *Epidemiol Psychiatr Sci*, 2019. 28(5): 544-562
3. Ulfvebrandetal S, Birgegård A, Noring C, Högdahl L, von Hausswolff-Juhlin Y. Psychiatric comorbidity in women and men with eating disorders results from a large clinical database. *Psychiatry Res*. 2015, 230(2): 294–299

4. Lu XY, Kim CS, Frazer A, Zhang W. Leptin: a potential novel antidepressant. *Proc Natl Acad Sci.* 2006.103(5):1593-8
5. Mitchell, J. E., & Peterson, C. B. Anorexia Nervosa. *N Engl J Med.* 2020; 382(14): 1343–1351
6. WHO Software for assessing growth and development of the world's children. In: WHO Anthro for Personal Computers. Manual. WHO, Geneva, 2007
7. Meng J, Hanze D, Yuelun Z, Huijuan Z, Ke X, Xianxian Y et al. Characteristics and reference values of fat mass index and fat free mass index by bioelectrical impedance analysis in an adult population. *Clin Nutr*, 2019, 38 (5): 2325-32
8. Pleplé, A., Lalanne, C., Huas, C., Mattar L., Hanachi M, Flament MF et al. Nutritional status and anxious and depressive symptoms in anorexia nervosa: a prospective study. *Sci Rep.*2021. 11: 771
9. Van de Veire NR, De Sutter J, Bax JJ, Roelandt JR: Technological advances in tissue Doppler imaging echocardiography. *Heart.* 2008, 94(8):1065–1074
10. Winston AP. The clinical biochemistry of anorexia nervosa. *Annals of Clinical Biochemistry.* 2012;49(2):132-143
11. Högberg G, Gustafsson SA, Hällström T, Gustafsson T, Klawitter B, Petersson M. Depressed adolescents in a case-series were low in vitamin D and depression was ameliorated by vitamin D supplementation. *Acta Paediatr.* 2012 Jul;101(7):779-83

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Bioenergetic Metabolism of Patients with Parkinson's Disease – a Pilot Study

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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 (Błaszczuk, 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.

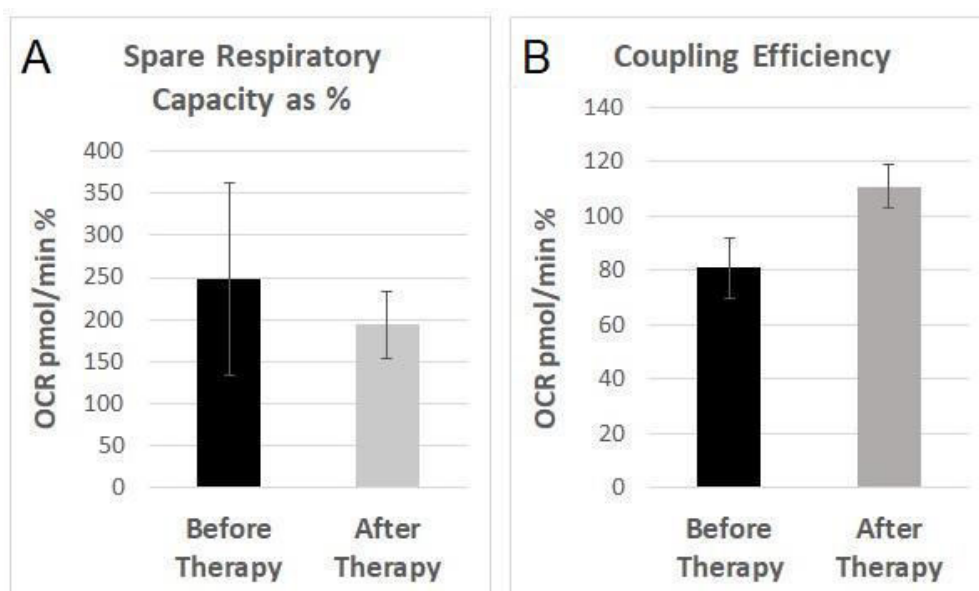


Figure 1. A) Difference in spare respiratory capacity in PD patients before and after therapy B) Difference in coupling efficiency in PD patients before and after therapy.

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.

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References

1. Błaszczyk JW. Parkinson's Disease and Neurodegeneration: GABA-Collapse Hypothesis. *Front Neurosci.* 2016;10:269. Published 2016 Jun 9. doi:10.3389/fnins.2016.00269
2. Malpartida AB, Williamson M, Narendra DP, Wade-Martins R, Ryan BJ. Mitochondrial Dysfunction and Mitophagy in Parkinson's Disease: From Mechanism to Therapy. *Trends Biochem Sci.* 2021;46(4):329-343. doi:10.1016/j.tibs.2020.11.007
3. Teves JMY, Bhargava V, Kirwan KR, et al. Parkinson's Disease Skin Fibroblasts Display Signature Alterations in Growth, Redox Homeostasis, Mitochondrial Function, and Autophagy. *Front Neurosci.* 2018;11:737. Published 2018 Jan 12. doi:10.3389/fnins.2017.00737.
4. Legmann R, Melito J, Belzer I, Ferrick D. Analysis of glycolytic flux as a rapid screen to identify low lactate producing CHO cell lines with desirable monoclonal antibody yield and glycan profile. *BMC Proc.* 2011;5 Suppl 8(Suppl 8):P94. Published 2011 Nov 22. doi:10.1186/1753-6561-5-S8-P94
5. Bell SM, De Marco M, Barnes K, et al. Deficits in Mitochondrial Spare Respiratory Capacity Contribute to the Neuropsychological Changes of Alzheimer's Disease. *J Pers Med.* 2020;10(2):32. Published 2020 Apr 29. doi:10.3390/jpm10020032
6. Gandhi S, Wood-Kaczmar A, Yao Z, et al. PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. *Mol Cell.* 2009;33(5):627-638. doi:10.1016/j.molcel.2009.02.013
7. Ludtmann MH, Angelova PR, Ninkina NN, Gandhi S, Buchman VL, Abramov AY. Monomeric Alpha-Synuclein Exerts a Physiological Role on Brain ATP Synthase. *J Neurosci.* 2016;36(41):10510-10521. doi:10.1523/JNEUROSCI.1659-16.2016

Potential antimicrobial activity of new metronidazole derivatives against pathogenic clinical isolates

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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) 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 million people all over the world. Antibiotics are the most effective treatment of all bacterial infections. Many of the bacteria, however, have become resistant to the antibiotics used in the clinic, which has led to the need for new agents to control pathogenic microorganisms [1]. 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).

	<i>S. aureus</i>			<i>C. albicans</i>		
	Solutions with CH ₃ OH					
µg/ml	50	100	200	50	100	200
Metronidazole	-	-	-	-	-	-
MTD1	-	12MM(±1)	13MM(±1)	-	14MM(±1)	14MM(±1)
MTD2	-	12MM(±1)	12MM(±1)	-	13MM(±1)	14MM(±1)
	Solutions with C ₂ H ₅ OH					
µg/ml	50	100	200	50	100	200
Metronidazole	-	-	-	-	-	-
MTD1	-	12MM(±1)	13MM(±1)	-	-	-
MTD2	-	12MM(±1)	12MM(±1)	-	-	-

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:

1. Dimitrova D., Lambev M., Hristova A., Mihaylova S., Valcheva-Kuzmanova S., Pajpanova T. Antimicrobial peptides (amps) - a potential solution against microbial resistance. *Varna Medical Forum*. 2018; 7(3): 195-202.
2. Jackson N., Czaplewski L., Piddock L.J. Discovery and development of new antibacterial drugs: learning from experience. *Journal of Antimicrobial Chemotherapy*. 2018 Jun; 73(6):1452–9. <https://doi.org/10.1093/jac/dky019>
3. Kabara J.J., Conley A.J., Truant J.P. Relationship of chemical structure and antimicrobial activity of alkyl amides and amines. *Antimicrob Agents Chemother*. 1972;2(6):492-8. doi:10.1128/AAC.2.6.492.
4. Antoszczak M., Maj E., Stefan´ska J., Wietrzyk J., Jan Janczak, Bogumil Brzezinski, Adam Huczyn´ski. Synthesis, antiproliferative and antibacterial activity of new amides of salinomycin. *Bioorganic & Medicinal Chemistry Letter*. 2014; 24(7). <https://doi.org/10.1016/j.bmcl.2014.02.042>.
5. Mihaylova S., Tsvetkova A., Arnaoudova M., Todorova A., Petkova V., Dimitrov M. et al. Current issues regarding approved peptide and protein drugs in Bulgaria. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2020; 7(4):708-17.
6. Sewald N., Jakubke HD. Peptides: Chemistry and Biology, Wiley-VCH, Weinheim, 2002 Search PubMed.
7. Seavill PW., Wilden JD. The preparation and applications of amides using electrosynthesis. *Green Chem*. 2020; 22:7737-59.
8. Roughley SD., Jordan AM. *J Med Chem*. 2011; 54:3451–79. [CrossRef CAS](#).
9. Wang X. *Nat Catal*. 2019; 2:98–102. [CrossRef](#).
10. Bray BL. *Nat Rev Drug Discovery*. 2003; 2:587–593. [CrossRef CAS](#).
11. Ivanova S., Mihaylova S., Tsvetkova A. Methods to enhance the metabolic stability of peptide drugs. *Varna Medical Forum*. 2021; 10(2):371-9.
12. Stamova S. Synthesis and characterization of substituted imidazole derivatives with potential biological effect. (*Doctoral dissertation, Medical University of Varna*).2021. <https://repository.mu-varna.bg/handle/nls/1035>.

Vitamin D status among patients with sarcopenic obesity

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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).

INDICATOR	Total (n=62)			Men (n=27)		Women (n=35)		Reference values (RV)
	Mean value	SD	Median	Mean value	SD	Mean value	SD	
LBMI (kg/m ²)*	18.18	2.73	18.37	20.53	2.08	16.37	1.51	men: 15.9–19.9 women: 13.1–16.3
FFMI (kg/m ²)*	19.19	2.76	19.36	21.60	2.05	17.34	1.53	BMI- and sex-based****
ALM (kg)*	23.26	5.91	21.43	29.28	2.88	18.61	2.30	men: 23.7–30.9 women: 14.0–21.4
ALMI (kg/m ²)*	7.92	1.35	7.52	9.23	0.83	6.91	0.59	men: 7.5–9.7 women: 6.4–8.2
ALM-to-BMI ratio*	0.70	0.17	0.66	0.86	0.11	0.57	0.09	men >1.109 women > 0.734
Appendicular Fat Mass (kg)**	17.78	4.47	18.63	16.00	4.38	19.20	4.08	–
FMI (kg/m ²)**	14.52	3.67	14.30	13.00	3.04	15.69	3.71	BMI- and sex-based***
A/G ratio*	1.11	0.15	1.11	1.24	0.11	1.01	0.10	< 1
FMR - Trunk/Total fat *	0.54	0.06	0.54	0.58	0.04	0.50	0.05	–
FMR- Legs/Total fat	0.34	0.05	0.34	0.30	0.04	0.37	0.04	–
FMR- Arms+Legs fat/Trunk fat	0.81	0.18	0.81	0.67	0.11	0.92	0.15	–

* statistically reliable sex-based differences ($p < 0.001$); ** statistically reliable sex-based differences ($p < 0.005$); *** RV for FMI (kg/m^2) men: normal levels – 3.0–6.0 kg/m^2 ; with class I obesity – between 9.0 and 12.0 kg/m^2 ; with class II obesity – between 12.0 and 15.0 kg/m^2 ; with class III obesity – more than 15.0 kg/m^2 . women: normal levels – 5.0–9.0 kg/m^2 ; with class I obesity between 13.0 and 17.0 kg/m^2 ; with class II obesity between 17.0 and 21.0 kg/m^2 ; with class III obesity more than 21.0 kg/m^2 ; **** RV for FFMI (kg/m^2): overweight: men – normal levels 21.8–24.4 kg/m^2 ; and women respectively – normal levels of 17.1–18.4 kg/m^2 ; obesity: men – normal levels $> 24.5 \text{ kg}/\text{m}^2$; and women respectively – normal levels $> 18.5 \text{ kg}/\text{m}^2$

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 $\frac{3}{4}$ (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:

1. Yao Y, Zhu L, He L, et al. A meta-analysis of the relationship between vitamin D deficiency and obesity. *Int J Clin Exp Med* 2015; 8(9): 14977-14984.
2. Didriksen A, Burild A, Jakobsen J, Fuskevåg OM, Jorde R. Vitamin D3 increases in abdominal subcutaneous fat tissue after supplementation with vitamin D3. *Europ J Endocrinol* 2015; 172: 235-241.
3. Seo JA, Cho H, Eun CR, et al. Association between visceral obesity and sarcopenia and vitamin D deficiency in older Koreans: the Ansan Geriatric Study. *J Am Geriatr Soc* 2012; 60: 700-706. doi: 10.1111/j.1532-5415.2012.03887.x.
4. Lenders CM, Feldman HA, Von Scheven E, et al. Relation of body fat indexes to vitamin D status and deficiency among obese adolescents. *Am J Clin Nutr* 2009; 90: 459-467
5. Chung JY, Kang HT, Lee DC, Lee HR, Lee YJ. Body composition and its association with cardiometabolic risk factors in the elderly: a focus on sarcopenic obesity. *Arch Gerontol Geriatr* 2013;56(1):270-8
6. Cipriani C, Pepe J, Piemonte S, Colangelo L, Cilli M, Minisola S: Vitamin d and its relationship with obesity and muscle. *Int J Endocrinol* 2014;2014:841248.
7. Hwang B, Lim JY, Lee J, Choi NK, Ahn YO, Park BJ. Prevalence rate and associated factors of sarcopenic obesity in korean elderly population. *J Korean Med Sci* 2012;27(7):748-55.
8. Kim TN, Park MS, Lim KI, Choi HY, Yang SJJ, Yoo HJ, et al. Relationships between sarcopenic obesity and insulin resistance, inflammation, and vitamin D sta

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

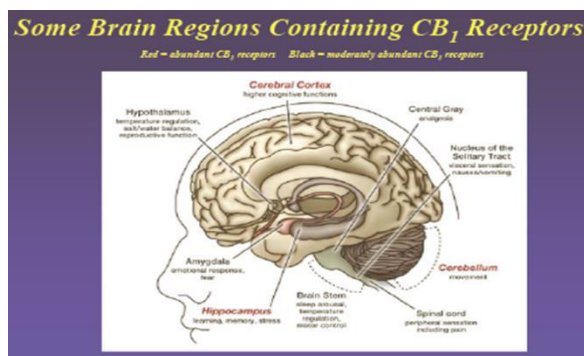
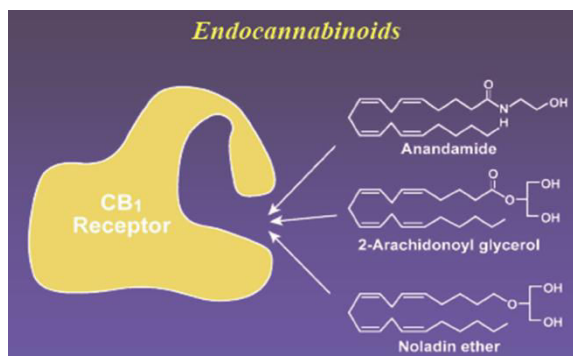
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Abstract

The bilateral olfactory bulbectomy (OBX) is a validated model of depression. The removal of olfactory bulbs 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 a 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 Δ^9 -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 diameter 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.
Exploratory and locomotor activity of rats were tested in an Opto-varimex apparatus for a 5 minute period 14 or 28 days 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).

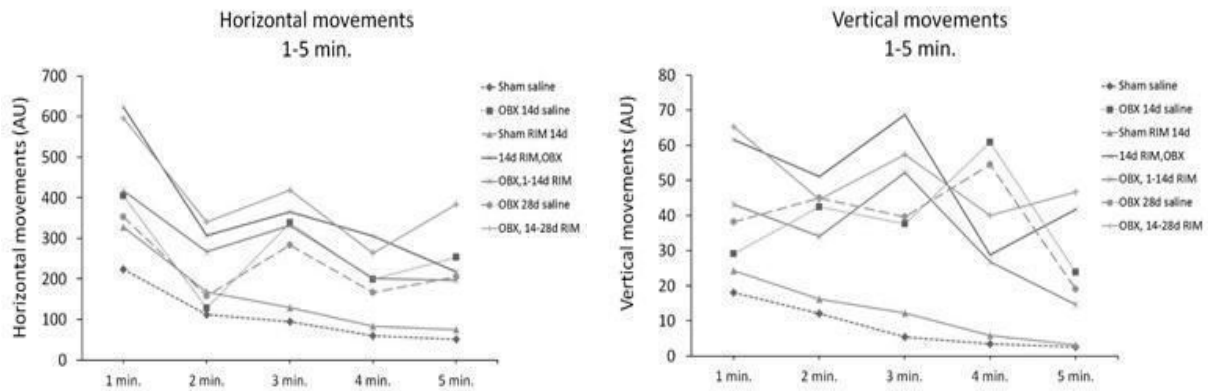


FIG. 1. A,B. Effect of Rimonabant (SR-141716A), on exploratory activity - horizontal (A) and vertical (B) movements in OBX rats (n = 8)

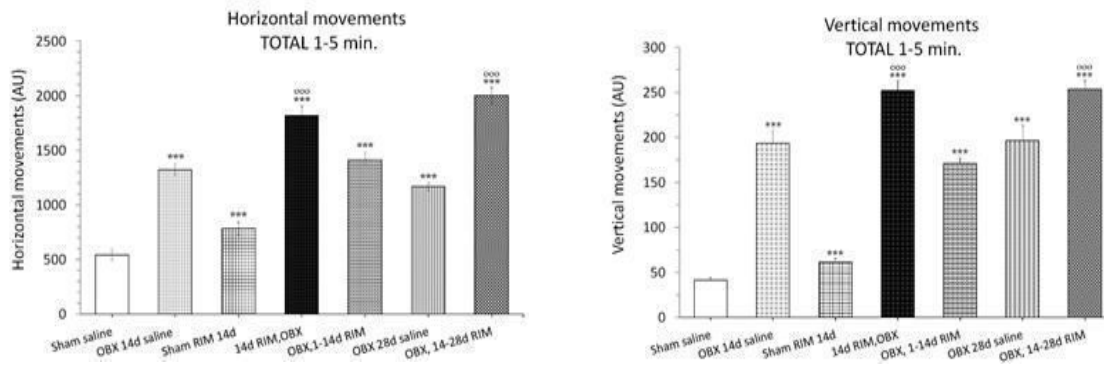


FIG. 2. A,B. Effect of Rimonabant (SR-141716A), on locomotor activity - total number of horizontal (A) and vertical (B) movements in OBX rats (14-day intragastral administration, (n = 8)

*** p<0.001 comparison with sham-operated controls; ⁰⁰⁰ 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 bulbectomy.

References:

- 1.Velikova M, Doncheva D, Tashev R. Subchronic effects of ligands of cannabinoid receptors on learning and memory processes of olfactory bulbectomized rats. *Acta Neurobiol Exp (Wars)*. 2020; 80(3):286-296 [PubMed - <https://pubmed.ncbi.nlm.nih.gov/32990286/>]
- 2.Velikova M, Doncheva D, Tashev R. Effects of Rimonabant on active avoidance learning in bulbectomized rats. *Journal of IMAB*, 2020, Jan-Mar;26(1):2936-2941, [Crossref-<https://doi.org/10.5272/jimab.2020261.2936>]

3. Busquets-Garcia A, Bains J, Marsicano G. CB₁ Receptor Signaling in the Brain: Extracting Specificity from Ubiquity. *Neuropsychopharmacology*. 2018; 43(1):4-20. [PubMed - <https://pubmed.ncbi.nlm.nih.gov/28862250/>]
4. Wang XF, Galaj E, Bi GH, Zhang C, He Y, Zhan J, et al. Different receptor mechanisms underlying phytocannabinoid- versus synthetic cannabinoid-induced tetrad effects: Opposite roles of CB₁/CB₂ versus GPR55 receptors. *Br J Pharmacol*. 2020, Apr;177(8):1865-1880 [PubMed - <https://pubmed.ncbi.nlm.nih.gov/31877572/>]
5. Li X, Hempel BJ, Yang HJ, Han X, Bi GH, Gardner EL, et al. Dissecting the role of CB₁ and CB₂ receptors in cannabinoid reward versus aversion using transgenic CB₁- and CB₂-knockout mice. *Eur Neuropsychopharmacol*. 2021, Feb; (43):38-51 [PubMed - <https://pubmed.ncbi.nlm.nih.gov/33334652/>]
6. Ettaro R, Laudermilk L, Clark SD, Maitra R. Behavioral assessment of rimonabant under acute and chronic conditions. *Behav Brain Res*. 2020; 390:112697 [PubMed - <https://pubmed.ncbi.nlm.nih.gov/32417279/>]
7. Antoniou K, Polissidis A, Delis F, Poulia N. The Impact of Cannabinoids on Motor Activity and Neurochemical Correlates. In: *In Vivo Neuropharmacology and Neurophysiology*. Neuromethods. Philippu A. (eds). Vol. 121. Humana Press, New York, NY. 2017; pp.341-365 [Crossref - https://doi.org/10.1007/978-1-4939-6490-1_15]

Investigation of antimicrobial activity of commercial essential oils of *Thymus vulgaris*

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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 via disk-diffusion method (zones of inhibition in mm).

Essential oil	<i>S. aureus</i> ATCC 29213			<i>E. coli</i> ATCC 25922			DMSO 1%
	10% stock solutions, µL						
	3	5	10	3	5	10	
<i>Thymus vulgaris</i> essential oil (internal use)	23 mm	30mm	40mm	20 mm	21mm	35mm	-
<i>Thymus vulgaris</i> (external use)	26mm	30mm	32mm	28mm	36mm	38mm	-

Legend: - no zones of inhibition

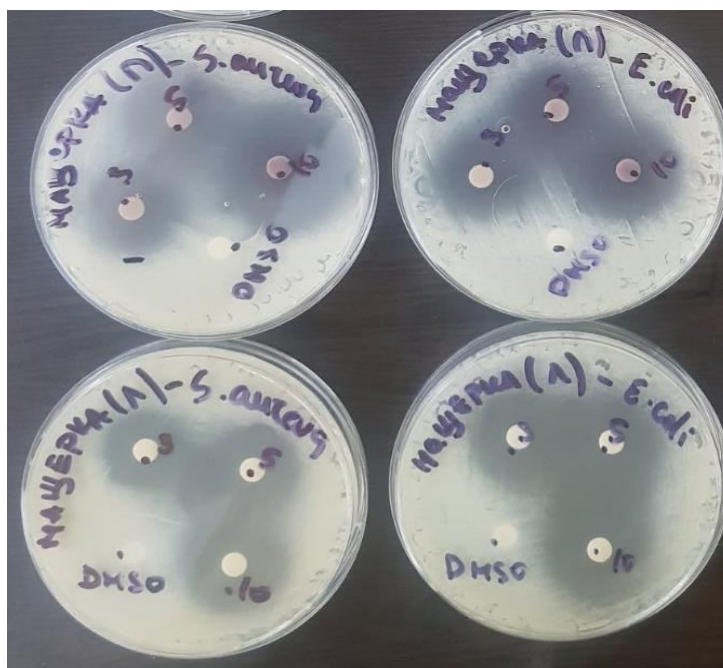


Figure 1. Antimicrobial activity of *Thymus vulgaris* essential oils (external and internal use) against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 determined by disk diffusion method.

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:

1. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils--a review. Food Chem Toxicol. 2008 Feb;46(2):446-75. doi: 10.1016/j.fct.2007.09.106. Epub 2007 Sep 29. PMID: 17996351.
2. Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ. Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. Food Control. 2008;19(7):681-687.
3. Thompson JD, Chalchat JC, Michet A, Linhart YB, Ehlers B. Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. J Chem Ecol. 2003 Apr;29(4):859-80. doi: 10.1023/a:1022927615442. PMID: 12775148.
4. J. Li, F. Ye, L. Lei, G. Zhao. Combined effects of octenylsuccination and oregano essential oil on sweet potato starch films with an emphasis on water resistance. Int. J. Biol. Macromol., 115 (2018), pp. 547-553.
5. Kulisic T., Radonic A., Milos M. Antioxidant properties of thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) essential oils. Italian Journal of Food Science (ITAL J FOOD SCI), 17(3):315-324.
6. Hamzeh Amiri, "Essential Oils Composition and Antioxidant Properties of Three *Thymus* Species", Evidence-Based Complementary and Alternative Medicine, vol. 2012, Article ID 728065, 8 pages, 2012. <https://doi.org/10.1155/2012/728065>.
7. Sienkiewicz M, Łysakowska M, Ciećwierz J, Denys P, Kowalczyk E. Antibacterial activity of thyme and lavender essential oils. Med Chem. 2011 Nov;7(6):674-89. doi: 10.2174/157340611797928488. PMID: 22313307.
8. Puškárová, A., Bučková, M., Kraková, L. et al. The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. Sci Rep 7, 8211 (2017). <https://doi.org/10.1038/s41598-017-08673-9>.
9. Hercules Sakkas, Chrissanthy Papadopoulou. Antimicrobial Activity of Basil, Oregano, and Thyme Essential Oils. J. Microbiol. Biotechnol. 2017; 27(3): 429-438. Published March 28, 2017 <https://doi.org/10.4014/jmb.1608.08024>.

An Organophosphorus Compound and its Physicochemical Characterization, In Silico Metabolite and Macromolecules Interaction Prediction and Cell Culture Testing

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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 \pm standard deviation of mean (Mean \pm SD) for MTT test and as mean \pm standard error of means (Mean \pm 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; $E_{\text{homo}}=-10.59397$ eV; $E_{\text{lumo}}=-0.5569724$ eV; Electronegativity $EN=-5.5754712$; Molecular dipole moment $\mu=5.505$; Total Energy $E_{\text{tot}}=-662792.7$ 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 $MR=66.36$ A; Polarization $\alpha=23.73$; Molecular reactivity $HARD=5.0184979$; 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 ($E_{\text{tot}}=-66279.7$ kmol/mol) capable to donate an electron couple to a donor acceptor bond ($E_{\text{homo}}=-10.59397$ eV). It passes through membranes ($\log P=1.62$) 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 vivo* 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].

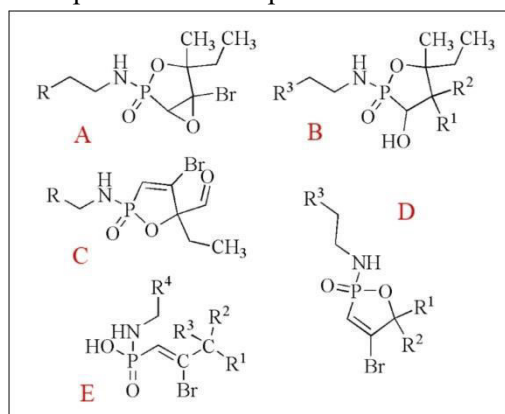


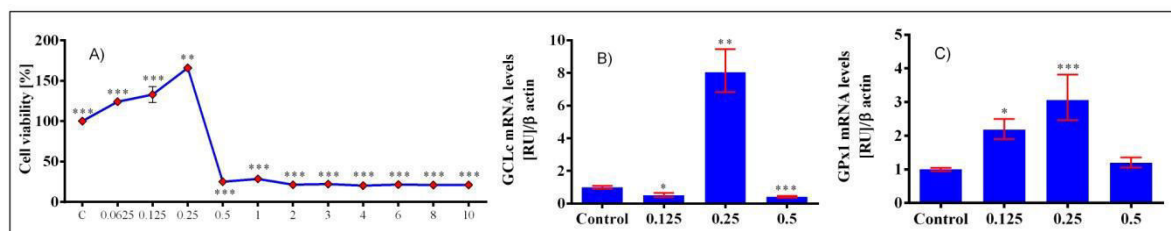
Fig. 1. Liver predicted metabolites of Br-oxph-1.

A: 1) -CH₂-CH₃, 15) -CH(OH)-CH₃; **B: 2)** R1: -Br; R2: -OH; R3: -CH₂-CH₃, **3)** R1: =O; R2: - R3: -CH₂-CH₃, **4)** R1: -H; R2: -OH; R3: -CH₂-CH₃, **16)** R1: -Br; R2: -OH; R3: -CH(OH)-CH₃; **C: 12)** R: -CH₂-CH₃, **23)** R: -CH(OH)-CH₃; **D: 5)** R1: -CH₃; R2: -CH₂-CH₃; R3: -CH₂-OH, **6)** R1: -CH₃; R2: -CH₂-CH₃; R3: -CHO, **7)** R1: -CH₃; R2: -CH₂-CH₃; R3: -COOH, **8)** R1: -CH₃; R2: -(CH₂)₂-OH; R3: -CH₃, **9)** R1: -CH₃; R2: -CH₂-CH₂O; R3: -CH₃, **10)** R1: -CH₃; R2: -CH₂-COOH; R3: -CH₃, **11)** R1: -CH₂-CH₃; R2: -CH₂-OH; R3: -CH₃, **13)** R1: -COOH; R2: -CH₂-OH; R3: -CH₃, **14)** R1: -CH₃; R2: -CH₂-CH₃; 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)CH₃, **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-CHO; R4:-CH₂-CH₃, **34**) R1:-OH; R2:-CH₃; R3:-CH₂COOH; R4:-CH₂-CH₃, **35**) R1:-OH; R2:-CH₃; R3:-CH₂-CH₃; R4:-CH₂-CH₂-OH, **36**) R1:-OH; R2:-CH₃; R3:-CH₂-CH₃; R4:-CH₂-CHO, **37**) 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 led 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 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:

1. Koleva V, Dragoeva A, Draganov M, Alafort L, Rosato, A, Uzunov N, Enchev D. Inhibition of growth and induction of apoptosis in human lung cancer cells by Br-oxph. *Genetika*. 2014; Jan; 46 (1): 1-10, [Crossref - <https://doi.org/10.2298/GENSR1401001K>]
2. Dragoeva A, Ivanova Ts, Koleva V, Nanova Z, Enchev D. Cytotoxic effect of Br-containing oxaphosphole on human melanoma cell line and pathogenic and symbiotic bacteria. *Int J Curr Microbiol App Sci*. 2014;3 (12): 1-7.
3. Enchev D, Koleva V, Dragoeva A, Behchet-Ibryam L, Mihaylov M, Uzunov N, Melendez-Alafort L, Rosato A, Brel V. Synthesis and cytostatic activity of 4-bromo-5-ethyl-2-(ethylamino)-5-methyl-5H-1,2-oxaphosphole 2-oxide. *J App Pharm Sci*. 2018; Jan 8 (1): 80-86. [Crossref - doi: 10.7324/japs.2018.8112]
4. Enchev D. Methyl-N-alkyl (phenyl)-P-(3-methylalka-1,2-dienyl) phosphonamidates, syntesis and and properties. *ASN*. 2015; March; 2(1): 6-11. [Crossref -<https://doi.org/10.1515/asn-2015-0001>]
5. Koleva V, Dragoeva A, Andreeva A, Burova M, Georgiev S, Enchev D. Comparative analysis of clastogen-induced chromosome aberrations observed with light microscopy and by means of atomic force microscopy. *Mutat Res*. 2013;753 (1): 29-35. [Crossref-<https://doi.org/10.1016/j.mrgentox.2012.12.014>]
6. Tasinov O, Vankova D, Nazifova-Tasinova N, Pasheva M, Kiselova Y, Sokrateva T, et al. Cytotoxicity of water from five Bulgarian wetlands contaminated by toxigenic cyanobacteria and cyanotoxins. *Bulg Chem Commun*. 2020; 52 (D): 257-262.
7. Tasinov O, Kiselova-Kaneva Y, Ivanova D, Pasheva M, Vankova D, Ivanova D. Ferrum phosphoricum D12 treatment affects J774A.1 and 3T3-L1 cells proliferation and gene expression of inflammation, oxidative stress and iron metabolism-related proteins. *Homeopathy*; 2020; 109(01): A1-A28 DOI: 10.1055/s-0040-1702084
8. Lipinski C, Lombardo F, Dominy B, Feeney P. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev*. 2001; March 46 (1-3): 3-26, [Crossref-[https://doi.org/10.1016/S0169-409X\(00\)00129-0](https://doi.org/10.1016/S0169-409X(00)00129-0)]

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Evaluation of academic stress among international students

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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 (\pm) 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 Alfa 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" ($M=1.72\pm 0.05$) and "Procrastination" ($M=1.55\pm 0.06$). The items causing sometimes stress (score 1.0) were "University/college environment" ($M=1.0\pm 0.05$) and "Parental expectations" ($M=1.04\pm 0.06$). The item comparison of the USS scale in the two periods of evaluation, November and May, indicated significant changes in four items (Fig.1).

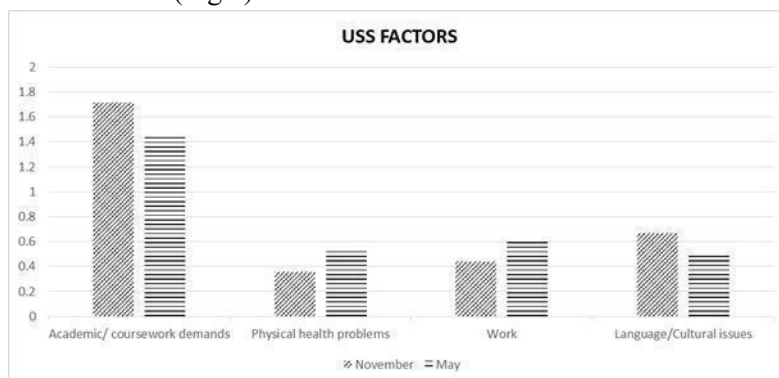


Fig.1. The item comparison of the USS in the two periods of evaluation.

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" ($M=0.66\pm 0.06$); "Physical health problems" ($M=0.53\pm 0.04$); "Work" ($M=0.6\pm 0.05$).

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" ($M=2.15\pm 0.06$); "Worrying about examination" ($M=1.91\pm 0.06$) "Difficulty in remembering all that is studied" ($M=1.67\pm 0.06$); "Lack of concentration during study hours" ($M=1.56\pm 0.06$), Examination syllabus too heavy ($M=1.5\pm 0.05$). Among the factors least related to stress (0.5 and below scores) were "Unable to discuss academic failures with parents" ($M=0.5\pm 0.05$); "Inadequate space or room for study" ($M=0.53\pm 0.06$); "Lack of fluency while speaking the English language" ($M=0.51\pm 0.05$); "Unable to find friends" ($M=0.33\pm 0.05$); "Inability to adapt to the new social & cultural environment" ($M=0.44\pm 0.05$) "Lack of support from parents" ($M=0.24\pm 0.04$).

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).

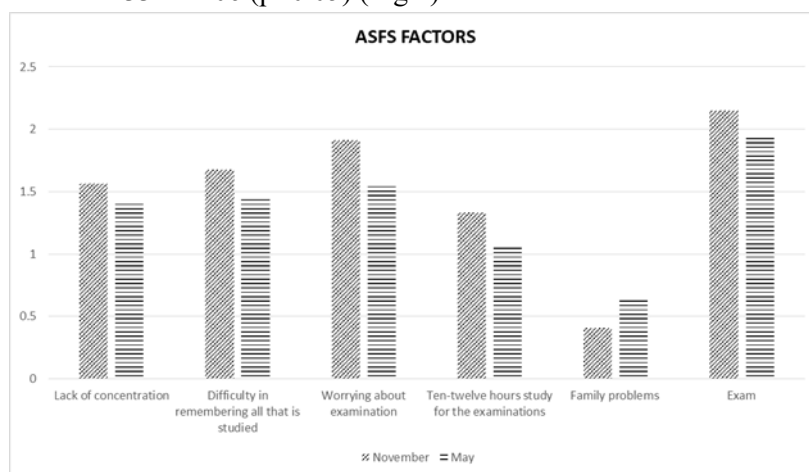


Fig. 2. The item comparison of the ASFS in the two periods of evaluation

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:

1. Reddy K. J, Menon K.R, Thattil A. Academic Stress and its Sources Among University Students. *Biomed. Pharmacol. J.* 2018;11(1). [Crossref-<http://biomedpharmajournal.org/?p=19485>]
2. Portoghese, I., Galletta, M., Porru, F. *et al.* Stress among university students: factorial structure and measurement invariance of the Italian version of the Effort-Reward Imbalance student questionnaire. *BMC Psychol* 2019;7,68 (2019).[Crossref <https://doi.org/10.1186/s40359-019-0343-7>]
3. Gold JA, Hu X, Huang G, et al. Medical student depression and its correlates across three international medical schools. *World J Psychiatry.* 2019;9(4):65-77. [PubMed <https://pubmed.ncbi.nlm.nih.gov/31799151/>]
4. Stallman H.M. and Camerton H.P. The University Stress Scale: Measuring Domains and Extent of Stress in University Students. *Australian Psychologist.* 2016;51(2):128-134. [Crossref - <https://doi.org/10.1111/ap.12127>]

Schizophrenic disorders in forensic psychiatric expertises in the civil process

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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)

Symptom	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
ICD-10															
20.0	8	-	-	-	1	81	46	74	41	41	4	-	80	43	7
20.1	-	-	-	-	-	1	1	1	-	1	-	-	1	-	1
20.6	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
22.0	-	-	-	-	-	3	-	3	3	2	-	-	3	-	-

Legend of the psychopathological symptoms:	
1. IQ 50 – 69	9. Disorders of orientation for personality and situation
2. IQ 35 – 49	10. Disorders of attention and memory, which disturb the reproduction of information, relevant to decision making
3. IQ 20 – 34	11. Severe disorders of identity
4. IQ below 20	12. Quantitative and qualitative disorders of consciousness
5. IQ undefined	13. Lack of insight
6. Disorders of thought: ideopsychic process, rigidity of thoughts, sperrung, manism, incoherency, disorganized thought	14. Negative symptoms
7. Hallucinations or delusional interpretations of reality	15. Parathymia and parabolia
8. Affective disorders with a predominance of pathological changes in emotions	

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.

References:

1. Testa M, West SG. Civil commitment in the United States. *Psychiatry (Edgmont)*. 2010 Oct;7(10):30-40. PMID: 22778709; PMCID: PMC3392176
2. Wangmo T, Seaward H, Pageau F, Hiersemenzel L-P and Elger BS (2021) Forensic-Psychiatric Risk Evaluations: Perspectives of Forensic Psychiatric Experts and Older Incarcerated Persons From Switzerland. *Front. Psychiatry* 12:643096. doi: 10.3389/fpsy.2021.643096
3. Horstead A, Cree A. Achieving transparency in forensic risk assessment: a multimodal approach. *Adv Psychiatr Treat*. (2013) 19:351–7. doi: 10.1192/apt.bp.112.010645
4. Pham T, Taylor P. The roles of forensic psychiatrists and psychologists: professional experts, service providers, therapists, or all things for all people? In: Goethals K, editor. *Forensic Psychiatry and Psychology in Europe: A Cross-Border Study Guide*. Cham: Springer International Publishing (2018). p. 155–63. doi: 10.1007/978-3-319-74664-7_10
5. Fazel S, Singh JP, Doll H, Grann M. Use of risk assessment instruments to predict violence and antisocial behaviour in 73 samples involving 24 827 people: systematic review and meta-analysis. *Bmj-Brit Med J*. (2012) 345:e4692. doi: 10.1136/bmj.e4692
6. Combalbert N, Andronikof A, Armand M, Robin C, Bazex H. Forensic mental health assessment in France: recommendations for quality improvement. *Int J Law Psychiat*. (2014) 37:628–34. doi: 10.1016/j.ijlp.2014.02.037
7. Dahle K-P, Lehmann RJB. Beiträge der deutschsprachigen forensischen verhaltenswissenschaft zur kriminalprognostischen methodenentwicklung. *Forens Psychiatr Psychol Kriminol*. (2016) 10:248–57. doi: 10.1007/s11757-016-0389-9
8. Howner K, Andiné P, Bertilsson G, Hultcrantz M, Lindström E, Mowafi F, et al. Mapping systematic reviews on forensic psychiatric care: a systematic review identifying knowledge gaps. *Front Psychiatry*. (2018) 9:452. doi: 10.3389/fpsy.2018.00452
9. Steinau S, Brackmann N, Sternemann U, Biller-Andorno N, Habermeyer E. Conflicting priorities between risk management and treatment of schizophrenia in swiss forensic services-a case report. *Front Psychiatry*. (2018) 9:680. doi: 10.3389/fpsy.2018.00680
10. Moulin V, Mouchet C, Pillonel T, Gkotsi GM, Baertschi B, Gasser J, et al. Judges' perceptions of expert reports: the effect of neuroscience evidence. *Int J Law Psychiatry*. (2018) 61:22–9. doi: 10.1016/j.ijlp.2018.09.008
11. Endrass J, Urbaniok F, Held L, Vetter S, Rossegger A. Accuracy of the static-99 in predicting recidivism in Switzerland. *Int J Offender Ther*. (2009) 53:482–90. doi: 10.1177/0306624X07312952
12. Urbaniok F, Endrass J, Rossegger A, Noll T. Violent and sexual offences: a validation of the predictive quality of the PCL: SV in Switzerland. *Int J Law Psychiat*. (2007) 30:147–52. doi: 10.1016/j.ijlp.2006.04.001
13. Джорджанова А. Съдебнопсихиатричната експертиза в гражданския процес. КОРС, 2021

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

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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 convalescent patients. A total number of 113 fully vaccinated individuals with two doses of mRNA vaccine and 40 convalescent 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 convalescent 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 2nd 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 6th 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 6th 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 2nd 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 2nd and 6th 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 6th 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 2nd month (VI - $R^2 = 0.41$; $p < 0.01$; RP - $R^2 = 0.64$; $p < 0.01$) and 6th month (VI - $R^2 = 0.48$; $p < 0.01$; RP - $R^2 = 0.42$; $p < 0.01$).

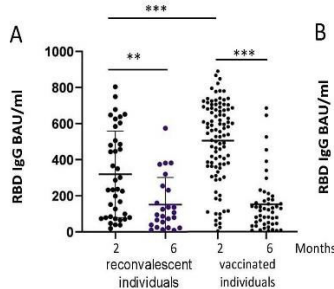


Fig.1.

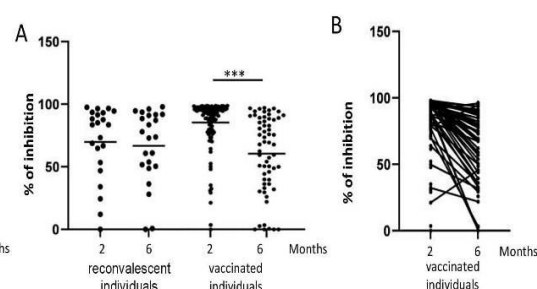


Fig.2.

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

Figure 2. (A) Levels of neutralizing antibodies by % of inhibition on 2nd and 6th month after vaccination and post symptoms onset (*** = $p < 0.001$), (B) % of inhibition data for each VI plotted on 2nd and 6th 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 2nd month in the group of VI, there was no significant difference on the 6th 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 6th 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 6th 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

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References

- 1.WHO Coronavirus Disease (COVID-19) Dashboard. 2020. <https://covid19.who.int/>
- 2.Sette A, Crotty S. Adaptive immunity to SARS-CoV-2 and COVID-19. *Cell*. 2021;184(4):861-80.
- 3.Sandberg JT, Varnaitè R, Christ W, Chen P, Muvva JR, Maleki KT, et al. SARS-CoV-2-specific humoral and cellular immunity persists through 9 months irrespective of COVID-19 severity at hospitalisation. *Clinical & translational immunology*. 2021;10(7):e1306.
- 4.Schiffner J, Backhaus I, Rimmel J, Schulz S, Möhlenkamp T, Klemens JM, et al. Long-Term Course of Humoral and Cellular Immune Responses in Outpatients After SARS-CoV-2 Infection. *Frontiers in public health*. 2021;9:732787.
- 5.Gussarow D, Bonifacius A, Cossmann A, Stankov MV, Mausberg P, Tischer-Zimmermann S, et al. Long-Lasting Immunity Against SARS-CoV-2: Dream or Reality? *Frontiers in medicine*. 2021;8:770381.
6. Anand SP, Prévost J, Nayrac M, Beaudoin-Bussièeres G, Benlarbi M et. al. Longitudinal analysis of humoral immunity against SARS-CoV-2 Spike in convalescent individuals up to 8 months post-symptom onset. *bioRxiv [Preprint]*. 25:2021.01.25.428097.
7. Israel A, Shenhar Y, Green I, Merzon E, Golan-Cohen A, Schäffer AA, Ruppin E, Vinker S, Magen E. Large-scale study of antibody titer decay following BNT162b2 mRNA vaccine or SARS-CoV-2 infection. *medRxiv [Preprint]*. 21:2021.08.19.21262111.
8. Cassaniti I, Bergami F, Percivalle E, Gabanti E, Sammartino JC, Ferrari A, Adzasehoun KMG, Zavaglio F, Zelini P, Comolli G, Sarasini A, Piralla A, Ricciardi A, Zuccaro V, Maggi F, Novazzi F, Simonelli L, Varani L, Lilleri D, Baldanti F. Humoral and cell-mediated response against SARS-CoV-2 variants elicited by mRNA vaccine BNT162b2 in healthcare workers: a longitudinal observational study. *Clin Microbiol Infect*. 25:S1198-743X(21)00536-X.
- 9.Chia WN, Zhu F, Ong SWX, Young BE, Fong SW, Le Bert N, et al. Dynamics of SARS-CoV-2 neutralising antibody responses and duration of immunity: a longitudinal study. *The Lancet Microbe*. 2021;2(6):e240-e9.
- 10.Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature medicine*. 2021;27(7):1205-11.
- 11.Johnson M, Wagstaffe HR, Gilmour KC, Mai AL, Lewis J, Hunt A, et al. Evaluation of a novel multiplexed assay for determining IgG levels and functional activity to SARS-CoV-2. *Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology*. 2020;130:104572.

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

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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 (*bla_{KPC}*, *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 – *bla_{KPC}* ($n=3$), *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].

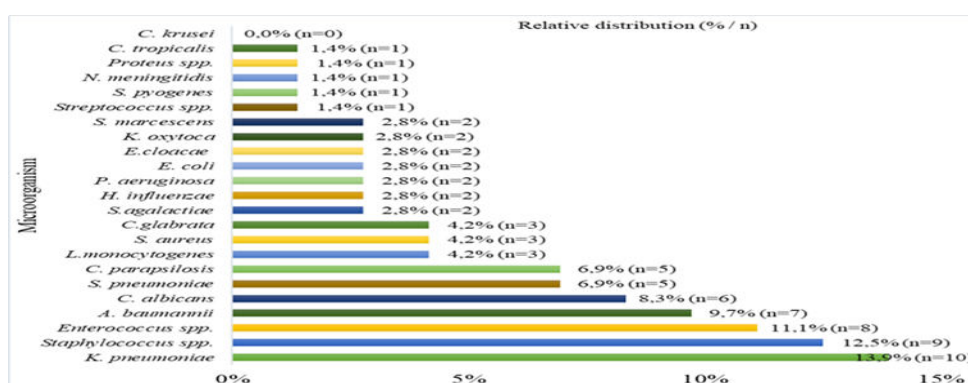


Fig 1. Relative distribution (%/n) of microorganisms from blood cultures detected by mPCR.

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.

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Bibliography:

1. Salimnia H. et al., Evaluation of the FilmArray Blood Culture Identification Panel: Results of a Multi-center Controlled Trial. *J Clin Microbiol.* 2016;54(3):687.
2. MacVane S. et al. Benefits of adding a rapid PCR-based blood culture identification panel to an established antimicrobial stewardship program. *J Clin Microbiol.* 2016;54(10):2455-246
3. Garnacho J. et al, Impact of adequate empirical antibiotic therapy on the outcome of patients admitted to the intensive care unit with sepsis. *Crit Care Med.* 2003;31(12):2742-2751.
4. Zaragoza R. et al. The influence of inadequate empirical antimicrobial treatment on patients with bloodstream infections in an intensive care unit. *Clin Microbiol Infect.* 2003;9(5):412-418.
5. Southern T., et al. Implementation and performance of the BioFire FilmArray® Blood Culture Identification panel with antimicrobial treatment recommendations for bloodstream infections at a midwestern academic tertiary hospital. *Diagn Microbiol Infect Dis.* 2015;81(2):96-101.
6. Bhatti M. et al. Evaluation of FilmArray and Verigene systems for rapid identification of positive blood cultures. *J Clin Microbiol.* 2014;52(9):3433-3436.
7. Rand K. et al. Direct identification of bacteria in positive blood cultures: Comparison of two rapid methods, FilmArray and mass spectrometry. *Diagn Microbiol Inf Dis.* 2014;79(3):293-297
8. Altun O., et al., Clinical evaluation of the Filmarray blood culture identification panel in identification of bacteria and yeasts from positive blood culture bottles. *J Clin Microbiol.* 2013 Dec;51(12):4130-4136.
9. Blaschke A., et al. Rapid identification of pathogens from positive blood cultures by multiplex polymerase chain reaction using the FilmArray system. *Diagn Microbiol Infect Dis.* 2012;74(4):349-355.

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