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Stojiljković. Scr Med 2020;51(2):67-8. DOI:10.5937/scriptamed51-27313
EDITORIAL

COVID-19 Pandemic, Passing of Professor Folke Sjöqvist and Other Topics

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Abstract

In Issues 1 and 2 the scientific biomedical journal *Scripta Medica* devoted a total of six articles to the aetiological, epidemiological and clinial aspects of serious acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the disease caused by this virus - coronavirus disease 2019 (COVID-19). The present Issue 2 also contains an obituary on the occasion of demise of Professor Folke Sjöqvist, one of the founding fathers of clinical pharmacology as a discipline and several other articles from the fields of quality assurance in healthcare, experimental and clinical medicine.

Key words: editorial, SARS-CoV-2, COVID-19, obituary, Folke Sjöqvist.

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On 11 March 2020 the World Health Organisation proclaimed a world pandemic caused by serious acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing the coronavirus disease 2019 (COVID-19).[1] The Editorial Board of the *Scripta Medica* decided to publish two articles on this phenomenon in Issue 1, Volume 51 that appeared in late March 2020 – the editorial containing general information on the virus, the disease – epidemiology and clinical aspects[2] and a letter to the editor containing some interesting data on the pharmacological aspects of the COVID-19 infection.[3]

The main accent that the Issue 2, Volume 51 of the Scripta Medica put on COVID-19 and it is conveyed by four articles covering this world-hottest topic. In their article, Bhandari et al[4] describe the management of patients infected by SARS-CoV-2 in the tertiary hospital settings in India. Aćimović et al[5] deal with the course of the epidemic in the Republic of Srpska, Bosnia and Herzegovina, while Stajić et al[6] bring an interesting case report of a false-negative patient with COVID-19 bilateral pneumonia. A global view of the current pandemic is presented in a review article by Professor Slavenka Janković.[7] In close connection with the COVID-19 pandemic is a very informative history of medicine article by Beštić-Bronza, covering the 1918-1919 pandemic of Spanish flu.[8]

The other main impression is contained in the obituary honouring late Professor Folke Sjöqvist, one of the founders of clinical pharmacology as a discipline in Europe,[9] to whom many of us and the entire discipline are indebted to. Since our journal covers all fields of medicine, the remaining articles bring the results of some basic pharmacological experiments on the role of $GABA_A$ receptors in vasodilation,[10] on the role of clinical pathways in quality improvement in patients undergoing total hip replacement[11] and describe some clinical studies performed in the fields of ophthalmology[12] and hepatic resections in metastatic colorectal cancer surgery,[13] followed by a case report on hypofibrinogenaemia in pregnancy.[14]

Of course, the Editorial Board will continue to follow the COVID-19 pandemic as it evolves, but at the same time will try to provide to its readers coverage of all other interesting topics.

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Conflict of interest

None.

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ORIGINAL ARTICLE

COVID-19 Related Mortality Profile at a Tertiary Care Centre: a Descriptive Study

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Abstract

Background/Aim: The recent pandemic of Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) is yet another scourge from the coronaviridae family that causes illnesses ranging from common cold to more severe diseases such as Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV). The numbers are still on the rise, despite a country wide lockdown and yet no definitive drugs and or/vaccines are available to manage the active COVID-19 cases.
Methods: The present research design was a hospital based observational descriptive study conducted at S. M. S. Medical College and Attached hospitals, Jaipur, that analysed data of all the patients with COVID-19 related mortality, admitted between 1 April to 4 May 2020. Patients included in this study were RT-PCR confirmed cases of SARS-CoV-2 using nasopharyngeal and oropharyngeal swab samples.
Results: The mean age of patients with COVID-19 related mortality was 53.41 ± 18.42 year with majority of patients belonging to age group of more than 60 years (41.18 %) followed closely by COVID-19 positive patients in age range of 45 to 60 years (33.33 %). The male to female ratio was 1.68: 1. Mean time lag between hospitalization and death reported was 6.18 days. Majority of the patients admitted (72.5 %) succumbed within 3 days of hospitalization. Eleven patients (21 %) were brought dead to the hospital who were tested COVID-19 positive after death. Most common comorbidity reported in patients with COVID-19 related mortality was hypertension (30 %) followed by diabetes mellitus (27.5 %).
Conclusion: Hypertension and diabetes mellitus might be independent risk factors making an individual susceptible to COVID-19 related death. Elderly patients also have a greater risk of mortality. The non-availability of definitive management protocol and/or vaccine against COVID-19 makes public health preventive measures of social distancing, use of masks and frequent handwashing an important modality in the fight against COVID-19.

Key words: age, comorbidity, COVID-19, hypertension, mortality.

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Introduction

The recent pandemic of Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) also known as Corona Virus Disease 2019 (COVID-19) is yet another scourge from the Coronaviridae family that causes illnesses ranging from common cold to more severe diseases such as Middle East respiratory syndrome (MERS-CoV) and severe acute respiratory syndrome (SARS-CoV).

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SARS-CoV-2 is a novel coronavirus that has not been previously identified in humans. The current pandemic of COVID-19 has affected 215 countries, areas, or territories worldwide as of 8 May 2020, and has infected 3,822,382 people worldwide, causing 263,658 confirmed deaths.[1] The spread of COVID-19 began from Wuhan, a city of Hubei province of China and was declared a pandemic by World Health Organization (WHO) on 11 March 2020.[2]

The clinical spectra of COVID – 19 is varied ranging from mild to moderate symptoms of cough, sore throat, headache, rhinorrhoea, vomiting and diarrhoea, fever and shortness of breath to signs and symptoms complex of severe pneumonia, acute respiratory distress syndrome, septic shock and/or multiple organ failure.[3] The disease is highly infectious with a reproductive number (R0) ranging from 2.2–3.5, that explains its rapid spread like wild fire throughout the world.[4]

India has been struggling to contain the spread of virus and has managed to flatten the curve at 41,472 active cases and 2,109 deaths as of 10th May 2020, since the first reported SARS-CoV-2 case on 30 January 2020.[5] The numbers are still on the rise, despite a country wide lockdown with yet no definitive management protocol inclusive of drugs and or/vaccines available to manage the active COVID-19 cases. State of Rajasthan is among the top five states of India with 3,708 confirmed cases of COVID-19 and 106 deaths reported till now. The present study was undertaken to appreciate and describe mortality profile of SARS-CoV-2 at one of premier Institute of Tertiary Care Medical College of Rajasthan and South-East Asia.

Methods

The present study, a hospital based observational descriptive study, was conducted at SMS Medical College and attached hospitals, Jaipur, sharing the highest load of patients in the Rajasthan that analysed and evaluated data of all COVID-19 related mortality of patients admitted between 1 April to 4 May 2020. A total of 51 mortalities were reported due to COVID-19 during this duration at this institute. All the patients were reverse – transcription polymerase chain reaction (RT – PCR) positive for SARS-CoV-2 using nasopharyngeal and oropharyngeal swab samples, tested at the Laboratory of Microbiology of the Institute. The data where collected and analysed preserving the anonymity of patients. Patients were categorised into five different age groups to evaluate the relation between age and COVID-19 related mortality. Patients were also categorized in three groups based upon the number of days stayed in hospitals. The COVID-19 patients were also categorised based upon the underlying comorbidities to evaluate its relation with COVID-19 related mortalities. Data was presented and compared as mean and percentage of distribution among different groups.

Results

During a period of 34 days from 1st April to 4th May 2020 there were 51 deaths reported due to COVID-19. The mean age of patients with

Table 1: Characteristics of patients with COVID-19 related mortality

	Number of patients with COVID-19 related mortality	Percent
Age Group (years, n = 51)		
0-15	1	1.96
15-30	8	15.67
30-45	4	7.84
45-60	17	33.33
> 60	21	41.18
Gender (n = 51)		
Female	19	37.26
Male	32	62.74
Hospital stay group (days, n = 40)		
1 - 3	29	72.5
4 - 6	6	15
> 6	5	12.5
Comorbidity (n = 40)		
No comorbidity	4	10
Hypertension	12	30
Diabetes Mellitus	11	27.5
Chronic Kidney Disease	2	5
Cardiovascular Disease	6	15
Respiratory Disease	6	15
Gastrointestinal Disease	4	10
Sepsis	6	15
MODS	3	7.5
Brought in status (n = 51)		
Brought Dead	11	21.57
Brought alive	40	78.43

MODS = Multiple organ dysfunction syndrome

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COVID-19 related mortality was 53.41 ± 18.42. The highest mortalities (n = 21, 41.18 %) were reported in the group above 60 years of age, closely followed by 45-60 years age group (n = 17, 33.33 %). The lowest mortality was observed in paediatric age group below 15 years with overall one death (1.96 %). Males (n = 32, 62.74 %) were affected more than the females (n = 19, 37.26 %) with a male to female ratio of 1.68 : 1. One death was reported in pregnant female positive for COVID-19. (Table 1).

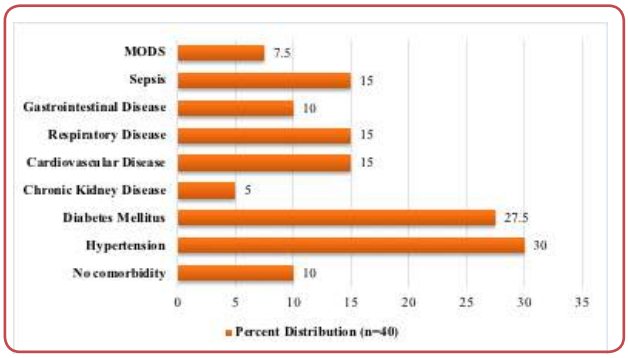

Figure 1: Comorbidities in patients with COVID-19 related mortality

MODS = Multiple organ dysfunction syndrome

Majority of patients (n = 29, 72.5 %) succumbed within a duration of 1-3 days during hospital stay. Six patients (15 %) survived for a duration of 4-6 days and only five patients (12.5 %) could survive for more than six days. The Mean time lag between hospitalization and death reported was 6.18 days. The comorbidity most prevalent in patients with COVID-19 related mortality was hypertension (n = 12, 30 %), closely followed by diabetes mellitus (n = 11, 27.5 %). Other comorbidities such as cardiovascular diseases (n = 6, 15 %), respiratory disease (n = 6, 15 %), sepsis (n = 6, 15 %), gastrointestinal disease (n = 4, 10 %), multiple organ dysfunction syndrome (n = 3, 7.5 %) and chronic kidney disease (n = 2, 5 %). Four patients (10 %) with COVID-19 related mortality did not have any associated comorbidities. There were 11 patients (21.57 %) who were brought dead and were reported positive for SARS-CoV-2 *post mortem* (Table 1 and Figure 1).

Discussion

The disease spectrum of COVID-19 may very from a mild illness to fatal complications like pneumonia, acute respiratory distress syndrome (ARDS), multi-organ failure, septic shock, disseminated intravascular coagulation and ultimately leading to death.[6, 7] Outcome of COVID-19 may be poorer in case of underlying comorbidities.[8] Therefore, identification of such underlying factors is of paramount importance in COVID-19 management. In the present study the mean age of patients with COVID-19 related mortality was 53.41 ± 18.42, suggesting a higher mortality among elderly individuals. Chen et al[9] also observed a similar average age profile of COVID-19 positive patients in the median age of 55.5.

Majority of patients afflicted with COVID-19 belonged to the age group of more than 60. Several studies have observed this association, indicating elderly age contributing to a severe outcome.[10-13] An elderly individual might be susceptible to COVID-19 due to age-related changes in pulmonary functions with obvious responsiveness and tolerance, subsequently leading to poor outcome in this age group.[14] COVID-19 exhibited preponderance for male individuals with a male to female ratio of 1.68 : 1, a finding that has been reported in various studies worldwide.[3, 15] Jin et al[16] also observed that the number of men with COVID-19 related mortality was 2.4 times higher than that of women.

In the present study hypertension was the most prevalent comorbidity in COVID-19 related mortality, closely followed by diabetes mellitus. This indicates a higher mortality risk in COVID-19 patients presenting with hypertension, followed by diabetes mellitus, a finding that supplants the observations of Guan et al from China[15] and Itelman et al from Israel,[17] supporting the premise that chances of morbidity and mortality in COVID-19 positive patients increase with associated comorbid conditions. Hypertension has been suggested as a risk factor for poor outcome in COVID-19 patients in several studies.[12, 13, 18] A similar finding in hypertensives was also observed in the present study. The exact mechanism underlying this observation is still unclear. However, the use of angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers used for treatment of hypertension has been linked to severe outcome in such patients. These drugs have been found to augment ACE2 mRNA expression.[19] SARS-CoV-2 has been found to utilis e this ACE2 receptor for invading the human cells.[20] A higher expression of these receptors facilitates entry of this virus, increasing the susceptibility of individuals to this infection and subsequently a

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severe disease. This receptor not only acts as the entry receptor of SARS-CoV-2, but also protects the lung from injury due to its anti-inflammatory effects.[21]

ACE2 exhibits anti-inflammatory and protective role that has been found downregulated in diabetics.[22] The downregulated anti-inflammatory response could be responsible for exaggerated immune response to SARS-CoV-2 virus in diabetics, with a severe and uncontrolled damage to the lungs and other tissues. This might explain the high mortality in diabetics observed in the present study. Respiratory and other cardiovascular diseases were also observed in patients with COVID-19 related mortality in this study. Acute respiratory distress syndrome with extensive inflammation, cell death, alveolar damage and oedema occurs in severe COVID-19 case leading to a hypoxic state due to reduced gaseous exchange.[23, 24] This could explain the possible severe outcome in patients with pre-existing respiratory diseases, as has been observed in this study.

Mortality in COVID-19 patients with cardiovascular diseases observed in this study could be attributable to a wide expression of ACE2 in cardiovascular system that is more pronounced in cardiovascular disease.[25, 26] Several researchers have pointed out the myocardial damage and related mortality in patients of SARS-CoV-2 infection.[3, 27, 28] In the present study around 3/4 of the patients succumbed within 3 days of hospitalization and surprisingly 1/5 of the patients was brought dead, who were tested positive post mortem. This finding indicates a rapid progression of COVID-19 in these patients.

The disease profile of COVID-19, inclusive of its behaviour, progress and severity scale, is crucial to determine appropriateness and adequacy of mitigation strategies and to enable planning and designing health-care needs and policies. Moreover, factors that might contribute to rapid progression of COVID-19, such as virulence, drug resistance, host factors or structural reformation of the virus, needs to be explored and should form the epicentre of focus especially in people without any risk factors. Country wide lockdown and social distancing has helped in containing the spread of virus to some extent, but the stumbling economy due to lock down poses a great challenge worldwide.

Conclusion

Hypertension and diabetes mellitus might be independent risk factors making an individual susceptible to COVID-19 related death. Elderly patients also have a greater risk of mortality. The non-availability of definitive management protocol and/or vaccine against COVID-19 makes public health preventive measures of social distancing, use of masks and frequent handwashing an important modality in the fight against COVID-19.

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Conflict of interest

None.

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ORIGINAL ARTICLE

Epidemiological Characteristics of COVID-19 Infection in the Republic of Srpska: a Hundred Days Survey

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Abstract

Background/Aim: The World Health Organization (WHO) declared the spread of a novel disease COVID-19 as a pandemic on 11 March 2020. As of 12 June, there have been more than 7.4 million COVID-19 cases and more than 418,000 COVID-19 deaths globally. This paper represents epidemiological analysis of the first 100 days of COVID-19 epidemic in the Republic of Srpska.
Methods: Data of all COVID-19 cases confirmed in the Republic of Srpska between 4 March and 12 June were collected from epidemiological and laboratory testing reports obtained from the Public Health Institute of the Republic of Srpska. This cross-sectional analysis was carried out on a sample of 1,607 laboratory-confirmed COVID-19 cases, which included: summary of patient characteristics, examination of age distributions and sex ratios, calculation of case fatality and mortality rates, incidence rates analysis, epidemiological curve construction and subgroup analysis.
Results: Over 100 days after the first case was confirmed, the total number of infected patients in the Republic of Srpska rose to 1,607 (31,471 persons had been tested). As of 12 June, 69.9 % of those cases has recovered. During that period there were 117 confirmed deaths (average age 72 years; 60.7 % males; 86 % older than 60 years; 94 % with at least one comorbidity). The sex ratio among the confirmed cases was 0.95:1 (48.7 % men vs 51.3 % women). Infections were less common in persons below 20 years of age (7.3 % of all confirmed cases) and the majority of the affected persons were in the group 40-69 years of age. As much as 86 % of all death cases occurred in persons older than 60 years (average age 72 years) and 94 % of all death cases had at least one underlying condition (mostly cardiovascular diseases, 79.5 %).
Conclusion: Evaluating the clinical data of COVID-19 patients, finding the source of infection and studying the behavior of the disease is crucial for understanding of the pandemic.

Key words: SARS-CoV-2, COVID, epidemic, epidemiology, analysis.

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Introduction

In December 2019, the newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in Wuhan, China, causing COVID-19, a respiratory disease presenting mostly with fever, cough and, very often, with pneumonia.[1, 2] The disease spread rapidly to other parts of China and then to other countries around the globe. The first cases in Europe were reported on 24 January 2020 in France.

The WHO Director General declared spread of COVID-19 as a Public Health Emergency of Inter-

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national Concern (PHEIC) on 30 January and, in view of the severity of the disease spread, as pandemic on 11 March 2020. According to the statistics of the WHO and the European Centre for Disease Control (ECDC), as of 12 June, there were more than 7.4 million COVID-19 cases and more than 418,000 COVID-19 deaths globally.[3]

In January and February, while the public focus of attention was on China, the number of new cases and fatalities worldwide increased day by day. At that time the Republic of Srpska was in the first of four epidemiological scenarios of potential COVID-19 outbreak, in which there were no confirmed cases, but it was necessary to undertake numerous activities in order to increase the level of preparedness and to be ready to respond properly in order to identify and manage the new cases of COVID-19 infection.[4] The first activities, led by the Public Health Institute of the Republic of Srpska (PHI RS), were aimed at setting-up the system for continuous monitoring of the epidemiological situation, as well as providing accurate information to the health authorities and general public related to SARS-CoV-2 onset and spread of the disease, the characteristics of the virus and the disease itself and recommending prevention measures to the whole population. The Ministry of Health and Social Welfare of the Republic of Srpska established coordination mechanisms to conduct activities pertaining to the outbreak of the novel coronavirus. Two medical faculties, Banja Luka (University of Banja Luka) and Foča (University of East Sarajevo), participated in providing adequate information to the public, through the established COVID-19 call centres. At that stage, it was necessary to undertake precaution measures at points of country border entry, with an aim of early detection of infected persons. At the same time, the PHI RS rapidly developed the set of guidance for healthcare system preparedness for the emergence of COVID-19 in the Republic of Srpska.

At the first phase, primary healthcare centres conducted the health surveillance of travelers arriving in the Republic of Srpska and at the same time prepared new protocols and trained staff to respond to the emergence of the novel virus. The hospitals prepared action plans for hospitalisation, isolation, and treatment of patients in infectious disease clinics and intensive care units. The capacities of two accredited laboratories in the Republic of Srpska have been strengthened for the reverse transcription polymerase chain reaction (RT-PCR) detection of the SARS-CoV-2 virus. Numerous activities have been undertaken to improve public health preparedness, as well as preparedness of other sectors, before the importation of the first cases of COVID-19 disease.

The aim of this study was to evaluate the epidemiological characteristics of the COVID-19 infection in the Republic of Srpska for the first 100 days of the infection outbreak.

Methods

This study was carried out retrospectively in the Republic of Srpska, which is one of two administrative entities in Bosnia and Herzegovina. The population of the Republic of Srpska is 1.2 million. All data of COVID-19 cases in the Republic of Srpska between 4 March and 12 June were obtained from epidemiological and laboratory reports of the PHI RS. Epidemiological data were obtained through the surveillance system (mandatory notification of the occurrence of cases of the selected infectious diseases). Data on laboratory tests (RT-PCR assays for the detection of SARS-CoV-2) were obtained from two microbiological laboratories, one at the University Clinical Centre of the Republic of Srpska (UCC RS), for testing of patients hospitalised or examined at the UKC RS and the second one at the PHI RS, for all other suspected cases in the Republic of Srpska. Only laboratory-confirmed cases were included in this study. This analysis was carried out on a sample of 1,607 laboratory-confirmed COVID-19 cases. Analyses included: summary of patient characteristics, age and sex ratios, case fatality and mortality rates, incidence rates analysis, epidemiological curve construction and subgroup analysis. All data were analysed using SPSS, statistical program for social sciences and the basic descriptive statistics was used for presentation of the results.

Results

One hundred days after the outbreak of COVID-19, the total number of confirmed cases in the Republic of Srpska was 1,607 (out of 31,471 people tested) and by the time 1,123 (69.9 %) of those cases have been already recovered. Among those infect-

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Table 1: The major characteristics of COVID-19 infected cases in Republic of Srpska on 12 June, 2020

Cases, characteristics	n	%
Number of infected patients	1,607	
Cumulative incidence rate/100.000	140.3	
Age in years, median	52.3	
(range)	(0.5-96)	
Female	825	51.3
Male	782	48.7
Age groups (years)		
0 - 9	45	2.8
10 - 19	72	4.5
20 - 29	178	11.1
30 - 39	217	13.5
40 - 49	249	15.5
50 - 59	272	16.9
60 - 69	235	14.6
70 - 79	192	11.9
≥ 80	147	9.1
Healthcare workers	109	6.8
Recovered	1123	69.9
Number of active cases	367	22.8

ed, 782 (48.7%) were man and 825 (51,3%) were women. The median age of patients was 52.3 years (range, 0.5-96 years; Table 1).

The number of confirmed cases differed by age. Most of the cases were in the age group 50-59 years (272 persons or 16.9 % of the total number of cases), and then in 40-49 (15.5 %) and 60-69 (14.6 %) age groups. The youngest person with confirmed SARS-CoV-2 infection was 6 months old and the oldest one was 96 years old (median 52). The total number of confirmed cases among children aged 0-9 years was 45, which accounts for 2.8 % of all confirmed cases. A total of 109 health care workers suffered from the infection, representing the 6.8% of all cases. (Table 1, Figure 1).

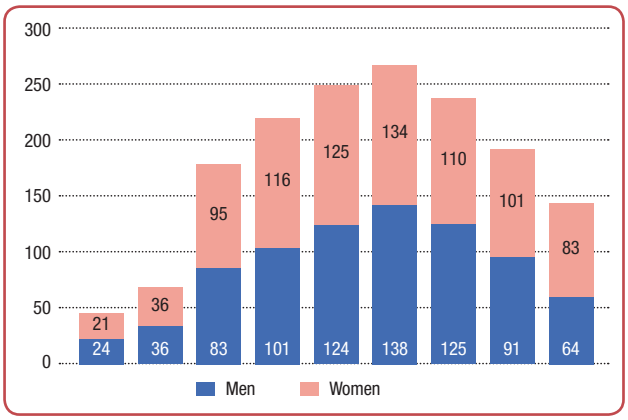

Figure 1: Age/sex distribution of COVID-19 cases in the Republic of Srpska presented in age groups.

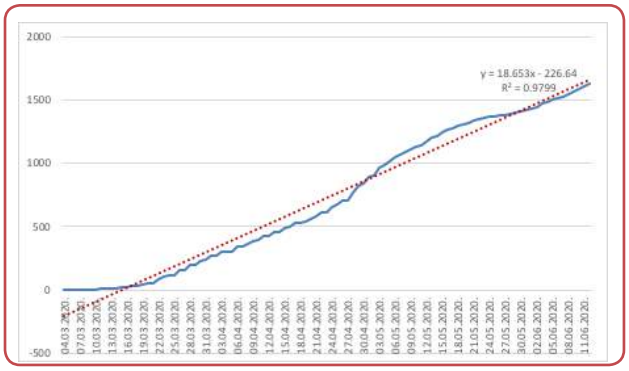

Figure 2: The cumulative number of confirmed COVID-19 cases in the Republic of Srpska during the 100 days after the first case was detected on 4 March 2020.

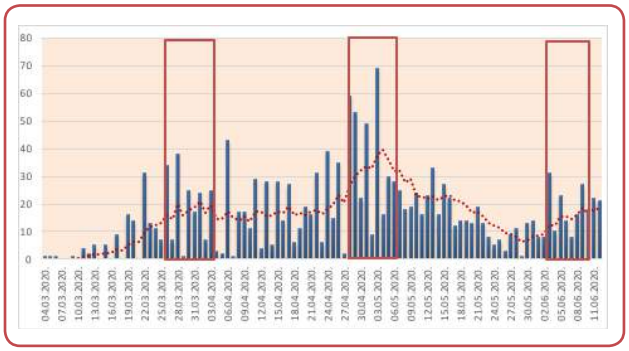

Figure 3: Daily number of COVID-19 cases in the Republic of Srpska (blue bars) and 7-day moving average trend line (dashed red line) by the June 12. The red rectangles represent the first (I), second (II) and third (III) peaks of significantly higher number of cases.

The epidemiological curve of cumulative number of cases in the Republic of Srpska indicates a moderate linear growth pattern (Figure 2). Although the number of cases has grown substantially, there were three peaks with significantly higher number of cases. The first one was observed between 25 March and 3 April with the number of 30-40 infected cases/day. The second peak was recorded at the last week of April with an average number of 40-66 confirmed cases/day, and the third one started at the beginning of June (Figure 3).

The cumulative incidence rate on 12 June was 140.3 cases per 100,000 inhabitants (HTI) which is significantly higher than in the whole Bosnia and Herzegovina (86/HTI). There were three significant increases in incidence rates/HTI per week; the first one was observed on week 4 with 11.27/HTI, the second one started on week 7 and continued for the following five weeks, reaching the peak on the week 9 with 21.66/HTI and the third one had begun to rise at the second week of June (13.71/HTI; Figure 4). The mortality rate per HTI was usually below 1, but during the weeks

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Figure 4: Weekly incidence and mortality rates of COVID-19 in the Republic of Srpska, starting from March 4th.

9-12 it significantly increased reaching the maximum of 2.62/HTI on week 10 (Figure 4).

In the South East European (SEE) Region, the highest cumulative incidence rate, recorded at the beginning of June, was in Serbia (165/HTI) and the lowest in Bulgaria (36/HTI) and Greece (28/HTI). However, well developed western countries, such as the USA and the EU countries (Italy, Spain, Belgium, UK, Sweden etc.) had scored 5-10 times higher incidence rates comparing to countries of SEE (Figure 5).

The first death case from COVID-19 in the Republic of Srpska was registered on 28 March, or 24 days after the first case was confirmed. Since that

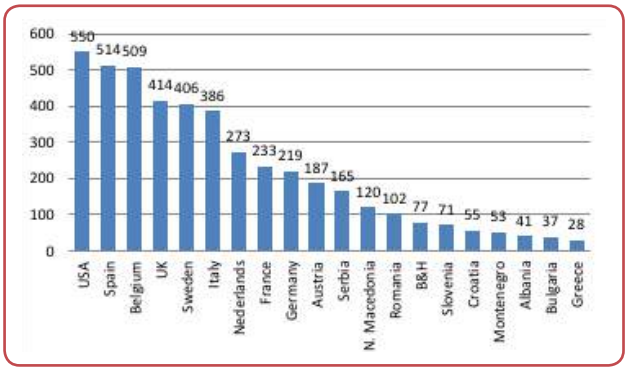

Figure 5: The comparison of cumulative incidence rates (number of cases per 100,000 inhabitants) of USA and some west European countries with east European countries.

time 117 people died due to the COVID-19. That represents a mortality rate of 101 per million inhabitants. Similar to the cumulative incidence rates, west European countries have had significantly higher mortality rates, comparing to countries of SEE (Figure 6).

Characteristics of COVID-19 death cases in the Republic of Srpska are described in the Table 2. The youngest patient who died due to COVID-19

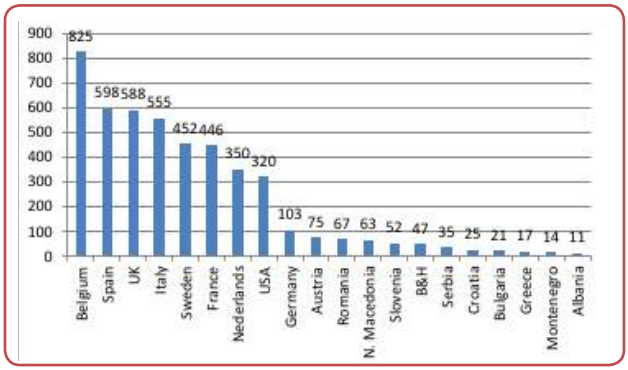

Figure 6: Comparison of cumulative mortality rates (number of death cases per million inhabitants) of some western European countries with eastern European countries.

infection was a 26 years old man with the Down syndrome, and the oldest was aged 96, with the median and average age among death cases of 72 years. Almost all (94 %) persons who died from COVID-19 infection had at least one comorbidity. The most common comorbidities belonged to cardiovascular diseases (79,5 % of all death cases; mainly hypertension) and diabetes (32,5 %). Data suggest that sex and age can also be considered as risk factors for COVID-19 related deaths. As much as 60.7 % of death cases were male (Table 2). The case fatality rate was 7.2 % and it was higher in men than in women; 9.1 % of men who contracted the COVID-19 infection died, comparing to 5.6 % of women. The difference is even higher in the age group ≥ 80 years, in which 36 % of men who contracted the virus died, comparing

Table 2: Major characteristics of COVID-19 related death cases in the Republic of Srpska by the June 12.

Deaths characteristics	n	%
Deaths	117	100.0
Age in years, median (range)	72 (26-96)	
Sex, male	71	60.7
Case fatality rate		7.2
Mortality rate/100.000	10.2	
At least one comorbidity associated to the	110	94.0
COVID-19 infection		
cardiovascular diseases	93	79.5
diabetes	38	32.5
lung disease	15	12.8
neurological disease/	19	16.3
dementia	20	14.5
renal disease	18	15.4
malignancy	11	9.4
obesity	9	7.7
liver disease	5	4.3
immune disease	32	27.3
other		

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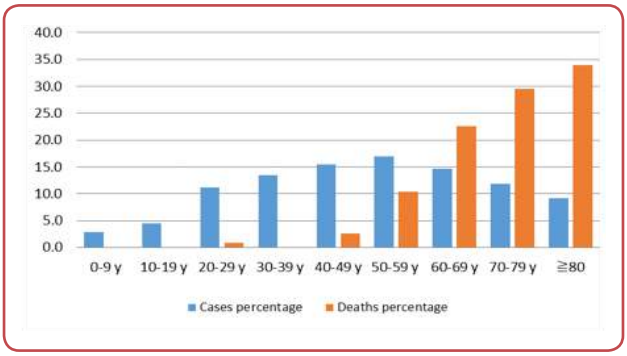

Figure 7: Percentage of COVID-19 infection cases (N=1607) and deaths cases (N=117) by age groups in the Republic of Srpska during 100 days of epidemic.

to 20 % of fatal outcomes among women from the same age group.

Age-specific case fatality rate was getting higher with the increase in age. The highest number of confirmed infection cases was within the age group 50-59 years, followed by the 40-49 and 60-69 age groups, but the highest number of deaths was among people aged ≥ 80, followed by the age groups 70-79 and 60-69 (Figure 7). The oldest age group represents only 9.8 % of all confirmed COVID-19 cases but comprised 34 % of all death cases (Figure 7).

Discussion

The first case of COVID-19 in the Republic of Srpska was confirmed on 4 March in the City of Banja Luka. That was a 43-year-old man who arrived from Italy and developed mild symptoms of the disease. Soon thereafter he was hospitalised at the University Clinical Centre of the Republic of Srpska.[2] Immediately after the laboratory test confirmed the SARS-CoV-2 infection, the epidemiologists from Banja Luka Health Centre started contact tracing, which is a critically important tool in counter-epidemic response.[4-6] All family members of the first patient, although asymptomatic, were promptly tested and the infection was confirmed in his 13-year-old son. In order to limit further transmission of the disease, the elementary school the boy attends was closed and disinfected and all children and teachers who were in contact with the boy were home-quarantined and tested. All measures were taken on time and no one from the school was infected.

At the same time, by tracing other contacts of the first patient, the infection was confirmed in a 46-year-old man from the neighbouring municipality of Čelinac, who also arrived from Italy in the same car with the first patient and then in his 17-year-old daughter. Among their numerous contacts, including girl's classmates and schoolteachers, there were no new infections. The first cluster in the Republic of Srpska was closed with only four people infected.

However, new imported cases very soon led to a greater spread of the disease. Like in other countries, transmission mostly occurred at social gatherings, with the important role of some super-spreading events, as well as at home and work settings, which directed us to the following epidemiological phases: phase of sporadic cases, clusters of cases and the phase of community transmission.[1, 4-6] Moving from phase to phase, public health response had to be adjusted in order to achieve the main goals: to slow down and stop transmission, prevent outbreaks and delay spread, provide optimised care for all patients, especially the seriously ill, and to minimise the impact of the epidemic on health systems, social services and economic activity.[7]

The Republic of Srpska was among the first countries in the region which, after the occurrence of local transmission of COVID-19, promptly introduced interventions on 10 March to suppress the spread of the virus, such as closing of schools, public places, restriction of public gatherings and border restrictions, advising citizens to work from home whenever is possible. Contact tracing followed by quarantine and isolation was carried out whenever new cases were identified. The purpose of these measures was to slow down the spread of the disease, and by controlling the infection to get "flattened the epidemic curve". Countries that had implemented the "lockdown" measures in earlier phases of the COVID-19 outbreak had lower incidence and mortality rates.[8] Despite all epidemiologic measures the virus spread rapidly and the City of Banja Luka has become the biggest COVID-19 cluster in the Republic of Srpska and the whole Bosnia and Hezegovina with more than 50 % of all cases.

One hundred days after the first case was confirmed, 31,671 people had been tested for COVID-19 in the Republic of Srpska, and 1,607

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have been found positive (5.1 %). Among those positive, the proportion of females had a slightly higher rate (51.3 %) compared with males (48.7 %). Similar observations were reported in other studies.[9-11] The mean age of patients was 52.3 and 47% of patients were from the age group 40-69, but older males and those with comorbidities, such as hypertension and diabetes, were more likely to have very severe symptoms and with the higher risk of death. As much as 86 % of all death cases occurred in persons older than 60 (average age of death cases was 72). A total of 34 % of all death cases were from the group of patients over 80 years and that group represented only 9.8 % of all COVID-19 cases. These findings are consistent with other studies from China suggesting a higher fatality rate among men compared to women.[9, 12] There is no clear explanation for this difference, but it is possible that men had higher proportion of comorbidities than women. On the other hand, younger people were less likely to get infection than the older ones.[13] As much as 94 % of all death cases in the present study had at least one chronic comorbidity (mostly cardiovascular diseases, 79.5 %), which is in accordance with systematic reviews that have shown that people with chronic comorbidities like hypertension, other cardiovascular disease or diabetes are at higher risk of progressing to more severe COVID-19 disease and death.[14]

There were 107 healthcare workers in this study that suffered SARS-CoV-2 infection, representing 6.8 % of all cases. Reports are different from different countries, ranging from 2.8 % in Iran,[11] 4.6 % in Wuhan (China),[9] 14 % in England[15] to 19.9 % in Kazakhstan.[16] The reason for obviously higher case rate in health care workers could be explained by initial lower awareness, shortage of medical resources and inadequate use of personal protective equipment, as well as the higher exposures to infected patients at the early stage of epidemic.

Timely implementation of measures in the Republic of Srpska was of utmost importance for epidemiological curve of cumulative number of cases indicating moderate linear growth pattern. In the last week of April, after a month and a half after the first COVID-19 case was confirmed, the Republic of Srpska faced a more intensive increase of number of cases, compared to the previous trend. The incidence rate per week was 21.66/HTI. By recognising the problem and after strengthening of infection prevention and control measures the number of cases declined and the epidemiological curve has gradually returned to the previous trend. Several factors have influenced that increase, among which are the occurrence of hospital-associated infections and the fact that infection had spread in long-term care facilities. It has been shown that virus can easily enter into the hospital settings and long-term care facilities and spread among vulnerable population, particularly elderly and people with chronic diseases.[17] According to the European Centre for Disease Prevention and Control (ECDC) the reported deaths in long-term care facilities account for 30–60% of all COVID-19 deaths in many European countries.[18]

Since 11 January 2020, when the first death case caused by COVID-19 was reported in China, the disease has become one of the leading infectious causes of death in number of countries around the world, with the total of 418.302 reported deaths globally, as of 12 June 2020. Although it is not possible to predict long term death toll of COVID-19, due to the ongoing nature of the pandemic, with an average of 2,300 deaths daily, it currently represents the third deadliest communicable disease, trailing tuberculosis and hepatitis and leaving behind pneumonia, HIV/AIDS, malaria, seasonal flu and all other vaccine-preventable communicable diseases.[19]

The developed countries, such as the USA and the EU countries, have experienced significantly higher cumulative incidence and mortality rates, comparing to SEE countries. There are no explanations for these facts, but some reports emphasised that the drastic social isolation measures, undertaken especially in Eastern European countries might contribute to these differences.6 Once the pandemic become over, it will be possible to make more specific analyses and to understand all the possible reasons why the SEE countries have provided better response to the first wave of COVID-19 pandemic, compared to developed EU countries.

By 12 June 2020, as much as 1,123 infected persons have recovered, representing 69.9 % of all infected cases in the Republic of Srpska. The number of active cases is 367 (22.83 %) and the epidemic is still far from fading out.

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Conclusion

It is clear that all age groups are susceptible to COVID-19, but older males and those with co-morbidities are more likely to develop severe form of disease. Even though COVID-19 is highly contagious, control measures have proven to be very effective and public health interventions were temporally associated with improved control of the COVID-19 outbreak. Evaluating the clinical data of COVID-19 patients, finding the source of infection and studying the behavior of the disease is crucial for understanding of the pandemic.

Acknowledgements

None.

Conflict of interest

None.

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ORIGINAL ARTICLE

The Vasorelaxant Properties of Novel Benzodiazepine-like Ligands on Isolated rat Thoracic Aorta

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Abstract

Background/Aim: In addition to well-established central effects, benzodiazepines, but also some other allosteric modulators of gamma-amino-butyric acid (GABA) receptor exhibit significant vascular effects. However, there are currently no elucidated mechanisms for manifested vasodilatory properties and very little is known about GABA gamma-amino-butyric acid function and $GABA_A$ receptor expression within peripheral blood vessels.
Methods: In the present study, we demonstrated the vasorelaxant properties of diazepam, GABA and novel imidazobenzodiazepine amide ligands GL-II-73 and GL-II-74, which are characterized as positive allosteric modulators of α5- containing $GABA_A$ receptor. Using isometric organ bath system, we examined the vascular responses to phenylephrine, in the presence and absence of various ligands, in the rat thoracic aorta.
Results: The observed significant and strong attenuation of the maximal contractile response of phenylephrine indicates a non-competitive antagonism of diazepam, GL-II-73 and GL-II-74 ($p < 0.001$), whereas GABA does not affect phenylephrine contraction.
Since the strongest inhibitory effect was observed with compound GL-II-74, that, compared to other tested ligands, exhibited a higher potentiation at α5 $GABA_A$Rs, it could be assumed that the α5 subunit plays a significant role in the structure of putatively present "vascular" $GABA_A$Rs.
Conclusion: This work emphasizes the importance of $GABA_A$Rs research in the periphery and also points to the possibility of using α5 selective $GABA_A$R modulators as potential therapeutic targets for novel vasodilators.

Key words: $GABA_A$ receptor, positive allosteric modulators, vasodilatation, rat thoracic aorta.

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Introduction

In addition to being a major inhibitory neurotransmitter in the central nervous system (CNS), GABA has a functional importance in many peripheral tissues. Peripheral GABA regulation of cardiovascular function has long been known,[1, 2] but to date no distinct roles or exact mechanisms have been established.

The first studies with isolated cerebral blood vessels had suggested that $GABA_A$ receptors ($GABA_A$Rs) exist in vascular smooth muscle, where GABA or GABA-agonists produced a dilatation of cerebral arteries.[3, 4] Even though $GABA_A$R subunit mRNA expression has been demonstrated in various rat peripheral organs, such as kidneys, adrenal gland, ovary, testis, uterus and ileum[5, 6] very lit-

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tle is known about $GABA_AR$ expression and GABA function within the peripheral vascular smooth muscle.

GABA levels in the peripheral vessels and activity of GABA-related enzymes, especially glutamic acid decarboxylase (GAD) and gamma-aminobutyric acid-transaminase (GABA-T), have been found to be up to 1 % of those in the brain[7] and such a modest expression can be regarded as insufficient to directly elicit vasoactivity of GABA. However, the finding that cultured human aortic and umbilical vein endothelial cells synthesize GABA, which further exhibits direct effects on endothelial cell metabolism[8] indicates the potential role of GABA as an autocoid for neighbouring smooth muscle cells.

Benzodiazepines (BZs) as positive modulators of $GABA_ARs$ have a wide range of acute effects, such as anxiolytic, sedative, hypnotic, skeletal muscle relaxant, anticonvulsant, anterograde amnesic and ataxic action. In addition to well-established central role, BZ's also exhibit vasodilatory properties.[9,10,11] However, there are currently no elucidated mechanisms of BZ`s vasoactivity and propensity to reduce the intracellular influx of calcium into the smooth muscle cell.

Vascular effects similar to those of diazepam are also exhibited by other $GABA_AR$ allosteric modulators, such as endogenous neurosteroids.[12] Considering that the peripheral benzodiazepine receptor (officially known as translocator protein, TSPO) has no role in regulating smooth muscle contractility,[10] the published results suggest that activation or positive modulation of $GABA_ARs$, such as that effected by diazepam, result in vascular dilation.[12] However, the receptor subtype substrate of that action is totally unknown.

Herein, the vasorelaxant properties of novel ligands with imidazobenzodiazepine (IBZD) amide structure GL-II-73 and GL-II-74 were demonstrated, which are characterised as positive allosteric modulators (PAMs) of $GABA_AR$ with preferential potentiation at α5 subunit-containing receptors.[13] In order to examine their possible vasoactivity, isometric organ bath study of vascular responses to phenylephrine was conducted. Diazepam and GABA were used in the same protocols, and in this way the manifested effects were compared and thus the possible mechanisms of vasoactivity were assessed.

Methods

Vessel preparation

Wistar rats were obtained from the Military Medical Academy and housed in vivarium facilities of the Faculty of Pharmacy, University of Belgrade (Belgrade, Serbia) under normal housing conditions (temperature: 22 ± 1 °C, relative humidity: 40-70 %, 12/12 h light/dark period). As a part of a wider national project led by the senior author, the experiments were approved by the Ethical Council for the Protection of Experimental Animals of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia. Male rats were anaesthetised with combination of ketamine hydrochloride (90 mg/kg, Ketamidor, Richter Pharma AG, Wels, Austria) and xylazine hydrochloride (10 mg/kg, Xylased, Bioveta, A. S., Ivanovice na Hane, Czech Republic). The descending thoracic aortas were dissected and cleared of surrounding adipose and connective tissue.

Aortic rings of approximately 3 mm length were obtained from isolated blood vessels bathed in Petri dish containing chilled (4 °C) modified Krebs-bicarbonate solution (composition: 118.3 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 11 mM glucose).[14] The aortic rings were rapidly placed for measurement of isometric contraction.

Experiments with isolated vascular rings

The aortic rings were suspended between two wire hooks in organ bath chambers filled with 15 mL modified Krebs-bicarbonate solution (37° C, pH 7.40) aerated with mixture of 95 % oxygen/5 % carbon dioxide. The upper hook was connected to the MLT0201 force displacement transducer (Panlab, Spain), and changes in isometric force were recorded using PowerLab/4SP data acquisition system (AD Instruments, Castle Hill, Australia) and software LabChart 7 Pro (AD Instruments). Experiments were performed on four organ baths in parallel.

The rings were placed under the optimal passive stretching tension of 4.0 g, defined previously.[15] The equilibration period of the preparation lasted 60 min and during that time the bathing solution was changed every 10 min. Each aortic ring was subjected first to the initial challenging contraction with potassium chloride (6×10^{-2} M) to assess the viability of preparations. The rings

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was then left to re-equilibrate for 40-50 min, before the appropriate protocol procedures were used.

Experimental protocol: experiments were aimed to investigate the effects of diazepam, GABA and novel imidazobenzodiazepine (IBZD) amide ligands (GL-II-73 and GL-II-74) on the contractile response induced by the α1 adrenoreceptor agonist phenylephrine (PE), in the endothelium-intact aortic rings.

At the beginning of the protocol, to obtain a reference contraction, the contractile response induced by potassium (6 x 10^{-2} M) was measured. After preparations were washed-out several times until tone returned to baseline, concentration-response curve of PE (control curve) was generated (10^{-9}-10^{-4} M). Aortic ring had been washed-out again and test compound (each at concentration 10^{-4} M and 10^{-5} M, except for diazepam with the applied concentration of 10^{-5} M) were added individually to the organ bath, 60 min before another PE-induced contraction was obtained. The effects of the test compound on the PE contraction were assessed by comparing the contractile response in the presence or absence of compound. Results were expressed in relation to the contraction achieved by the same ring previously contracted with isotonic potassium.

Drugs and solutions

Phenylephrine hydrochloride and GABA were purchased from Sigma-Aldrich (St. Louis, USA). Diazepam was generously supplied by Galenika (Belgrade, Serbia).

The ligands GL-II-73 ((R)-8-Ethynyl-6-(2-fluorophenyl)-N,N,4-trimethyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide) and GL-II-74 ((R)-N-Ethyl-8-ethynyl-6-(2-fluorophenyl)-4-methyl-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxamide) were synthesised at the Department of Chemistry and Biochemistry, University of Wisconsin, Milwaukee, USA.

All drugs were prepared as concentrated stock solutions 10^{-1} M in 100 % ethyl alcohol, with exception for PE and GABA, the stocks of which were prepared in distilled water. The subsequent dilutions were carried out in mixture of solvent and distilled water, so that the final solvent concentration was never higher than 0.3 % in the 15 mL-organ bath.

Statistical analysis

Statistical analysis and graphs were prepared using LabChart 7 Pro software (AD Instruments) and SigmaPlot 11 (Systat Software Inc.) Results were summarised as the mean ± standard error of n replicates, where n is the number of aortic rings tested in one protocol, each obtained from a separate animal. The negative logarithm of the ligand concentration (pEC_{50}) producing 50% of the maximum response was calculated in LabChart 7 Pro software. Statistical analyses were performed using Student's paired t-test (p values less than 0.05 were considered statistically significant).

Results

Diazepam (10^{-5} M) produced a significant attenuation ($p < 0.001$) of the maximal contractile response of PE (117.24 ± 5.30 % vs 66.62 ± 3.71 %), while it did not affect the pEC_{50} value of PE (Figure 1).

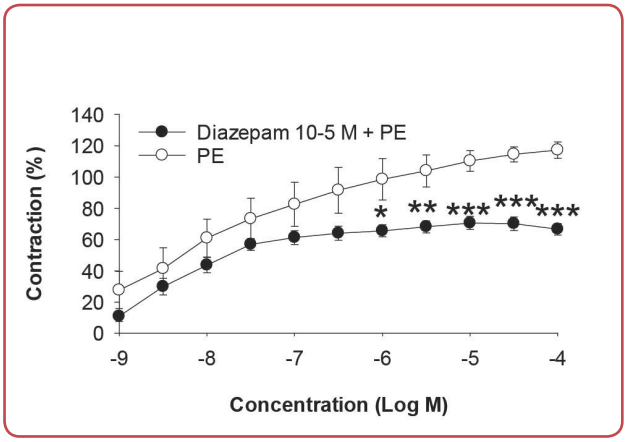

*Figure 1. Effect of diazepam on the phenylephrine concentration-response curve when aortic rings were pre-incubated with diazepam (10^{-5} M, n = 6). Results (mean ± SEM) are expressed with reference to the contraction induced by potassium (6 x 10^{-2} M). *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (significantly different E_{max} values). Parentheses indicate the number of preparations studied, each obtained from a separate animal.*

Although applied at a very high concentration (10^{-4} M), GABA did not shift the PE concentration-response curve or affect the PE-induced maximal contraction (Figure 2A). GABA used at concentration of 10^{-5} M also did not affect the PE contraction (data not shown).

The ligand GL-II-73 (10^{-5} M) significantly decreased ($p < 0.05$) the maximal contractile re-

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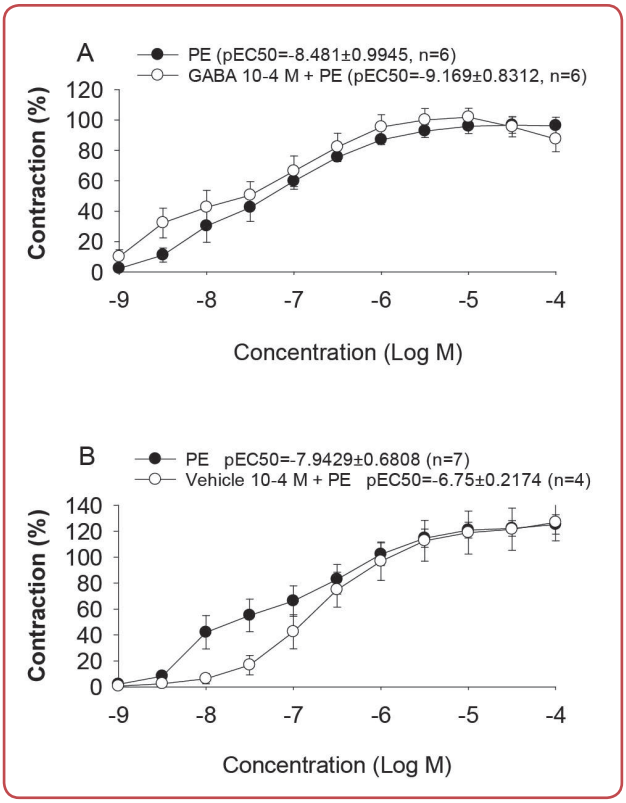

Figure 2. Cumulative log concentration-relaxation curves for pheny-lephrine (PE) in the absence and presence of A) GABA 10^{-4} M (n = 6) vehicle 10^{-4} M (n = 4). Results (mean ± SEM) are expressed with reference to the contraction induced by potassium (6 x 10^{-2} M). Parentheses indicate the number of preparations studied, each obtained from a separate animal.

sponse to PE (82.36 ± 16.41 % vs 125.17 ± 7.45 %), but had no effect on the PE potency (there was no significant differences between pEC_{50} values). Pre-treatment with GL-II-73 at a ten-fold higher concentration (10^{-4} M) strongly attenuated ($p < 0.001$) the maximal PE contraction (35.03 ± 9.60 % vs 125.17 ± 7.45 %), whereas had no significant effect on the pEC_{50} value of PE (10^{-9}-10^{-4} M) (Figure 3A, B).

The ligand GL-II-74 used at the higher concentration (10^{-4} M) strongly decreased ($p < 0.001$) the PE-induced maximal contraction, compared with untreated rings (18.08 ± 4.48 % vs 125.17 ± 7.45 %). When aortic rings were pre-treated with a lower concentration of GL-II-74 (10^{-5} M) there also was no effect on the pEC_{50} value for PE, while the maximal contractile response was significantly ($p < 0.001$) decreased (42.99 ± 11.63 % vs 125.17 ± 7.45 %) (Figure 3C, D).

The influence of vehicle was obtained when the same volume, as for ligand additions at concentration (10^{-4} M), was added in pre-incubation period. There was no altered pharmacological activity on aortic rings in presence of vehicle (Figure 2B).

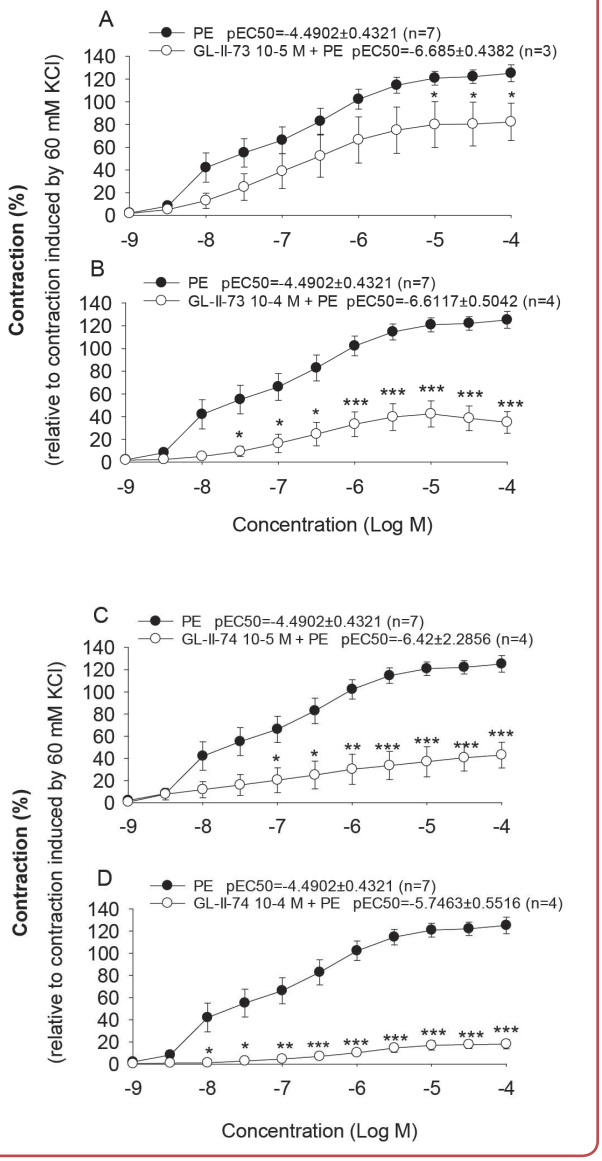

Figure 3. Cumulative log concentration-relaxation curves for phenyl-ephrine (PE) in the absence and presence of A) GL-II-73 10^{-5} M (n = 3); B) GL-II-73 10^{-4} M (n = 4); C) GL-II-74 10^{-5} M (n=4); D) GL-II-74 10^{-4} M (n=4). Results (mean ± SEM) are expressed with reference to the contraction induced by potassium (6 x 10^{-2} M).
**** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ (significantly different E_{max} values). Parentheses indicate the number of preparations studied, each obtained from a separate animal.*

Discussion

The differential expression of total of nineteen $GABA_AR$ subunits (α1-6, β1-3, γ1-3, δ, ε, θ, π, ρ1-3) has been demonstrated in various peripheral organs, indicating that $GABA_AR$ subunits are expressed in a tissue-specific manner.[6, 16] Immunohistochemical analyses, western blotting and real time reverse transcription polymerase chain

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reaction (RT-PCR), had revealed the presence of functional $GABA_A$Rs within the gastrointestinal tract,[16] airway smooth muscle of trachea,[17,18] pancreatic β cells.[19] However, there is still no clear evidence for the expression of functional $GABA_A$Rs on vascular smooth muscle cells. This study was based on the hypothesis that positive allosteric modulation of $GABA_A$Rs that contain the α5 subunit contributes to vasodilating effects of BZs. The inhibiting influence of diazepam on the contractile activity of phenylephrine in isolated rat aorta was demonstrated, thus confirming the previous *in vitro* studies, where diazepam inhibited PE- induced calcium oscillations,[20] attenuated the PE- induced contractions in the rat aorta[10] and produced vasodilation in the PE-precontracted rat aortic rings.[9] The observed significant and strong attenuation of the maximal contractile response of PE indicates a non-competitive antagonism of diazepam, in terms of signalling mechanisms of contraction in vascular smooth muscle cells.

Concentration of GABA in the systemic circulation of humans was found to be between 0.5 to 3 μM.[8] It has been suggested that apart from GABA produced by the pancreatic beta cells, adrenal gland and certain immune cells, an important source of GABA in circulation may be that related to endothelial cells of blood vessels.[8] The examination of the effect of GABA on vascular response to PE in isolated rat aorta indicated that GABA did not affect PE contraction, even when applied in high concentration (100 μM). Findings of GABA indifference on contracted aortic rings found in this study may correlate with earlier data that no vasodilating effects on peripheral blood vessels have been reported for GABA.[8,11,21] Nevertheless, the results from other studies with isolated blood vessels have shown that GABA has relaxatory effect on rat mesenteric bed.[22,23]

Diazepam, a standard non-selective PAM of $GABA_A$Rs, was used as the reference ligand, in order to investigate the vasorelaxant properties of GL-II-73 and GL-II-74. Previously performed electrophysiological and binding studies showed that ligands GL-II-73 and GL-II-74 acted as PAMs with primary efficacy and affinity at α5-containing $GABA_A$Rs,[13] whereas diazepam modulates $GABA_A$R activity as a non-selective PAM, with high affinity and efficacy at α1, α2, α3 or α5–containing $GABA_A$Rs.[24] Both ligands (GL-II-73 and GL-II-74) reduced the maximum contraction induced by PE, compared to the untreated rings, indicating similarity to the effects of diazepam in the same protocol.

It was also shown that the vascular responses to PE in the isolated aortic rings vary significantly, depending on concentrations of GL-II-73 and GL-II-74 used during incubation. When aortic rings were pre-treated with a higher concentration (10^{-4} M), the maximal contractile response was approximately 20-30 % of the corresponding control maximal contraction ie (without the presence of ligand), while at lower concentrations of tested ligand, the inhibitory effects were weaker (approximately 50 % reduction in contraction). This clearly indicates a concentration-dependent inhibitory effects of the tested IBZDs.

Concentrations of compounds used in this study were in accordance with those in studies of vascular effects of BZs on isolated blood vessels. Although these concentrations are too high to correspond with the clinical use of BZs, they can still be reached in cases of overdose or other abuse.[11] In this regard, their vascular effects should not be neglected. Interestingly, a stronger inhibitory effect on the PE concentration-response curve was observed with compound GL-II-74 than with GL-II-73. This might be explained by the observed differences in their modulatory properties, taking into account that GL-II-74 exhibited a higher potentiation at α5 $GABA_A$Rs than GL-II-73.[13] Accordingly, it could be assumed that the presence of the α5 subunit in the structure of putatively present "vascular" $GABA_A$Rs may play a substantial role in the overall observed vasoactivity.

Conclusion

The present work highlights the importance of $GABA_A$Rs research in the periphery and also opens the possibility of using α5 selective $GABA_A$R modulators as potential therapeutic targets for novel vasodilators.

Acknowledgements

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CONTENT

Conflict of interest

None.

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ORIGINAL ARTICLE

The Role of Clinical Pathways on Healthcare Quality Improvement in Hospitals for Patient Undergoing Total Hip Replacement

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Abstract

Background: Clinical pathways are important tools to achieve better quality of care and to reduce the costs for healthcare system. The total hip replacement (THR) is among the most expensive procedures in health system and the number of these operations has greatly increased in the past decade in the Republic of Srpska.
Aim: The aim of the present study was to determine how the implementation of a clinical pathway for THR can influence the length of stay and postoperative complications in hospitals in the Republic of Srpska.
Methods: This prospective and comparative study was performed on 2,485 patients who underwent the THR over a 3-year-period in 2012 (prior to the introduction of the clinical pathways, baseline), in 2013 (first evaluation period) and in 2014 (second evaluation period), one and two years after its implementation, respectively. The study was conducted in 10 hospitals in the Republic of Srpska, where the effects of the clinical pathways on length of stay and postoperative complications after THR were measured.
Results: The introduction of THR clinical pathways significantly decreased the length of stay in hospital from 14.53 ± 7.03 days measured at baseline, to 12.79 ± 4.81 days and 11.19 ± 4.11 days at first and second evaluation period, respectively. At the same time, the number of early postoperative complications such as death and venous thromboembolism significantly decreased in both groups, while the number of dislocations, as parameter of late complications, decreased just after the second evaluation period. For all other complications, such as revision procedures, infections and periprosthetic fracture, there were no statistical differences after the implementation of clinical pathways.
Conclusion: The introduction of clinical pathways was successful in reducing the length of stay in hospitals as well as the postoperative complications after THR.

Key words: clinical pathways, total hip replacement, length of stay, quality of care.

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Introduction

Total hip replacement (THR) is an orthopaedic surgery performed to reduce pain and improve function in patients suffering from hip fractures or hip arthritis. In recent decades, there has been a growing need for this procedure, which is the result of an aging of population, an increase in the prevalence of osteoarthritis and the presence of obesity epidemic.[1] If this trend continues in the future, an even greater increase in the need for intervention such as THR can be expected.[2]

Although the THR is a cost-effective treatment, both from the clinical and patients' perspective, this operation produces a significant cost for hos-

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pitals, which could be a critical issue due to limited funds available for healthcare systems. Having that in mind, it would be extremely important to introduce certain instruments for costs control without compromising the quality of patient care. There are several strategies that are being applied for quality of care improvement in hospitals, but introduction of clinical pathways, also known as care pathways (CP), have been seen as the most successful instrument for quality of care improvement and reduction of costs in healthcare worldwide.[3, 4] There is a considerable amount of evidence that implementation of CP can reduce both the length of stay in hospital and cost of treatment, without undesirable outcomes and postoperative complications.[5-9] However, these studies have never been performed in health system of the Republic of Srpska.

Vanchaecht et al[10] studied the effects of CPs on total knee arthroplasty in a large teaching hospital in Belgium and concluded that CPs effectively reduce the length of stay (LOS) by 33 % without affecting the short-term functional outcomes. Results of studies conducted in hospitals in the Netherlands showed that using a CP approach for patients with hip fracture tends to be more effective than usual care.[11] In another study conducted in 11 US hospitals, it was demonstrated that the implementation of THR clinical pathway was successful in reducing the patient's LOS in hospital, as well as with increasing discharges to home what consequently led to cost reduction.[12]

The aim of this study was to determine how the implementation of a CP for THR can influence the LOS and postoperative complications in hospitals in the Republic of Srpska, Bosnia and Herzegovina.

Methods

Study period

This prospective and comparative study was performed in all patients who underwent the THR over a 3-year period. This period covered the 12 months (January – December 2012) prior to the introduction of the CPs, and the 24 months follow-up period (January 2013 - December 2013 and January 2014 -December 2014) after its implementation.

Development and implementation of the clinical pathways

The CPs were developed by a multidisciplinary team of healthcare professionals involved in the THR patients care in accordance with recommendations of international clinical guidelines. The team was comprised of an orthopaedic surgeon, a clinical quality coordinator, a chief nurse and a physiotherapist. The objectives of this CP were the reduction of LOS in hospital and the reduction in post-operative complications. In this regard, the following key interventions and outcomes were defined: (1) admission criteria; (2) admission date; (3) prophylactic use of antibiotics; (4) thromboembolic prophylaxis; (5) start of physiotherapy procedure on postoperative day 1; (6) discharge criteria and (7) discharge date. The structure and the identification of other activities in the process (nursing assessment, evaluation by a physiotherapist, daily monitoring, etc) in addition to responsibility for these activities were left to each hospital to be adapted according to organisational and working conditions.

Sample size

The study was conducted in 10 hospitals in the Republic of Srpska, at the departments for orthopaedic surgery that regularly perform THR procedure. The clinical pathway project began in January 2013. Each patient underwent the THR gave the informed consent to participate in the study. Before the implementation of the CP, a baseline measurement was performed from January 2012 to December 2012 in one group of patients (n = 849; 288 men and 561 women). First evaluation of the CP was done from January 2013 to December 2013 in a second group of patients (n = 802; 284 men and 518 women). Second evaluation was done from January 2014 to December 2014 in a third group of patients (n = 834; 262 men and 572 women). Therefore, the total number of patients included in this study was 2,485. Patients excluded from this study were those with THR revision (replacement of previously fitted prosthesis), as well as the patients suffering from cancer as a primary diagnosis.

Indicators

To measure the effects of CPs the two groups of indicators were defined, known as the process and the outcome indicators. The process indicator was measured by the average LOS in hospital, while the outcome indicators were measured through early complications, as a rate of various surgical complications within 90 days (venous thromboembolism, death) and late complica-

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tions, as the outcomes within one year after surgery (infection, dislocation, periprosthetic fracture, revision of surgery).[13]

Measurements were taken at three time points: the baseline measurement, the second measurement taken 12 months after the implementation of the pathway and the third measurement taken 24 months after the implementation of the pathway. The outcomes were registered during the hospital stay and throughout the follow-up period. For all three groups of patients the follow-up period was 1 year after surgery.

Data collection and monitoring

Data collection and monitoring were carried out continuously, by entering data into the on-line application form for routine monitoring of quality indicators designed by the Agency for Certification, Accreditation and Healthcare Quality Improvement of the Republic of Srpska (ASKVA). The electronic system for collection, monitoring, analysis and presentation of quality indicators results in hospitals of the Republic of Srpska has been successfully introduced at the beginning of 2011 as an online application. The responsible hospital staff enters the required information from CP for all patients no later than on the fifth day of each month for the previous month. The reliability of data was adequate since data were used to categorise patients according to the Diagnosis Related Group system (DRG) and the consequent payment of each hospital by the Health Insurance Fund.

Statistical analyses

Data analysis were performed using the commercial statistical software SPSS Statistics 18. Verification of the normality of parameters distribution was performed using the Kolmogorov-Smirnov test. Statistical significance between groups was verified using the Kruskal-Wallis test and the post hoc comparison by using the Mann-Whitney test. Individual variables were presented in the form of frequency of individual characteristics (categories) and the statistical significance of the differences is determined using the chi-square test. The significance level was set at $p < 0.05$.[14]

Results

The study included a total of 2,485 patients with performed THR. The first group, before the CP was implemented, included 849 patients with a mean age of 70. The second group of 802 patients, with an average age of 68, underwent the primary THR based on CP and was assessed during the first evaluation period. Eight hundred thirty-four patients, with a mean age of 68, had THR surgery based on CP and was assessed during second evaluation period. All three groups were similar regarding the age and gender (Table 1).

Average length of stay

The implementation of THR pathway corresponded with the decreased LOS in hospitals by 22.9 % during the overall observational period (first group compared to second evaluation group; $p < 0.001$). Compared to the years of observation (2012-2013 and 2012-2014), the average length of stay decreased by each year (12.79 and 11.19 days, respectively; Table 1).

Table 1: Demographics and clinical data regarding years of observation

Parameters	Baseline evaluation (n = 849)	First evaluation group (n = 802)	Second evaluation group (n = 834)
Age	70. 69 ± 23.69	67.92 ± 25.11	68.60 ± 23.64
Gender, n (%)			
female	561 (66.08)	518 (64.59)	572 (68.59)
male	288 (33.92)	284 (35.41)	262 (31.41)
Average length of stay	14.53 ± 7.03	12.79 ± 4.81*	11,19 ± 4.11*

*All data (except gender) are presented as mean value ± SD. * p < 0.001*

The differences between the average LOS were significant in both evaluation periods. The average LOS was significantly decreased in both observational periods; 2012 to 2013 ($p < 0.001$), as well as 2012 to 2014 ($p < 0.001$) (Table 2).

Table 2: Paired statistical comparison of average length of stay data according to the evaluation period

Evaluation period	z value	p value
First evaluation (2012 vs 2013)	6.04	p < 0.001
Second evaluation (2012 vs 2014)	13.57	p < 0.001

Postoperative complications

The implementation of CPs significantly reduced the total number of postoperative complications in the second and third group of patients; from 134 at baseline (first group) to 92 (second group) and 79 (third group) measured at the first and second evaluation period, respectively. In the first group, 15.9 % of patients had at least one postoperative

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Table 3: The rates of postoperative complications after THR

A. Postoperative complications	Baseline (2012) n = 849		First evaluation (2013) n = 802		χ^2	p-value
Early complications	**n**	**%**	**n**	**%**		
Death	57	6.71	32	3.99	5.999	0.014 *
Venous thromboembolism	28	3.30	12	1.50	5.663	0.017 *
Late complications						
Revision procedures	15	1.77	24	2.99	2.687	0.101
Infections	9	1.06	9	1.12	0.015	0.903
Dislocation	13	1.53	4	0.50	3.360	0.067
Periprosthetic fracture	13	1.53	11	1.37	0.073	0.786
Total of complications	**135**	**15.9**	**92**	**11.47**	**6.456**	**0.011**
B. Postoperative complications	**Baseline (2012) n = 849**		**First evaluation (2014) n = 834**		χ^2	**p-value**
Early complications	**n**	**%**	**n**	**%**		
Death	57	6.71	27	3.24	10.722	0.001 ***
Venous thromboembolism	28	3.30	11	1.32	7.280	0.006 **
Late complications						
Revision procedures	15	1.77	21	2.52	1.134	0.286
Infections	9	1.06	5	0.60	1.082	0.298
Dislocation	13	1.53	2	0.24	7.943	0.004 *
Periprosthetic fracture	13	1.53	13	1.56	0.002	0.963
Total of complications	**135**	**15.9**	**79**	**9.47**	**15.092**	**0.001**

*THR – total hip replacement; * p < 0.05; **p < 0.01; ***p < 0.001*

complication and the most frequent one within 90 days after surgery was death, which occurred in 57 of all cases (6.71 %). During the first evaluation period, 11.47 % of patients in second group had at least one of postoperative complications and the most common one within 90 days after surgery in this period was also death (32 cases; 3.99 %). At the second evaluation period complications were observed in 9.47 % of patients in the third group and the most common one within 90 days after surgery was again death, but that number was significantly lower than in the previous two groups (27 cases; 3.24 %). The incidence of other postoperative complications observed in all three groups are presented in Table 3.

For each of the early observed complications there were comparative differences in terms of their reduction, from baseline to the first year of observation, but the statistically significant differences were present only for two major complications such as death and venous thromboembolism ($p < 0.05$). However, comparing the impact of CPs on post-operative complications after two years of its implementation, the difference was even more significant, particularly in complications such as: death ($p < 0.001$), venous thromboembolism ($p < 0.01$) and dislocation ($p < 0.05$), (Table 3).

Concerning the late postoperative complications there was no significant improvement after the first and second evaluation periods, although the number of revision procedures even increased, but not significantly (Table 3).

Discussion

The present study evaluated the role of CPs on quality of care for patients undergoing the THR surgical procedure. The implementation of CPs significantly decreased the LOS by 22.9 %. Although the LOS in this study has been decreased, it is still longer than in other hospitals as it was confirmed in similar studies, mainly from USA and Europe.[5, 7, 10, 11, 15] It is well known that CPs contribute to reducing the LOS in general, the same result in terms of the existence of a positive relationship between CPs and reduced hospital stay was also observed for patients with THR surgery.[4, 5] Beside the improvement of process indicators, the reduced number of postoperative complications is the most important finding of this study confirming that the full implementation of the CPs significantly improved the healthcare quality in the hospital settings. Postoperative physical rehabilitation is a very important component in

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the postoperative recovery of patients after THR. The CP in this study includes early rehabilitation which also contributes to faster discharge from the hospital, followed by a process of further rehabilitation at a rehabilitation hospital or at a community-based rehabilitation (CBR) centre. Timely discharge from the hospital and subsequent referral to rehabilitation centres is another value of clinical pathways because they represent a good logistical support throughout the process.

Indicators of treatment outcomes were significantly improved after the introduction of CPs. Most importantly, the mortality significantly decreased from 6.71 % at the baseline evaluation to 3.24 % at the second evaluation. Similarly, the frequency of another two important postoperative complications, venous thromboembolism and dislocation, also decreased due to the implementation of CPs. However, this positive effect can simultaneously lead to some negative effects such as long-term complications. The ultimate goal of CPs is to optimise the quality and certainly not only to decrease the length of stay. For example, Mauheran et al. found that implementation of a CPs decreased the length of hospital stay, but at the same time it increased the rate of dislocations following THR surgery, underscoring the need to consider the long-term effects of CPs.[16]

A systematic review done by Mufarrih et al showed that there is a positive effect of hospital volume on outcomes following THR, that is, "high-volume" hospitals had superior outcomes compared with "low-volume" hospitals (LOS, early and late postoperative complications).[17] Establishing a system for measurement and monitoring the process of providing health care, as well as the system for their improvement and in accordance with the defined Deming principles of quality, it is possible to achieve improvements in the "low volume" hospitals. In other words, implementing the CPs taken from the experiences of "high volume" hospitals can realise the benefit for the wider population of patients.[18] This study showed that the implementation of CPs can reduce the unwanted deviations and process variations for complex interventions such as THR allowing "low-volume" hospitals to achieve better quality of care and help "high-volume" hospitals to improve their quality.

The present study has some limitations. First, the patient sample is large enough, but the baseline measurement occurred a year before the CPs were implemented. Therefore, it cannot be excluded that some of the observed improvements represent a natural drift toward higher performance. A different study design, such as a randomised controlled trial, could improve the strength of these findings. Another limitation of the study is the lack of measurement of patient satisfaction. The combination of outcome indicators with patient satisfaction could provide more accurate information about the quality of care which is achieved by implementing CPs.

In addition, the introduction of CPs does not necessarily have a positive effect on all patient-related outcomes. In contrast, cost-effectiveness from the point of view of health care providers may even be extremely reduced, as indicated by the research of Krummenauer et al[19] and Cochrane systematic review and meta-analysis by Rotter et al.[5] This study did not include the cost implications of the implementation of CPs, which would certainly be desirable for future research in the field of clinical pathways.

Conclusion

The introduction of CPs in hospitals in the Republic of Srpska significantly reduced the LOS in hospital and also reduced the postoperative complications in patients after THR. Significant improvements in different groups of patients suggest that the implementation of the CPs does have an impact on the quality of care.

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None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Influence of Vitreal Reflux on Intraocular Pressure After Intravitreal Application of Bevacizumab

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Abstract

Background/Aim: Intravitreal drug injection cause an increase in intraocular pressure (IOP). The increase in IOP is directly proportional to the volume of drug injected into the eye and inversely to the vitreal reflux. The mixture of fluid, presents as a subconjunctival bleb, composed of vitreous and injected drug is called vitreal reflux. In this study changes in IOP after intravitreal injection of bevacizumab in relation to the vitreous regurgitation were observed.
Methods: This prospective study involved 50 patients (57 injections). Bevacizumab was administered intravitreally at a dose of 1.25 mg/0.05 mL. According to the type of disease, patients with diabetic macular oedema, proliferative diabetic retinopathy, age-related macular degeneration, retinal vein occlusion, choroidal neovascularisation and central serous chorioretinopathy were included. They were divided into two groups – the first one without vitreal reflux and the second one with vitreal reflux. IOP values were measured at baseline, right after drug application and 20 min thereafter.
Results: The reference value range of IOP values were 10-21 mmHg and so were the average baseline values in both experimental groups of patients (15.81 ± 3.50 mmHg). In the experimental group without reflux post application mean values of IOP at 0 and 20 min were 50 ± 9.65 and 18.54 ± 5.06 mmHg, respectively. In the experimental group with reflux post application mean IOP values after 0 and 20 min were 36 ± 8.68 mmHg and 18.91 ± 4.82 mmHg, respectively.
Conclusions: Following intravitreal bevacizumab application, a significant increase in IOP occurs. After 20 minutes the IOP values spontaneously decreased below 25 mmHg in both groups and there was no significant difference in comparison with the baseline values. Vitreus reflux is a major factor in short-term post-injection IOP elevation, but not from the longer-term perspective.

Key words: intraocular pressure, intravitreal injection, anti-VEGF, bevacizumab, vitreal reflux.

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Introduction

Intravitreal drug delivery is a very efficient method for treatment of many different ophthalmic diseases, including diabetic retinopathy, diabetic maculopathy, senile macular degeneration or any other macular disease that may benefit from anti-vascular endothelial growth factors (anti-VEGF) therapy.

The volume of the drug and vitreal reflux directly affects the increase in intraocular pressure and indirectly decrease the circulation in nerve fibres. Some animal experiments show that the acute rise in IOP blocks axonal nerve transport and damages the optic nerve in direct proportion to IOP rise.[1] Besides, acute increase in IOP causes

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decreased retinal circulation around the papilla and within the papilla itself, in proportion to the size of the pressure.[2] There are a lot of reports about levels of IOP that are safe after intravitreal anti-VEGF injection.[3–7] Vitreal reflux was the major factor that impacts short term post injection IOP rise.[8] Studies of ranibizumab (Lucentis®, Novartis/Genentech Inc, USA) showed at two-year follow-up that there were no long-term changes in IOP values.[9] This study also reported that at 60 min post-injection, less than 20 % of patients had an elevated IOP of 30 mmHg or higher. The report on escalating doses of ranibizumab showed no changes in IOP in the follow-up period of at least 4 months.[10] Bevacizumab (Avastin®, Roche/Genentech Inc, USA) is an off-label drug for intraocular treatment of many retinal diseases. Although bevacizumab causes an increase in IOP it never causes complete occlusion of the central retinal artery.[5]

The aim of the present clinical trial was to observe changes in intraocular pressure after intravitreal application of anti-VEGF drug related to the presence or absence of the vitreal reflux.

Methods

This was a prospective clinical study in which patients received an intravitreal drug of anti-VEGF at the Eye Clinic "Svjetlost" Banja Luka, the Republic of Srpska, Bosnia and Herzegovina, from March to June 2019. Patients were asked to participate in this study if they were over 18 years of age. This clinical study was approved by the Ethics Committee of the Eye Clinic "Svjetlost" (Decision No 04/2019). Exclusion criteria were: patients with active uveitis, patients who have received intravitreal or subtenon triamcinolone in the last 6 months and patients diagnosed with any type of glaucoma.

Every patient was subjected to the following tests before entering the operating room for intravitreal drug injection: best corrected visual acuity (BCVA), slit lamp examination and IOP examination.

Intravitreal drugs application was performed in the operating room by using aseptic techniques. Topical anaesthesia in the form of tetracaine was applied, then the eye was sterilised with povidone iodine (5 %) and a lid speculum was inserted. Intravitreal injection of bevacizumab 0.05 mL was instilled with a 30-gauge needle through the *pars plana* area, inferotemporal 3.5-4.0 mm of limbus. After the needle entered into the eye 1.0-1.5 cm, the drug was administrated. Studies have shown that the frequency of vitreal reflux does not depend on whether a 30 or 32 guage needle was used.[11] A vitreal reflux is defined as any accumulation of subconjunctival fluid after intravitreal drug application. After injection, a sterile cotton swab was placed over the injection site. IOP was measured at baseline, immediately after drug injection and 20 min thereafter. The IOP was measured in the same position and by the same examiner with an Icare® PRO rebound tonometer model (Icare, Tiolat Oy, Helsinki, Finland).

The baseline values and individual values of IOP at each time-point were analysed using a paired t-test.

Results

Data from 57 eyes of 50 patients were analysed. Mean patient age was 68 ± 8. According to the prevalence of retinal diseases: 18 eyes (31.5 %) with diabetic macular oedema, 15 eyes (26.31 %) with proliferative diabetic retinopathy-vitreous haemorrhage, 15 eyes (26.31 %) with exudative age-related macular degeneration, 6 eyes (10.52 %) with retinal vein occlusion-related macular oedema, 1 eye (1.75 %) with chronic central serous chorioretinopathy and 2 eyes (3.50 %) with idiopathic choroidal neovascularisation. Intravitreal injection of bevacizumab 0.05 mL was performed. Post-injection vitreous regurgitation was confirmed in 33 (57.9 %) of 57 eyes (Table 1).

Table 1: Incidence of disease and vitreal reflux after intravitreal injection of bevacizumab

Disease	Number of eyes (%)	Number of eyes with reflux	Number of eyes without reflux
Diabetic macular oedema	18 (31.5)	10	8
Proliferative diabetic retinopathy	15 (26.31)	9	6
Age-related macular degeneration	15 (26.31)	7	8
Retinal vein occlusion	6 (10.52)	5	1
Choroidal neovascularisation	2 (3.50)	1	1
Central serous chorioretinopathy	1 (1.75)	1	0
Total	**57 (100.00)**	**33**	**24**

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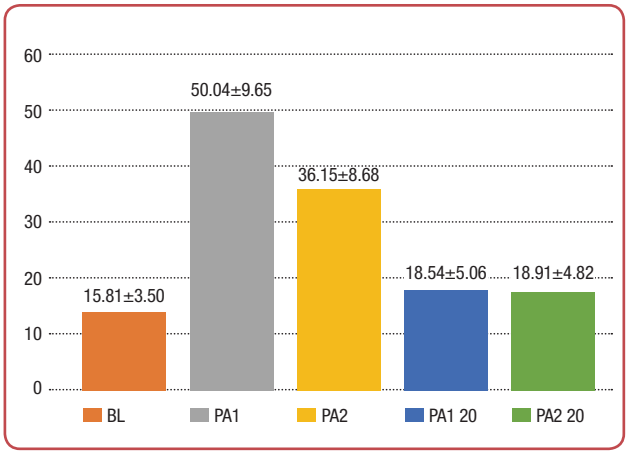

Figure 1: Intarocular pressure (IOP) before and after intravitreal application of bevacizumab

BL - baseline value before application; PA1 - post application value in the group 1; PA2 - post application value in the group 2; PA1 20 – 20 minutes post application value in the group 1; PA2 20 – 20 minutes post application value in the group 2

Baseline IOP values did not deviate from normal values which are within the range of 10 - 21 mmHg. The mean pre-application value of IOP was 15.81 ± 3.50 mmHg. The mean value of IOP immediately after application was 50.04 ± 9.65 mmHg in the group 1 (group without vitreal reflux) and 36.15 ± 8.68 mmHg in the group 2 (group with vitreal reflux) immediately after the injection. The IOP value immediately after intravitreal drug injection was significantly higher than the pre-injection IOP value ($p < 0.001$); there was also a significant difference in post-application IOP between the reflux and non-reflux groups. After 20 min there was no significant difference between the both groups, as well as no significant clinical difference from the pre-application IOP values (18.54 ± 5.06 mmHg in the group 1 and 18.91 ± 4.82 mmHg in the group 2) (Figure 1).

Discussion

Intravitreal drug injection of anti-VEGF is a common and widespread way of treatment of various ophthalmic diseases. The mean difference between IOP value before and immediately after drug application was 32.64 ± 17.40 mm Hg in the group 1 and 26.21 ± 9.50 mmHg in the group 2. The IOP changes that occur after intravitreal anti-VEGF drug application are usually transient and the values return to the almost normal values after a short period (< 25–30 mm Hg within 20 min), irrespective of whether there was a reflux or not. A prolonged elevation of IOP was not found. Some researchers report that there is a risk of increasing IOP in patients who received more than 29 injections, compared with those who received less than 12 injections.[12]

The results obtained in the present study are consistent with those of similar studies.[5, 6] In these studies, IOP values were monitored 30 min after drug application and in the following days. Other studies have also shown that a transient IOP spike occurs, and that additional IOP-lowering interventions such as paracentesis are not required.[4–9]

The results indicate that the presence or absence of vitreal reflux does not participate in the ultimate value of IOP, which in both cases is within the allowed value range. Tamponade with a cotton swab after intravitreal drug injection does not always cause a complete blockage of reflux. Even when cotton swabs were used for tamponade, there was sometimes a vitreous reflux, while in other cases, without a tamponade, there was no vitreous reflux. Irrespective of whether the tamponade was used or not and no matter whether there was a vitreous reflux or not, the ultimate IOP values are similar, with no significant difference between them after 20 min.

This study has its limitations in the form of a short follow-up time and a small number of intravitral injections.

Conclusion

Intravitreal drug injection causes a significant increase in IOP. Increased IOP values are spontaneously lowered to the reference values without leading to a central retinal artery occlusion. This outcome was found irrespective of the absence or presence of the post-injection vitreal reflux.

Acknowledgements

None.

Conflict of interest

None.

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ORIGINAL ARTICLE

Surgical Treatment of Colorectal Cancer Metastases

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Abstract

Background/Aim: Colorectal metastatic liver tumours are the most common secondary liver tumours. During the life of patients with colorectal tumorous, this liver metastases will develop either synchronously or metachronously in half of the patients. Approximately 25 % of patients with colorectal cancer diagnosis have secondary deposits in the liver and the additional 25 % of patients will develop metastases within five years. The objective was to investigate whether anatomic resections of the liver present a method of choice in surgical treatment of colorectal liver metastases compared to metastasectomy surgery.
Methods: A total of 65 patients were divided into two groups. Patients in the first group underwent metastasectomies consisting in the removal of metastases and the surrounding liver parenchyma no more than 1 cm by Kelly clamp crushing technique or LigaSure vessel-sealing system. Patients in the second group were subjected to the anatomic resection of the liver where not only metastases were removed, but also the associated anatomical segment or section or half the liver, depending on the number and localisation of metastases.
Results: The mean values (± standard deviation) of the overall survival for the first and the second group were 36 ± 4.8 months and 36 ± 2.6 months, respectively. The mean values (± standard deviation) of the disease-free survival in the first and in the second group were 18 ± 2.22 months and 22 ± 0.74 months, respectively. None of the found inter-group differences were statistically significant.
Conclusion: It can be concluded that metastatic surgery for colorectal liver metastases and anatomic resections have almost the same results and are irreplaceable methods in the treatment of colorectal liver metastases.

Key words: colorectal cancer, liver, metastasis, metastasectomy, anatomical resection.

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Introduction

Colorectal cancer is the most common gastrointestinal malignant tumour that gives rise to metastatic liver tumours.[1] Approximately 25 % of patients with colorectal cancer at time of diagnosis have presence of secondary liver metastases and in 25 % of patients metastases are generated within the next five years.[2] The first liver resection for colorectal liver metastases was performed by Catell in 1940. By improvement of surgical techniques, by introducing of new cytostatic drugs and by reducing the resection margin to less than 1 cm, the application of interventional radiology, portal venous embolisation allowed more patients in the unresectable group to move to the resectable tumour group. The goal of treatment is surgical removal of all metastatic tumours in the liver, because, based on the experience, 25-60 % of the operated patients survive for five years.[2] Seventy percent of the conservatively treated patients with metastases in the liver do not survive even a year.[3]

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Metastasectomies belong to the group of nonanatomic resection surgeries where the excision of the hepatic parenchyma within 0.5-1 cm of the metastases is performed according to Kelly clamp crushing technique[4] or LigaSure vessel-sealing system.[5] Anatomical liver resections are based on the principles of segmental and sector anatomy of the liver. The extent of anatomical surgery goes from segmentecomies, bisegmentectomies, sectionectomies and hemihepatectomies.

The aim of this study was to investigate whether anatomic resection of the liver is the method of choice in surgical treatment of colorectal liver metastases compared to metastasectomy surgery.

Methods

The survey was conducted at the University Clinical Centre of Banja Luka. The study covered the period from January 2007 to January 2014. The study was conducted in 70 patients divided into two groups (group A and B). Criteria for inclusion in the study were patients who were technically able to be operated by either a surgical method of metastasectomy or by a method of anatomical resections. Metastatic disease had to be localised only in the liver.

The first group of patients N = 35 (group A) underwent surgical metastasectomies of the tumour and the second group of N = 30 (group B) underwent anatomical resection of the tumour. In group A metastasectomy consisted in removing metastases and surrounding liver parenchyma no more than 1 cm from the metastasis by the Kelly clamp crushing technique or the LigaSure vessel sealing system. The patients in group B underwent the anatomical resection of liver metastases that consisted of the removal of metastases in the corresponding anatomical segment or section of half of a liver depending on the number and position of metastases.

The following surgical approaches were used: medial, J incision and Makuch laparotomy. After opening, mobilisation of the liver was performed, followed by the intraoperative ultrasonography (IOUSA), which provided the precise tumour position. Liver resection was performed at reduced central venous pressure (CVP) of 0-5 cm H_2O) and with the patient being in the Trendelenburg position. Parenchymal transection begun with diathermy tag of the resection line; the section parenchyma was performed by LigaSure or Kelly clamp crushing technique. Biliovascular structures were secured with clips and vascular sutures.

Results

Patients in group A who underwent metastatectomy had mean overall survival based on Kaplan-Meier curves 36 ± 4.8 months and a one-year, two-year and three-year survival was 85.3 %, 68.3 % and 50 % (Figure 1).

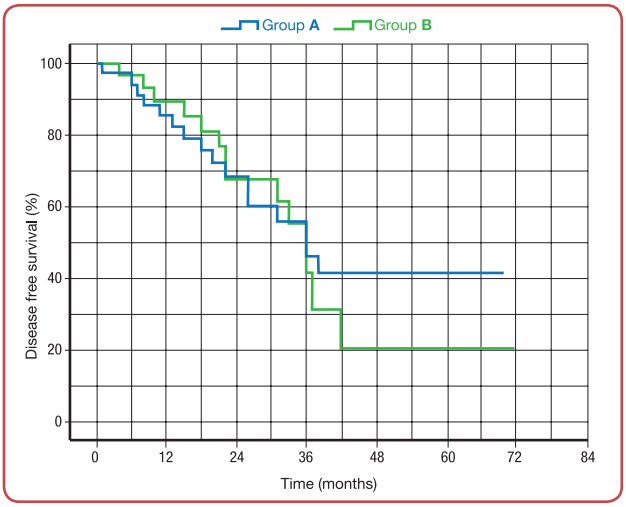

Figure 1. Mean overall survival in patients with colorectal carcinoma with liver metastases treated by metastasectomy (Group A) or with anatomical resection of the liver (Group B)

The mean overall of survival in patients of Group B who had anatomical hepatic surgery was 36 ± 2.6 months and the one-year, two-year and three-year survival was 89.3 %, 67.5 % and 50 %, respectively.

After the statistical comparisons of Kaplan Meier curves of overall survival by Mantel-Cox test (χ^2 = 0.167; p = 0.683), it can be concluded that there was no significant difference between the study groups A and B when it comes to overall survival (p > 0.05).

The mean value of disease-free survival in group A was 18 ± 2.22 months, with the one-year, two-year and three-year disease-free survival 64.7%, 29.1% and 29.1%, respectively.

In group B, the value if disease-free survival was

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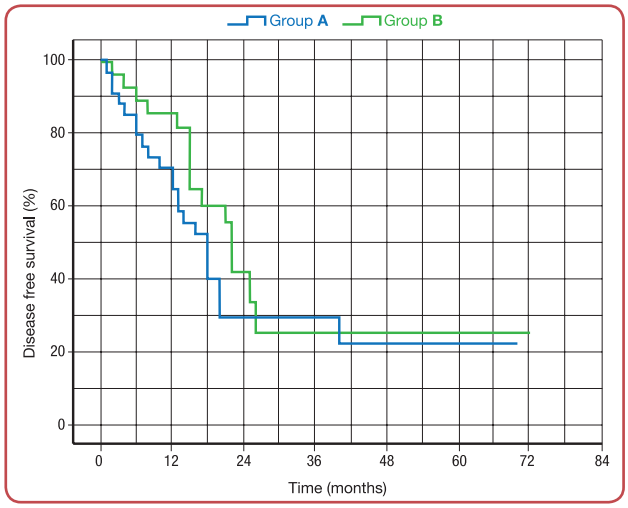

Figure 2. Mean disease-free survival in patients with colorectal carcinoma with liver metastases treated by metastasectomy (Group A) or with anatomical resection of the liver (Group B).

22 ± 0.74 months, with one-year, two-year and three-year disease-free survival percentage of 85.7 %, 41.6 % and 25 %, respectively (Figure 2).

After the statistical comparisons of Kaplan Meier disease-free survival curves performed with Mantel-Cox test ($\chi^2 = 1.357$; $p = 0.244$), it can be concluded that there was no statistically significant difference ($p > 0.05$) between the study groups A and B when it comes to disease-free survival.

Discussion

In a study published by Scheele and his associates, 902 patients with metastatic colorectal liver cancer without resection were monitored and only 21 patients or 2.3 % survived for three years, while there was no record of five-year survival.[3]

In the study published by Fong and his associates in 1,001 patients who underwent surgical removal of metastatic colorectal cancer in the liver five-year survival of 37 % and an average life expectancy of 42 months were found.[6] In this study, two basic surgical treatment methods of metastases in the liver were compared and the following results were found: mean survivals in anatomic and metastasectomic surgery were 39 and 46 months, respectively.6 While the one-, three- and five-year survival rates for anatomical surgery was 85 %, 53 % and 37 %, respectively,6 in the present study the results for the anatomical surgery were similar - 89.3 % after one-year and 50 % after three years.

According to the Fong et al study,[6] the data for metastasectomic surgery for survival after one, two and three years amounted 93 %, 75 % and 40 %, respectively, while in the present study the patients that underwent metastasectomy had one- and three-year survival rates of 85.6 % and 50 %, respectively.

DeMatteo and associates monitored 267 patients operated in the period from 1985 to 1998. Among 119 patients operated by metastasectomic surgery and 148 patients operated anatomically, mean overall survival was 53 months in the anatomical surgery versus 38 months for metastasectomic surgery.[7] In the present study that included 65 patients, ie 35 metastasectomic surgeries and 30 anatomical surgeries, overall survival was 36 months for both types of surgeries. When data for one-, two- and three-year survivals in the anatomical surgeries and in metastatesectomic surgery were 89 %, 67 %, 50 % and 85 %, 68 %, 50 %, respectively, without any significant difference.

Scheele in the study in 436 surgically treated patients for liver metastases of colorectal cancer states after five-year a disease-free interval was reached in 33.6 % patients.[3] In the present study that includes 65 patients, mean disease-free interval was 22 months for anatomical surgery and 18 months for metastasectomic surgeries.

These percentages of 5-year overal survival associated with careful selection and operating techniques are in accordance with the general trend of increasing the overall survival from early 5 % to 40-50 % nowadays.[8] One of the important factors affecting the overall survival in these patients is the type of the metastasectomy – the R0 type (excision of the tumour with a 1-cm-breadth of normal surrounding liver tissue), like the one in the present study - assures more favourable outcomes than the R1 or R2 type. In the clinical study by Allard et al[9] the 3- and 5-year overall survival in patients undergoing R0/R1 liver colorectal metastasectomy was 61 % and 39 % and in those ones that had R2 resection or no metastasectomy at all it was 29 % and 5 %, respectively.

Some surgeons proposed the so-called liver-first approach for treatment of the liver colorectal metastases, which means that the patient with colorectal cancer and liver metastases should first receive pre-operative chemotherapy, then have their liver metastases removed and finally

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undergo the colorectal surgery aimed at resection of the primary tumour. In a systematic review by Lam and co-workers[10] it was shown that the median overall survival in these patients was 40 (range 19-50) months, with a recurrence rate of 52 %. However, a meta-analysis by Kelly et al[11] that included 18 studies and 3,605 patients found no significant difference among the three compared techniques: colorectal-first, liver-first and the combined approach.

Additional factor that should be taken into account is the localisation of the primary colorectal cancer. It was noted that the right-sided colorectal cancer with liver metastases was associated with significantly shorter mean overall survival than the left-sided tumour (44.1 vs 55.3 months).[12] It seems however that one of the main predictive factors remains the tumour biology and presence of KRAS mutations[13] – the tumour cells from the right-sided colorectal tumours had significantly more mutated-type KRAS than the left-sided colorectal tumours (48.8 % vs 28.8 %, respectively).[12]

Conclusion

It can be concluded that metastatic surgery for colorectal liver metastases and anatomic resections have almost the same results and are both irreplaceable methods in the treatment of colorectal liver metastases.

Acknowledgements

None.

Conflict of interest

None.

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REVIEW ARTICLE

Current Status and Future Perspective of Coronavirus Disease 2019: a Review

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Abstract

A novel infectious disease named coronavirus disease-2019 (COVID-19) first emerged in Wuhan, China, in December 2019 and rapidly spread worldwide. In March 2020 the World Health Organization declared a global pandemic which is the worst global public health crisis in over 100 years. On 14 June 2020, almost eight million confirmed cases of COVID-19 and more than 430,000 deaths globally were reported. Over 200 countries have been affected so far. COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In the absence of specific therapy and vaccine, effective infection control intervention, particularly self-hygiene and social distancing, is the only way to prevent the spread of SARS-CoV-2. The lessons learned from this pandemic will be useful for successful management of the second wave of COVID-19 which is expected in autumn or winter.

Key words: SARS-CoV-2, COVID-19, coronavirus, pandemic, world, epidemiology.

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Introduction

Human infections with zoonotic coronaviruses (CoVs) are emerging public health problems worldwide. In the last two decades, two major coronavirus outbreaks have occurred, the severe acute respiratory syndrome (SARS) in 2002,[1] and the Middle East respiratory syndrome (MERS) in 2012.[2]

The recent outbreak of novel coronavirus disease-2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan, China, in December 2019[3] and rapidly spread throughout the world. At the end of January 2020, the World Health Organization (WHO) declared the novel coronavirus epidemic a public health threat of international importance.[4] On 11 March 2020, the WHO declared the coronavirus outbreak a pandemic.[5]

Causative agent

COVID-19 is caused by the SARS-CoV-2, a novel betacoronavirus.

CoVs are RNA viruses that infect primarily birds and mammals. In humans, CoVs are mainly associated with upper respiratory tract infections, but can cause severe diseases. However, some CoVs can be transmitted from animals to humans causing outbreaks. The human SARS-CoV, aetiologic agent of SARS, originated from bats in China,[6] and MERS-CoV, etiologic agent of MERS, originated from camels or bats in the Middle East.[7]

Bats are most likely the natural reservoir of SARS-CoV-2, while the intermediate animal host between bets and humans is not yet known.[8] Also, it is not known whether this virus in a future will adapt fully to humans and circulate within them without an animal reservoir or intermediate host.[9] SARS-CoV-2 has similar charac-

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teristics with SARS-CoV. It is confirmed that both viruses use the same cell entry receptor – angiotensin-converting enzyme 2 (ACE2).[10] However, SARS-CoV-2 is more transmissible, but less pathogenic than SARS-CoV.

There is evidence that SARS-CoV can remain infectious on inanimate surfaces for several days and that surface disinfection with broad-spectrum disinfectants of proven antiviral activity (eg 70 % ethanol) significantly reduces its infectivity after short exposure time. Researchers expect a similar effect for the SARS-CoV-2.[11]

Epidemiology

Geographic distribution

At the time of writing (14 June 2020), the SARS-CoV-2 continues its global spread, with almost eight million confirmed cases and more than 430,000 deaths worldwide.[12]

Cumulative cases and deaths of COVID-19 across the world from 1 January to 13 June 2020, are presented in Figure 1.

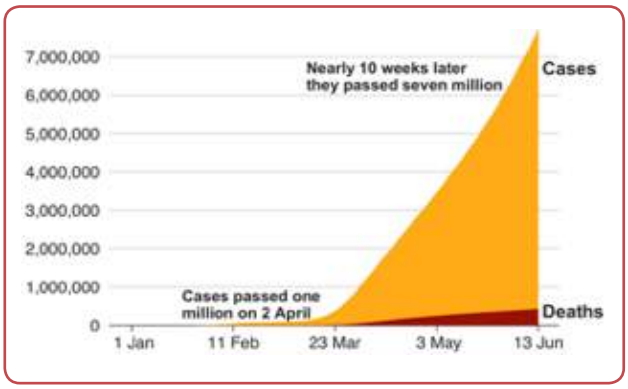

Figure 1: Global coronavirus cases and deaths
Source: Johns Hopkins University, National Health Agencies. BBC[12]

All continents, except Antarctica, are affected by SARS-CoV-2. Total confirmed COVID-19 cases and deaths across continents, on 14 June 2020, are presented in Table 1.

Until now, COVID-19 cases have been confirmed in almost all countries worldwide.[13]

Total confirmed COVID-19 cases and deaths in 20 most affected countries in the world are presented in Table 2.

Table 1: Total confirmed COVID-19 cases and deaths worldwide on 14 June 2020, by continent

Continent	Total cases	Total deaths
Asia	1,617,030	40,246
Africa	236,929	6,306
Europe	2,205,339	182,855
Oceania	8,931	124
North America	2,469,848	144,789
South America	1,402,112	59,604
Total	**7,940,189**	**433,924**

Source: Worldometer. COVID-19 Coronavirus pandemic.[13]

Many factors make international comparison difficult: different ways of recording COVID-19 cases and deaths, different population sizes, different percentages of the elderly in different countries, different density of populations, as well as different stages of a pandemic in any single country.

It is worth noting that the total number of people infected with SARS-CoV-2 is unknown since it depends on the number of people who have been tested.

Age and sex

People of all ages and both sexes can be affected by SARS CoV-2. Middle-aged adults and the elderly are most commonly affected, while children are rarely affected. The course of the disease is severe in the elderly.[14] Based on the recently reported data, COVID-19 affects men and women almost equally. However, compared to women, men have more severe disease and a higher risk of death, potentially due to sex-based immunological or gendered differences.[15-18]

Transmission

SARS-CoV-like viruses are sought to have been introduced into the human population from wildlife hosts. Since the first cases of the COVID-19 disease in Wuhan, China, were linked to direct exposure to seafood market that sold live animals, it was thought that the main mode of transmission was animal to human spread.[19] However, with the increase in the number of infected persons, it became clear that the main route of transmission is person-to-person spread, by direct contact and respiratory droplets.[20]

It is evident that SARS-CoV-2 is capable of person-to-person transmission with a secondary attack rate of 3 to 10 %.[8] It is worth noting that the expected number of secondary cases of COVID-19 may change as further information becomes avail-

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Table 2: Total confirmed COVID-19 cases and deaths worldwide on June 14, 2020, by country

Continent	Total cases	Total deaths	Total cases/ 1M popul.	Deaths/ 1M popul.	Total tests	Tests/ 1M population
USA	2,152,112	117,676	6,504	356	24,562,632	74,227
Brazil	852,785	42,837	4,013	202	1,499,041	7,055
Russia	528,964	6,948	3,625	48	14,880,172	101,967
India	332,901	9,520	241	7	5,658,614	4,102
UK	295,889	41,698	4,360	614	6,772,602	99,789
Spain	291,008	27,136	6,224	580	4,465,338	95,507
Italy	236,989	34,345	3,919	568	4,620,718	76,419
Peru	225,132	6,498	6,833	197	1,338,477	40,623
Germany	187,621	8,868	2,240	106	4,694,147	56,035
Iran	187,427	8,837	2,233	105	1,244,074	14,821
Turkey	178,239	4,807	2,114	57	2,632,171	31,226
Chile	174,293	3,323	9,121	174	840,150	43,968
France	156,813	29,398	2,403	450	1,384,633	21,215
Mexico	142,690	16,872	1,107	131	401,755	3,118
Pakistan	139,230	2,632	631	12	868,565	3,936
Saudi Arabia	127,541	972	3,666	28	1,106,398	31,806
Canada	98,735	8,146	2,617	216	2,113,924	56,033
Bangladesh	87,520	1,171	532	7	504,465	3,065
China	83,132	4,634	58	3		
Qatar	79,602	73	28,350	26	290,714	103,538

M: million Source: Worldometer. COVID-19 Coronavirus pandemic.[13]

able.[21] Close contact between persons is necessary. The spread of virus occurs primarily where there is close contact – in the family, hospitals[22] and long-term care facilities.[23] Reported clusters of cases after social or work gatherings also suggest the risk of transmission through close contact.

Symptomatic patients are the most important source of SARS-CoV-2 spread. Transmission from infected persons during the incubation period has been documented.[24] Although one study reported that coronavirus may have been transmitted by the asymptomatic carrier,[25] the relative contribution of asymptomatic carriage or transmission to the overall disease burden remains unclear.[21] Since the SARS-CoV-2 is detected in newborns, they might play a role in transmission.[26]

Contaminated surfaces may be a source of infection if susceptible persons touch these surfaces and then transfer the virus to eyes, nose or mouth. The frequency and relative importance of this type of transmission remain unclear.[14] The risk of transmission by contaminated paper (eg paper documents and paper money) is low.[27]

Till now, airborne spread, faecal-oral transmission, and bloodborne transmission have not been documented.[28]

The period of communicability is not yet completely understood.[29] It appears that SARS-CoV-2 can be transmitted before the development of symptoms and throughout the course of illness. Some studies have suggested transmission as early as five days before symptom onset. Latest time of communicability is unknown at this time.[30]

Initial estimates suggested that the basic reproductive number – Ro (the mean number of newly infected from an infected person in a susceptible population) for the SARS-CoV-2 was 2.7.[31] However, Sanche et al.[32] calculated much higher Ro (5.7) in the same population. Besides, some evidence indicates that several COVID-19 patients were recognised as "super-spreaders" because of the high number of persons secondarily infected. SARS-CoV-2 is more transmissible than severe influenza pandemic viruses in the past.[33]

Immunity and risk of reinfection

Antibodies to the SARS-CoV-2 are induced in people who have become infected. However, it is unknown how long any protective effect will last. Till now, there is no evidence that people who have recovered from COVID-19 are protected from reinfection.[14] It is also not known yet whether mild or asymptomatic cases will develop antibodies.

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Clinical features

Incubation period

The incubation period for COVID-19 is estimated to be between 2 and 14 days (median 5–6 days), with isolated reports of a longer period.[14] Some persons can be contagious during incubation. Therefore, transmission from a pre-symptomatic case can occur before the symptom onset.[29]

Clinical manifestation

Early signs and symptoms of COVID-19 are consistent with influenza-like illness, with a spectrum of disease ranging from milder presentation to severe respiratory illness. Pneumonia is the most frequent serious manifestation of infection. Most patients experience fever, cough, fatigue, anorexia, shortness of breath, and myalgia. Other symptoms, like sore throat, nasal congestion, headache, diarrhoea, nausea, vomiting and smell or taste disorders can also be present.[22, 34]

A serious complication in critically ill patients is acute respiratory distress syndrome (ARDS) which can occur suddenly. Other complications include arrhythmia, shock, acute cardiac injury and multiple organ dysfunction syndrome (MODS).[22, 34] Most patients who required hospitalisation were older persons with underlining comorbidities. About 20 % of hospitalised patients required admission to the intensive care unit for critical management. Cases can become severe quickly, progressing to ARDS or MODS, leading to death.[35-37]

Symptomatic infections in children are rare and usually mild.[26] Asymptomatic infections are common, but the precise frequency is unknown.[14]

Risk factors for severe illness

Based on the literature review, elderly, people with underlying diseases (cardiovascular diseases, diabetes, hypertension, chronic lung disease, cancer, chronic kidney disease, severe obesity, etc) and those immunocompromised are at higher risk for severe form of COVID-19[38, 39] and have a higher risk of dying from this disease.[40] Healthcare workers and patients in the hospital setting have a higher risk of becoming infected with SARS-CoV-2 than people in the community.[41]

Laboratory and imaging findings

Laboratory findings in patients with COVID-19 include lymphopenia and elevated levels of aminotransaminases, dehydrogenase and markers of inflammation such as C-reactive protein and erythrocyte sedimentation rate.[34]

Pneumonia caused by SARS-CoV-2 manifests with a specific chest computed tomography imaging, even in asymptomatic patients.[42]

Case fatality rates and recovery

Case fatality rates from COVID-19 vary widely between countries from 0.2 % in Germany to 7.7 % in Italy.[43] The overall case fatality rate is 2.3 %.[14] Recovery time for mild infections is two weeks, while for a severe disease it lasts three to six weeks.[44]

Diagnosis

Diagnosis of COVID-19 is based on laboratory confirmation of SARS-CoV-2, specific clinical and imaging criteria compatible with COVID-19 and epidemiological criteria.

According to the latest case definition for COVID-19, as of 29 May 2020:

- Confirmed case of COVID-19 is any person meeting the laboratory criteria (detection of SARS-CoV-2 RNA in a clinical specimen);
- Probable case of COVID-19 is any person meeting the clinical criteria (at least one of the following symptoms: cough, fever, shortness of breath, sudden onset of anosmia, ageusia or dysgeusia) and epidemiological criteria (close contact with a confirmed COVID-19 case in the 14 days prior the onset of symptoms) or any person meeting the diagnostic imaging criteria (radiological evidence of lesions compatible with COVID-19); and
- Possible case of COVID-19 is any person meeting the clinical criteria mentioned above.[45]

Diagnostic tests for SARS-CoV-2

Two types of diagnostic tests for SARS-CoV-2 infections are available: molecular tests to detect viral RNA and serological tests to detect IgG and IgM antibodies.

Molecular tests

The main technique for diagnosis of current infection with SARS-CoV-2 is viral RNA detection by reverse transcription-polymerase chain reaction (RT-PCR) during the first or second week

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of disease. The WHO recommends collection of nasopharyngeal or oropharyngeal specimens for testing and, if clinical symptoms and signs remain and tests are negative, collection of specimens from the lower respiratory tract (sputum or bronchoalveolar lavage in a ventilated patient) when available.[46, 47]

The test reliability depends on the type of collected specimens, sampling techniques, extraction and detection of viral RNA, and the quality of diagnostic reagents. Due to the low sensitivity of real-time RT-PCR assay, the persistence of clinical symptoms and signs where tests are negative requires the repetition of testing.[48]

Serological tests

Serological tests detect antibodies to the SARS-CoV-2 in blood. They are particularly useful for monitoring and responding to COVID-19 pandemic through detection of asymptomatic or mild cases of infection in the population.[49]

The earliest and most sensitive serological marker is level of total antibodies. IgM and IgG antibodies may be positive early after the symptom onset, with higher levels occurring in the second and third week of disease. After that IgM antibodies gradually decline, while IgG antibodies start to increase and may persist over seven weeks.[50]

It is not known yet whether antibodies to SARS-CoV-2 can protect against reinfection and how long that protection might last. Scientists worldwide are doing studies to answer those questions.[51]

Rapid immunodiagnostic tests can only indicate the presence or absence of SARS-CoV-2 antibodies. However, they need further validation to determine their accuracy and reliability. In addition, antibodies to SARS-CoV-2 may have cross-reactivity with antibodies to other CoVs.[52]

Prevention and control

Global public health measures

The WHO advises all countries to take action to detect COVID-19 infection and prevent spread, emphasizing following priorities for countries: protection of health workers and those at highest risk of severe disease (eg elderly and people with comorbidities) and supporting vulnerable countries in containing infection.[44]

The WHO recommends screening for international travellers from areas with ongoing outbreak of COVID-19 to identify particularly persons with fever and cough and those with high-risk exposure.[53]

Other public health measures including social distancing efforts and stay-at-home decrees, aggressive contact tracing ("test, test, test...") and quarantine, restricting and policies on face masks or coverings in public, have been variably employed in different countries. Despite affording only limited evidence, all studies found quarantine to be important for controlling the spread of severe coronavirus disease. Decision makers worldwide should monitor the outbreak situation all the time and the impact of the measures they implement.[54]

Preventing exposure in the community

Currently, the best prevention is to avoid being exposed to the SARS-CoV-2. People should be suggested to practice social (spatial) distancing[55] by staying at home and maintaining two metres distance from others in the community. In particular, individuals should avoid crowds and close contact with potentially ill people.

The measures that are recommended to reduce transmission of virus are:

- Proper hand washing, especially after touching surfaces in public places or use of alcohol-based hand sanitiser when hand washing is not available.
- Respiratory hygiene (covering the cough or sneeze with disposable tissues, and hand washing).
- Avoiding touching the eyes, nose, and mouth.
- Cleaning and disinfecting surfaces that are touched often (at home, workplace, school).

The WHO does not recommend wearing a medical mask in the community for those without respiratory symptoms.[56] Recommendations on use of masks by healthy people in the community vary by country.[57]

Management of asymptomatic persons with potential exposure

For people returning from international travel and those who have had close contact with a patient with suspected or confirmed COVID-19, the following measures are suggested:[58, 59]

- Self-quarantine at home for 14 days following the last exposure;
- Avoiding contact with persons at high risk for

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severe illness (eg the elderly and those with serious medical conditions);

• Temperature checks and monitoring for symptoms such as fever, cough, or dyspnoea. If individuals develop such symptoms, they should continue to stay at home isolated from other household members and contact their physicians.

Some institutions are testing asymptomatic persons after an exposure. However, a negative test does not rule out subsequent development of infection with SARS-CoV-2 and therefore infection control precautions should be continued for the duration of the incubation period, despite a negative result.

Management of the immediate environment

There is evidence that SARS-CoV-2 may remain viable for hours to several days on different surfaces. Frequent touching of contaminated surfaces in public areas is therefore a potential route of SARS-CoV-2 transmission. Cleaning of visibly dirty surfaces with water and detergents followed by disinfection with 0.1 % sodium hypochlorite, or 62–71 % ethyl alcohol or 0.5 % hydrogen peroxide, reduces the virus infectivity.[11]

Infection control in the healthcare setting

Infection control measures to reduce spread of COVID-19 in a healthcare setting are warranted. They include universal source control, early identification and immediate isolation of patients with suspected disease, use of personal protective equipment (eg N95 or other respirators or FFP3 masks, goggles or a face shield, gloves and gowns) and environmental disinfection.[60]

Healthcare workers should monitor themselves for symptoms of COVID-19 and stay home if they are ill; wear a medical mask or respirator while in the hospital setting and performed hand washing immediately after any contact with the face covering.

Patients should be screened for clinical manifestations consistent with COVID-19 before entry into a hospital. In some institutions, when N95 respirators are not available for routine use, patients with COVID-19 are asked to wear a medical mask during face-to-face contact with healthcare workers, even when they are in their room, to lower their risk of transmitting infection. When this is done, healthcare workers must still use appropriate personal protective equipment, as described above. In patients with COVID-19, aerosol-generating procedures should be avoided when possible to reduce the potential risk of transmission to health care workers.[60]

Herd immunity

In addition to public health measures mentioned above, development of herd immunity could protect against COVID-19. However, data on the immune response to COVID-19 are lacking.[61] It is still unknown how much of the population has been infected. Results from a few serological studies suggest that a relatively small part of the population has been infected (5 % of the Spain's population and 4.4 % of the French population). A household survey conducted in England found that only 0.3 % of the population is currently infected with the virus.[62] Given the transmissibility of SARS-CoV-2, around 65 % of the population might need to be immune to reach a threshold of herd immunity to end the pandemic[63] and this will take time. We also do not know how long the post immunity lasts – a few months or several years.

Vaccines – what do we know so far?

According to WHO, 83 potential COVID-19 candidate vaccines are being assessed, of whom seven have been approved for human testing in clinical trials.[64] Three approved candidate vaccines are being tested in China. Other vaccine candidates are testing in the UK (one), in the USA (two), and in Germany (one that has been approved but is yet to start).[64] Although clinical trials have begun, the medical community and the public will have to wait. The proposed time frame for the development of an effective vaccine is at least 12 to 18 months.

Post-exposure prophylaxis

Based on experiences with post-exposure prophylaxis (PEP) with antimicrobial drugs of other infections, clinical trials are also being conducted to evaluate the safety and efficacy of PEP against COVID-19.[65]

Management of patients

Patients with mild COVID-19 disease must be isolated at a designated COVID-19 health or community facility or at home (self-isolation). WHO recommends symptomatic treatment and monitoring for clinical worsening, which should prompt hospitalisation.[46] Patients with suspected or confirmed moderate COVID-19 (pneumonia) should be isolated in hospitals.[46]

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Management of patients with ARDS depends on its severity. In patients with COVID-19 with mild ARDS non-invasive ventilation may be used. Patients with severe ARDS need intubation and invasive mechanical ventilation.[46] In case the disease results in MODS, therapy to support the functions of the organs is required.[43]

Clinical care of suspected patients must focus on early recognition, prompt separation and implementation of necessary prevention and control measures.[66, 67]

There is no approved specific treatment for COVID-19.[68, 69] The recommendations for using antiviral drugs to treat COVID-19 have been recently revised by the US FDA. Remdesivir is recommended for use only in hospitalised adults with severe disease, while chloroquine and hydroxychloroquine, due to their potential for toxicity, are recommended only in clinical trials.[70]

Development and evaluation of drugs and their combination to treat COVID-19 will take several years.[71] The multinational trial to further evaluate remdesivir, chloroquine and lopinavir-ritonavir has already launched by the WHO.[14] Convalescent plasma, collected from donors who have recovered from COVID-19, might be useful in treatment of COVID-19 patients. However, randomised controlled trials are necessary to confirm this.[72]

Future perspectives

Scientists worldwide agree that the global COVID-19 pandemic could have several different endings. The first scenario may be COVID-19 spontaneously disappearing, as it happened with SARS in 2003. The second scenario is that SARS-CoV-2 may continue to sporadically appear over many years with the occasional outbreak as MERS has done. The third, most likely scenario, is that COVID-19, like the 1918 Spanish influenza, may take root in populations worldwide with Covid-19 outbreaks through the end of 2022.[73, 74] If an effective vaccine does not appear during that time, SARS-CoV-2 will continue to circulate with important activity in human population until at least half of the population has been infected.[73]

Conclusion

COVID-19 is a novel coronavirus disease that has been declared in March 2020 as a pandemic. It is rapidly spreading throughout the globe, infecting almost eight million people and killing more than 430,000. People of all ages and both sexes can be affected. The disease is severe in elderly and those with comorbidities. To date, vaccine and specific treatment are lacking. Currently, effective infection control intervention, particularly self-hygiene and social distancing, is the only way to prevent the spread of SARS-CoV-2.

When the pandemic wanes, it will be possible to assess its impact on the health, economy and social life. The lessons learned from this worst global public health crisis in the last 100 years, especially in terms of international public health, will be useful for successful management of the second wave of COVID-19 which is expected in autumn or winter or any future similar pandemics. Governments across the world will have to balance the needs of the economy and social life with suppressing the spread of the virus.

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Conflict of interest

None.

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HISTORY OF MEDICINE

Spanish Flu 1918-1919 – Aspects of Demographic Implications

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Abstract

The usual perception of the influenza pandemic that ravaged the world throughout 1918–19 (and in a much less manner in 1920) is structured into analyzing three different waves that touched almost the whole of humanity in quick succession from the spring of 1918 onwards. Results from pandemics regarding the statistical records of influenza morbidity and mortality are the focus of medical science for a long time. Although the evolution of estimations across recent decades almost always tended towards higher and higher estimates, has led to visions of over 100 million deaths throughout definitely the most deadly influenza epidemic, it is clear that such numbers should be taken with a huge dose of skepticism. The approach of this paper is centred on the methods used in determining demographic estimations, and especially evaluations, of historical developments in the situation where the influenza pandemic overlapped with World War I.

Key words: Spanish flu, pandemics, World War I, demographics, influenza.

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Introduction

In history, as a science in general, and in the history of medicine especially, the study of the historical medical aggregation or cluster known as Spanish Flu 1918-1919 has become the ultimate place for particularly inventive excursions and inspirational theories often during the last decades. The challenges that have emerged on the medical horizon in recent times have been an impacting inspiration on medical history to explain the mechanisms of the occurrence, spread, and consequences of that major pandemic now more than a century old. However, the situation in which Spanish Flu 1918-1919 is discussed in the midst of the current COVID-19 pandemic - the first pandemic in over 100 years that by its demographic implications can be compared to the events developed at the end of the World War I - certainly represents a completely new dimension of challenge. All aspects of comparative analysis are self-evident, and the possibilities for observing the mechanisms related to the pandemic phenomena of 1918-1919 are now incomparably more illustrative. Of course, the reverse is also true, within the current pandemic humanity is turning more than ever to the experiences gained during Spanish Flu, in order to find solutions from the then implemented measures for an adequate response to all unknowns that a new wave of pandemics instantly presents.

In this sense, it is especially interesting and illustrative to follow the mechanisms of the spread of Spanish Flu, its mortality and all the demographic consequences, because in this segment the lessons that can be learned are very useful. It is clear that such comparative analysis should be more precise only after the COVID-19 pandemic is over. That is the time when clear comparisons of the pandemic's reach can be made at the planetary level, especially in the context of demographic

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loss calculations. However, the current moment provides a much better insight into the reality of 1918 and 1919, especially in terms of comparing the existence of knowledge about the dangers that people encountered, or the misconceptions that resulted in the loss of precious time and, with it, many lives as well.

Ignorance and silence

In understanding the consequences caused by the events of 1918, one of the key dimensions is in having adequate knowledge of how the public and medicines of that time reacted to the appearance of a deadly epidemic, i.e. what did the doctors and researchers know about the disease that suddenly emerged: what and how could previous experiences help them. In this context, the very fact that in 1918, during the first encounter with the patients, some doctors still wondered if they were actually facing the outbreak of a new plague epidemic[1] speaks quite illustratively of the widespread ignorance regarding the elementary aspects of danger that loomed over humanity. Basically, medicine and medical practice in 1918 still did not have a clear idea of how to deal with bacteriological infections, and of course it did not have the slightest idea about viral epidemics. The crucial knowledge that existed in confronting the flu epidemic of 1918 came from the results of previous epidemics. The most recent and illustrative experience being the epidemic of 1889–1890, known as the "Asiatic flu" or "Russian flu", because it too resulted in many casualties (nearly a million worldwide).[2]

It was in the context of the analysis of this last great pandemic of the 19th century that the German doctor and bacteriologist Richard Pfeiffer (1858-1945) discovered the bacterium Haemophilus influenzae and developed the theory that this germ was the main cause of influenza.[3] His theory prevailed until the end of World War I and remained current during the great epidemic of 1918-1919. Only in the following years and decades did scientists come to realise that this theory was completely wrong. A major step in the discovery of the Influenza A virus in 1934 was in the development of the electron microscope in Germany, in 1931.[4]

However, the operational use of the electron microscope, its availability outside of Germany, as well as the circulation of knowledge, understanding and ideas about the Influenza virus took several years to emerge into theories and medical practice. The onset of World War II additionally slowed the process. It was not until the early 1950's that a complete system of knowledge about diagnostics and treatment of influenza became established as part of the science of medicines. The reality of the extent to which medicine and medical practice had little realization and no clear idea about the Influenza A virus subtype H1N1 in 1918 is very often completely suppressed in the context of later analysis of events during the Spanish flu period.


WEAR A MASK

AND SAVE YOUR LIFE!

The Emergency That Now Confronts Our City Is Beyond the Facilities of the Health Department

The RED CROSS

has come to the assistance of the Board of Health. Doctors and nurses can not be obtained to take care of the afflicted. You must wear a mask, not only to protect yourself but your children and your neighbor from influenza, pneumonia and death

A GAUZE MASK IS 99% PROOF AGAINST INFLUENZA

Doctors wear them. Those who do not wear them get sick. The man or woman or child who will not wear a mask now is a dangerous slacker.

WEAR MASKS

GOING TO WORK AT HOME GOING HOME AT HOME

This statement was authorized at a meeting of the undersigned, who are convinced that it is the only way to stamp out the epidemic. You must do your part

Alameda County Relief Committee

County of Alameda
City of Oakland
Board of Health of Oakland

Oakland Chapter American Red Cross
Oakland Clearing House Association
Oakland Chamber of Commerce

Associated Charities
Retail Dry Goods Association

Figure 1: Newspaper Ads, October 1918.
https://www.pinterest.ca/pin/444800900701088612/

The very high levels of morbidity and mortality of Spanish flu, already at initial stage, prompted some doctors to make comparisons with outbreaks of plagues from previous centuries. Those experiences gained during earlier confrontations with plague pandemics, especially in the context of isolation, by building sanitary cordons at borders and through quarantine stations,[5] were of uppermost importance in battling against the spread of the disease. Unfortunately, some of those measures, although successfully

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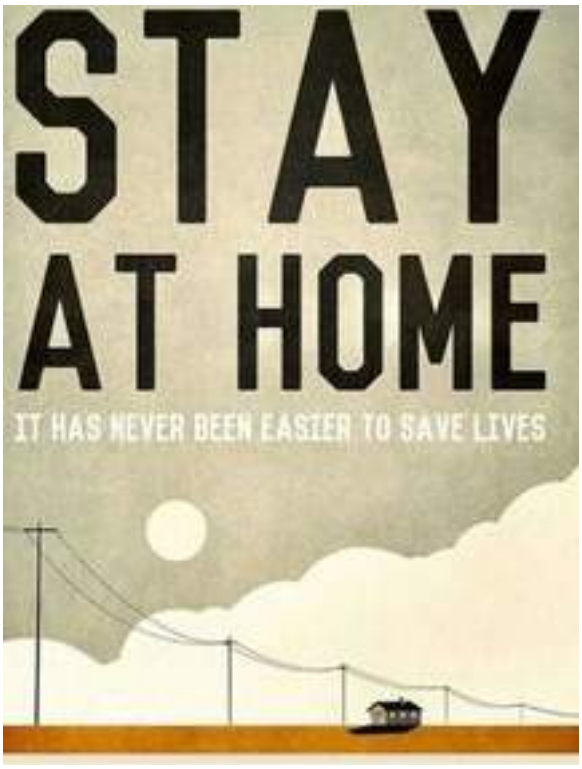

Figure 2: Influenza Poster
https://www.pinterest.ca/pin/508343876694359021/

Figure 3: Seattle police wearing masks in December 1918
https://en.wikipedia.org/wiki/Spanish_flu#/media/File:Spanish_flu_in_1918,_Police_officers_in_masks,_Seattle_Police_Department_detail,_from-_165-WW-269B-25-police-l_(cropped).jpg

implemented throughout the 18th century, were largely forgotten in 1918. The only means of protection during the crisis were face masks, but with limited use only in the United States (US) and some countries in Western Europe. Masks in use throughout 1918-1919 were exclusively double layered gauze masks, made of cotton.[6] In most countries and situations everywhere they were found to be of poor quality and often used in an inappropriate way, and in general were ineffective. It was not before the 1960's that surgical

Figure 4: Family wearing masks during the Spanish flu pandemic.
https://www.pinterest.ca/pin/591104325342816 5/

Figure 5: „Flu fashion“
https://www.pinterest.ca/pin/798263102686610385/

masks were manufactured and became available, and even then, only in western countries (Figures 1-6).[7]

All aspects about the lack of knowledge in situations of confronting the new and very lethal strain of influenza virus are the foundation for the same

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Figure 6: Street car conductor in Seattle not allowing passengers aboard without a mask. (1918)
https://designyoutrust.com/2009/04/influenza-pandemic-worlds-history-1918/#BML8dKyxgyjSomD9.

lack of adequate knowledge about the dynamics of the spread of the disease and its consequences, particularly in regard to the level of mortality. That was especially important in the first years and decade that followed the First World War and the Spanish flu. Throughout the 1920's and 1930's it was almost impossible to find studies on the biggest pandemics of the last several centuries. The effects of the Second World War added more into the story of suppressed memories and delay in adequate scientific reaction. In fact, it was not before the 1970's that Spanish flu began to gain ground within the history of medical science. Even then, results were poor in regard to quality and quantity.

Rising attention

The historiography of the Spanish flu only commenced in 1998, when the first international conference on the history of the pandemic under the title 'Reflections on the Spanish Flu Pandemic after 80 Years: Causes, Course & Consequence' was held in South Africa. Even at that event the subject attracted little attention beyond the group of 36 scholars who gathered in Cape Town to discuss it. However, the participants and the volume of conference papers which resulted from the meeting,[8] produced a survey of the historiography of the pandemic up to that time. The event contributed in identifying distinct ways in which Spanish flu had been conceived over previous decades, such as for example a significant episode of epidemiological history, or as a crisis in social and public health. A major inspiration for the conference came from the then newest scientific discoveries made by Jeffery Taubenberger within the field of viral archaeology, which was completely new.[9]

The efforts of Jeffery Taubenberger and his team were with the aim of a full restoration of the virus that caused the Spanish flu pandemics by the recovery of tissues from flu victims. This technique was made possible during the 1990's. Initial tests throughout 1996 and 1997 on specimens that originated from the corpses of American soldiers who died in army camps in South Carolina and Upton, New York, respectively, around the time that the second wave of pandemics peaked in September 1918, provided possibilities for the first encouraging results.[10] Further fieldwork, in the following years mainly on tissues dug up in permafrost in Alaska, where corpses could have been well preserved all the way back to 1918, gave a clear pattern towards amassing enough viral ribonucleic acid (RNA) segments to implement a complete sequencing of the influenza virus from 1918.[11] Another several years of genetic work in laboratories resulted in the publication of the complete genome sequence in 2005.[12] Without any doubt this was one of the crucial breakthroughs in the history of medicine during the last few decades.

At the same time as the breakthrough in the new field of viral archeology, the original group of scientists, who met at the international conference in Cape Town, gathered again. They provided further assurance regarding theories about the importance of pandemics, now with new and very significant levels of scientific attractivity. So, results made in the field of viral archeology suddenly led to shaking the foundations built around historical knowledge regarding some key events from human history, such as the outcome of First World War. Questions on the importance of Spanish flu pandemics, in the entire scope of the first

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global encounter in human history with them, came to the surface pretty fast.

The usual human inclination to greatness, meaning towards great numbers, brought about a series of new articles where estimations about numbers of victims connected with the pandemics of Spanish flu tended to increase constantly.[13] Earlier assumptions that the number of deaths should have been around 20 million, were quickly substituted with much bigger estimations, where total mortality was presented as somewhere between 50 and 100 million, or perhaps even more.[14] Estimations like that followed the trend of a gradual rise of global interest in the scope of pandemics, especially the possibilities for the emergence of new pandemics, capable to emulate or even surpass the dreadful numbers that Spanish flu posted early in 20th century.

The rise in this interest was actually quite complex. The reasons for such phenomenon at the end of the 20th and beginning of the 21st century have been very diverse. It is an undeniable fact that history as a science was in huge turmoil, especially with the rise in importance of social and economic history against the old dominance of political history. In addition, environmental history became more and more attractive and, in general, an inter-disciplinary approach in science gained momentum like never before. With the crushing of rigid disciplinary foundations and walls, other scientific disciplines were provided with possibilities of utilizing this experience and methodological approach in studying historical phenomena.

Next to that, real public pressure was apparent over a new pandemic threat, which was embodied in the increasing danger of AIDS throughout the 1980's and 1990's. Also fears of possible new epidemics of flu influenza were fueled by the emergence of the H5N1 strain influenza in Hong Kong, at the end of 1997. The same strain occurred several more times in the following years, especially in Asia. The problem of perspective related to new influenza pandemics was especially underlined in 2009, with the outbreak of swine flu in Mexico. Fears about it have grown since mainly because of the fact that the causative virus was found to be of the H1N1 family strain, the same one responsible for millions of deaths during Spanish flu.[9]

The new dimension in confronting viruses and fears of respiratory disease pandemics, was also faced in 2002-2003 by the outbreak of Severe Acute Respiratory Syndrome (SARS), caused by a new type of corona virus (severe acute respiratory syndrome-related coronavirus, SARS-CoV-1). The speed in which that disease spread from Asia to North America, causing the outcome of about 800 deaths and with a mortality of almost 10 per cent, appeared to be the realisation of all the fears amassed from previous years.[15] Luckily, after that pandemic was definitely contained in 2004, SARS-CoV-1 never emerged again.

All these examples of dealing with epidemics have additionally influenced the public in becoming interested in the history of previous epidemics, and thus in the Spanish flu as well. In parallel with that came the fear that developed from potential bioterrorism, especially in the USA, following the terrorist attack of 11 September 2001, as well as subsequent anthrax attacks,[9] where the use of bacteria (in this case *Bacillus anthracis*) as bioweapon was clearly demonstrated. An earlier event in Japan, where doomsday sect Aum Shinrikyo released one form of toxic compound sarin in the Tokyo Metro, in March 1995,[16] especially illustrated the possibilities for a massive dispersion of lethal poisonous agents.[17]

In general, attention towards the heritage of Spanish flu mostly increased in the US. Some scientist and authors definitly used the special kind of American attention for their own promotion. In 2004 John Barry, American author and historian published a book about Spanish flu, entitled: 'The Great Influenza: The Epic Story of the Deadliest Plague in History'. In the preface he clearly stated how the recent outbreak of influenza in Asia inspired him to explore history of Spanish flu in order to present 'how American society reacted to an immense challenge, a war of nature launched against man...'. Adding that: '[M]y own interests have always focused on people who try to exercise some kind of control over events', he emphasised how the focus in the book was clearly only upon the best American doctors and scientists from the period of Spanish flu, who had battled disease with lot of heroism.[18] It was no wonder, after all, that his book was a New York Times best seller.

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Estimations and overestimations

With the passing years the number of books and articles about Spanish flu has seen a further rise. Authors almost constantly tended to point out how whole story regarding the influenza pandemics from 1918-1919 was pretty much swept under the carpet from the side of 'classic history' because of the significance of the First World War.[19] Some of them made specific social research in trying to answer how it was possible that immediately after Spanish flu its heritage was so strongly surrounded by silence. The American author Nancy Bristow made the first steps in the territory of gender in confronting the history of medical treatment in the US, during 1918 and 1919. She concluded how female nurses and their whole profession 'came through the epidemic with their heads held'. This was because they had been able to fulfil all aspects of their professional purpose and to show how important their role was in the medical systems and in society in general. Their work was 'a meaningful opportunity for service that only enhanced their confidence as women and as nurses'. On the other hand, most doctors of that time had been males. It was their inability to successfully combat and overcome the disease. She, therefore, concluded how 'For many doctors the epidemic would always remain the low point in their professional lives'.[20] That led to the assumption of how such a feeling became the basis for silence about the lethal episode arising from the Spanish flu.

Nevertheless, authors mainly suggested how the primal aspects of negligence in dealing with the history of Spanish flu contributed to a constant under estimation in the number of victims. The usual perception that there were three waves in the history of the influenza pandemic of 1918–19 is already firmly entrenched in historical knowledge, where it is clear how the first wave started in late spring and early summer of 1918, in the northern hemisphere, that the second wave, at its height between September and November 1918, was by far most lethal, and that the third wave, from early spring of 1919, was much less significant. Those who claim significant underestimates in the recorded statistics of influenza morbidity and mortality mainly like to point out the limitations of those data, connected with missing records or misdiagnosis. Initial estimations from the 1920's produced a calculation of somewhere about 21.5 million deaths around the world. One article from 1991 revised those numbers, claiming that the number of victims was in the range of 24.7–39.3 million.[13]

Probably most famous example of any modern estimation is an article with the title 'Updating the Accounts: Global Mortality of the 1918–1920 "Spanish" Influenza Pandemic', published (in 2002) by Niall P. A. S. Johnson and Juergen Mueller.[14] This paper proposed how the number of outcomes in deaths during the Spanish flu pandemics was of the order of 50 million. Some authors even claimed that the number can be much higher. As one of them stated: 'However, it must be acknowledged that even this vast figure may be substantially lower than the real toll, perhaps by as much as 100 per cent underestimated'. As the basis for such estimations those authors refer to very possible mistakes made in under reporting cases. They especially point to colonial areas in the world, which could 'also occur because of the deadlines placed on reporting by (colonial) authorities and reporting agencies, as well as inconsistent coverage or reporting of the population (often overlooking rural and/or native populations)'.

Authors also introduced aspects of censorship into the calculations, claiming how 'these factors are all in addition to the widespread problem of the restriction of reporting on the major wave of the pandemic, ignoring influenza mortality before and after this wave'.[14] The situation in regard to censorship was one of the key factors in the early perceptions about Spanish flu. First of all, the name 'Spanish flu' resulted from the fact that Spain was the first country in Europe and the world where reports about the outbreak of the disease openly circulated in newspapers and in public opinion. This was enabled by the neutrality of Spain during the First World War. Almost all other countries in Europe, deeply entrenched in the fierce war battles of 1918 that were decisive, were made to resort to concealing information about the outbreak, because of the possibility that the enemy could exploit any sign of weakness shown in operations of the war.[19]

Mortality rates in the US were probably the most correctly calculated of all the countries in the world immediately after the pandemic. They were, nevertheless, based on estimations, where the usual system included a perception of 'ex-

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cess deaths' in the calculations. This is where the measurement of deaths during a certain shorter period for a certain area is produced in comparison to the average level of deaths during a longer period in that same area. Based upon this form of estimation the total number of deaths connected with the Spanish flu among American citizens was 675,000 (the US population at that time was around 103 million). Mortality rates in many European countries from the 1918–1919 influenza pandemic are also based on estimations. So, estimations that circulated during the 1920's gave approximate rates for the biggest European countries: Great Britain 230,000 deaths (population 39 million), France 240,000 (population 33 million), Russia/USSR 450,000 (population 184 million), Germany 240,000 (population 58 million), Italy 390,000 (population 36 million) and Spain 260,000 (population 21 million). From those estimations the total number of outcomes in deaths for all European countries, was about 2.3 million.[14]

It is, of course, of utmost importance to recognise the demographic relations of the world in 1918, especially when some comparisons with the actual COVID-19 pandemic for the year 2020 are eventually made.[21] World population in 1918 is estimated to be around 1.8 billion. That number is about 4.3 times or 6 billion smaller than the world's population today, just over a century later (of about 7.8 billion). This also means the population density level on the planet was 4.3 times smaller than now. However, just as it is today, in 1918 the world population was mainly concentrated in Asia. About 60 per cent of all inhabitants lived on the biggest continent (that proportion remains approximately the same), with the most important concentrations in China and India.[22]

Unfortunately, so far, the historiography about influenza pandemic has always been closely connected to national boundaries, while pandemic being a phenomenon, logically have a transnational global character. It is very noticeable that the estimations made in the majority of the articles stated here tend to be largely US-centred and, in a much less manner, also West European-centred. In this context the big differences in estimating outcomes in deaths throughout epidemics are mainly connected with the fluctuations in estimating the number of deaths in Asia. Based upon estimates for the number of deaths in the developed world, the authors always tend to display much bigger numbers for countries in Africa and especially Asia. While the historical basis for such estimations is very unstable, records of data for those areas from the period of 1918-1919 are also quite scarce. For example, in their article Niall P. A. S. Johnson and Juergen Mueller came to conclude that estimates for the then area of India (at that time under British colonial rule, which encompassed the contemporary area of Pakistan and Bangladesh) was in the order of 18.5 million deaths.[14] This result was obviously heavily implied by the fact that India, in the then territorial frame, originally had a population of about 306 million.

For several years one of the most important indicators for population movement within historic demographic relations is the Human Mortality Database (HMD), a specific tool published online,[23] produced through cooperation between the University of California, Berkeley (US) and the Max Planck Institute for Demographic Research (Germany), assisted by the Centre on the Economics and Development of Aging (CEDA) at French Institute for Demographic Studies (INED). The HMD contains data on population and death for several countries, by age and by year. Numbers for cases of deaths are extracted from national registries, while population counts are the product of periodic censuses and official population estimates.

In their recent article 'Reassessing the Global Mortality Burden of the 1918 Influenza Pandemic',[24] authors Peter Spreeuwenberg, Madelon Kroneman and John Paget, from NIVEL (Netherlands Institute for Health Services Research), in Utrecht, also included the 'Statistical Abstract for British India 1915–16 to 1924–25', published in London in 1926. The analysis of data from this period reveals that the demographic los-ses in the area of South and Southeast Asia in the early decades of the 20th century was from the much greater impact of malaria epidemics, than the impact and consequences of Spanish flu. The final estimations by those authors for India are of about 6 million outcomes in deaths corelated with the Spanish flu in 1918 and 1919.

Also, just to note the global estimations. Those have been presented in three scenarios, with the focus on the middle version of the estimations: about 15 million deaths in 1918 and about 2.5 million deaths in 1919.[24] The ultimate computa-

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tion, therefore, is the number of 17.5 million victims from the biggest and deadliest pandemic in recent history. To reiterate again, that those results are based upon estimations and which, mainly, are indirect estimations. Although this is so, they seem to me to be much more in accordance with demographic reality at that time than suggestions of about 100 million or even more deaths.

In his article 'Die „Spanische Grippe" 1918/19. Verlauf, Folgen und Deutungen in Deutschland im Kontext des Ersten Weltkriegs' ('Spanish Flu 1918/19. Course, Consequences and Interpretations in Germany in the Context of the First World War')[1] the German historian Eckard Michels emphasised how small the real influence of Spanish flu actually was in all aspects of German society in comparison to the developments that occurred during the First World War, especially over the last months of military operations. From the first news about the spread of the disease in Spain at the end of May, all the way through to the end of the most deadliest second wave of influenza in mid-November – almost exactly the time when the German Reich capitulated on the Western front and the war was ended (11 November 1918) – the focus of public opinion was much more concentrated on political and military developments than on the raging disease. Although this claimed many lives, they were definitely much less than the war that had ravaged Germany and Europe already for four years. A similar situation, related to the hierarchy of importance, can be found all over Europe during that time. For example, in descriptions about the setback of Austro-Hungarian forces in the area of Bosnia and Herzegovina in November 1918, there is not one single line about the impact of influenza pandemics in documents from the Austrian archives.[25]

Michels, in conducting his deep research of German archives discovered that it is impossible to have one unique picture of the development of the disease in Germany. Some cities and regions had suffered a huge impact (such as Freiburg im Breisgau, Marburg, Recklinghausen, Schleswig or Stralsund), while some have been largely spared (Hamburg, Augsburg, Berlin, Dortmund or Karlsruhe).[1] A geographical pattern was not absolutely decisive in the case of Germany. For example, in areas of the Baden region, where it was seen that Karlsruhe used to have very mild epidemics, Freiburg (only about 100 kilometres south), was quite severely affected. (It is a very interesting coincidence that similar e developments occurred during the current COVID-19 pandemic. Although the city of Freiburg has about 30 % less population than Karlsruhe, it has suffered about six times more outcomes in deaths).

In general, it should be concluded that Germany managed to navigate through the waves of the Spanish flu pandemic a little bit better than its counterparts further west. While the geographic spread of the disease implied that western parts should have suffered a bigger impact, as transmission of the disease was mainly happening at the Western front through contact with foreign soldiers (mostly prisoners of war), that proved not to be the case. On the contrary, for Germany it is clear that the Western front tended to have the function of a defensive wall, that enabled the country to be isolated from a worse impact of influenza.[1] Along the other side of the front French, British and American soldiers suffered much more from the lethal waves during the summer and autumn of 1918. It is also apparent that civilian populations suffered more, especially in the US, than in Germany.[9]

This example once again emphasises the connections that are made between the First World War and the Spanish flu. There has always been a very strong claim as to how the pandemics that broke out so strongly out during 1918 was definitely caused by the course of the war. This was mainly connected with the fact that first outbursts of disease, both in the US and in Western Europe, were observed among soldiers, at the front (in Europe) and inside of the camps for preparation for the front (in the US).[18] However, that claim is very questionable. Illustrative presentation of the disease, including the usual perception of 'cytokine storm'[26] – where immune systems of young, strong bodies of soldiers between 20 and 40 years overreacted and released very large numbers of cytokines, that eventually led to fast drowning in their own blood with dramatic scenes of blood coughing and painful deaths – were so deeply entrenched in the memory of those involved (soldiers, doctors, nurses) that it was almost impossible to let other possibilities enter into calculations on the causes of death or descriptions of the developments of the disease. The facts that the excessively high levels of deaths, especially in 1918, was by far highest in among the 20-40 years age group of the pop-

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ulation was almost always connected with a view of the influenza 'genetically programmed' to trigger 'cytokine storm'. Many tend to forget how bloody were summer and autumn months of military operations initiated along the Western front, where millions of lives were lost in brutal grinding of the war since 1914. This inability to separate victims of war and victims of disease is one of the most crucial problems and challenges in estimations of the real impact of the deadliest pandemics in recent history.

Conclusion

In the general scope of historiography related to the Spanish flu pandemics estimations about the number of victims always play a key role. The essential perception that is of significance about the disease that shook the world in 1918 and 1919 was that it was, and is, almost exclusively connected with numbers. The analysis of scientific (and less scientific) documentations and views throughout the last several decades clearly shows the presence of battle between 'historians' and 'medical historians'. It is where one side tends to overlook the impact of pandemics and to stick with the usual narrative about the First World War with all (mainly political, economic and military) mechanisms that led to its conclusion in November 1918, and where the other side tends to point out the unique significance of the Spanish flu, focusing on the impact of that pandemic on the course of the war, even its outcome, and especially on the huge number of deathly cases, with emphasis on the fact 'that more people died from the Spanish flu than during all of the military operations during World War I'.

How many more? That question became an obsession for some medical historians, and estimations were developed as a purely defensive reaction, for understanding that only huge bold projections can justify an increase and forcing of further rise of attention towards Spanish flu totally prevailed. The result was that some of the estimations clearly evolved into becoming overestimates, out of touch with the demographic reality of 1918 or 1919. Those efforts were, unfortunately, mainly attributed to a general loss of focus, time and resources. For a proper understanding of the history of Spanish flu it is much more important to comprehend how this pandemic emerged (the how and from where this strain of avian flu began its spread among human beings was never clarified!), and what were the devices of its dynamics in its infectious circulation around the globe, rather than only to calculate if the number of the victims was in the range of 10-20 million or 50-100 million.

The overlap between the Spanish flu and final months of World War I is actually a very good opportunity to research both events from an interdisciplinary point of view. Modern historiography still has lot of opportunities in providing a much more detailed picture about the developments from 1918 and 1919. Now it is clear that evolution of Spanish flu should not be seen only as a derivative product of war, but, on the other side, it is also clear that the impact of the Spanish flu at the outcome of war was not of huge significance.

Future research must concentrate more on archives and looking for mechanisms that enabled the emergence and the spread of the disease. In his final opinion after the publication on the the restored genome of H1N1 influenza virus, Jeffery Taubenberger concluded how the virus from 1918 was in fact the genetic ancestor of all subsequent influenza viruses in 20th and 21 century and how Spanish flu virus indeed is 'the “mother” of all pandemics'. But even he was not at all able to give answers of how that virus emerged and what made it so lethal. All other influneza pandemics in the 20th and 21st century ('Asian flu' 1957, 'Hong Kong flu' 1968, 'Swine flu' 2009) resulted in far fewer victims and were insignificant regarding the impact on demographic and social relations in comparison with the Spanish flu. Huge discrepancies in the level of its lethality are still not understood. There is a huge field of opportunity, but also clear feeling of necessity, for common work of history and medicine in solving those puzzles.

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Conflict of interest

None.

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CASE REPORT

COVID-19 Pneumonia: When Negative RT-PCR Testing Does Not Rule out the Disease

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Abstract

In diagnosing COVID-19, false negative findings from the biological sample taken from a mucosal swab of the upper respiratory tract and tested with the real-time reverse-transcription polymerase chain reaction (RT-PCR) technique have been reported. This patient has had a proven contact with an infected person, clear symptoms of viral respiratory disease, yet negative test results on the fifth day of self-isolation. On repeated test after 48 hours, on the 7th day of isolation, due to persistence of some symptoms, he tested positive for SARS-CoV-2. The existence of symptoms and characteristic sings after laboratory and radiological analysis of the patient prompted the repetition of the tests, which at the end led to the confirmed diagnosis and the possibility for adequate treatment of the patient as well.

Key words: COVID-19, SARS-CoV-2, pneumonia, false negative RT-PCR.

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Introduction

COVID-19 represents a disease caused by the novel corona virus (first named 2019-nCoV, later renamed to SARS-CoV-2), which initially emerged in the Chinese town Wuhan, at the end of 2019.[1] It is a case of a new virus from the Coronaviridae family, which was not recorded before, so the clinical features and diagnostics are different from the diseases caused by the already known viruses from this family, severe acute respiratory syndrome (SARS) and Middle-East respiratory syndrome (MERS).[2, 3] COVID-19 represents a highly contagious respiratory disease with, in most cases, mild clinical presentation.[4, 5] False negative results received from the mucosal swab of the upper respiratory tract and tested with real-time reverse-transcription polymerase chain reaction (RT-PCR), especially in early stages of the disease, have been reported.[6-9]

This is a case report of a patient with symptoms of the upper respiratory tract infection who had initially tested negative for COVID-19 on the fifth day of self-isolation and then positively when the test was repeated two days later.

Case history

A male patient, aged 62, contacted the medical authorities by phone because of the symptoms that included mild dry cough, slightly increased body temperature (37.5 °C), languor, subjective feeling of shortness of breath and loss of senses of smell and taste. Previous health problems like long-lasting diabetes treated with oral hypoglycaemic agents were revealed from the patient's medical history. He was a non-smoker, overweight (BMI = 28.3 kg/m^2) and moderately physically active.

The symptoms appeared on the third day of self-isolation that he started when his son tested positive for SARS-CoV-2. On the fifth day of self-isolation, on 23 March 2020, he was admitted to the Department of Infectious Diseases, General Hospital Doboj, after which a nose swab was taken from him and sent for further testing to Banja Luka University Clinical Centre of the Republic of Srpska (BL-UCCRS). The RT-PCR method then showed that the test had been negative. The patient was taken back home to continue his self-isolation.

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During the next two days, his body temperature fell to normal values during the day but it kept rising to 37.5 °C in the evening. His cough was less frequent, but the feeling of shortness of breath persisted and the patient was driven to a medical institution on 25 March. It has been ascertained by observation that the patient is conscious, oriented and eupnoeic while sitting in the upright position. Physical examination revealed: on lung auscultation exaggerated, but symmetrical vesicular breathing sounds, mild hyperaemia of the throat and normal blood pressure. The tests that were performed were complete blood count, sedimentation of erythrocytes (SE), C-reactive protein (CRP), pulse oximetry, electrocardiogram (ECG) and posteroanterior roentgenogram (X-ray) of the lungs.

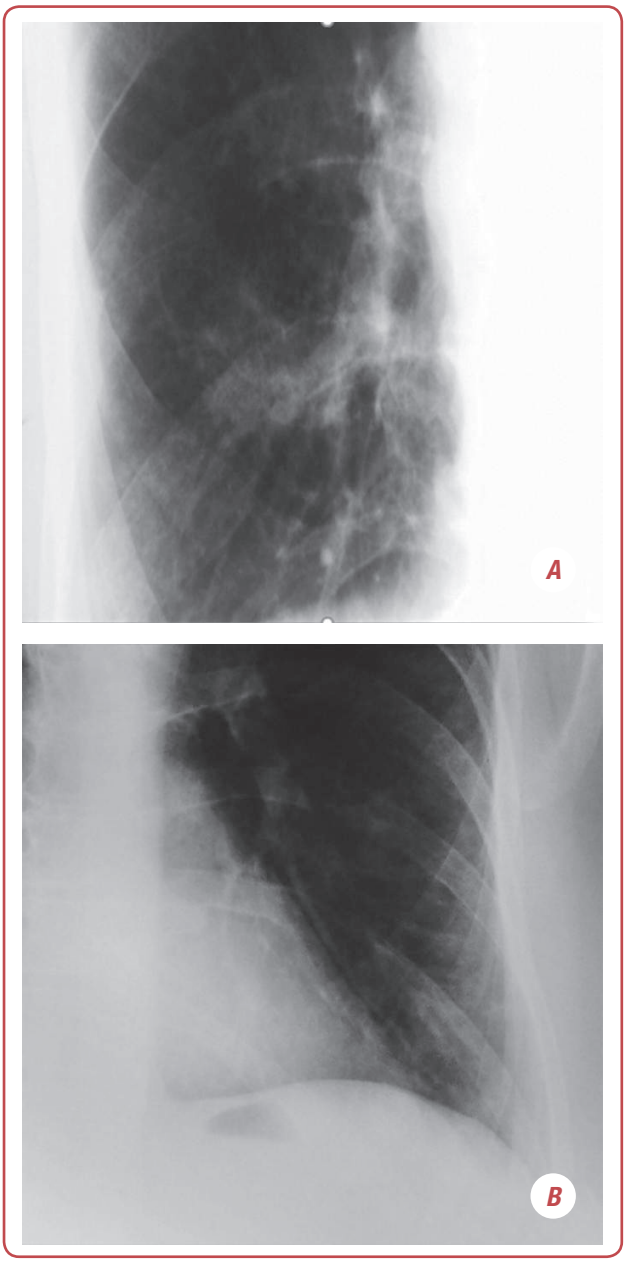

Figure 1. Chest X-ray of patient with COVID-19-induced pneumonia: (A) Right lung with ground glass opacities, with accentuated hilar shadows. (B) Left lung with basal consolidation.

Complete blood count revealed a lymphopenia, with normal values of the leukocytes, erythrocytes and thrombocytes. SE was 68 mm after 30 minutes, the value of CRP was 75.6 mg/L. Pulse oximetry showed a lower oxygen saturation (SaO_2) of 81 %. The ECG showed sinus tachycardia, with the heart rate of 105 per minute, with left axis deviation, expressed muscle artefact and ST segment in the isoelectric line. On the posteroanterior chest X-ray, the following was observed: oval inhomogeneous opacity in the middle third of the right lung wing of peripheral localisations with accentuated hilar shadows. Basal left part showed a shadowing in the lower third section with the shadowing of the costophrenic angle. Interlobar pleural effusion was noticed on the right (Figure 1).

All of these findings suggest that this is the case of bilateral pneumonia, most likely due to viral aetiology, in a patient that was in direct contact with a person that was suffering from COVID-19 and who tested negative two days before this examination, on the fifth day after contact.

The patient was sent to be re-tested and he was admitted to the Infectious Disease Clinic of the BL_UCCRS, on the same day, ie on the 7th day after contact with SARS CoV-2-infected person. The repeated test was positive for SARS-CoV-2 and a definitive diagnosis of a bilateral bronchopneumonia was established. The patient was hospitalised for additional ten days with moderately severe clinical manifestations of the disease and was treated with hydroxychloroquine tablets according to treatment guidelines for COVID-19 infection.

Discussion

Currently, the RT-PCR method of detection of viral ribonucleic acid (RNA) in the respiratory tract is the gold standard in diagnosing COVID-19.[10] The swab from the upper respiratory tract mucosa is taken most frequently, but it is recommended to take the sample from the lower respiratory tract whenever possible.[7] RT-PCR has high specificity, but its sensitivity is between 60 and 70 %, which requires retesting in up to 30 % of the cases.[11] Our patient with the negative test result on the 5th day of isolation and with present identical symptoms of shortness of breath like on the 7th day when he

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was retested (this time positively), points out to the need of radiologic and laboratory evaluation in parallel with the PCR processing. Several individual or group case reports have been recorded where the patients had false negative results, very similarly to the patient in this case report.[12, 13] The experience so far points to the fact that negative test results of SARS-CoV-2 do not completely exclude the disease.[13]

Alongside this method, laboratory parameters and radiological techniques, predominantly lung computerised tomography (CT) scan, are additionally used to establish the diagnosis.[14] On computerised tomography, changes in lungs induced by COVID-19 are presented in the form of ground glass opacities, spread along the lung parenchyma (medial, lateral and posterior lung fields), localised predominantly peripherally, with the central axis parallel with the pleura.[15, 16] In some cases pleural effusion was present as well.[16] In absence of a CT scanner, this patient underwent a postero-anterior chest X-ray, which was in correlation with the global data on radiological presentation of the COVID-19-induced lung lesions.[17]

Regarding the laboratory diagnostics, in patients suffering from COVID-19, lymphopenia was noticed, as well as a negative correlation between the lymphocytaemia and the degree of tissue damage.[18, 19] CRP and SE, as inflammatory parameters were increased.[18] It was the case with this patient as well, who had a confirmed COVID-19 pneumonia, lymphopenia with normal values of total leucocytes, accompanied with the increased values of CRP and SE.

In this case report, we presented a patient who was in direct contact with a SARS-CoV-2- infected person for a longer time period, after which he developed symptoms of a febrile respiratory tract infection. This was the indication for testing.[7] First testing was done without radiological and laboratory processing on the fifth day after contact. RT-PCR showed negative test results, which is relatively common in the early stage of the disease (RT-PCR sensitivity is 60-70 %).[11] Due to the persistence of symptoms, the test was repeated two days later, after noticeable radiological and laboratorial abnormalities had been discovered. This time the patient tested positive for SARS-CoV-2.

At the time of writing this article, there were 8 out of 710 hospitalised patients at the Infectious Disease Clinic of BL-UCCRS having clear clinical manifestations of COVID-19 pneumonia, but with false negative RT-PCR test. Although they have been regularly retested the SARS-CoV-2 infection was never confirmed in these patients. In many cases, the time of exposure to virus infection is unknown and testing is usually based on time of symptom onset. According to some recent findings, the false negative rate is lowest 3 days after onset of symptoms, or approximately 8 days after exposure to infected patients.[20] Before or after that period the false negative rate is higher and clinicians should be aware of it. It is clear that serial testing would certainly reduce the frequency of false negative results.

Conclusion

The interpretation of RT-PCR tests for SARS-CoV-2 infection must be taken with caution, particularly at early course of infection. The infection should not be ruled out on the basis of RT-PCR testing alone. It is necessary to perform radiological and laboratorial processing of every patient suspected to COVID-19 infection, no matter the results of the RT-PCR testing. Due to low sensitivity of RT-PCR testing, persistence or impairment of clinical features should lead to re-testing.

Acknowledgements

None.

Conflict of interest

None.

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Simeonova Krstevska et al. Scr Med 2020;51(2):124-8. DOI:10.5937/scriptamed51-26499

CASE REPORT

Multidisciplinary Approach to Management of Hypofibrinogenaemia in Pregnancy, a Case Report

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Abstract

Inherited fibrinogen disorders introduce risk for recurrent abortions, sub-chorionic haematoma, placental abruption and postpartum haemorrhage. This is a case report of a successful pregnancy outcome in a 37-year old woman with hypofibrinogenaemia. She was referred to a coagulation test in the first trimester because of history of preeclampsia and HELLP syndrome in previous pregnancy. Hypofibrinogenaemia was diagnosed with fibrinogen level of 0.7 g/L. During the pregnancy she was regularly monitored for fibrinogen levels and multiple cryoprecipitate concentrates were given. She delivered at 39th gestation week, with elective caesarean section under general anaesthesia. There was one episode of postpartum haemorrhage treated with 2 units of red blood cells, repeated infusions of cryoprecipitate to obtain the level of fibrinogen of 2 g/L. She was discharged on the 6th postpartum day in a good condition. In these disorders levels of fibrinogen should be higher than 1 g/L during pregnancy or 2 g/L in case of caesarean section for successful prenatal and peripartal management.

Key words: Hypofibrinogenaemia, pregnancy, peripartal management.

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Introduction

The coagulation system has one final goal: convert fibrinogen to fibrin and form a clot. During pregnancy changes in the system of coagulation and fibrinolysis occur. Thrombin and fibrin generation increase to facilitate placental implantation and prevent excessive haemorrhage during delivery. Normal pregnancy causes progressive increase in maternal plasma D-dimer concentration from conception until delivery.[1]

Fibrinogen (normal concentration in blood between 2.0 and 4.5 g/L) supports pregnancies by maintaining haemostatic balance and stabilising the foetal-maternal junction and embryo implantation.[2] Maternal fibrinogen and factor XIII are essential after 4-5 gestational week in decidual stroma.[3] Fibrinogen plays a positive role in the process of implantation. Coagulation changes of pregnancy help in regulating attachment of placenta and stabilising some placental detachments that may happen. Low fibrinogen may cause rupture affecting embryonic trophoblast infiltration and leading to haemorrhage.[4]

Inherited fibrinogen disorders introduce risk for: menorrhagia, menometrorrhagia; recurrent foe-

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tal loss, spontaneous abortions, bleeding in early gestations, sub-chorionic hematoma, placental abruption; increased incidence and severity of postpartum hemorrhage.[5] There are two types of hereditary fibrinogen abnormalities: quantitative defects like afibrinogenaemia or hypofibrinogenaemia and qualitative defects like dysfibrinogeneamia or hypodisfibrinogenaemia (HD). First reported in 1935, prevalence of afibrinogenemia is extremely rare, around 1/1,000,000. Prevalence of hypofibrinogenaemia is hard to say as there are many asymptomatic patients.[6]

Congenital defects of fibrinogen are caused by mutations in the FGA, FGB, or FGG genes, located on the q arm of 4th chromosome at position 31.3. Hypofibrinogenaemia and dysfibrinogenaemia are autosomal dominant or recessive. There are more than 100 fibrinogen mutations that have been associated with hypofibrinogenaemia.[7] Hypofibrinogenaemia could be: a) mild (fibrinogen levels > 1 g/L): asymptomatic, excessive bleeding can occur after injuries, surgery or delivery; b) moderate (fibrinogen levels between 0.1-1 g/L): bleedings can be spontaneous or caused by injuries, surgery, or delivery; c) severe (undetectable clot): spontaneous, severe, and even life-threatening bleeding may occur.[8]

Dysfibrinogenaemia results in abnormal fibrinogen function. It can be asymptomatic in 55% of the cases, can cause haemorrhage in 25 % and thrombosis in 20 %.[9] Dysfibrinogenaemias are result of defect of fibrinogen molecule which impairs polymerisation of fibrin associated with irregular cross linking with factor XIIIa. Dysfibrinogenaemias can be associated with hypofibrinogenaemia and congenital trombophylia.[10, 11] As first such case was diagnosed in 1958 until today over 100 mutations have been reported. Dysfibrinogenemia increase the risk of spontaneous abortions, placental abruption, thrombosis and haemorrhage.[12]

If possible, during pregnancy fibrinogen levels should be more than 1 g/L and in case of caesarean section 2 g/L. Best choice for treatment of acute bleeding episodes are fibrinogen concentrates.[13] If unavailable, cryoprecipitate (a fibrinogen-rich plasma fraction) should be used.[14]

Case history

A thirty-seven-year old woman went to a gynaecologist to perform a routine exam due to the new pregnancy (gravida 2, para 1). There were no haemorrhagic and thrombotic events in the personal or family medical history.

Her previous pregnancy resulted in premature labour in 30th gestation week by caesarean section due to HELLP syndrome under general anaesthesia. The newborn had a body weight of 1,300 g and length of 42 cm. It was admitted and treated in ICU and had a good outcome.

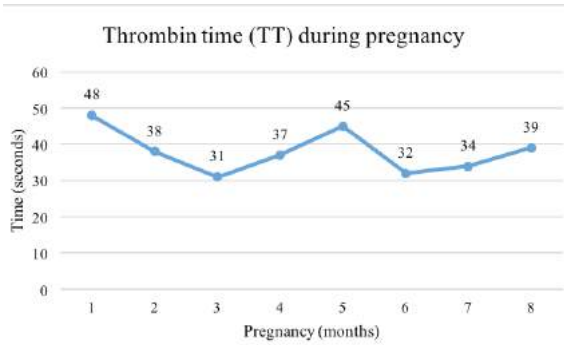

Figure 1a: TT Thrombin time during pregnancy

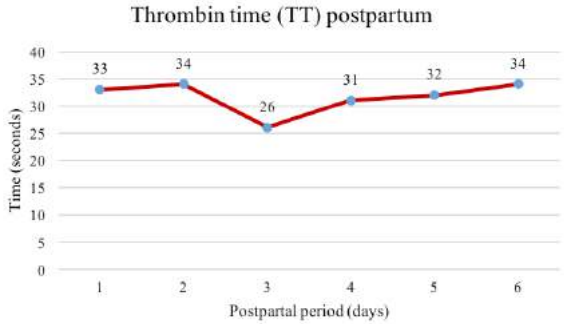

Figure 1b: Thrombin time postpartum

Figure 1: Thrombine time values during pregnancy and postpartum

During this pregnancy she was referred for a coagulation test in the 1st trimester (7th gestational week) when hypofibrinogenaemia was diagnosed with fibrinogen level of 0.7 g/L. Analysis for determining mutations/polymorphisms of the thrombophylic genes revealed: heterozygotic mutation of factor V H1299R, factor XIII V34L, PAI-1 5G/4G, MTHFR C677T, MTRR A66G polymorphisms.

Due to previous history of severe preeclampsia and HELLP syndrome, serum placental angiogen

levels were analysed and risk for preeclampsia was ruled out (PLGF 103, sFLT 4708, sFLT 1/PLGF 4.6).

Non-invasive prenatal screening test was done due to maternal age and it came out as low risk for aneuploidy (chromosome 21, 18, 13, sex chromosomes, microdeletions).

Monitoring of fibrinogen levels on a two week or monthly interval was made by a specialist in transfusion medicine. She received multiple transfusions of cryoprecipitate (20 units each) during the 1st and 2nd trimester, as well as multivitamin supplementation with B_6, B_{12}, methylfolic acid.

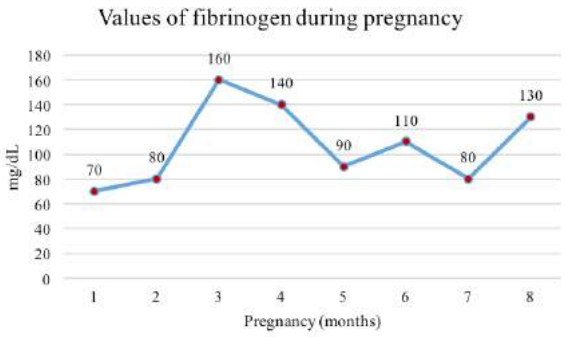

Figure 2a: Fibrinogen values during pregnancy

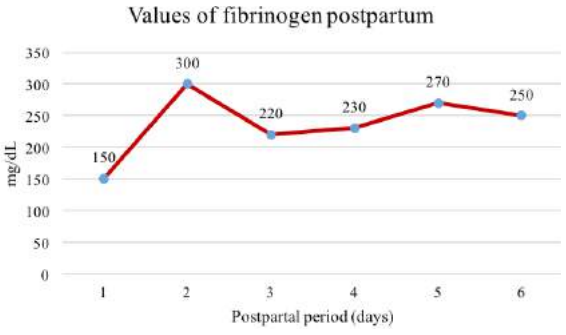

Figure 2b: Fibrinogen values postpartum

Figure 2: Fibrinogen level during pregnancy and postpartum

Platelet count ranged from 200 x 10^9/L at the beginning to 150 x 10^9/L at the end of pregnancy. Haematocrit was maintained above 30 % during the pregnancy (42.7 % - 30.9 %).

Screening haemostasis parameters were as follows: prothrombin time (PT) was in the normal range (12-14 s) during whole pregnancy; activated partial thromboplastin time (aPTT) was shortened according to the gestational age (29-24 s) and thrombin time (TT) was significantly prolonged (48-31 s) according to the cryoprecipitate substitution (Figure 1a). During the pregnancy the level of fibrinogen varied from 0.7 to 1.6 g/L according to the cryoprecipitate substitution (Figure 2a). D-dimers were increased according to the gestational age (from 227 to 2,900 ng/ml).

Foetal growth was adequate for gestational age, placental echogenicity and doppler of the feto-placental unit was closely monitored through the pregnancy by ultrasound. Pregnancy was uneventful with no signs of bleeding. Patient received thromboprophylaxis with enoxaparin in the late second and third trimester.

She was admitted at the University Clinic for Obstetrics and Gynaecology at 38th gestation week for adequate preparation for delivery. Anaesthesiology consultation was made with physical exam, ECG and cholinesterase analysis (history of prolonged wakening after general anaesthesia in previous caesarean section, results in reference values 6,389 U/L).

Blood products were supplied: erythrocyte concentrate, fresh frozen plasma, cryoprecipitate. Thromboprophylaxis with enoxaparin was discontinued 12 hours before delivery. Cryoprecipitate infusion was administered with 10 units/8 h interval until concentration of fibrinogen reached 3 g/L before scheduling the caesarean section.

The patient was delivered at 39th gestation week by elective caesarean section under general anaesthesia. The newborn had weight of 3,550 g, length of 50 cm and Apgar score 9/10. Operative period was uneventful.

Postpartum screening haemostasis parameters were analysed daily: PT and APTT were in the normal range (12.1-13.2 s) as well as aPTT (24-30.7 s), while D-dimers were decreasing. Platelet count ranged from 122 up to 361 x 10^9/L. Postpartum TT and fibrinogen level is shown on Figure 1b and 2b.

There was one episode of postpartal haemorrhage with estimated blood loss of 700 mL treated with standard uterotonics (oxytocin, methylergometrine, carboprost tromethamine). The haemoglobin levels were reduced from 114 g/L to 74 g/L, haematocrit from 0.34 to 0.21. She received two units of red blood cells (RBC), 3x20 units of cryoprecipitate (the level of fibrinogen was main-

CONTENT

tained above 2 g/L), which improve haemoglobin up to 96 g/L and haematocrit to 0.33. Thromboprophylaxis with enoxaparin 2x40 mg was administered according to the protocol.

The patient was discharged from the clinic on the 6th postpartum day in good condition.

Discussion

According to the genetic testing, dysfibrinogenemia was not confirmed in combination with the hypofibrinogenaemia in this case report. However, genetic testing revealed heterozygous mutation for FXIII V34L which is important for the interplay of FXIII and fibrinogen.[15]

In pregnancy with hypofibrinogenaemia levels of fibrinogen should be more than 0.6 g/L and higher than 1.5 g/L at delivery.[11] Even though case reports on this topic are few, pregnancy risk is connected to HD. Management of pregnancy with HD is appropriate to be on an individual basis considering the analysis, individual and family history. There have been case reports of good pregnancy outcome achieved with fibrinogen therapy 2-3 times a week or without any therapy.[13] Low molecular weight heparin prophylaxis can be administered in pregnancy and postpartum if there is a case of thrombotic phenotype.

As quantitative and qualitative fibrinogen disorders are rare, publication of cases like this is necessary. This patient had no previous haemorrhagic or thrombotic episodes in her lifetime. The previous pregnancy resulted in severe preeclampsia, so the coagulopathy was assumed to be connected to the HELLP syndrome. Nevertheless, this was the reason why coagulation testing was ordered in the very beginning of the second pregnancy.

Multidisciplinary management including specialist in transfusion medicine, haematologist, obstetrician/gynaecologist specialised in maternal-foetal medicine, anaesthesiologist and blood bank cervices is essential especially when fibrinogen concentrate is not available and antenatal care and postpartum haemorrhage treatment included cryoprecipitate. Combination of cryoprecipitate and anticoagulation helped the stability between the risk of bleeding and clotting in pregnancy.

The level of functional activity of fibrinogen below 0.7 g/L is suggestive of dysfibrinogenaemia, which was not the case in this report. Was there the impact of the FXIII V34L heterozygous mutation on the fibrinogen function in combination with decreased fibrinogen level (not less than 0.7 g/L) as it was in this case, is hard to say. However, the cryoprecipitate transfusion enabled successful haemostasis and together with the absence of bleeding history, suggested that the genetic defect of FXIII had no influence on the haemostatic balance in this particular case. Having in mind that inherited fibrinogen disorders are very rare, further studies and more precise laboratory investigation are necessary for diagnostics of these conditions and for the prediction of obstetrics complications. [13]

Conclusion

Congenital fibrinogen disorders carry pregnancy risk, but it can be successfully managed by engaging the multifunctional team of specialists. As there are no randomised controlled studies, management is made on expert consensus. Successful perinatal outcomes can be accomplished by analysis of the fibrinogen levels and supportive therapy. Management must be individualised considering the personal history and the specific clinical situation. Interdisciplinary approach and close collaboration between the institutions is necessary since it was crucial to the positive outcome in this case.

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None.

Conflict of interest

None.

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OBITUARY

In Memory of the Late Professor Folke Sjöqvist (1933– 2020)

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It is with great sadness that we have to inform our medical society that Professor Folke Sjöqvist, one of the founding fathers of modern clinical pharmacology as a discipline, passed away on 30 March 2020, aged 86.

Professor Sjöqvist was born on 28 May 1933 in Västervik, Sweden. His alma mater was Karolinska Institute in Stockholm, where he earned his MD and DSc degrees in 1959 and 1962, respectively. After spending several years in the United States, Folke Sjöqvist served from 1964 to 1970 as associate professor in clinical pharmacology at the Karolinska Institute. In 1970, he became the first full professor of clinical pharmacology, not only at Linköping University, but also in the whole of Scandinavia. In 1972, Professor Sjöqvist became the first professor of clinical pharmacology at the newly established Huddinge University Hospital, where he remained chief of the department for 27 years, until his retirement in 1999. Folke Sjöqvist's main scientific interest was in pharmacogenetics, where he established a world-known research team and school. He spent many years as a leader and source of inspiration within the International Union of Basic and Clinical Pharmacology (IUPHAR). The list of scientific publication is very impressive; he published more than 300 peer-reviewed publications that have been, until now, cited more than 16,000 times, giving him the h-index of 69.

Folke Sjöqvist is survived by his wife Margareta, four children and twelve grandchildren.[1]

His numerous publications, countless awards[2] and utmost respect that he had been receiving from his colleagues over the past five decades still cannot describe the magnitude of the void created by his death. The reason for this is his unique approach to medicine, clinical pharmacology, science and teaching in general.[3] Simply, Folke, as we used to call him, lived for clinical pharmacology – for him, it was never just a job, but a true calling. He was emanating his contagious positive energy, accepting everything and even the problems with an optimism and smile. This is why he was not only respected, but liked and loved by his peers, younger colleagues and students.

Although being almost thirty years his juniors and knowing him only from congresses, symposia and the European Association of Clinical Pharmacology and Therapeutics (EACPT) meetings, we were also exposed to his positive energy and relentless fight for the advancement of clinical pharmacology. By doing so, Folke and his Scandinavian colleagues helped our predecessors and us to establish this discipline in former Yugoslavia and the countries that emerged from it in the 1990s and this is something that we will never forget, as well as his hospitality and generosity. It was him who established the so-called Scandinavian model of clinical pharmacology that became widely accepted in the South-East Europe.

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The pharmacogenetic laboratory which has been recently developed within the Centre for Biomedical Research at the Banja Luka University Faculty of Medicine is based on principles and structure developed by Professor Sjöqvist at Huddinge University Hospital. We can still remember Folke's radiant lecture and participation at the Fourth European School of Clinical Pharmacology and Therapeutics that we organised in Vršac, Serbia, in September 2006. We were truly honoured to be in a position to host him then.

Professor Sjöqvist's passing marks an end to an era, but his spirit will remain living among us throughout the world as a beacon obliging us to carry on the torch of clinical pharmacology.

Professor Ranko Škrbić
Professor Miloš P Stojiljković

Society for Clinical Pharmacology and Pharmacotherapy
Banja Luka, the Republic of Srpska, Bosnia and Herzegovina

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