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Staff of the Koševo State Hospital (1939) with the director Prim. Dr. Asaf Šarac. Source: The anthropological collection of the Museum of Sarajevo.

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Knowledge and Attitudes regarding Covid-19 Vaccination among Medical and Non-medical Students in Bosnia and Herzegovina

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Abstract

Objective. The aim of this study was to investigate students' knowledge, attitudes and hesitancy regarding COVID-19 vaccination. **Methods.** A cross-sectional questionnaire-based survey was conducted among a total of 1282 medical students and 509 non-medical students at four public universities in Bosnia and Herzegovina: Tuzla, Sarajevo, Banja Luka, and Mostar. **Results.** A significantly higher rate of vaccination was observed in the group of medical students as well as a higher level of knowledge about vaccination in general and vaccines against the COVID-19 disease. Students who received the COVID-19 vaccine had a higher level of knowledge about vaccination in general and COVID-19 vaccines in particular compared to the non-vaccinated students in the medical and non-medical groups, respectively. Furthermore, vaccinated students, regardless of the course they are taking, showed generally stronger positive attitudes compared to non-vaccinated students, regarding the safety and effectiveness of the COVID-19 vaccine. Both groups of students believe that the rapid development of the vaccine is contributing to refusal or hesitancy to receive a vaccine against COVID-19. Social media/networks were the main sources of information about the COVID-19 vaccine. We did not find any contribution of social media to the reduced level of COVID-19 vaccine coverage. **Conclusion.** Education of students about the benefits of the COVID-19 vaccine will lead to its better acceptance as well as the development of more positive attitudes towards vaccination in general, especially having in mind that students are the future population of parents, who will make decisions about vaccinating their children.

Key Words: COVID-19 Vaccine Students ▪ Knowledge ▪ Attitudes ▪ Hasitancy.

Introduction

At the beginning of the current COVID-19 pandemic, the development of a safe and effective vaccine against COVID-19 was expected to be a long-term solution to control the pandemic (1). According to the World Health Organization's report, as of March 16, 2023, a total of 760,360,956 infection cases had been confirmed globally, including 6,873,477 deaths (2). On December 31, 2020, the World Health Organization (WHO) announced that the Pfizer/BioNTech mRNA vaccine had been approved for emergency use, making it the first to be approved since the beginning of the pandemic (2).

Immunization programs face significant challenges in achieving targeted vaccination rates, and outbreaks of vaccine-preventable diseases are still frequently reported (3). Health experts attribute vaccine-preventable disease outbreaks to increasing hesitancy and negative attitudes toward

vaccination, suggesting that vaccination coverage rates are partly a reflection of individual vaccination attitudes and behaviors (4-6). One theory is that individuals will adopt a negative attitude towards vaccination when they feel threatened (7). In many cases, however, the necessity of vaccination is questioned due to the lack of awareness of the disease, poor knowledge of its potential consequences, and the perception of low susceptibility to the disease (8-10). A study of COVID-19 vaccination acceptance rates in different parts of the world found high acceptance rates in Malaysia, Indonesia, and China, whereas low acceptance rates were found in Italy, Russia, the United States, and France (11).

The decision to vaccinate can be influenced by doubts about the safety and protective effect of the vaccine, as well as the perceived benefits of vaccination (7). Such distorted attitudes can be formed due to misinformation regarding the risks posed by vaccination (12). Social norms and networks exert a strong influence on attitudes and behaviors related to vaccination, and individuals may align their vaccination decisions with the decisions of family members and members of their social network (4). This explains why negative attitudes towards vaccination sometimes cluster geographically (13).

While many studies have examined parents' knowledge, beliefs, and attitudes toward vaccination, perceptions and behaviors toward vaccination among young adults remain poorly described, with the exception of a few published studies focusing on specific vaccines for HPV infection and influenza (10, 14, 15). Young people represent the population of future parents who will make decisions about vaccinating their children. Also, young people represent future health workers who will educate the public about the benefits of vaccines, and counsel individuals who express doubts about vaccines. Regarding the student population, certain studies show that educational background affects attitudes about vaccination in general as well as about the vaccine against COVID-19 in particular.

The objective of this study was to examine and contrast the COVID-19 vaccination-related knowledge and attitudes of medical and non-medical students in Bosnia and Herzegovina. Additionally, the study aimed to identify the primary reasons why individuals were hesitant or refused to receive the COVID-19 vaccine.

Materials and Methods

Participants

This cross-sectional study was conducted in April and May 2022, and included a total of 1282 students from the medical group of faculties (faculties of pharmacy, medicine, veterinary medicine, dental medicine and health science) and 509 students from the non-medical group of faculties (faculties of electrical engineering, mechanical engineering, economics, science and mathematics) at four public universities in Bosnia and Herzegovina: the University of Tuzla, the University of Sarajevo, the University of Banja Luka and the University of Mostar.

Questionnaire

The survey questionnaire comprised four sections: socio-demographics, inquiries regarding knowledge of and attitudes towards the COVID-19 vaccine, and reasons for vaccine hesitancy or refusal. The criteria for the question selection were based on previously published articles (16-19). Socio-demographic characteristics comprised the first part of the questionnaire, and included the students' gender, age, university status, and questions about COVID-19 vaccination and sources of information about COVID-19 vaccines. The second part of the questionnaire consisted of ten main questions regarding the participants' general knowledge of vaccines and specific knowledge of COVID-19 vaccines. The participants provided answers to ten questions related to the COVID-19 vaccine, with the answer options "*Yes/No/I don't know*" (seven questions) and multiple answers (three questions). For the purposes of statistical data processing, their answers were translated into true/false answers.

The third part of the questionnaire consisted of nine questions regarding the participants' attitudes toward the COVID-19 vaccine, and students rated the answers on a Likert scale, with numbers from 1 (representing the lowest degree of agreement) to 5 (representing the highest degree of agreement) (20). In order to check the reliability of the measurement scale, the calculation of the Cronbach alpha coefficient was applied. Cronbach's alpha for the group of questions that assessed students' attitudes about the COVID-19 vaccine was 0.662.

The fourth part of the questionnaire, regarding the participants' hesitancy/refusal of the COVID-19 vaccines, consisted of twelve questions, 6 medically-based and 6 non- medically based. Students rated the answers using a Likert scale with numbers from 1 (representing the lowest degree of agreement) to 5 (representing the highest degree of agreement). Cronbach's alpha for the group of questions examining the reasons for hesitancy/refusal of the COVID-19 vaccine was 0.822.

Data Collection

The study was created as a cross-sectional study, based on filling out a survey questionnaire. The clarity, content comprehensibility and layout acceptability of the questionnaire were pretested on a small sample of students (N=10). For this purpose, the questionnaire was piloted with 10 medical students from the University of Tuzla.

Participation in the study was voluntary. The survey was conducted online, using the *SurveyMonkey* platform. The link to access the survey was delivered to students via email or a *Viber* group, along with an information leaflet and informed consent. The respondents were asked if they had read and understood the information leaflet, and gave consent for their anonymous answers to be saved and analyzed together with all the other answers. After the students had agreed to participate in the study, they could fill out the survey. The survey was set up by software so that it was not mandatory to answer all the questions in order to be able to submit the survey. Seven days after the initial invitation, students were sent an additional e-mail/message in the *Viber* group inviting them to fill out the survey, if they had not already done so. When the survey was closed, all the anonymous responses were entered into a single database. All data collected for this study were securely stored and will be destroyed five years after the end of the project.

Ethical Statement

The study protocol and questionnaires were previously approved by the Ethics Committee for Scientific Research of the University of Tuzla (number 03/7-2185-1/22, April 18, 2022.)

Statistical Analysis

The collected data were analyzed using the Statistical Package for Social Sciences (SPSS)/ WIN program (Release 21.0 SPSS Inc., Chicago, IL, USA). Qualitative data are presented as frequencies and percentages. The Chi-square test was used to examine differences between groups of categorical variables. The t-test was used to compare average knowledge score between the groups. The Mann–Whitney U test was used to compare the medians of two or more independent groups, when assessing attitudes and reasons for refusing/ hesitating regarding the COVID-19 vaccine. In all tests, values of $P \leq 0.05$ were considered statistically significant.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on request.

Results

The socio-demographic characteristics of the participants are shown in Table 1. Females aged 21 to 22 were the main participants in both groups of students. Out of 1791 students surveyed, 1502 students (83.86%) answered the question whether

Table 1. Socio-demographic Characteristics and Vaccination Status of the Survey Participants

Participant characteristics		Medical group of faculties N (%)	Non-medical group of faculties N (%)
Gender	Female	1030 (80.40)	315 (62.0)
	Male	229 (17.88)	188 (37.0)
	Prefer not to say	22 (1.72)	5 (1.0)
Age	18-20	433 (33.80)	176 (34.58)
	21-22	626 (48.87)	213 (41.85)
	23-25	159 (12.41)	69 (13.55)
	>25	63 (4.92)	51(10.02)
University	Tuzla	676 (52.73)	165 (32.48)
	Sarajevo	331 (25.82)	102 (20.08)
	Banja Luka	49 (3.82)	131 (25.79)
	Mostar	226 (17.63)	110 (21.65)
Study year	I	320 (24.96)	155 (32.63)
	II	351(27.38)	133 (28.0)
	III	306 (23.87)	101 (21.26)
	IV	151 (11.78)	86 (18.11)
	V	137 (10.69)	-
	VI	17 (1.33)	-
Have you been vaccinated against COVID-19?	Yes	601 (54.88)	157 (38.57)
	No	494 (45.12)	250 (61.43)

they were vaccinated. In the group of medical faculties, 601 students (54.89%) were vaccinated out of the 1095 who answered this question. In the group of non-medical faculties, 157 students (38.57%) were vaccinated out of the 407 who answered this question. The vaccination rate in the group of students from medical faculties was significantly higher than the group of students from non-medical faculties (χ^2=31.58, P<0.001).

Overall, medical students showed a higher level of knowledge about vaccines in general and COVID-19 vaccines in particular (mean correct response: 6.74/10, 67.4%, SD=0.69) compared to non-medical students (4.82, 48.19%, SD=0.54) (t=112.99, P<0.001) (Table 2). In addition, a higher level of knowledge was found in vaccinated students compared to non-vaccinated students in medical (vaccinated 4.16, 41.6%, SD=0.11 *vs* non-vaccinated 2.58, 25.83%, SD=0.10; t=613.32, P<0.001) and in the non-medical group (vaccinated 2.60, 25.96%, SD=0.075 *vs* non-vaccinated 2.34, 23.37%, SD=0.13; t=53.49, P<0.001) respectively.

Students rated their attitudes about COVID-19 vaccination on a Likert scale, with numbers from 1 (representing the lowest degree of agreement) to 5 (representing the highest degree of agreement). Out of nine attitudes evaluated, students from the medical group of faculties had a statistically significant higher average level of agreement in seven attitudes compared to students from non-medical faculties. When it comes to attitude 3 (*The best way to carry out COVID-19 vaccination is allowing free choice to receive the vaccine or not*) both groups showed a high degree of agreement in the sense that a person has the free choice to receive the vaccine or not.

However, in this case, students from non-medical faculties expressed a statistically significant higher degree of agreement with this attitude compared to students from the medical group of faculties (Table 3). Similarly in attitude 7 (*I am worried about the long-term side effects of COVID-19 vaccines*), students from non-medical faculties expressed a statistically significant higher level of concern when it comes to the long-term

Table 2. Distribution of the Answers to the Questions That Assessed Students' Knowledge and Differences between Medical and Non-medical Students

Questions used to assess students' knowledge	Correct answer	Students		Total number	Chi square	P value
		Medical N (%)	Non-medical N (%)			
Smallpox and polio have been eliminated in Europe thanks to vaccination	Yes	929 (80.02)	235 (52.81)	1606	118.03	<0.001
	No	232 (19.98)	210 (47.19)			
Vaccines are only important for children	Yes	1034 (88.99)	335 (75.28)	1607	47.89	<0.001
	No	128 (11.01)	110 (24.72)			
Vaccines can protect me from diseases that are quite dangerous	Yes	1036 (89.16)	293 (65.70)	1608	123.71	<0.001
	No	126 (10.84)	153 (34.30)			
A larger part of the population needs to be vaccinated against a certain disease in order to prevent an outbreak of that disease	Yes	1019 (88.0)	273 (61.35)	1603	87.32	<0.001
	No	139 (12.0)	172 (38.65)			
The production and marketing of vaccines is a safe and controlled process	Yes	753 (64.80)	137 (30.79)	1607	149.30	<0.001
	No	409 (35.20)	308 (69.21)			
The vaccine against COVID-19 can cause infection with the corona virus	Yes	418 (35.88)	98 (21.97)	1611	28.02	<0.001
	No	747 (64.12)	348 (78.03)			
The vaccine against COVID-19 weakens human immunity	Yes	612 (52.58)	137 (30.79)	1609	60.56	<0.001
	No	552 (47.42)	308 (69.21)			
What type of vaccine is Pfizer vaccine?	Yes	887 (77.67)	282 (66.51)	1556	19.77	<0.001
	No	255 (22.33)	142 (33.49)			
What type of vaccine is Astra Zeneca vaccine?	Yes	624 (54.98)	202 (47.98)	1566	5.76	<0.05
	No	511 (45.02)	219 (52.02)			
What type of vaccine is Sinopharm vaccine?	Yes	435 (38.84)	107 (24.76)	1540	23.33	<0.001
	No	685 (61.16)	313 (75.24)			

side effects of COVID-19 vaccines (Table 3). What needs to be emphasized is the response of both examined groups of students in attitude 6 (*The rapid development of the COVID-19 vaccine contributes to the refusal or hesitation of the population regarding vaccination using this vaccine*). Both groups show an extremely high degree of agreement with this attitude, i. e. both groups believe that the rapid development of a vaccine contributes to refusal or hesitancy regarding the COVID-19 vaccine, although students from the medical group of faculties expressed a statistically significant higher degree of agreement with this attitude.

When the attitudes of vaccinated medical students were examined in comparison to unvaccinated students, it was found that vaccinated medical students evaluated six attitudes statistically significantly more positively, except attitudes 3, 6 and 7 where the non-vaccinated students showed a statistically significantly higher degree of agreement with the attitudes that students should have free choice to be vaccinated (Mann-Whitney U=86703.50, P<0.001), that the rapid development of the vaccine contributes to the hesitancy of the population regarding vaccination with this vaccine (Mann-Whitney U=135633.00, P<0.05) and the attitude related to concern due to the long-term side effects of COVID-19 vaccines (Mann-Whitney U=86819.50, P<0.001).

When the attitudes of vaccinated non-medical students were examined in comparison to unvaccinated students, it was found that vaccinated non-medical students evaluated six attitudes statistically significantly more positively, except attitudes 3 and 7 where non-vaccinated students showed a statistically significantly higher degree of agreement with the attitude that students should have the free choice to get vaccinated (Mann-Whitney

Table 3. Descriptive Statistics in Attitudes regarding the Covid-19 Vaccination and Differences in Attitudes between Medical and Non-medical Students

Student's attitudes	Students	Mean	SD*	Median	IQR†	Mann -Whitney U	P value
The vaccine against COVID-19 is safe	Medical	3.17	1.11	3	1	182304.50	<0.001
	Non-medical	2.76	1.31	3	2		
The COVID-19 vaccine is effective	Medical	3.19	1.08	3	1	181798.00	<0.001
	Non-medical	2.78	1.23	3	2		
The best way to carry out COVID-19 vaccination is to choose freely whether to receive the vaccine or not	Medical	3.57	1.36	4	2	186552.00	<0.001
	Non-medical	3.95	1.33	5	2		
The best way to implement COVID-19 vaccination is the mandatory vaccination of the entire population	Medical	2.87	1.42	3	2	171293.50	<0.001
	Non-medical	2.27	1.36	2	2		
The best way to carry out COVID-19 vaccination is mandatory vaccination of certain groups of people (health workers, people with chronic diseases, people over 60 years old, etc.)	Medical	3.22	1.35	3	2	197082.00	<0.001
	Non-medical	2.92	1.38	3	2		
The rapid development of the COVID-19 vaccine contributes to the refusal or hesitation of the population regarding vaccination with this vaccine	Medical	3.85	1.19	4	2	198670.50	<0.001
	Non-medical	3.59	1.29	4	2		
I am concerned about the long-term side effects of the COVID-19 vaccines	Medical	3.26	1.31	3	2	204286.00	<0.01
	Non-medical	3.45	1.39	4	3		
Mass vaccination of COVID-19 may lead to the end of the pandemic	Medical	3.38	1.25	3	1	177607.50	<0.001
	Non-medical	2.9	1.35	3	2		
The COVID-19 vaccine will bring life back to pre-pandemic levels	Medical	3.09	1.24	3	2	184599.50	<0.001
	Non-medical	2.69	1.30	3	2		

*Standard deviation; †Interquartile range.

U=12678.50, P<0.001) and with the attitude related to concern about the long-term side effects of the COVID-19 vaccine (Mann-Whitney U=12237.50, P<0.001). Vaccinated and non-vaccinated students at non-medical faculties showed an equally high level degree of agreement with attitude six (that the rapid development of the vaccine contributes to the hesitancy of the population regarding vaccination against COVID-19).

During the survey, students who were not vaccinated answered the question about the reasons for not being vaccinated. Only 9.54% had contraindications for vaccination and the others were hesitant (29.84%) or did not want to receive the vaccine (60.62%). Students rated the contribution of the reasons for reluctance/refusal to receive the vaccine on a Likert scale from 1 (representing the least degree of agreement) to 5 (representing the highest degree of agreement). Twelve questions, that is, the reasons why students were hesitant to receive the vaccine, were divided into two groups, medically based (reasons 1-4, reasons 6 and 7) and medically unfounded reasons (reason 5, reasons 8-12) (Table 4).

As can be seen from Table 4, the reasons that most contribute to students' reluctance/refusal to receive the COVID-19 vaccine are the first four reasons, which can be considered medically based (reason 1: *the COVID-19 vaccine is not safe due to its rapid development;* reason 2: *the COVID-19 vaccine can cause a fatal outcome*; reason 3: *the COVID-19 vaccine can cause long term genetic defects*; reason 4: the *adverse effects of the COVID-19 vaccine are not well known due to the relatively short time of administration of the vaccine*). In fact, reason 4, in both investigated groups, contributed the most to students' reluctance/refusal to receive the vaccine, and no statistically significant difference

Table 4. Descriptive Statistics on Reasons of Refusal/reluctance COVID-19 Vaccine and Differences in Reasons between Medical and Non-medical Students

Reason of refusal/reluctance	Students	Mean	SD*	Median	IQR†	Mann -Whitney U	P value
The COVID-19 vaccine is not safe due to its rapid development	Medical	3.50	1.12	3	1	44049.00	>0.05
	Non-medical	3.66	1.24	4	2		
The COVID-19 vaccine can cause death	Medical	3.29	1.11	3	1	39861.00	<0.001
	Non-medical	3.64	1.28	4	2		
The COVID-19 vaccine can cause long term genetic defects	Medical	3.33	1.01	3	1	38840.00	<0.001
	Non-medical	3.68	1.13	4	2		
The adverse effects of the COVID-19 vaccine are not well known due to the relatively short time of administration of the vaccine	Medical	3.89	1.11	4	2	47422.00	>0.05
	Non-medical	3.88	1.21	4	2		
Anti-vaxxer theories spreading on social media influence my attitudes towards the COVID-19 vaccine	Medical	2.35	1.20	2	2	47663.00	>0.05
	Non-medical	2.36	1.31	2	2		
The COVID-19 vaccine can cause autism	Medical	2.65	1.01	3	1	40129.50	<0.001
	Non-medical	2.98	1.10	3	0		
The COVID-19 vaccine can cause sterility	Medical	3.08	1.00	3	0	39347.00	<0.001
	Non-medical	3.41	1.02	3	1		
People around me told me not to get vaccinated against COVID-19	Medical	2.53	1.26	2	2	48017.50	>0.05
	Non-medical	2.52	1.33	2	2		
The COVID-19 pandemic was created in order for pharmaceutical companies to make huge profits from vaccines	Medical	3.02	1.16	3	2	36233.00	<0.001
	Non-medical	3.56	1.25	4	2		
The SARS COV-2 virus is a biological weapon	Medical	3.23	1.07	3	1	40273.50	<0.001
	Non-medical	3.56	1.09	3	2		
Authorities are fabricating death tolls and implementing vaccinations against COVID-19 to control the population	Medical	3.15	1.13	3	2	38187.50	<0.001
	Non-medical	3.56	1.17	3	2		
5G antennas are linked to the COVID-19 pandemic	Medical	2.17	1.21	2	2	43997.50	>0.05
	Non-medical	2.37	1.29	2	2		

*Standard deviation; †Interquartile range.

in the average values of students' answers was found regarding this reason.

Both medical and non-medical students showed the lowest degree of agreement with the medically unfounded reasons (reason 5: *Anti-vaxxer theories spreading on social media influence my attitudes towards the COVID-19 vaccine;* reason 8. *People around me told me not to get vaccinated against COVID-19;* and reason 12: *5G antennas are linked to the COVID-19 pandemic*). At the same time there was no statistically significant difference in the average values of their answers regarding these reasons.

The main sources of information about the COVID-19 vaccine were social media/ social networks (Figure 1). When the students were classified according to the type of study (medical, non-medical) and according to whether they had received the COVID-19 vaccine, social networks remained the main source of information in all subgroups. It was found that vaccinated students gave greater importance to the WHO as a source of information compared to non-vaccinated students in both groups of students (69.88% vaccinated students vs. 50.51% unvaccinated in the medical group; 67.52% vaccinated students vs. 41.60% unvaccinated in

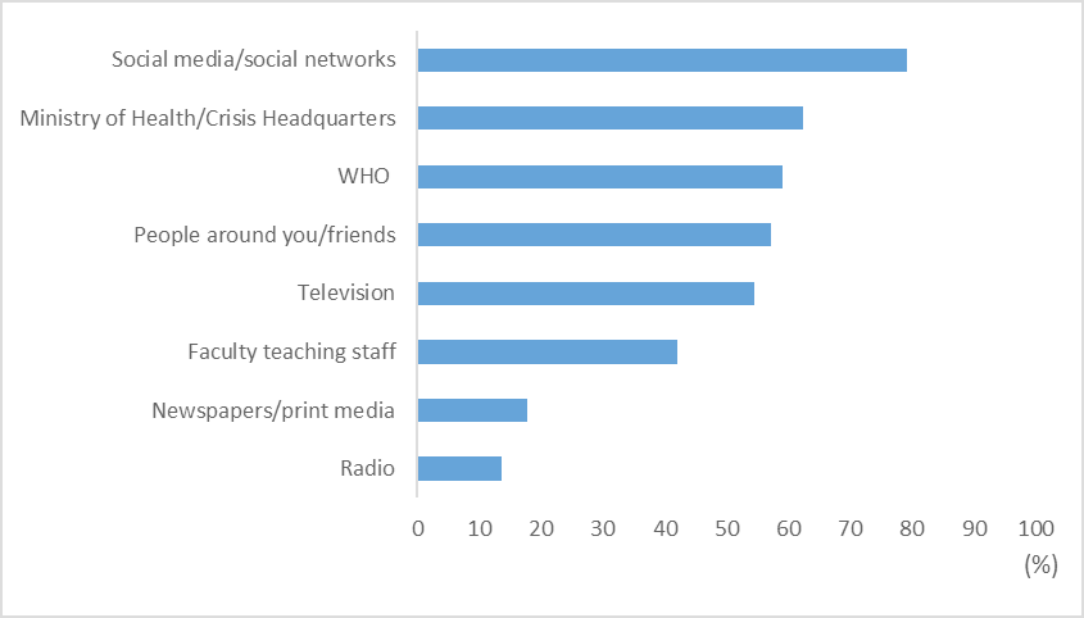

Figure 1. Sources of information about the COVID-19 vaccine.

the non-medical group). Also, vaccinated students gave greater importance to the WHO as a source of information compared to non-vaccinated students in both groups of students. Medical students gave more importance to teaching staff as a source of information than non-medical students, which is to be expected given the nature of the studies they attend (53.91% of vaccinated and 51.52% unvacinated medical students vs. 15.29% vaccinated and 14.80% unvaccinated non-medical students).

Discussion

This study analyzed the differences in knowledge and attitudes regarding COVID-19 vaccination between medical and non-medical students. Out of 1791 students surveyed, 83.86% answered the question about whether they had been vaccinated. The vaccination rate in the group of medical faculties was significantly higher compared to the group of students from non-medical faculties. Medical students showed a higher level of knowledge about vaccines in general and COVID-19 vaccines in particular compared to non-medical students, which was to be expected given the nature of the studies they attend. Furthermore, our study shows that a higher level of knowledge is positively reflected in a higher rate of vaccination, regardless of whether it is a question of medical or non-medical students. This result is consistent with the result showing vaccination rates among medical and non-medical students, according to which medical students have a higher vaccination rate compared to non-medical students. In addition, a higher level of knowledge was found amongst vaccinated students compared to non-vaccinated students in both the medical and non-medical groups. Research conducted in Bulgaria showed that medical students had a more positive attitude towards the COVID-19 vaccination, a higher rate of vaccination against COVID-19 and showed a higher rate of trust in information about the COVID-19 vaccines provided by the WHO and national health organizations, in relation to non-medical students (21). A study conducted in China on a student population showed that the approval rate of medical students towards the safety and effectiveness of the vaccine against COVID-19 was higher than that of non-medical students (22). A cross-sectional study conducted among the students at a tertiary care center in North India, showed that medical students had

sufficient knowledge, an optimistic attitude, and moderate levels of concern towards COVID-19. Vaccine hesitancy was much lower among medical students when compared to non-medical students (23). The Coronavirus Disease 2019 (COVID-19) pandemic has persisted despite reductions in disease severity, hospitalizations and deaths since the introduction of multiple vaccines that protect against COVID-19 and pharmaceuticals to treat its symptoms (24, 25). However, vaccine hesitancy and refusal continue to impede the effectiveness of these interventions (26, 27). Drivers of vaccine hesitancy include lower education, mistrust in science and governments (28-30), and misinformation (31, 32).

Medical students, as well as vaccinated students regardless of the course they are taking, showed more positive attitudes compared to non-medical students and unvaccinated students, regarding the safety and effectiveness of the COVID-19 vaccine, regarding the attitude that mass vaccination will lead to the end of the pandemic, and the attitude that COVID-19 vaccine will bring life back to pre-pandemic levels. However, both medical and non-medical students showed a high level of agreement with the attitude that the COVID-19 vaccination should be based on free choice. Both medical and non-medical students, regardless of whether they had been vaccinated, expressed a high degree of agreement with the attitude that the rapid development of COVID-19 vaccines contributes to vaccine refusal or hesitancy, and with the attitude related to concerns about possible long-term side effects of COVID-19 vaccines.

A survey of medical students at two universities in Egypt (N=2133) showed that the most frequently reported barriers to COVID-19 vaccination were insufficient information about the adverse effects of the vaccine (74.4%) and insufficient information about the vaccine itself (72.8%) (33). A study among medical students (N=167) in southeast Michigan found that 98% believed that COVID-19 vaccination was critical to reducing community spread. Although 98% of students believed they were most likely to be exposed to COVID-19, 23% said they would not receive a COVID-19 vaccine immediately after U.S. Food and Drugs Administration (FDA) approval. Medical students' concerns about the serious side effects of the COVID-19 vaccine were closely related to their confidence in the information they received regarding the COVID-19 vaccine (34). Vaccine hesitancy is a critical barrier to COVID-19 vaccine uptake in high-income countries or regions, where vaccine-specific factors associated with increased vaccine hesitancy have been found to lead to beliefs that the vaccine is not safe/effective, and increased concern about the rapid development of COVID-19 vaccines (35). Balan et al.'s survey conducted in Romania found that more than 88% of students expressed a favorable attitude towards COVID-19 vaccination (36). However, healthcare students in Romania who declined to receive the SARS-CoV-2 vaccine, cited the rapid development of the vaccine as the primary reason ($P<0.001$). According to Bagić et al.'s study conducted in Croatia, the primary reasons for vaccine hesitancy were concerns over the safety of SARS-CoV-2 vaccines (reported by 82% or 627/765 of participants), as well as a general lack of trust in vaccines (reported by 71% or 543/765 of participants) (37). Our research shows that the reasons given by the students for hesitation/refusal to receive the COVID-19 vaccine are similar to other research, i.e. distrust in the vaccine due to its rapid development, and a lack of information about side effects.

Extensive anti-vaccine content is frequently shared across social media (38-40). The existing evidence suggests that exposure to such content may directly influence vaccination opinions and drive up vaccine hesitancy (41). Betsch et al. and Nan et al. have demonstrated that exposure to vaccine-critical websites and blogs negatively impacts the intention to be vaccinated (42, 43). When it comes to our research, the leading source of information about the COVID-19 vaccine were social media (N=1390 or 79.16%) followed by the Ministry of Health (N=1095 or 62.36%), the WHO (N=59.00% or 1036), friends (N=57.18% or 1004) and television (N=54.50% or 957) (Figure 1). These results could have been expected having in mind

that the survey participants were young people who also are the main social media users. When the students were classified according to the type of study (medical, non-medical) and according to whether they had received the COVID-19 vaccine, social media/networks remained the main source of information in all subgroups. Our research did not identify the link between social media and the decline in COVID-19 vaccine coverage that was reported by Marinos et al. (44) The authors found that respondents who received information on COVID-19 vaccines from social media had lower COVID-19 vaccine coverage. Riad et al. (45) also found that higher dependence on media and social media platforms was significantly associated with lower COVID-19 vaccine acceptance ($P<0.01$).

Conclusion

This study examined the knowledge and attitudes of medical and non-medical university students regarding COVID-19 vaccination in Bosnia and Herzegovina. The results showed that medical students had a significantly higher vaccination rate and better knowledge about vaccines, including COVID-19 vaccines, than non-medical students. In terms of attitudes, medical students had a statistically significant higher level of agreement in seven out of nine attitudes compared to non-medical students. Both groups showed a high degree of agreement that the rapid development of the COVID-19 vaccine contributes to hesitancy regarding vaccination, although medical students expressed a statistically significantly higher degree of agreement with this attitude. Concerns about the long-term side effects of COVID-19 vaccines were also expressed, particularly by non-medical students. The main reasons for not being vaccinated were hesitation and a lack of willingness to receive the vaccine. The study provides unique insights into the factors influencing vaccination decisions among university students in Bosnia and Herzegovina, highlighting the need for targeted educational interventions to increase vaccine uptake.

What Is Already Known on This Topic:

Previous studies in several countries have found that vaccine hesitancy remains a concern despite the high rate of a declared positive attitude towards COVID-19 vaccination. Some of the factors that have been identified as contributing to vaccine hesitancy among students include concerns over vaccine safety, distrust of the healthcare system, and information circulating on social media. Additionally, research has indicated that medical students tend to have a better understanding of vaccine efficacy and safety, as well as a higher likelihood of getting vaccinated, compared to non-medical students.

What This Study Adds:

The study provides novel insights into the trends and beliefs regarding vaccination among students, specifically in the context of COVID-19 vaccination in Bosnia and Herzegovina. The results highlight that medical students have a significantly higher vaccination rate compared to non-medical students, and also have a higher level of knowledge and more positive attitudes towards COVID-19 vaccination. The study also revealed that both groups of students share a concern regarding the rapid development of the COVID-19 vaccine, which contributes to vaccine hesitancy. Additionally, societal and cultural factors, such as personal beliefs, mistrust in authorities, and misinformation play a role in vaccine hesitancy among the surveyed students. Overall, the study sheds light on the need to address these factors in order to promote vaccination uptake and mitigate the negative impact of vaccine hesitancy on public health.

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Family Physicians' Perceptions of Primary Health Care Use in Bosnia and Herzegovina during the Covid-19 Pandemic, a Cross-sectional Study

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Abstract

Objectives. The main objective of this paper was to examine the perceptions of family physicians on the use of primary health care in Bosnia and Herzegovina during the COVID-19 pandemic. **Materials and Methods.** A cross-sectional study was conducted using a short online questionnaire that was sent to primary care physicians in Bosnia and Herzegovina from April 20th, 2022, to May 20th, 2022. **Results.** The research sample consisted of 231 doctors of primary health care from Bosnia and Herzegovina, with an average age of 45 years and 85% women. About 70% of participants reported having COVID-19 at least once from March 2020 to March 2022. Participants had an average of 1,986 registered patients and approximately 50 encounters per day. The study revealed a high degree of reliability between test-retest measurements, with a single measure Intraclass correlation coefficient of 0.801, and internal consistency determined using Cronbach's alpha of 0.89. Participants reported that the following health services were most affected during the COVID-19 pandemic: care for patients with chronic diseases, home visits, navigating the health system with patients making appointments with specialists, cancer screening, and preventive health services. The study also found statistically significant perceived differences in the use of these health services based on age, gender, postgraduate education in family medicine, participation in COVID-19 clinics, and personal history of Covid-19. **Conclusion.** There were significant disturbances to the use of primary health care during the COVID-19 pandemic. Future research could investigate patient outcomes compared to family physician perceptions.

Key Words: COVID-19 ▪ Primary Health Care Utilization ▪ Routine Care Disruption ▪ Bosnia and Herzegovina.

Introduction

The World Health Organization declared coronavirus disease (COVID-19) a global pandemic over two years ago. After the first COVID-19 cases in Bosnia and Herzegovina (BiH), on March 5th, 2020, the government announced the implementation of preventive measures on March 16, 2020, with several key restrictions (e.g., closure of school institutions, universities, cafes, bars, and restaurants; public and city transport ban; and comprehensive patient care reorganization in the public health system) (1). All European countries imposed similar restrictions, including curfews (2).

Primary care is where most health care takes place, and where most people have trusted health-related relationships, making its physicians the 'eyes and ears' of the health system. Primary care is frequently at the mercy of the government's policies and laws resulting in job losses and difficulty in providing patient care. Clinicians were relocated to COVID-19-only clinics or other specialist departments, because of primary care workforce adjustments. Because of primary care's generalist nature, physicians were directed to wherever they were required, with little control over daily scheduling decisions (3). Furthermore, clinicians were subjected to additional stress and uncertainty when they were required to work double shifts to fill in for nursing staff shortages or other physicians placed under lockdown protocols, or

who were required to quarantine after being exposed or infected. Health Care workers declared that they felt anxious during the pandemic. Most of them declared they were upset due to routine changes, and significantly affected by the Covid-19 pandemic (4). Globally, the pandemic created significant disruptions to healthcare systems. Several countries' healthcare systems encountered major staffing issues, reflecting both an increase in demand and a loss in workers (5).

Healthcare workers were reassigned, volunteers were sought, and non-emergency healthcare services were reduced or eliminated in response. Teleconsultations and remote monitoring are now available at several hospitals in Hong Kong, Singapore, South Korea, Norway, and the United Kingdom, allowing patients to receive care without having to travel. Furthermore, shortages of personal protective equipment have forced medical personnel to work without proper protection in some nations (6).

As a respond to the COVID-19 pandemic, all outpatient clinics were closed, and only emergency cases and refills of medications were treated. Patients' fears that they might get Covid-19 or pass it on, as well as their worries about breaking the lockdown rules, led to significant changes in family medicine practices during this time compared to the same time in 2019. In response to the COVID-19 epidemic, all family medicine clinics were halted, and only urgent care visits and prescription refills were processed (7). As a result of giving priority to the provision of acute and urgent treatment, other services, most notably the management of chronic diseases and preventative care, were disrupted. This was especially problematic in light of the fact that the situation was deemed an emergency (8).

The COVID-19 epidemic globally altered healthcare needs and requests at all levels of the organization's healthcare system, including primary health care and its family medicine concept of healthcare. This concept of patient treatment was shattered during the pandemic, and family medicine teams were preoccupied with the treatment of COVID-19 patients and their post-COVID treatment, as opposed to the treatment of typical patient requests (the most common diseases, especially in elderly patients with existing chronic diseases, mental illness, and malignant diseases) (9).

A recent study in Sarajevo Canton found that all of family medicine's principles have been adversely affected. Regular access to health care was jeopardized for three primary reasons: difficult access for patients to family medicine clinics as a result of the crisis headquarters' decision and lockdowns, decreased physician and nurse staffing as a result of at least 20% – 30% of physicians and nurses working in COVID-19 outpatient clinics and approximately 10% on sick leave at all times due to COVID-19, and extremely difficult telephone access to family physicians as a result of the same lines being used by COVID-19 patients (10).

The set aim of our research was to examine the perceptions of family physicians on the use of primary health care in BiH during the COVID-19 pandemic and to find differences between family medicine perceptions of use in relation to participants' level of postgraduate education, location of medical practice, COVID-19 infection status, number of registered patients, the average number of patient encounters, and the amount of time spent away from their primary medical practice.

Methods

A cross-sectional study design was used. All members of the research team reviewed and revised the survey questions to ensure clarity. The final web-based survey included 12 demographic-based questions and 10 statements that aligned with the study objectives.

The research used a questionnaire that was constructed through a focus group of 7 doctors of family medicine who are involved in research, in order to collect data to examine the perceptions of family physicians on the use of primary health care in BiH during the COVID-19 pandemic and to find differences between family medicine perceptions of use in relation to participants' level of postgraduate education, location of medical practice, COVID-19 infection status, number of registered

patients, the average number of patient encounters, and the amount of time spent away from their primary medical practice. The questionnaire was finalized after ambiguous and unsuitable questions were modified based on the comments of four independent family medicine physicians.

The final survey was pilot tested with multiple healthcare professionals who were not involved in the initial creation of the surveys. The same group of 20 physicians repeated the survey after ten days for test-retest validity purposes. Approval was provided by the Scientific Teaching Council of the Faculty of Medicine at the University of Sarajevo. All study activities were conducted in accordance with the Declaration of Helsinki.

An online survey was distributed to email addresses of family physicians working in primary care clinics in BiH. The respondents were required to choose whether the pandemic: did not disturb, caused minimal disturbance, caused moderate disturbance, caused much disturbance, or caused maximal disturbance. A convenience sampling technique was used. The study population included physicians working in family medicine who were able to complete a web-based survey in Bosnian, and self-reported as being employed within a Primary Healthcare Centre in Bosnia and Herzegovina during the period from March 2020 to March 2022. Healthcare professionals who did not work in that period were excluded. Two types of physicians can work in family medicine in Bosnia and Hercegovina: medical doctors with or without postgraduate education in the field. Bosnia and Herzegovina offers two options for postgraduate training in family medicine: a one-year programme of additional training in the field and a four-year family medicine specialty programme (11). Post-graduate education is organized according to the a WONCA global standards for postgraduate family medicine education (12). Initially, 400 physicians were contacted by email, and snowball recruiting was encouraged. The publicly available contact information of the medical practices was also used. Of those contacted, 169 declined to participate in the study due to lack of interest or time or did not respond after initial contact. Consent was provided by all participants before starting the survey. Physicians with additional training in family medicine were grouped with family medicine specialists during the analysis. Primary Healthcare Providers were sent an invitation to participate in the web-based survey through recruitment emails. The web-based survey was open for 30 days from April 20, 2022, until May 20, 2022. No recruitment incentive was used.

Statistical Analysis

Frequencies and proportions were used to present participant demographic data. The participant responses for the 5-point Likert scales were converted into a number-based system where (1=No disturbance), (2=Minimal disturbance), (3=Moderate disturbance), (4=Significant disturbance), and (5=Maximal disturbance). Using graphical methods (histograms) and the Shapiro-Wilk test, a normal distribution of variables was determined. Since the data was not normally distributed, nonparametric tests were used. The Mann-Whitney U test was employed to assess whether t a statistically significant difference existed in the dependent variable for independent variables. Dependent variables were part of the health care utilisation scale (14 questions): Routine medical care, Management of patients with chronic disease, Management of patients with acute disease (not including COVID), Patients' ability to get in contact with their family doctor, Patients' ability to visit their primary healthcare provider, Home visits, Patients' ability to receive their medications on time, Arranging specialist appointments, Preventative health services, Cancer screening, Navigating the healthcare system with the patient, Patients' ability to contact the family medicine office by telephone, Staff Cohesion and Overall functioning of the family medicine office. Independent variables were Age, Gender, Postgraduate training in family medicine, Work Experience, Location of practice, Number of registered patients, Work in COVOD-19 clinics, and Personal history of COVID-19. A P-value less than 0.05 was considered statistically significant.

The questionnaire's reliability was evaluated utilizing internal consistency and test-retest reliability. Typically, internal consistency is measured with an alpha coefficient (Cronbach's alpha), which reveals the degree to which questionnaire items are interrelated or whether they measure the same construct consistently. Cronbach's alpha coefficient was deemed acceptable (values between 0.6 and 0.7) and exhibited high internal consistency (values between 0.7 and 0.9) (13, 14). A test-retest reliability analysis is conducted to ascertain the questionnaire's consistency in measuring subject performance. Since standard limits for test-retest reliability have not been clearly defined, all conclusions should be made with caution. We considered Intraclass Correlation Coefficient (ICC) <0.5 to be poor, 0.50 to 0.75 to be moderate, 0.75 to 0.90 to be acceptable, and >0.90 to be excellent (15, 16). We also calculated Spearman's rank-order correlation (rs) to measure test-retest reliability. The statistical analyses were performed using IBM SPSS Statistics V25.

Table 1. Demographic Data of Study Participants (N=231)

Demographic	N (%)
Gender	
Female	196 (85.3)
Male	35 (14.7)
Age ($\bar{x}$±SD)	44.9±10.2
Education	
Medical Doctors	64 (27.7)
Postgraduate education in Family Medicine*	167 (72.3)
Work Experience ($\bar{x}$±SD)	15.6±10.1
Number of registered patients ($\bar{x}$±SD)	1986.4±511
Average daily encounters from March 2020 - March 2022 ($\bar{x}$±SD)	50.5±21.4
Participants reported having covid at least once	161 (69.7)
Time Spent Away from Primary Office because of work in the COVID-19 clinic	
Did not work in COVID clinics	34 (14.7)
Up to 30% time	110 (47.6)
≥30% time	87 (37.7)

*Program of additional training in family medicine (N=11; 4,8%) and family medicine specialization (N=156; 67.5%).

Results

The study sample consisted of 231 primary health care doctors: 64 medical doctors, and 167 family medicine physicians with postgraduate education in family medicine from all over BiH. The response rate was 58%. The average age of the respondents was 45 years old and approximately 85% were women. All participants worked in primary care and had a mean professional work experience of 16 years. In total 81% worked in urban locations and 98% worked in the public sector. About 88% of respondents claim to have registered patients and of those, they had an average of 1986 registered patients. During that same period, the respondents averaged about 50 encounters per day.

Roughly 70% of the participants reported having COVID-19 at least once from March 2020 to March 2022. Up to 34% claimed to have had COVID-19 at least twice. About 15% spent no time away from their primary office, whereas almost half spent up to a third of their time away from their primary office. Regarding having registered patients, only 10% out of the all participants do not have registered patients. Furthermore, 49% had <1900 patients, and 41% had >1900 patients. About half of the participants had roughly 50 patient encounters per day (Table 1).

According to reports from the Public Health Institutes, there are 1,744 physicians in family medicine in Bosnia and Herzegovina. Out of the total number, 746 (42.8%) were family medicine specialists, while the remaining physicians were medical doctors without postgraduate training in the discipline. The average age of specialists in family medicine was 49.6 (±12.7), and 82.6% were female (16, 17).

Reliability

Internal consistency was determined using Cronbach's test α=0.89 which is considered as a good internal consistency. There was not a question that needed to be delayed to improve Crombach's α (Table 2).

Twenty participants of the total sample were asked to complete the questionnaire twice with 7-10 day intervals to assess the test–retest

Table 2. Questionnaire Item-Total Statistics

Question	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item-Total Correlation	Squared Multiple Correlation	Cronbach's Alpha if Item Deleted
Routine medical care	43.63	85.273	0.650	0.676	0.877
Management of patients with chronic disease	43.57	84.796	0.729	0.719	0.874
Management of patients with acute disease (not including COVID)	44.40	83.288	0.615	0.501	0.879
Patients' ability to get in contact with their family doctor	44.32	82.176	0.712	0.647	0.873
Patients' ability to visit their primary healthcare provider	44.15	82.863	0.642	0.557	0.877
Home visits	43.33	85.682	0.527	0.436	0.883
Patients' ability to receive their medications on time	45.21	87.286	0.510	0.462	0.883
Arranging specialist appointments	42.97	91.379	0.444	0.350	0.886
Preventative health services	42.77	92.065	0.375	0.515	0.888
Cancer screening	42.86	89.661	0.492	0.506	0.884
Navigating healthcare system with patient	43.16	90.127	0.553	0.456	0.882
Patients' ability to contact the family medicine office by telephone	44.60	83.559	0.579	0.518	0.881
Staff Cohesion	44.24	89.722	0.441	0.248	0.886
Overall functioning of family medicine office	44.04	85.858	0.684	0.511	0.876

Table 3. Intraclass Correlation Coefficient

Measures	Intraclass correlation[a]	95% Confidence interval		F Test with True value 0			
		Lower bound	Upper bound	Value	df1	df2	Sig
Single	0.801[b]	0.563	0.916	9.027	19	19	0.000
Average	0.889	0.720	0.956	9.027	19	19	0.000

Two-way random effects model where both people effects and measures effects are random: a. Type C intraclass correlation coefficients using a consistency definition. The between-measure variance is excluded from the denominator variance, b. The estimator is the same, whether the interaction effect is present or not.

reliability. Mean age of test-retest responders was 49.55 (±7.94), and 17 (85%) of them were women. A high degree of reliability was found between test-retest measurements. The single measure ICC was 0.801 with a 95% confidence interval from 0.563 to 0.916 ($F(19,19)= 9.027$, $P<0.01$). Table 3 Correlation between two tests was considered good. ($r_s =0.790$, $P<0.01$).

The mean response of the overall use of primary healthcare is shown in Table 4. A patient's ability to receive their medication on time was the least affected with an average response of minimal disruption. However, participants found that arranging specialist appointments, cancer screening, and preventative health services proved to be the most affected.

Table 4. Participants' Perceptions of Primary Health Care Services Use during the Covid-19 Pandemic

Primary Health Care Service	1* N (%)	2† N (%)	3‡ N (%)	4§, N (%)	5‖ N (%)	(x̄±SD)
Patients' ability to receive their medications on time	114 (49.4)	49 (21.2)	40 (17.3)	21 (9.1)	7 (3)	1.97±1.16
Patients' ability to contact the family medicine office by telephone	76 (32.9)	35 (15.2)	48 (20.8)	54 (23.4)	17 (7.4)	2.57±1.35
Management of patients with acute disease (Excluding COVID)	52 (22.5)	48 (20.8)	55 (23.8)	53 (22.9)	23 (10)	2.78±1.31
Patients' ability to get in contact with their family doctor	41 (17.7)	47 (20.3)	74 (32.0)	44 (19)	25 (10.8)	2.86±1.24
Staff Cohesion	33 (14.3)	24 (10.4)	113 (48.9)	47 (20.3)	14 (6.1)	2.94 ±1.06
Patients' ability to visit their primary healthcare provider	34 (14.7)	51 (22.1)	58 (25.1)	51 (22.1)	37 (16)	3.03±1.30
Overall functioning of family medicine office	15 (6.5)	38 (16.5)	100 (43.3)	57 (24.7)	21 (9.1)	3.13±1.01
Routine medical care	15 (6.5)	15 (6.5)	81 (35.1)	70 (30.3)	50 (21.6)	3.55±1.10
Management of patients with chronic disease	7 (3.0)	25 (10.8)	69 (29.9)	82 (35.5)	48 (20.8)	3.61±1.03
Home visits	18 (7.8)	20 (8.7)	41 (17.7)	55 (23.8)	97 (42)	3.84±1.27
Navigating healthcare system with patient	1 (0.4)	7 (3.0)	53 (22.9)	97 (42.0)	73 (31.6)	4.01±0.84
Arranging specialist appointments	1 (0.4)	11 (4.8)	34 (14.7)	79 (34.2)	106 (45.9)	4.21±0.89
Cancer screening	4 (1.7)	11 (4.8)	28 (12.1)	54 (23.4)	134 (58)	4.32±0.97
Preventative health services	5 (2.2)	9 (3.9)	19 (8.2)	56 (24.2)	142 (61.5)	4.41±0.94

*No disturbance; †Minimal disturbance; ‡Moderate disturbance; §Significant disturbance; ‖Maximal disturbance.

Age

Participants under the average age of 45 considered that COVID-19 significantly negatively affected the patients' ability to contact the family medicine office by telephone (U=5640.000, P<0.05) and the patients' ability to get in contact with their family doctor (U=5383.000, P<0.01). While participants above the average age were more likely to perceive that Staff Cohesion (U=5510.500, P<0.05) and Preventive health services were significantly disrupted (U=5667.500, P<0.05) (Table 5).

Gender

The results indicated that women had the perception that the COVID-19 pandemic significantly disrupted Cancer screening (U=2351.50, P<0.01) and Preventative health services (U=2305.00, P<0.01) (Table 6).

Table 5. Participants' Age Differences in Perception of Primary Health Care Services

Primary Health Care Service	Age		Mann-Whitney U	P
	<45	≥45		
	(N=106)	(N=125)		
Patients' ability to contact the family medicine office by telephone	125.51	106.94	5640.000	0.046
Patients' ability to get in contact with their family doctor	125.29	108.12	5383.000	0.005
Staff Cohesion	106.97	123.66	5510.500	0.029
Preventative health services	104.28	125.94	5667.500	0.043

Table 6. Gender Differences in Perception of Primary Health Care Services Use

Primary Health Care Service	Gender		Mann-Whitney U	P
	Women	Men		
	(N=197)	(N=34)		
Cancer screening	121.30	85.29	2351.50	0.001
Preventative health services	121.06	86.66	2305.00	0.001

Postgraduate Education

The results indicated that medical doctors had a significantly higher perception that the COVID-19 pandemic has impeded the patients' ability to contact the family medicine office by telephone (U=4231.500, P<0.05) than family physicians. In contrast, family physicians perceived that home visits (U=4455.500, P<0.05), cancer screening (U=4342.000, P<0.05), and preventative health services were significantly more disrupted (U=4458.000, P<0.05) (Table 7).

Participation in Covid-clinics Work

Participants who spent less than 30% of their time away from their workplaces working at COVIDid-19 clinics were more likely to assume that the pandemic i significantly mpacted the following services: Patients' ability to contact the family medicine office by telephone U=3995.500, P<0.05), Patients' ability to get in contact with their family doctor (U=3865.000, P<0.05), Overall functioning of the family medicine office (U=4085.500, P<0.05) and Cancer screening (U=3956.500, P=0.05)(Table 8).

Table 7. Differences in Perception of Primary Health Care Services Use between Participants with Postgraduate Education (Family Physicians) and Medical Doctors

Primary Health Care Service	Level of Education		Mann-Whitney U	P
	MD*	FP†		
	(N=64)	(N=167)		
Patients' ability to contact the family medicine office by telephone	133.38	109.34	4231.500	0.012
Home visits	102.12	121.32	4455.500	0.040
Cancer screening	102.16	121.31	4342.000	0.011
Preventative health services	100.34	122.00	4458.000	0.028

*Medical doctor (no postgraduate education in the field); †Family physician (postgraduate education in the field).

Table 8. Differences in Perception of Primary Health Care Services Use between Participants who worked up to 30% and ≥30% Working Time Spent in Covid-19 Clinics

Primary Health Care Service	Work in Covid19 clinics*		Mann-Whitney U	P
	<30% of time	≥30% of time		
	(N=110)	(N=87)		
Patients' ability to contact the family medicine office by telephone	106.178	89.93	3995.500	0.038
Patients' ability to get in contact with their family doctor	107.36	88.46	3865.000	0.018
Overall functioning of the family medicine office	106.53	89.48	4085.500	0.046
Cancer screening	105.36	90.96	3956.500	0.027

*The statistical analysis excluded 34 participants, which accounted for 14.7% of the total sample, due to their non-involvement in Covid-19 clinics.

Personal History of Covid-19 Illness

Participants with a personal history of COVID-19 illness considered that the pandemic had a significant effect on the following variables: Management of patients with acute disease (Excluding COVID) (U=4085.500, P<0.05), Patients' ability to get in contact with their family doctor (U=4085.500, P<0.05), Staff Cohesion (U=4085.500, P<0.05), Patients' ability to visit their primary healthcare provider (U=4085.500, P<0.05)(Table 9).

The study found no statistically significant differences in the dependent variables in relation to the rural and urban locations of participants or among participants with 1900 or more registered patients and those with fewer than 1900 registered patients. The cut-off value 1900 was chosen to correspond to the median number of self-reported registered patients in this study.

Discussion

Our study revealed that family physicians working in BiH perceived there to be disruptions in all aspects of primary healthcare use caused by the COVID-19 pandemic especially in services such as arranging specialist appointments, cancer screening, and preventative health services. This highlights the challenges faced by primary health care doctors in providing comprehensive care during the pandemic. Similarly, to our results, research conducted across the globe revealed that the COVID-19 pandemic has disrupted the use of primary healthcare in many ways. The findings of research conducted in Sweden (18), China (19), and Iran (20) indicate that the COVID-19 pandemic has resulted in a significant decrease in the quantity of services provided by primary health care facilities. According to their findings, there was a substantial drop in the total number of outpatient visits.

The results of a qualitative study among primary care practitioners in Belgium showed that respondents' perceptions of the impact of the COVID-19 pandemic on primary care was similar to those shown in our research. All participating practices reported drastic changes in organization with a collective shift to care for COVID-19, a reduction in chronic care activities and fewer consultations in primary health care (21).The perspectives of health workers in primary health care on the influence of the pandemic on comprehensive health care were investigated as part of the cross-sectional PRICOV-19 study, which was carried out in 38 different countries. The findings of this study similar to our results, indicate that healthcare professionals working in PHC were constrained in their ability to provide high-quality care, the possibility of home treatment and home visits, consultations with urgent acute care facilities or the prescription of medications, and that this circumstance put the comprehensive approach of PHC in jeopardy (22).

Our results showed that the participants felt that a patient's ability to receive their medications on time had the least disruptions. On the contrary, participants perceived cancer screening and preventive services to have been the most disrupted. In Bosnia and Herzegovina, there is an opportunistic screening available for breast and cervical

Table 9. Differences in Perception of Primary Health Care Services Use between Participants with a Personal History of Covid-19 Illness and Those Which Not Had Covid-19

Use of Primary Health Care	Personal history of Covid-19		Mann-Whitney U	P
	No	Yes		
	(N=70)	(N=161)		
Management of patients with acute disease (Excluding COVID)	99.28	123.27	4464.500	0.010
Patients' ability to get in contact with their family doctor	96.86	124.32	4295.500	0.003
Staff Cohesion	99.68	123.10	4492.500	0.009
Patients' ability to visit their primary healthcare provider	98.72	123.51	4425.500	0.008

cancer, and guidelines have been issued for the early detection of childhood cancers. However, it is still lacking routine cancer screening programmes and general cancer management guidelines, but this does not diminish the importance of this area of activity and the role of family physicians in promoting and referring to their implementation (23). A study in the Netherlands found that during the first three months of the epidemic, the number of cancer cases was about 75% of what they usually are. The national breast, colorectal, and cervical cancer screening programs have been suspended temporarily to reduce the burden on the healthcare system caused by COVID-19. In the Netherlands, it's possible that about 5,000 new cancers haven't been found (yet) because care has been put off. If the number of cancer cases in the Netherlands is the same as in Europe, 245,000 new cancers are not diagnosed (24).

Based on the level of postgraduate training, there were statistically significant differences in the perceptions of primary healthcare use. The participants with postgraduate training in family medicine in our study perceived there to be greater disruptions in preventative health services, cancer screening, and home visits. Similarly to our study, the study in the United States found that the actual problem was the long-term repercussions of failing to recognize, prevent, and treat illnesses like diabetes and hyperlipidaemia-related heart risks (25). In Canada and South Africa, all cancer and cardiovascular disease screenings have been discontinued. Home visits and care have ceased, and there were decreased number of chronic care for non-communicable diseases (3).

The location of the family medicine practice was divided into rural and urban. There were no statistically significant differences in the perceptions of primary healthcare use based on the location of the practice. Opposite to our results, the study on physicians in rural Germany found that family physicians stated that they did not believe their patients would suffer any health consequences because of the pandemic. Practice organization and healthcare delivery changed quickly. Telephone, home, and practice window consultations increased. Family physicians developed personal relationships to promote healthcare and prevent health problems (26).

Similar to the findings of our study, general practitioners in the Netherlands shifted from face-to-face to telephone contact for chronic respiratory disease care during the COVID-19 pandemic. In family medicine practices, the proportion of face-to-face contacts decreased substantially, while the proportion of telephone contacts increased significantly (27, 28). During the pandemic, family physicians in Croatia encountered changes in work organisation and an increase in workload. Due to the increase in virtual contacts and telephone consultations, the workload has increased despite the reduction in face-to-face consultation time (29).

A like our results, a study in Greece found that hospital service rationalization slowed diagnostic testing and review of referred patients; referring patients was difficult for general practitioners who frequently failed (30). Furthermore, international reviews found that primary care remained the first point of contact, with telemedicine being used to handle acute non-COVID and COVID-related presentations that did not necessitate in-person management (8, 31).

In our study, participants who did not have COVID-19 perceived there to be fewer disruptions in the management of acute diseases (excluding COVID-19), their patients' ability to get in contact with their family doctor, staff cohesion, and their patients' ability to visit the primary healthcare provider. Researchers in Türkiye, looking into the experiences of family physicians who were infected, found that family physicians' social relationships with their co-workers had eroded because of the lack of safety in their work environment due to the high risk of infection, making them feel increasingly lonely at work (32). Since many primary care doctors were asked to fill different roles away from their primary practice, the participants were asked how much time they spent away from their primary office.

Similar to our results, the multinational study found this to be the case around the world, and some clinicians in Italy took up the tasks of various types of primary care clinicians, such as nursing

procedures from home health care personnel who were no longer able to work in-house due to governmental restrictions, while others shifted departments to handle COVID-19 patients. In Bosnia and Herzegovina COVID-19 clinics and call centres with family medicine staff were established. More than 20% of all family physicians and nurses worked in these facilities (3).

Limitation of this Study

The major limitation of this study is the presence of recall bias since the pandemic began over two years ago, so there may be errors caused by inaccurate or incomplete recollections from study participants regarding their experiences from March 2020 to March 2022. Another limitation is the use of Likert scales since two respondents may give the same value having had different experiences. Another limitation of the Likert scale is that respondents tend to agree with the statements shown, which is known as acquiescence bias. The study is also limited by the fact that perception is very subjective, and therefore our results are not able to be standardized and generalized.

Conclusion

This study brought light to the perceptions of family physicians in BiH on the use of primary health care during the pandemic. The use of primary health care was perceived to be disrupted, especially the preventative services, cancer screening, the patient's ability to get in contact with their family doctor, home visits, management of patients with chronic disease, arranging specialist appointments, patient's ability to contact the family medicine office by telephone, the management of patients with acute disease (excluding COVID-19), patients ability to visit their primary healthcare provider, and staff cohesion. Further research into the negative perceptions is needed to investigate which other factors contribute to those perceptions and if they can be improved upon, so that family physicians can enhance the quality of patient care and be better prepared for the next pandemic.

What Is Already Known On this Topic:
It is now well known that the COVID-19 pandemic created massive disruptions to the provision and quality of primary health care around the world while causing a great amount of stress for family physicians. It is also known that family physicians have been on the front lines of the pandemic and have adjusted their practices in many ways throughout the pandemic.

What This Study Adds:
This study brings light to family physicians' perceptions toward the use of primary health care. It shows that certain factors have a role in how family physicians perceive different aspects of the use of primary health care, while other factors do not have an impact on their perceptions.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Narrative Review Article
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P53/MDM2 Complex-Based Targeted Strategies in Colon Adenocarcinoma

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Abstract
In the current molecular review, we describe the mechanisms of *TP53/MDM2* deregulation and their impact on the colon adenocarcinoma molecular substrate and phenotype. Among the genes that are critically altered in carcinogenesis, the *TP53* tumor suppressor gene is of major importance. The *TP53* gene (gene locus: 17p13.1) regulates the cell cycle by controlling the G1/S and G2/M checkpoints securing the normal sequence of cell cycle phases. Furthermore, it is involved in apoptosis programmed cell death. The gene is mutated or epigenetically altered in all epithelial malignancies, including colon adenocarcinoma. Additionally, Mouse Double Minute 2 Homolog (*MDM2*), a proto-oncogene (12q14.3), acts as a major negative regulator for p53 expression in the p53-MDM2 auto-regulatory pathway. MDM2 binds directly to p53 and represses its transcriptional activity, promoting p53 degradation. **Conclusion.** In colon adenocarcinoma, *MDM2* oncogene overexpression directly influences p53 oncoprotein expression levels.

Key Words: Colon ▪ Carcinoma ▪ p53-MDM2 ▪ Immunohistochemistry ▪ Genetics.

Introduction

Carcinogenesis is a multiple-step procedure based on a variety of different chromosome and gene imbalances and modifications (1, 2). Gross numerical chromosome alterations, known as Chromosome Instability (CI), include polysomy/aneuploidy and monosomy, whereas point mutations/substitutions, deletions and amplifications comprise specific gene numerical abnormalities, respectively (3). Interestingly, combinations of these genetic alterations lead to aggressive phenotypes in the majority of malignancies (4). Detection and isolation of specific genetic signatures in solid malignancies provide a rational way for oncologists to handle sub-groups of patients on the basis of suitable and relatively efficient, targeted chemotherapeutic regimens (5). *TP53* and *Mouse Double Minute 2 Homolog (MDM2)* are highly significant genes, critically involved in the carcinogenetic process of colon adenocarcinoma (6). They comprise an intracellular complex. Deregulation of the *TP53/MDM2* genes' auto-regulatory pathway is observed in various solid malignancies, including colon adenocarcinoma. Concerning colon adenocarcinoma, the Knudson two-hit hypothesis seems to be perfectly fitted regarding *TP53* suppressor gene inactivation (7).

In the current molecular review, we describe mechanisms and targeted strategies for *TP53/MDM2* deregulation, and their impact on the colon adenocarcinoma molecular substrate and phenotype.

The P53/MDM2 Auto-Regulatory Pathway: Anatomy and Function

The evolution of molecular biology in the past three decades has revealed a galaxy of genes/proteins,

their interactions and functional mechanisms inside the normal or transformed/altered cellular microenvironment (8). Extensive molecular analyses have concluded that *TP53* is a key regulator gene securing genome stability and function, involved in specific signaling transduction pathways, such as p53-sirtuin1 (SIRT1), a conserved nicotinamide adenine dinucleotide (NAD^+) (9). The gene is located on the short (p) arm of chromosome 17 at position 13.1 (17p13.1) and encodes for a nuclear phosphoprotein (molecular mass of 53 kDa). This protein acts as a strong transcription factor that negatively regulates cell proliferation (10). In fact, p53 regulates the cell cycle by causing arrest at stages of the G1/S and G2/M checkpoints (11). This function prevents DNA damage from being inserted in the S phase of DNA replication. Besides its prominent function, *TP53* acts as a positive regulator of histone de-acetylation and apoptosis, and a negative regulator for telomerase activity, proteolysis and helicase activity (12, 13). Additionally, *TP53* is a strong modulator for gene transcription, and is also implicated in biochemical mechanisms including cellular response to hypoxia, response to glucose deficit, protein oligomerization and base-excision repair, even in mitochondrial DNA (14). Interestingly, cell cycle arrest has been noticed as a result of P53-mediated indirect transcriptional repression due to activation of the P53/P21/DREAM/E2F/CHR pathway (15). Concerning P53 protein expression levels, it is expressed in low and moderate levels in normal cells that are visualized by immunohistochemistry assays as a nuclear staining pattern (16). Strong mutated P53 nuclear expression is detected in 50% to 60% of the solid malignancies examined of different histo-genetic origin (17).

MDM2 (also known as E3 ubiquitin-protein ligase) is a proto-oncogene (gene locus: 12q14.3) that encodes a nuclear-localized protein. Enzyme and zinc ion binding to specific intra-cellular substrates and ligase/transferase activity represent the main biochemical MDM2-mediated functions (18). Combined with P53, it forms an auto-regulatory pathway (Figure 1). MDM2 binds directly to p53, acting as a major negative regulator by repressing its transcriptional activity, and promotes p53 proteasomal degradation (19). In fact, MDM2 binds to the N terminus of p53, enhancing p53 ubiquitination and finally degradation. Amplification is the major mechanism of *MDM2* gene transformation to oncogene, and its overexpression in solid malignancies, mainly sarcomas, is frequently associated with more aggressive phenotypes in the corresponding patients (20). *MDM2* mutations have also been reported to impair the ability to degrade P53 oncoprotein efficiently (21, 22).

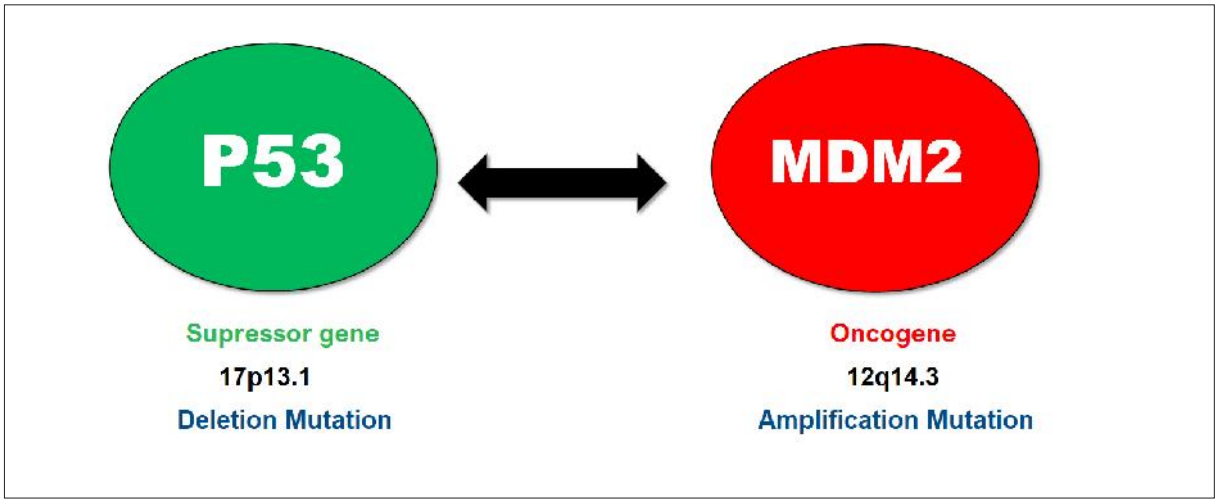

Figure 1. P53 and MDM2 form an auto-regulatory pathway. MDM2 binds directly to p53, acting as a major negative regulator by repressing its transcriptional activity, and promotes p53 proteasomal degradation.

P53/MDM2 Alterations in Colon Adenocarcinoma

Combined normal P53 and MDM2 expression secures cell cycle stability and functionality, partially under the influence of ubiquitin ligases (23). In neoplastic, pre- and malignant tissues this balance is aborted, leading to excessive cell proliferation. Concerning colon adenocarcinoma, there are significant new data based on the influence of MDM2 and also MDM4 on P53 degradation. One study group reported MDM2/MDM4/mitogen-activated protein kinase kinase (MEK) anti-P53 synergistic activity in colon adenocarcinoma. They concluded that nutlin-3, acting as an MDM2-p53 inhibitor, combined or ,not combined with a chimeric small interfering RNA and trametinib, induced activation of wild type *TP53* and simultaneously inhibition of the *KRAS* mutant oncogene (24). In fact, trametinib enhanced G1 phase arrest and promoted induction of apoptotic death. Additionally, another study focused on the causes of increased chemo resistance in sub-groups of colon adenocarcinoma patients. The researchers showed that elevated resistance to paclitaxel, a cytostatic agent combined with nutlin-3a, previously referred to as a P53/MDM2 inhibitor, could be a result of a universal efflux defense mechanism (25). Interestingly, specific peptides, such as PNC-27, seem to be strong agents implicated in P53/MDM2 inhibition in colon adenocarcinoma, destroying colon carcinoma stem cells by blocking the membrane H/MDM-2 (26). In conjunction, another study group explored the role of another agent in P53/MDM2 inhibition. Combined application of the RITA agent with cisplatin in colon adenocarcinoma cell cultures led to P53 activation by suppressing *MDM2* function (27). Furthermore, Tripartite motif-67 (TRIM67), a member of the TRIM protein family responsible for cell cycle regulation (arrest), DNA repair and apoptosis, restores P53 normal expression, thereby sensitizing *in vitro* colon adenocarcinoma cell series to specific chemotherapeutic regimens (28). For this reason, the P53/TRIM67 axis seems to be of significant importance regarding novel targeted therapeutic strategies. Similarly, the HS-1793 resveratrol analog has been found to disrupt the P53-MDM2 complex effectively (29). Another micro-genetic marker, the *lncRNA MIR4435-2* host gene (*MIR4435-2HG*), located on chromosome 2, is implicated in a broad spectrum of intracellular signaling transduction pathways, including Wnt/β-catenin, Hippo, PI3K/AKT/m TOR/PTEN, MAPK/ERK, TGF-β and the P53-MDM2 complex. The marker blocks a series of approximately 20 micro-RNAs and, especially in colon adenocarcinoma, enhances cisplatin activity (30). Similarly, hinokiflavone is a natural biflavonoid promoting pre-mRNA splicing. Interestingly, the agent acts as a potential anti-MDM2 inhibitor, suppressing its mRNA synthesis at the transcriptional level. Concerning colon adenocarcinoma, a study group revealed that the molecule enhanced G2/M phase arrest and apoptosis induction in a series of malignant colon cell cultures, by activating *TP53 gene* in parallel (31).

Multi-target oncoprotein blocking by specific tyrosine kinase inhibitors (TKIs) is a novel, very promising oncological approach in solid malignancies, including colon adenocarcinoma. One study group showed that application of combined selumetinib (a MEK inhibitor) with KRT-232 (a MDM2 inhibitor) *in vivo* in patient-derived xenograft (PDX) colon carcinoma models induced P53 activity, promoting apoptosis (32). Furthermore, the combination of rigosertib and 5-FU in colon adenocarcinoma cell culture-based models positively regulates P53, e-cadherin and CD31 expression, also inhibiting *MDM2* oncogenic activation independently of the presence of *KRAS* mutations (33). Decreased metastatic potential and neo-angiogenesis are the results of rigosertib influence in the corresponding colon cell series. In conjunction, diarylpentanoids act as MDM2/X ligands. In a molecular study, the corresponding researchers investigated their effect on P53-MDM2/X interaction. They observed that diarylpentanoids demonstrated significant anti-proliferative effects in HCT116 cell series (34). Another agent that seems to critically affect the P53-MDM2 pathway is the zinc finger protein SNAI2 (Slug). The molecule increases

MDM2 oncogenic activity, and promotes P53 and P21 cellular expression deficiency by degrading them. A study group analyzing its effect *in vitro* on HCT116 cells showed that a Slug-dependent P53 decrease is an important genetic event that crucially desynchronizes cell cycle phase succession (35). Moreover, DJ-1 has been found to modulate the *TP53/MDM2* signaling pathway by disrupting their interaction and reducing BCL2-, BAX, and CASPASE-3 activity, leading to increased cell proliferation and decreased apoptotic rates (36). This imbalance negatively affects the normal function of the P53/MDM2 complex. Additionally, DJ-1 demonstrates strong oncogenic activity in SW480 and HCT116 malignant cell lines by promoting cell proliferation, invasion and migration. All of these actions are mediated by over activation of the cyclin-D1/MDM2-p53 signaling pathway. In contrast, another agent that seems to affect not only the P53/MDM2 complex but also the PI3K/AKT signaling transduction pathway is costunolide, a natural sesquiterpene lactone. One study group observed that this molecule activated and stabilized P53 by inhibiting its MDM2-mediated ubiquitination, also providing *in vitro* AKT's phosphorylation suppression (37).

Finally, P53/MDM2 involvement in immune response and stromal microenvironment modifications is a very promising field of research in solid malignancies, including colon adenocarcinoma molecular mechanism. Another study group analyzed the potential interactions of the complex with the PD-1/PD-L1 pathway (38). The researchers reported a new mechanism that joins anti-PD-L1 checkpoint blocking immunotherapy strategies with MDM2 inhibitors in patients with normal wild-type P53 expression, claiming a new approach in abnormal intracellular pathway disruption.

Additionally, concerning the clinical relevance of MDM2 in colon adenocarcinoma, small interfering LINC00342 (siLINC00342) was found to be co-overexpressed with MDM2 oncoprotein deregulating the miR-545-5p/MDM2 axis (39). The study group showed that targeting LINC00342, cancerous cell proliferation was decreased, combined with increased apoptotic activity. Furthermore, TP53 mutated protein overexpression is involved in resistance to specific chemotherapeutic-based strategies, including oxaliplatin. An experimental study suggested that targeting aurora-A, a significant kinase in G2/M phase could provide elevated response rates to the corresponding patients with adenocarcinoma (40).

Conclusion

In conclusion, understanding the molecular nature and deregulation mechanisms of the P53/MDM2 complex in solid malignancies, and particularly in colon adenocarcinoma, is a challenge for further investigation. P53 suppressor activity antagonizes MDM2 oncogenic activity in neoplastic and malignantly transformed cells. P53/MDM2 interaction regulates most crucial cell cycle phase successions, and their desynchronization negatively affects the equilibrium between normal cell survival and apoptotic death, leading to the aberrant cell proliferation of malignant cells. Development of targeted anti-MDM2 strategies combined with P53 enhancement should open new horizons in handling colon adenocarcinoma patients rationally on the basis of specific genetic signatures.

Review Highlights

This review study represents a rigid and updated multi-synthesis of all novel molecular knowledge in the field of P53/MDM2 in colon adenocarcinoma, especially focused on modern oncological approaches for targeting this significant autoregulatory pathway for cellular homeostasis.

Conflict of Interest: The authors declare that they have no conflict of interest.

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SARS-CoV-2 Vaccination IgG Antibody Responses in Patients with Hematologic Malignancies in a Myeloid Enriched Cohort: A Single Center Observation

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Abstract

Objective. Patients diagnosed with hematologic malignancies are at increased risk for severe SARS-CoV-2 infection. We evaluated the serological IgG response following two doses of the SARS-CoV-2 vaccine in patients with hematologic malignancies. **Methods.** Patients treated at UT Southwestern Medical Center with a diagnosis of a myeloid or lymphoid neoplasm were included. SARS-CoV-2 vaccination response was defined as a positive quantifiable spike IgG antibody titer. **Results.** Sixty patients were included in the study and 60% were diagnosed with a myeloid neoplasm. The majority (85%) of the patients with a myeloid malignancy and 50% of the patients with a lymphoid malignancy mounted a serological response after receiving two doses of the vaccine. **Conclusion.** Vaccination should be offered irrespective of ongoing treatment or active disease. Findings require validation in a larger cohort of patients.

Key Words: SARS-CoV-2 Vaccination ▪ Hematologic Malignancy ▪ Antibody Response.

Introduction

Patients with a hematologic malignancy are at increased risk for severe SARS-CoV-2 infection due to their immunocompromised status and as a result of receiving immunosuppressive treatments. Depending on the underlying disease, mortality rates have been reported as high as 30–40% (1, 2). While the BNT162b2 (Pfizer/BioNTech) and the mRNA-1273 (Moderna) COVID-19 vaccines both have been shown in large phase 3 clinical trials to be more than 90% effective at preventing lab-confirmed COVID-19 illness and severe infections, data on vaccine efficacy and safety in immunocompromised patients remain scarce (3, 4). In order to generate optimal protective immunity following vaccination, intact host immunity is needed. The American Society of Hematology and American Society of Transplant and Cellular Therapy COVID-19 vaccine guidelines indicate that certain immunocompromised patient populations could have an attenuated response to the SARS-CoV-2 vaccine. However, most pivotal SARS-CoV-2 vaccination trials required patients to be off immune suppression for a certain period to be eligible, and patients with hematologic malignancies were therefore often excluded.

In this study, we aimed to evaluate the serological response of Pfizer/BioNTech and Moderna vaccination after administration of two doses in patients with hematologic malignancies.

Methods

Patient Population

This retrospective observational study was approved by the institutional review board of the University of Texas Southwestern Medical Center

(Dallas, TX, USA). Patients diagnosed with a myeloid or lymphoid malignancy that had a quantitative SARS-CoV-2 IgG spike antibody measured between December 2020 and November 2021 after receiving two doses of the Moderna or Pfizer/BioNTech SARS-CoV-2 vaccine were included in the study. Inhouse testing for a quantitative IgG antibody titer detection was performed for the majority (N=40) of the patients in accordance with the manufacturer's package insert (AdviseDX SARS-CoV-2 IgG II/ SARS-CoV-2 IgG II Quant assay, Abbott Alinity i platform, Abbott Laboratories, Chicago, IL, USA) (5). Briefly, SARS-CoV-2 antigen-coated microparticles bind to the IgG antibodies that attach to the virus's spike protein. Subsequently, an anti-human IgG conjugate is added and the degree of chemiluminescence is measured, reflecting the quantity of IgG present. The remaining patients had send-out testing performed either using an immunoassay that uses a recombinant protein that represents the nucleocapsid virus antigen (Roche Elecsys® anti-SARS-CoV2 reagent assay, Roche Diagnostics, Indianapolis, IN, USA (N=16)) or an assay that selects the receptor-binding domain of the S1 spike antigen to detect neutralizing IgG antigens (Atellica® IM SARS-CoV-2 IgG (COV2G) Siemens Healthcare Diagnostics, Terrytown, NY, USA (N=4)). Vaccine response was defined as having a positive quantifiable spike IgG antibody titer per the laboratory reference range.

Ethics Statement

Statistical Analysis

Categorical variables were compared using Fisher's exact test and summarized as count with percentage. Continuous variables were compared using the Wilcoxon signed-rank test and summarized as median with interquartile range. P-value <0.05 was considered statistically significant. Statistical analyses were performed in R Version 4.2.2 (2022-10-31), (RStudio, Inc., Boston, MA).

Results

A total of 60 patients were included in the study. Patients had a median age of 72 year at time of first vaccination, and the large majority were non-Hispanic (88%) and were white (83%). Baseline patient and disease characteristics are shown in Table 1. Twenty patients (33%) received a complete vaccination series with the mRNA-1273 (Moderna) COVID-19 vaccine and 67% received the BNT162b2 (Pfizer/BioNTech) vaccine. Sixty percent of the patients were diagnosed with a myeloid neoplasm including acute myeloid leukemia (AML) (15%), myelodysplastic syndrome (MDS), chronic myelomonocytic leukemia (CMML) or clonal cytopenia or undetermined significance (CCUS) (23%), a myeloproliferative neoplasm (MPN) (15%) or chronic myeloid leukemia (CML) (7%). The remaining patients were diagnosed with chronic lymphocytic leukemia (17%), lymphoma (10%) or other (N=5). At the time of first vaccine administration, 41 (68%) of patients were on active therapy or were treated within the past 12 months, and 41 (68%) of patients had active disease. The median number of days between administration of the first and second vaccine was 22 days (range 17-32) and 54 (range 26-277) days between second vaccine and IgG SARS-CoV-2 spike antibody collection.

Most patients (73%) mounted a serological response with quantifiable IgG SARS-CoV-2 spike antibodies after receiving two doses of the vaccine; 85% of the patients with a myeloid disease vs. 50% with a lymphoid malignancy (P=0.01). All patients (100%) with MDS, CMML, CCUS or CML showed a positive serological immune response, followed by 78% of the AML patients (Figure 1). Five of the nine patients diagnosed with MPN developed a positive spike antibody, including none of the two polycythemia vera patients. Additionally, one patient with AML secondary to *JAK2* mutated MPN remained seronegative after vaccination. Forty percent (N=4) of the CLL patients mounted a positive response. Among the responders and the non-responders, 64% and 81% were on active therapy respectively (P=0.35), and 63% compared to 70% had active disease, respectively (P=0.55).

Table 1. Baseline Patient Characteristics

Characteristics	Patients			P*
	Total: N (%)	Negative: N (%)	Positive: N (%)	
	60 (100)	16 (23)	44 (73)	
Diagnosis				
AML	9 (15)	2 (13)	7 (16)	0.001
CLL	10 (17)	6 (38)	4 (9)	
CML	4 (7)	0 (0)	4 (9)	
Lymphoma	6 (10)	2 (13)	4 (9)	
MDS/CMML/CCUS	17 (28)	0 (0)	17 (39)	
MPN	9 (15)	4 (25)	5 (11)	
Other	5 (8)	2 (13)	3 (7)	
Patient Characteristics				
Gender (Female)	28 (47)	5 (31)	23 (52)	0.24
Age (Years and ranges)	72 (22-85)	75 (22-84)	72 (23-85)	0.67
Race (White)	50 (83)	15 (94)	35 (80)	0.26
Ethnicity (non-Hispanic)	53 (88)	13 (81)	40 (91)	0.37
Active disease status	41 (68)	10 (63)	31 (70)	0.55
Treatment (active, or within the past 12 months)	41 (68)	13 (81)	28 (64)	0.35
Stem cell transplantation	7 (12)	2 (13)	5 (11)	1.00
Vaccine (Pfizer)	40 (66)	11 (69)	29 (66)	1.00
Blood counts at time of first vaccine (median, IQR)				
Blood counts	Total; N=60	Negative: N=16	Positive; N=44	P†
WBC	5.9 (4.0 – 8.5)	7.7 (4.9– 10.9)	5.6 (4.0–7.4)	0.24
Hemoglobin	11.6 (10.4–13.2)	12.8 (11.4–13.5)	10.9 (10.3–12.5)	0.06
Platelets	172.5 (112.5–281.8)	203.0 (147.5– 275.0)	163.0 (68.0–281.0)	0.32
ANC	3.1 (1.9–4.9)	4.8 (2.1– 6.5)	2.8 (2.0–4.0)	0.13
ALC	1.6 (1.0–2.4)	1.2 (0.7–2.1)	1.7 (1.1–2.5)	0.25
AMC	0.5 (0.3–0.6)	0.5 (0.3– 0.6)	0.5 (0.3–0.7)	0.77

*Fisher's exact test; †Wilcoxon signed-rank test. AML=Acute myeloid leukemia; CLL=Chronic lymphocytic leukemia; CML=Chronic myeloid leukemia; MDS=Myelodysplastic syndrome; CMML=Chronic myelomonocytic leukemia; CCUS=Clonal cytopenia of undetermined significance; MPN=Myeloproliferative neoplasm; WBC=White blood cell count; ANC=Absolute neutrophil count; ALC=Absolute lymphocyte count; AMC=Absolute monocyte count; BTK=Bruton's tyrosine kinase; HMA=Hypomethylating agent; TKI=Tyrosine kinase inhibitor; IQR=Interquartile range.

All (100%) patients who had received hypomethylating agent (HMA) therapy (N=4) or a tyrosine kinase receptor inhibitor (N=4) at the time of vaccination or within the past 12 months resulted with a positive IgG SARS-CoV-2 seroconversion, vs. 38% of the patients receiving a Bruton's tyrosine kinase (BTK) inhibitor (ibrutinib (N=2), acalabrutinib (N=1)), 50% of the patients who had received rituximab and 57% of the patients on ruxolitinib mounted an IgG SARS-CoV-2 response. While two of the patients treated with rituximab that had a positive response were off the rituximab for more than 5 months, another patient who was off therapy for 5+ months did not. Out of the four patients receiving venetoclax-based combination therapy, 50% mounted a response; 2/3 AML patients and 0/1 CLL patient. The CLL patient has had several lines of therapy, including B-cell depleting therapy, but none within the past 12 months.

Five patients with a documented positive IgG response and one patient without positive IgG antibody response developed COVID-19 infection

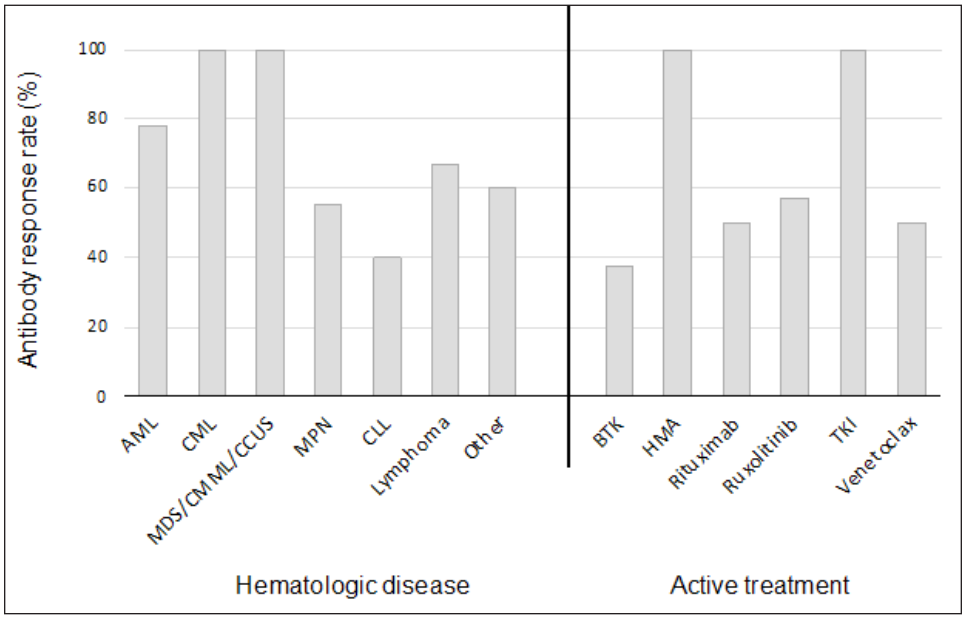

Figure 1. SARS-CoV-2 IgG antibody response rates. Left: Response rates in patients diagnosed with AML=Acute myeloid leukemia; CML=Chronic myeloid leukemia; MDS=Myelodysplastic syndrome; CMML=Chronic myelomonocytic leukemia; MPN=Myeloproliferative neoplasm; CLL=Chronic lymphocytic leukemia; Lymphoma; Other. Right: Antibody response rates in patients treated within the past 12 months with BTK=Bruton's tyrosine kinase inhibitor; HMA=Hypomethylating agent; Rituximab, Ruxolitinib, TKI=Tyrosine kinase inhibitor; Venetoclax.

after two vaccinations (median 317 days), two patients had COVID-19 infection prior to the first dose of vaccination, both patients had IgG titers >10,000. No differences in response rates were seen between patients vaccinated with the BNT162b2 (Pfizer/BioNTech) and the mRNA-1273 (Moderna) vaccine. No statistical differences were observed in complete blood counts between responders and non-responders.

Discussion

In this retrospective single center observational study, we evaluated the IgG SARS-CoV-2 antibody response post two doses of SARS-CoV-2 vaccination in patients diagnosed with a hematologic malignancy. We observed a positive IgG antibody response in 73% of the patients, with a lower response rate in patients diagnosed with a lymphoid malignancy (50%) compared to patients diagnosed with a myeloid malignancy (85%). This observation is similar to what has been previously described in B-cell lymphoid malignancies (6), which given that they often have profound impaired humoral and cellular immune function is not surprising.

As published by several others, CLL is found to be associated with attenuated SARS-CoV-2 IgG response with CLL seroconversion rates ranging from 47-64% (7-9). A large multicenter international study that included 198 CLL patients with a symptomatic COVID-19 infection showed a high case fatality rate of 33% for all patients, and 37% for hospitalized patients (6, 10). This association is likely exacerbated by B-cell depleting therapy, such as anti-CD20 monoclonal antibodies and BTK-inhibitors, which further limits the protective response. Thakkar et al. studied IgG SARS-CoV-2 antibody response in patients with cancer (N=200) that had received full dosing of COVID-19 vaccine. While they found a seroconversion rate of 85% in hematologic malignancies, lowest positive response rates were associated with targeted CD20 therapy, BCL-2 inhibitors and BTK inhibitors (11). Others reported a cohort of 167 CLL patients with none of the 22 patients

who had received anti-CD20 therapy within the past 12 months mounted a positive response (9), and Rotterdam et al. showed therapies including rituximab and BTK inhibitors to be significantly correlated with negative seroconversion rates (12). Although we observed a positive response rate of 50% among the patients that had received rituximab, rituximab was discontinued at least 5 months prior to first vaccination date in three of the four patients, which may potentially have mitigated the effect.

While most studies have been focused on lymphoid malignancies, a limited number of studies have been published for myeloid diseases, in particular in the context of different treatment agents. One of the first published studies on COVID-19 in the setting of myeloid neoplasms, reported comparable antibody titers in patients with AML and MDS vs. healthy controls. However, when they compared titers obtained in AML patients in complete remission on maintenance therapy to patients in remission on treatment-free observation, they noted IgG levels to be lower in the treatment group. Most of these patients received an HMA. A similar observation was done for MDS patients on treatment showing lower titers compared to healthy controls (13). More recently, several other reports came out showing the antibody response rates were favorable in AML and MDS with seropositive rates ˜90% both after treatment with HMA with or without venetoclax, although the latter remains controversial and most studies were performed using small cohorts (13, 14). Here we report seven patients treated with HMA, of which two received a combination with venetoclax, all with a positive antibody titer.

Other myeloid diseases associated with lower response rates were primary myelofibrosis and patients treated with ruxolitinib, whereas CML showed seroconversion in almost all cases, even in the setting of TKI treatment (12, 13). It may be debatable whether the disease or treatment predisposes to a low seroconversion rate. In a study that included 20 primary myelofibrosis patients (JAK2+ (N=15)), ten patients received ruxolitinib and the other 10 received hydroxyurea or supportive therapy with no significant differences seen between both groups in positive seroconversion rate or IgG spike levels (15). Interestingly, Rotterdam et al. reported one patient with JAK2+ CML patient on ruxolitinib who mounted a negative response (N=1/101), as did our patient with secondary AML with a history of JAK2 MPN on ruxolitinib therapy.

Limitations of Study

This study has several limitations including the small sample size and the large heterogeneity in hematologic disease subtypes and treatment regimens, which could perhaps result in statistical bias and low statistical power. Due to the different assays that we used to measure qualitative IgG COVID-19 SARS-CoV-2 antibody response, each with a different laboratory normal reference range and upper limit of detection level, qualitative IgG spike cannot be correlated with reported disease variables. Findings require validation in a larger, prospective and multi-institutional cohort of patients.

Conclusion

In this study we demonstrated that the majority of patients with a hematologic neoplasm mount a positive response following SARS-CoV-2 vaccination, suggesting that vaccination should be offered irrespective of ongoing treatment or active disease. This study further helps to identify higher-risk patients for negative seroconversion, including patients with CLL or MPN, and patients receiving anti-CD20 therapies, BTK-inhibitors or JAK2+ inhibitors.

What Is Already Known on This Topic:

Patients with a hematologic malignancy are at increased risk for severe SARS-CoV-2 infection and data on SARS-CoV-2 vaccination efficacy and safety in immunocompromised patients remain scarce. Particularly, chronic lymphocytic leukemia has been associated with attenuated SARS-CoV-2 IgG response, whereas patients diagnosed with a myeloid malignancy often show high seroconversion rates comparable to the healthy population.

What This Study Adds:

We demonstrated that the majority of patients with a hematological neoplasm mount a reasonable response following SARS-CoV-2 vaccination, suggesting that vaccination should be offered irrespective of ongoing treatment or active disease. Our findings help to identify higher-risk patients for negative seroconversion.

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Conflict of Interest: Y.F.M. has received honoraria/consulting fees from BluePrint Medicines, GERON, OncLive and MD Education. Y.F.M. participated in advisory boards and received honoraria from Sierra Oncology, Stemline Therapeutics, Blueprint Medicines, Morphosys, Taiho Oncology and Novartis. YFM received travel reimbursement from Blueprint Medicines and Morphosys. F.T.A. has provided consultancy to Genentech, Astrazeneca, Abbvie, Janssen, Pharmacyclics, Gilead sciences, Kite pharma, Celgene, Karyopharm, MEI Pharma, Verastem, Incyte, Beigene, Johnson and Johnson, Dava Oncology, BMS, Merck, Cardinal Health, ADCT therapeutics, Epizyme, Caribou Biosciences, Cellecter Bisosciences, and received research funding from Pharmacyclics. P.R.G. has provided consultancy services to Kite Pharma, Bristol Myers Squibb (BMS) and Rafael Pharma and served on the advisory boards of Pharmacyclics LLC, ADC Therapeutics and Cellectar Biosciences. G.K. has provided consultancy to Cellectar Biosciences and BMS. P.A.P. has served on the advisory boards of Servier and BMS Celgene and is currently employed by Servier. None of these relationships are related to this work.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Factors Predictive of In-Hospital Mortality in Patients with Systemic Lupus Erythematosus: A Single-Centre Retrospective Analysis

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Abstract

Objective. We aimed to investigate the causes and factors predictive of in-hospital death among patients with systemic lupus erythematosus (SLE) admitted to a tertiary care hospital in Thailand. **Materials and Methods.** We retrospectively reviewed the records of patients with SLE admitted between 2017 and 2021. We collected data related to age, sex, body mass index, comorbidities, disease duration, medication usage, clinical symptoms, vital signs, laboratory results, evidence of infection, presence of systemic inflammatory response syndrome, quick sepsis-related organ assessment scores, and SLE disease activity on the date of admission. The length of hospitalization, treatment administered, and subsequent clinical outcomes (including in-hospital complications and death) were also recorded. **Results.** Among 267 enrolled patients, the overall in-hospital mortality rate was 25.5%, and infection was the most common cause of death (75.0%). Multivariate analysis revealed that prior hospitalization within 3 months (odds ratio [OR]: 2.311; 95% confidence interval [CI]: 1.002–5.369; P=0.049), initial infection on admission (OR: 2.764; 95% CI: 1.006–7.594; P=0.048), use of vasopressor drugs (OR: 2.940; 95% CI: 1.071–8.069; P=0.036), and mechanical ventilation (OR: 5.658; 95% CI: 2.046–15.647; P=0.001) were independent risk factors for in-hospital mortality. **Conclusion.** Infection was the major cause of mortality in patients with SLE. Prior hospitalization within 3 months, initial infection on admission, vasopressor use, and mechanical ventilation during admission are independent risk factors for in-hospital mortality in patients with SLE.

Key Words: Risk Factor ▪ In-Hospital Death ▪ Mortality ▪ Predictor ▪ Systemic Lupus Erythematosus.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by multiple organ system involvement and patterns of relapse and remission, resulting in major morbidity and mortality. Despite improvements in both diagnosis and treatment modalities over the past decade (1), the SLE mortality rate remains high, at approximately 2–3 times that observed in the general population (2-4).

Primary reasons for hospitalization among patients with SLE include disease activity, infection, and concurrent infection during disease flares (5, 6). Given the cumulative organ damage and high rate of infection, patients with SLE are considered to have an increased risk of mortality during hospitalization (7, 8). The in-hospital mortality during SLE-related hospitalization maybe up to 70% (8). Understanding these outcomes and their prognostic factors is necessary for optimal management of patients with SLE. However, differences in geographic region, ethnicity, and socioeconomic status can influence SLE disease patterns and outcomes (9-11). Most studies conducted in developed or high-income countries report improved survival outcomes (9, 10). whereas studies conducted in developing countries report relatively worse outcomes (12). To date, there is a paucity

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of clinical data regarding the factors influencing in-hospital mortality in patients with SLE in developing countries, where healthcare resources are relatively limited.

To address this issue, we aimed to investigate the causes and factors predictive of in-hospital mortality in patients with SLE admitted to a tertiary care hospital in Thailand.

Methods

Study Design and Patient Population

This retrospective study was conducted at Hatyai Hospital (a regional tertiary center in southern Thailand). The study protocol was approved by the institutional ethics committee (protocol number 57/2564) and was conducted in accordance with the Declaration of Helsinki. The need for informed consent was waived because patient information was anonymized prior to analysis.

The study included patients with SLE aged >18 years admitted to the Department of Internal Medicine at Hatyai Hospital between January 2017 and December 2021. The exclusion criteria were as follows: (a) coexistence of other connective tissue diseases, (b) coexistence of antiphospholipid syndrome (APS), (c) pregnancy or lactation, (d) concurrent malignancy, (e) elective admission for diagnostic procedures (e.g., kidney biopsy) or scheduled intravenous administration of SLE-specific therapy (e.g., intravenous methylprednisolone and cyclophosphamide), and (f) insufficient data. SLE was diagnosed based on the 1997 American College of Rheumatology revised criteria for SLE and/or Systemic Lupus International Collaborating Clinics (13, 14).

Data Collection and Definitions

Data from each patient's medical records were manually collected and reviewed retrospectively. Each admission was treated as a separate observation for the analysis. For each patient, we collected data related to age, sex, body mass index, comorbidities, disease duration, medication usage, history of prior hospitalization within three months, clinical symptoms, vital signs, laboratory results, evidence of infection, presence of systemic inflammatory response syndrome [SIRS], quick sepsis-related organ assessment (qSOFA) scores (15), and SLE disease activity (measured using the modified SLE Disease Activity Index -2 K [Modified SLEDAI-2K] score) (16) on the admission date. In addition, the length of hospitalization, treatment administered, need for admission to the intensive care unit (ICU), and subsequent clinical outcomes (including in-hospital complications and death) were recorded.

Active SLE was defined as a modified SLEDAI-2K score >4. SIRS was considered to be present in patients exhibiting two or more of the following: (a) body temperature >38°C or <36°C; (b) heart rate >90 beats per minute; (c) respiratory rate >20 breaths per minute; and (d) white blood cell count >12,000 cells/mm^3, <4,000 cells/mm^3, or >10% of band forms (17). Infections were diagnosed based on either bacteriological, radiological, or serological findings and on the treatment response to antibiotic therapy. Patients who met both the definitions of active SLE and infection were classified as having infection concurrent with disease flare. Prior hospitalization included only admissions for SLE-related reasons, such as disease flare and/or infection, and not diagnostic procedures or scheduled intravenous medication administration. The primary outcome was in-hospital mortality, which was defined as any death that developed during the admission period.

Statistical Analysis

Categorical variables were evaluated using frequency statistics and compared using Pearson's chi-square or Fisher's exact test, as appropriate. Continuous variables are presented as means with standard deviations (SD) or medians with interquartile ranges (IQR) and were compared using Student's t-test and the Wilcoxon rank-sum test. The associations between the variables and in-hospital mortality were determined using a logistic regression analytic model that was adjusted for possible confounders. In the first model (model 1), only age and sex were included. Then, additional multiple regression modeling was performed by including

clinical characteristics (age, sex, Modified SLEDAI-2K score, organ damage comorbidity, preadmission medication) that were known to influence patients' prognosis (model 2). Finally, we performed additional multiple regression modeling to identify predictors of in-hospital mortality using the variables in model 2 and other variables that were significantly associated with in-hospital mortality (P-values <0.05) in the univariate analyses (model 3). Analyses were performed using STATA version 15.1 software (StataCorp LLC, College Station, TX, USA). Statistical significance was set at P<0.05.

Results

Baseline Characteristics and Causes of Death

Among 503 patients identified during the study period, 267 patients met the inclusion criteria and were enrolled in the study. The average age among the included patients was 34.4±13.2 years, 93.3% of whom were female, and the median duration between disease onset and admission was 15 (IQR: 2–50) months (Table 1). Two hundred and thirty-six patients were excluded because of undergoing scheduled intravenous medication administration

Table 1. Baseline Clinical Characteristics of Lupus Patients (N=267) and Clinical Outcome during Admission

Baseline characteristics	Value N (%)
Female sex	249 (93.3)
Age (years), mean ± SD*	34.4±13.2
Classification diagnostic criteria	
ACR97†	225 (84.2)
SLICC12‡	235 (88.0)
Comorbidities	
Hypertension	37 (13.9)
Dyslipidemia	27 (10.1)
Diabetes mellitus	17 (6.4)
Chronic kidney disease	74 (27.7)
Lupus nephritis	132 (49.4)
Use of corticosteroid prior to admission	226 (84.63)
Use of antimalarial agents prior to admission	119 (44.6)
Use of immunosuppressive agents prior to admission	
None	158 (59.2)
Azathioprine	25 (9.45)
Cyclophosphamide	54 (20.2)
Mycophenolate mofetil	30 (11.2)
Duration of disease before admission (months), median (IQR§)	15 (2–50)
Cause of admission	
Disease activity	109 (40.8)
Infection	57 (21.3)
Infection concurrent with disease flare	101 (37.8)
Symptom or organ involvement at admission	
Fever	174 (65.2)
Mucocutaneus	47 (17.6)
Musculoskeletal	17 (6.4)
Neuropsychiatric lupus	29 (10.9)
Cardiovascular-pulmonary	41 (15.4)
Gastrointestinal	10 (3.7)
Leukopenia	20 (7.5)
Autoimmune hemolytic anemia	35 (13.1)
Thrombocytopenia	32 (12.0)
Vasculitis	10 (3.7)
Renal	157 (58.8)
Presence of SIRs at admission	152 (56.9)
Length of hospitalization (days), median (IQR§)	10 (5–19)
In-hospital mortality	68 (25.5)

*Standard deviation; †American College of Rheumatology revised criteria for SLE 1997; ‡Systemic Lupus International Collaborating Clinics 2012; §Interquartile range.

and kidney biopsy (N=157), coexistence of other connective tissue diseases or APS (N=11), concurrent malignancies (N=5), and incomplete information (N=63). The reasons for admission included disease activity, infection, and concurrent infection with active disease, accounting for 40.8%, 21.3%, and 37.8%, respectively. The mean modified SLEDAI-2k scores at admission across hospitalization type were as follows: 7.0 for admission due to SLE disease flare, 3.2 for admission due to initial infection, and 7.0 for admission due to concurrent infection with SLE disease flare. Among all patients, 155 had lupus nephritis (58.8%); 54.1%, 11.5%, and 34.4% of patients were admitted due to SLE disease flare, initial infection, and concurrent infection with SLE disease flare, respectively.

There were 68 in-hospital deaths, corresponding to an overall in-hospital mortality rate of 25.5%. Infection was the most common cause of death (N=51; 75.0%), followed by SLE disease flare (N=17; 25%). Infection could be classified as concurrent infection with SLE disease flare (N=39; 57.4%) and initial infection on admission (N=12; 17.6%).

Comparison of Baseline Characteristics and Clinical Outcomes Among Survivors and Non-Survivors

Tables 2 and 3 show the baseline characteristics, treatment modalities, and clinical outcomes for survivors and non-survivors. Mean age (P=0.003)

Table 2. Comparison of Characteristics of Patients Who Survived (N=199)Versus Those Who Did Not Survive (N=68)

Variables	Survivors N (%)	Non-survivors N (%)	P value
Female sex	185 (93.0)	64 (94.1)	1.000
Age (years), mean±SD*	33.1±13.0	38.5±12.8	0.003
Comorbidities			
Hypertension	20 (10.1)	17 (25.0)	0.002
Dyslipidemia	17 (8.5)	10 (14.7)	0.146
Diabetes mellitus	10 (5.0)	7 (10.3)	0.150
Chronic kidney disease	46 (23.1)	28 (41.2)	0.004
Lupus nephritis	95 (47.7)	37 (54.4)	0.400
Use of corticosteroid prior to admission	169 (84.9)	57 (83.8)	0.846
Use of antimalarial agents prior to admission	93 (46.7)	26 (38.2)	0.224
Use of immunosuppressive agents prior to admission			
None	123 (61.8)	35 (51.5)	0.134
Azathioprine	22 (11.1)	3 (4.4)	0.147
Cyclophosphamide	34 (17.1)	20 (29.4)	0.029
Mycophenolate mofetil	20 (10.1)	10 (14.7)	0.294
Duration of disease before admission (months), median (IQR†)	15 (3–50)	17.5 (2–58)	0.697
Modified SLEDAI-2K score††, median (IQR†)	8 (2–14)	8 (2–16)	0.780
Modified SLEDAI-2K score†† >4	122 (61.3)	46 (67.6)	0.350
Equivalent dose to prednisolone dose, mg/day, median (IQR†)	20 (5–60)	20 (5–60)	0.750
Prior hospitalization within 3 months	67 (31.8)	42 (58.3)	<0.001
SLE** flare at admission	94 (47.2)	15 (22.1)	<0.01
Mucocutaneus	18 (9.0)	5 (7.4)	0.668
Lupus nephritis	120 (60.3)	37 (54.4)	0.394
Hematological	47 (23.26)	18 (26.5)	0.636
Neurological	23(11.6)	12 (17.6)	0.199
Others	15 (7.5)	7(10.3)	0.475

Continuation of Table 2.

Variables	Survivors N (%)	Non-survivors N (%)	P value
Initial infection on admission	105 (52.8)	53 (77.9)	<0.001
Bacteremia	27 (13.6)	16 (23.5)	0.059
Urinary tract infection	34 (17.1)	49 (25.0)	0.152
Pneumonia	24 (12.1)	22 (32.4)	<0.001
Deep skin infection	26 (13.1)	19 (27.9)	0.005
Infective diarrhea	16 (8.0)	5 (7.4)	0.856
Unidentified source infection	5 (2.5)	2 (2.9)	1.000
Laboratories test at admission			
Hemoglobin (g/dL), mean±SD*	9.0 ± 2.4	8.3 ± 2.1	0.020
White blood cell count ($\times10^3$/μL), median (IQR†)	8.3(5.0–13.0)	8.6 (4.0–13.5)	0.980
Proportion of white blood cell count <4×10^3	32 (16.1)	15 (22.1)	0.264
Platelet count ($\times10^3$ /μL), median (IQR†)	918 (122–283)	148 (113–235)	0.008
Proportion of platelet count <50×10^3/μL	7 (3.5)	5 (7.4)	0.189
Creatinine (mg/dl), median (IQR†)	1.3 (0.7–2.8)	1.9 (1.0–4.14)	0.006
UPCR‡ (mg/g), median (IQR†)	2275 (250–4,924)	2498 (365–5840)	0.908
Albumin (g/dL), mean±SD*	2.6 ± 0.8	2.4 ± 0.6	0.037
qSOFA§ score >2	16 (8.0%)	19 (27.9%)	<0.001
SIRS‖ >2 score	99 (49.7%)	53 (77.9%)	<0.001

*Standard deviation; †Interquartile range; †† Modified SLE Disease Activity Index -2 K; **Systemic lupus erythematosus; ‡Urine protein creatinine ratio; §Quick sepsis-related organ assessment;‖Systemic inflammatory response syndrome. P value= For bivariate two-sided comparisons between the two groups, Pearson chi-square test or Fisher's Exact test was used for categorical variables, and the Student's t-test and Wilcoxon rank-sum test was used for continuous variables.

Table 3. Comparison of Treatment Modality and Outcome between Patients Who Survived (N=199) Versus Those Who Did Not Survive (N=68)

Variables	Survivors N (%)	Non-survivors N (%)	P value
Vasopressors use	15 (7.5)	41 (60.3)	<0.001
Mechanical ventilator	40 (20.1)	55 (80.9)	<0.001
Renal replacement therapy	22 (11.1)	24 (38.3)	<0.001
Median dose equivalent to prednisolone (mg), median (IQR*)	225 (775–1,120)	365 (60–1,150)	0.683
Pulse methylprednisolone pulses (>500 mg)	71 (35.7)	27 (39.7)	0.552
Cyclophosphamide use	30 (15.1)	1 (1.5)	0.002
Intravenous immunoglobulin G use	6 (3.0)	3 (4.4)	0.697
Plasma exchange	10 (5.0)	11 (16.2)	0.003
Nosocomial bacteremia	6 (3)	8 (11.8)	0.010
Length of stay (day), median (IQR*)	9 (5–18)	12 (6–28)	0.175

*Interquartile range. P value=For bivariate two-sided comparisons between the two groups, Pearson chi- square test or Fisher's Exact test was used for categorical variables, and the Wilcoxon rank-sum test was used for continuous variables.

and rates of underlying hypertension (P=0.002), chronic kidney disease (P=0.004), and cyclophosphamide use within 3 months prior to admission (P=0.029), history of admission within 3 months prior to the current admission (P<0.001), and initial infection on admission (P<0.001) were higher among non-survivors than among survivors. Serum creatinine levels (P=0.006), the frequency of SIRS on admission (P<0.001), and the proportion of patients with qSOFA scores >2 (P<0.001) were also higher among non-survivors than among survivors. In contrast, hemoglobin (P=0.020) and serum albumin levels (P=0.037) were higher among survivors than among non-survivors.

During admission, patients in the non-survivor group were more often require vasopressor agents, mechanical ventilation, renal replacement therapy, and plasma exchange and were more experience nosocomial infection, while those in the survivor group more often received cyclophosphamide administration.

Factors Predictive of In-Hospital Mortality

Logistic regression analysis was performed to identify risk factors for in-hospital mortality (Table 4). Age over 45 years was positively associated with in-hospital mortality in model 1 (odds ratio [OR]: 1.953, 95% confidence interval [CI]: 1.036–3.683; P=0.038) but not in model 2. According to model 3, prior hospitalization within 3 months (OR: 2.311; 95% CI: 1.002–5.369; P=0.049), initial infection on admission (OR: 2.764; 95% CI 1.006–7.594; P=0.048), use of vasopressor drugs (OR: 2.940; 95% CI 1.071–8.069; P=0.036), and mechanical ventilation (OR: 5.658; 95% CI: 2.046–15.647; P=0.001) were independent risk factors for in-hospital mortality.

Table 4. Predictor Factors for Mortality by Univariate and Multivariate Logistic Regression Analyses

Variables	Univariate analysis		Multivariate analysis					
	OR (95% CI)	P value	Model 1		Model 2		Model 3	
			OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Female sex	1.211 (0.385–3.812)	0.744	1.115 (0.323–3.846)	0.863	1.115 (0.323–3.846)	0.863	2.210 (0.284–17.216)	0.449
Age >45 years	1.956 (1.046–3.659)	0.036	1.953 (1.036–3.683)	0.038	2.004 (0.964–4.164)	0.063	1.630 (0.609–4.367)	0.331
Modified SLEDAI-2K score* (every 1 unit increased)	1.013 (0.977–1.051)	0.487	-	-	1.024 (0.982–1.063)	0.267	1.029 (0.972–1.089)	0.321
Hypertension	2.983 (1.456–6.114)	0.003	-	-	2.033 (0.882–4.683)	0.096	2.264 (0.725–7.072)	0.160
Chronic kidney disease	2.328 (1.298–4.178)	0.005	-	-	1.603 (0.793–3.243)	0.189	1.056(0.337–3.315)	0.925
Lupus nephritis	1.307(0.752–2.270)	0.343	-	-	1.063(0.542–2.083)	0.859	1.222 (0.468–3.187)	0.682
Medication within 3 months prior to admission								
Corticosteroid	0.920 (0.433–1.954)	0.828	-	-	0.669 (0.266–1.685)	0.394	0.750 (0.211–2.661)	0.656
Azathioprine	0.317 (0.108–1.282)	0.117	-	-	0.427 (0.111–1.644)	0.216	0.882 (0.164–4.728)	0.883
Cyclophosphamide	2.022 (1.067–3.831)	0.031	-	-	1.818 (0.823–4.014)	0.319	1.751 (0.580–5.281)	0.320
Mycophenolate mofetil	1.543 (0.683–3.485)	0.297	-	-	1.624 (0.628–4.200)	0.317	1.757 (0.461–6.894)	0.409
Antimalarial	0.706 (0.402–1.239)	0.225	-	-	0.972 (0.506–1.863)	0.932	0.927 (0.378–2.273)	0.867
Prior hospitalization within 3 months	2.710 (1.542–4.763)	0.001	-	-	-	-	2.311 (1.002–5.369)	0.049
Laboratory at admission								
Hemoglobin <8 (g/dL)	1.423 (0.813–2.491)	0.216	-	-	-	-		
Creatine >1.5 (mg/dL)	2.262 (1.282–3.985)	0.005	-	-	-	-	1.323 (0.488–3.587)	0.582
Albuminemia <3.5 (g/dL)	1.863 (0.821–4.224)	0.136	-	-	-	-		
Present of SIRS† on admission	3.569 (1.888–6.748)	<0.001	-	-	-	-	1.153 (0.445–2.987)	0.770
qSOFA‡ >2 scores	4.435 (2.215–9.258)	<0.001	-	-	-	-	1.825 (0.581–5.732)	0.303
Initial infection	3.163 (1.673–5.982)	<0.001	-	-	-	-	2.764 (1.006–7.594)	0.048
Nosocomial infection	10.904 (5.362–22.172)	<0.001	-	-	-	-	2.268 (0.819–6.285)	0.115

Continuation of Table 4.

Variables	Univariate analysis		Multivariate analysis					
	OR (95% CI)	P value	Model 1		Model 2		Model 3	
			OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Vasopressor drugs	18.627 (9.101–38.123)	<0.001	-	-	-	-	2.940 (1.071–8.069)	0.036
Mechanical ventilator	16.816 (8.378–33.758)	<0.001	-	-	-	-	5.658 (2.046–15.647)	0.001
Plasma exchange	3.647 (1.474–0.926)	0.005	-	-	-	-	0.857 (0.197–3.736)	0.838
Intravenous cyclophosphamide use during admission	0.085 (0.011–0.629)	0.016	-	-	-	-	0.213 (0.023–1.981)	0.174

*Modified SLE Disease Activity Index -2 K; †Systemic inflammatory response syndrome; ‡ Quick sepsis-related organ assessment.

Discussion

In this study, we investigated factors predictive of in-hospital mortality among patients with SLE. Our analysis indicated that infection was the most common cause of death in this population, followed by disease activity. Initial infection on admission, prior hospitalization within 3 months, and the use of vasopressors and mechanical ventilators during admission were positively associated with the in-hospital mortality rate.

Our study, which was based on data collected from a government hospital in Thailand, reflects "real-life" outcome data from a developing country with relatively limited medical resources. The overall mortality rate among included patients was 25.5%, and the most common cause of death was infection (including infection on admission and concurrent infection with SLE flare). This result is consistent with the findings of previous studies conducted in developing countries, which have reported relatively higher mortality rates than studies conducted in developed countries. Such studies have also reported infection and disease flares as the leading causes of in-hospital death (12, 18-20). Differences in ethnicity, socioeconomic status, regional cultural background, and the availability of medical resources have been shown to influence disease patterns and treatment outcomes in patients with SLE (11). The current study was conducted in a hospital in an urban area of Thailand, and most patients had a low socioeconomic status, which has been associated with non-adherence to medical care and poor outcomes. Identifying risk factors in these populations can aid in the development of more effective strategies based on regional and cultural variations.

Previous studies revealed different patterns of mortality by age of onset in patients with SLE. SLE patients with younger age of onset tended to die due to active disease or infection (21, 22), while those with older age of onset tended to die due to cardiovascular causes and malignancy (23). In our study, the study population had younger onset SLE (mean age of participants of 34.4 years) and most patients died due to active disease or infection, thus confirming the results of previous studies.

In contrast to current guidelines that mention that antimalarial agents should be prescribed for all SLE patients unless contraindicated (24), the rate of antimalarial drugs use in this study was relatively low (44.6%). There are many possible reasons to explain this finding. First, for patients who were newly diagnosed during admission, physicians tended to commence antimalarial drugs in the outpatient setting after the disease had subsided. Second, some patients had contraindications for antimalarial drugs. Third, decision making can vary depending on the physician's specialty. Finally, this result may be influenced by the socioeconomic factors in the population studied. We plan to conduct further research to assess the rate of adherence to guidelines regarding antimalarial drugs use, explore the reasons for non-adherence, and identify the factors affecting non-adherence. The use of antimalarial agents has established beneficial effects on long term outcomes (25, 26). Thus, antimalarial drugs use could have an important influence on the course of the disease during admission. However, antimalarial agents use was not associated with better short-term survival as evidenced by in-hospital mortality rates in this study.

Previous studies have identified several risk factors for in-hospital mortality among patients with SLE. Another study conducted in Thailand reported that, among patients with SLE admitted to medical ICUs, the need for vasopressor therapy and ventilator-associated pneumonia was associated with higher mortality during ICU admission (27). In accordance with this finding, the use of vasopressors and the need for mechanical ventilator treatment were identified as independent risk factors for in-hospital death in the current study, with odds ratios of 2.94 and 5.66, respectively. Patients with SLE meeting the criteria for admission to a critical care setting usually require more complex treatment modalities, including vasopressor therapy, mechanical ventilation, and renal replacement therapy, which are associated with a longer duration of hospitalization and a higher incidence of treatment-related complications, thereby increasing the risk of death.

Prior hospitalization within 3 months was identified as an independent predictor of mortality in the current study. Consistent with previous findings, patients with SLE required frequent readmission, with disease activity as the primary reason for readmission (28). Previous studies have identified numerous risk factors associated with repeated remission, including disease flares, lupus nephritis, serositis, and thrombocytopenia. In addition, patients who required readmission within a short period of time had more severe clinical manifestations than those who did not (6, 29). Both disease activity and intensive immunosuppressive therapy make patients with SLE more susceptible to infection, contributing to increases in in-hospital mortality (28). Taken together, the available evidence highlights the importance of outpatient care, adherence to treatment, and patient education to reduce the risk of readmission and death.

In our study, qSOFA >2, SIRS on admission, initial infection on admission, and nosocomial infection were associated with in-hospital mortality in univariate analyses. However, after multivariate analysis, only initial infection on admission was positively associated with in-hospital mortality. This finding underscores the impact of infection as major presenting problem in patients with SLE. Regular surveillance of SLE patients with infection-related risk factors (e.g., lupus nephritis, immunosuppressive therapy, and use of high dose glucocorticoids) and prompt recognition of infections (as soon as possible) may reduce the risk of development of disease complications and in-hospital mortality.

The strength of the present study is that it has been conducted in a regional tertiary government hospital in Thailand, which is a developing country where medical resources are limited. The results of our study reflect the outcomes of real-world treatment of SLE patients better than those of studies conducted in university affiliated hospitals. However, this study had several limitations. First, the study was retrospective in nature. All information was manually evaluated via a review of each patient's medical charts, which may have resulted in misclassification bias and missing data. Lupus nephritis-related variables, which could interfere with in-hospital mortality, especially the time to diagnosis of lupus nephritis, its duration, and specific treatments, were not obtained. Second, this was a single-center study, which limits the generalizability of the study results. Third, some therapies (including vasopressor support and mechanical ventilation) may lead to higher mortality rates because these variables are surrogate markers of disease severity. Therefore, these factors must be interpreted carefully. Fourth, we included only patients admitted to the Department of Internal Medicine and excluded some conditions such as the coexistence of Sjogren syndrome, APS, pregnancy, and malignancy; this may result in selection bias. Fourth, the lack of information regarding the site, foci, and microbiological etiology of the infections may affect the outcomes. Finally, we did not investigate the predictors of disease activity or infection. Future trials investigating in this aspect are needed. While multi-center trials with larger sample sizes are required to investigate this association as well as potentially protective factors, our results may aid in clinical decision making and management of SLE during admission.

Conclusion

The present results indicated that infection was the most common cause of death among hospitalized patients with SLE. The clinical variables of underlying, prior hospitalization within 3 months, initial infection on admission and the use of vasopressors and mechanical ventilators during admission were positively associated with in-hospital mortality. While multicenter trials with larger sample sizes are required to investigate this association as well as potentially protective factors, our results may aid in clinical decision making and management of SLE during admission.

What Is Already Known on This Topic:

The clinical complexity of systemic lupus erythematosus (SLE) makes accurate prediction of hospital outcomes difficult. Most studies reporting improved survival outcomes have been conducted in developed or high-income countries, while studies from developing countries have reported relatively worse outcomes. Furthermore, clinical data related to the factors that influence in-hospital mortality in patients with SLE in developing countries, where healthcare resources are relatively limited.

What This Study Adds:

In Thailand, infection was the most common cause of death in this population, followed by disease activity. Prior hospitalization within 3 months, initial infection on admission, and the use of vasopressors and mechanical ventilators during admission were positively associated with the in-hospital mortality rate.

Authors' Contributions: Conception and design: SL and AC; Acquisition, analysis and interpretation of data: SL, AR and AC; Drafting the article: SL and AR; Revising it critically for important intellectual content: AC; Approved final version of the manuscript: SL, AR and AC.

Conflict of Interest: The authors declare that they have no conflict of interest.

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Case Report
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The Coexistence of an Incomplete Superficial Palmar Arch and a Berrettini Anastomosis: A Case Report

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Abstract

Objective. The aim of this study is to present a relatively rare case of the coexistence of an incomplete superficial palmar arch and a Berrettini anastomosis, identified in a cadaveric specimen, and further discuss the potential clinical implications of such anatomical variations. **Case Report.** The variation was found in the left hand of a formalin-fixed male cadaver of Greek origin, that was dissected under an operating microscope (×4, ×10 magnification) in our Anatomy Department. In the specimen, we found an incomplete superficial palmar arch, formed only by the superficial branch of the ulnar artery, and a Type 1 Berrettini Anastomosis, originating from the ulnar nerve and joining a branch of the median nerve. **Conclusion.** To avoid iatrogenic damage and permanent loss of sensation, hand surgeons and microsurgeons should be aware of the presence of a BA, and the potential coexistence of this variation with vascular abnormalities in the hand that may complicate surgical procedures.

Key Words: Median Nerve ▪ Ulnar Nerve ▪ Superficial Palmar Arch ▪ Microsurgical Anatomy ▪ Berrettini Anastomosis.

Introduction

Both the descriptive and microsurgical anatomy of the human hand are of undeniable complexity. Specifically, the vascular anatomy of the hand is of paramount importance for radiologists and hand surgeons (1).

The vascularization of the hand is dependent on the superficial and deep palmar arches. The superficial palmar arch (SPA) is formed in most cases by the superficial branch of the ulnar artery (SUA) completed by a superficial palmar branch of the radial artery (SRA). The SPA normally lies superficially to the long flexor tendons and lumbricals, and beneath the palmaris brevis, as well as the palmar aponeurosis. It provides the three common palmar digital arteries (CDA) and a fourth digital artery that supplies the ulnar half of the 5th finger, the so-called Digiti Minimi Artery (DMA) (1-3). However, the formation of the SPA is subject to non-negligible variability (1, 4). There have been many reported patterns of SPA formation. The first described classification, and one of the most frequently used, is the classification of Coleman and Anson (1). According to this classification, the classical formation pattern of the SPA, with equal contributions from the SUA and SRA, is Type A. Type B SPA refers to an arch formed solely by the SUA, Type C to a mediano-ulnar SPA, Type D to a radio-mediano-ulnar SPA, and Type E SPA to an arch initiated by the SUA and finished by an enlarged arterial branch from the deep palmar arch (1).

Two nerves contribute to the sensory and motor innervations of the palmar surface of the hand: the median nerve (MN) and the ulnar nerve (UN). From the MN the first three common digital nerves arise (CDN) that provide sensory innervation of the first 3 and a half fingers, and the fourth CDN comes from the UN, innervating the ulnar half of the 4th finger and the radial half of the 5th finger

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(2). Two of the most prevalent communications that occur between the MN and UN in the hand are the Riche-Cannieu Anastomosis between the 1st and 4th CDNs, and the Berrettini Anastomosis (BA) between the 3rd and 4th CDNs, or between the 3rd CDN and the main stem of the UN (5-7). Specifically, the BA was first described in 1741 by Berrettini, and includes 3 subtypes. According to Roy et al., Type 1 BA is a communicating branch from the UN towards the MN, Type 2 is a branch from the MN towards the UN, and Type 3 includes diffuse interconnecting communication branches between the UN and the MN (7).

Our aim is to present a relatively rare case of the coexistence of an incomplete SPA and a BA, identified in a cadaveric specimen, further discuss the potential clinical implications of such anatomical variations, and highlight the significance of deep knowledge of the complex anatomy of the hand.

Case Report

The left hand of a formalin-fixed (10% v/v solution) male cadaver was dissected under a Carl Zeiss™ operating microscope (×4, ×10 magnification) using microsurgical instrumentation, for research purposes, in the Dissection Hall of our Anatomy Department. The cadaver was of Greek origin and obtained through body donation, with the written and informed consent of the donor, according to the relevant legislation (8). A Würth™ digital Vernier caliper (0.01 mm, accuracy) was used for the measurements of distances and the vessels' outer diameters (d), calculated at their point of origin.

In the dissected hand, we found an incomplete superficial palmar arch, formed only by the SUA (d: 1.48mm). This branch gave rise to the 1st, 2nd and 3rd CDAs (d: 1.13 mm, 1.18 mm, 1.04 mm) the DMA (d: 0.64 mm) and a small thenar branch (d: 0.61 mm) (Figure 1). The thumb was supplied by a princeps pollicis branch, originating from the deep palmar arch. In the part of the SUA between the origin of the 2nd and 3rd CDA, it followed a curly course, being in quite a close relationship with the 3rd CDN.

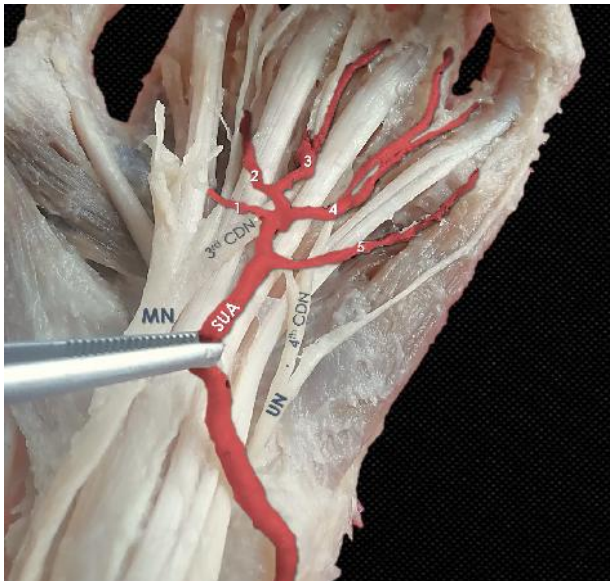

Figure 1. The incomplete Superficial Palmar Arch; MN=Median Nerve; UN=Ulnar Nerve, 3rd CDN= 3rd Common Digital Nerve; 4th CDN=4th Common Digital Nerve; SUA=Superficial branch of the Ulnar Artery; 1= Small thenar branch (cut); 2-4=1st -3rd Common palmar Digital Arteries; 5=Digiti Minimi Artery.

Figure 2. The Berrettini Anastomosis (white arrow); The branches of the Superficial Palmar Arch have been retracted; MN=Median Nerve, UN=Ulnar Nerve; 3rd CDN=3rd Common Digital Nerve; 4th CDN=4th Common Digital Nerve, TCL=Transverse Carpal Ligament.

In addition, a BA was identified (between the 3rd and 4th CDNs). It followed an oblique trajectory, from proximally and medially to distally and laterally (Type I BA) (7), and was 17.88 mm in length and 0.72mm in width (Figure 2).

Discussion

Embryology

The seventh cervical intersegmental artery of the upper limb, the axis artery, continues, initially, as the anterior interosseous artery. It extends along the ventral axial line, and ends in the palmar surface of the hand as a deep capillary plexus. When the latter recedes, the median artery expands distally and joins the superficial palmar capillary plexus. The superficial palmar capillary plexus gives rise to the digital arteries of the hand. The median artery finally recedes and is replaced by the radial artery (RA) and UA. The RA appears first and joins the deep palmar arch, while the UA later joins the SPA (3).

Embryologically, variations in the blood vessels of the hand, may be explained as resulting from the persistence of vessels that are normally obliterated, the disappearance of vessels that are normally retained, incomplete development of the vessels, and the abnormal merging and absorption of elements that are usually distinct. Thus, the variance in SPA formation could be attributed to any of the aforementioned mechanisms (3).

The embryological basis of the BA (and every intercommunicating neural branch in general) is related to the neural fibers of the MN or the UN, that have abnormally fused with another nerve (UN or MN) at its rising point on the brachial plexus and, after covering some distance with that nerve, they finally split at the hand and rejoin the UN or the MN (2).

Prevalence

The pattern reported of incomplete SPA formation, i.e. stemming entirely from the SUA without supplying the thumb, is Type – F SPA, according to the Gellman et al., classification (4). The frequency of this SPA type varies between studies. Gellman et al. reported a frequency of 11.1% in 45 cadaveric specimens (4). Joshi et al. (2014) reported that 66% of 100 upper limbs presented a SPA formed exclusively by the SUA; however, in this study there were no specific data about the thumb supply and the termination of the SUA in the thenar (9).

The BA is the most frequent (60.9%) type of anastomosis between the UN and the MN. The second most frequent type is a Riche-Cannieu anastomosis, the prevalence of which has been found to be up to 55.5% (7). However, the prevalence of each of the three subtypes of BA varies. Type 1 BA, like the one described in the current report, is the most common of the three subtypes, accounting for 86.2 % of all BA cases. The other two subtypes are significantly less common, with the prevalence of Type 2 at 9.4% and Type 3 at 4.4% (7).

The prevalence of the coexistence of these two anatomical variations is considered rare, given that there are only a few descriptions of this condition (10). One such description is the case study by Sirasanagandla et al. (10).

Clinical Considerations

The perfusion of the hand is bifid. The normal presence of both the deep and superficial palmar arches offers collateral perfusion via a wide anastomotic network, that protects the hand from ischemia. In cases in which the deep or superficial palmar arch is incomplete, occlusion of the RA or the UA, e.g. due to thrombosis following cannulation, can lead to severe ischemia of the hand (3). In addition, the UA is more vulnerable than the RA, given its course around the hook of the hamate bone, so, in cases like the one reported in this paper, partial hand ischemia may occur as a result of an UA injury.

Several authors have discussed the clinical implications of the BA. A case was described of a patient with traumatic laceration of the BA, leading to sensory loss between the 3rd and 4th fingers. The symptoms improved after the laceration was surgically repaired. Additionally, carpal tunnel release has been reported to involve injuries of the

communicating branch in BA cases (7). A "danger zone" has been described, which extends to the middle half of the hypothenar eminence, and which is limited distally by the proximal transverse crease of the palm, and radially by the longitudinal crease between the eminences of the thenar and hypothenar (6). To avoid iatrogenic damage and resulting permanent loss of sensation, hand surgeons and microsurgeons should be aware of the presence of a BA, with the greatest risk emerging in procedures such as carpal tunnel release, Dupuytren fasciectomy, 4th finger flexor tendon surgery, as well as mobilization of neurovascular island flaps (7).

Conclusion

The case described is a combination of two relatively common anatomical variations of the hand. However, being aware of such structural abnormalities is of non-negligible practical significance for hand surgeons, as vascular and neural abnormalities in the hand that may complicate surgical procedures.

What Is Already Known on This Topic:

The vascular and neural anatomy of the hand is of indisputable complexity. Berrettini anastomosis between the ulnar and median nerves is a well-described anatomical variation in the hand. An incomplete superficial palmar arch is also a relatively common anatomical entity.

What This Study Adds:

This study describes (also using detailed dissection images) a relatively rare case of the coexistence of a Berrettini anastomosis and an open superficial palmar arch, and highlights the clinical significance of such variations that may complicate surgical procedures in the hand.

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Case Report
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The Brachiocephalic Trunk Variant Origin and High-Riding Course: Two Cadaveric Cases

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Abstract

Objective. The current report describes two rare cadaveric findings of a left sided brachiocephalic trunk (BCT) in relation to the trachea, and its high-riding course above the suprasternal notch (SN). **Cases Descriptions.** In two elderly body donors dissected after death, a left-sided BCT was identified with a high-riding course (0.5 and 0.8 cm above the SN). The BCT originated from the aortic arch, in common with the left common carotid artery, more distally than the typical left-side location and crossed in front of the trachea. In the 1st case, the ascending and descending aortae, and the left subclavian artery had aneurysmal dilatation. In both cases, the trachea was displaced to the right side and had a stenosis due to the chronic compression. **Conclusion.** A high-riding BCT is of paramount clinical importance, as it may complicate tracheotomy, thyroid surgery and mediastinoscopy, leading to fatal complications. BCT injury leads to a massive bleeding during neck dissection (level VI), when the vessel crosses the anterior tracheal wall.

Key Words: High-Riding Brachiocephalic Trunk ▪ Innominate Artery ▪ Variant ▪ Compression ▪ Trachea.

Introduction

The typical aortic arch (AA) three-branch pattern, with a 77% prevalence (1) consists of the brachiocephalic trunk (BCT), alternatively called the innominate artery (1st branch), that further divides into the right common carotid artery (RCCA) and the right subclavian artery (RSA). The 2nd and 3rd branches are the left common carotid artery (LCCA) and the left subclavian artery (LSA). AA variant branching patterns (23% prevalence) have been associated with dysphagia, vascular diseases, and aortic dissection (1). The increase in cardiovascular interventions requires a deep and detailed understanding of AA anatomy (2). The importance of the evolution of computed tomography (CT) scans in AA variant detection was highlighted in a recent meta-analysis (1). Among the atypical AA variants, the brachiocephalico-carotid trunk (BCCT), is the most common variant (36% prevalence) (1) and is composed of the fused vessels BCT and LCCA. This arterial complex in humans was falsely characterized as "bovine AA". The true "bovine AA" is identified only in animals with deep chests, and corresponds to a single great vessel originating from the AA, giving rise to both the RSA and LSA and a common trunk of the RCCA and LCCA (the so-called bicarotid trunk) (3). A correlation was identified between BCCT occurrence and thoracic aortic disease onset (4). In addition, the aneurysmal presence, and the high risk of rupture with concomitant death is highlighted (5). Therefore, this variant was characterized as a "true

silent killer" (5). Typical and variant BCT locations: Typically the BCT is located across the anterior tracheal wall, between the 6th and 13th rings (the 5th and 6th tracheal rings are the most common locations) (6). A high BCT location has been identified between the 1st and 5th tracheal rings (6-8) and is of clinical importance, as it may complicate tracheotomy (6, 8), thyroid surgery (9) and mediastinoscopy (7), leading to death. During neck dissection, massive bleeding may occur after BCT injury (10).

The current report describes two rare cadaveric findings of an ectopic BCT (left-sided BCT) related to the trachea and its high-riding course. The possible clinical impact is highlighted on the basis of clinical studies. Coexisting variants are also described.

Case Presentations

Case 1. An 80-year-old donated male cadaver was identified as having a left-sided BCT (28mm diameter) originating in common with the LCCA. The BCT had a typical elongation of 6.25cm and a high-riding course, reaching up to the right side of the 5th tracheal ring (uppermost border point). The BCT crossed in front of the trachea, and posterior to the right sternoclavicular joint, dividing into the RSA and RCCA. The RCCA had an atypical course, anterolateral to the trachea. The ascending aorta had an aneurysmal dilatation (18 cm perimeter and 3cm diameter) and a bicuspid aortic valve. The LSA, and the remaining part of the descending aorta, from the isthmus downwards, also presented aneurysmal dilatation. The trachea was displaced to the right, and had a stenosis due to its chronic compression by the abnormal vessel (Figure 1). A pacemaker was implanted into the left cephalic vein (drained in common with the axillary vein to the subclavian vein) to help heartbeat control. Coexisting variants: a common origin of the BCT with the LCCA, and the bilateral common origin of the internal thoracic artery from the thyrocervical trunk.

Case 2. A 77-year-old donated female cadaver had a left-sided BCT originating from a common trunk with the LCCA, the so-called BCCT. The BCT had an aberrant high-riding route up to 1.5cm beneath the lower border of the thyroid isthmus. The RCCA and RSA originated after characteristic coiling (angulation of the BCT, where the superior border compresses the trachea). The LCCA route was tortuous just after its point of origin (Figure 2). The right recurrent laryngeal nerve originated at the point of the BCT division into the RSA. *Coexisting variants:* a common trunk of the BCT with the LCCA (BCCT).

Both cadavers derived from the body donation programme of both Universities after written informed consent (donation before death).

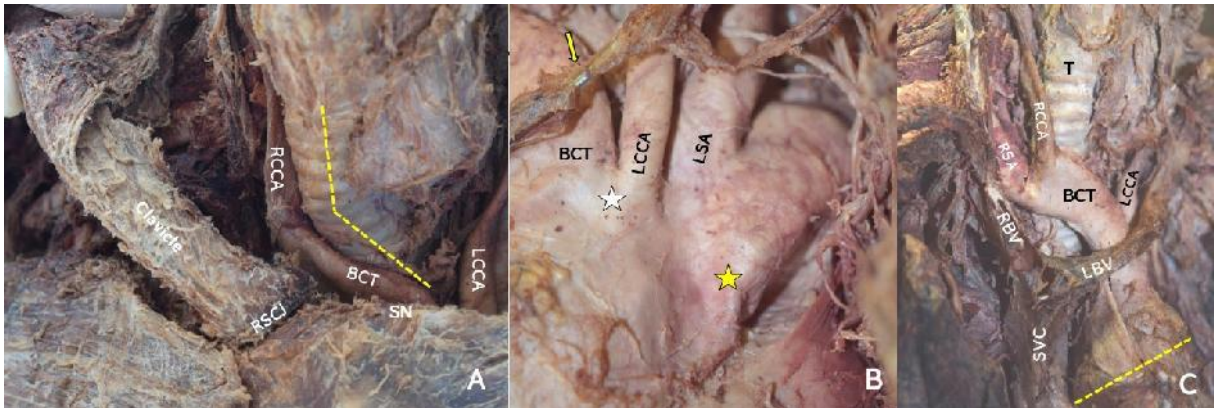

Figure 1. **A.** Trachea (T) compression, brachiocephalic trunk-BCT bifurcation into the right common carotid and right subclavian artery (RCCA and RSA) posterior to the right sternoclavicular joint (RSCJ). SN- sternal notch, dotted yellow line- T inclination after compression. **B.** White asterisk- the BCT-LCCA common origin, yellow asterisk aneurysm dilation, yellow arrow- catheter of the pacemaker into the vein, LCCA-left common carotid artery, LSA-left subclavian artery. **C.** The BCT on the left of the T, yellow dotted line-the aneurysm diameter, RBV-right brachiocephalic vein, LBV-left brachiocephalic vein, and SVC-superior vena cava.

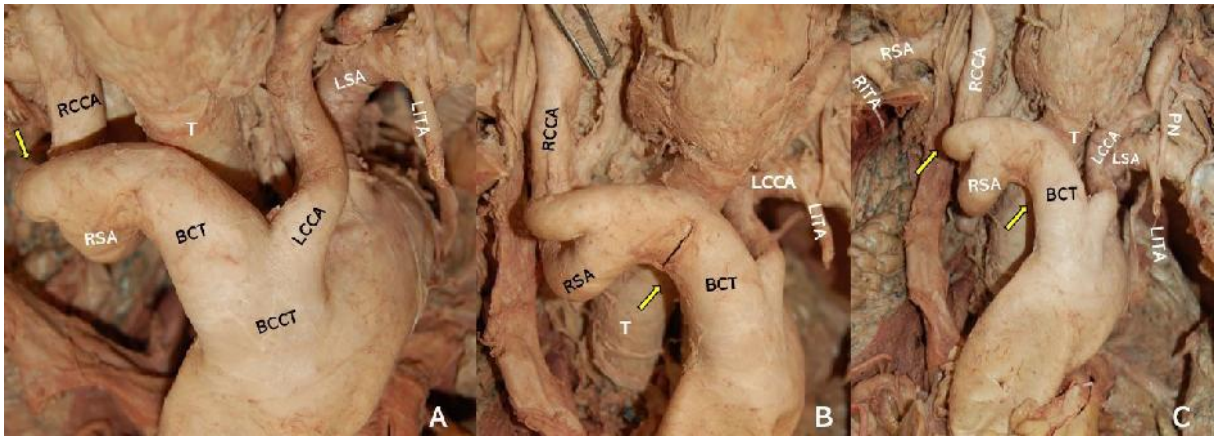

Figure 2. **A**. The brachiocephalico-carotid trunk (BCCT) after the brachiocephalic trunk (BCT) fusion with the left common carotid artery (LCCA). Trachea (T) compression by the elongated BCT, yellow arrow-kinking vessel. **B**. yellow arrow curvature of the elongated BCT and division into the right common carotid and right subclavian artery (RCCA and RSA), LITA-left internal thoracic artery. **C**. Yellow arrows indicate the vessels' tortuosity, RITA-right internal thoracic artery, LSA-left subclavian artery.

Discussion

The AA branching pattern configuration is related to the different developmental grades of the various branches' "migration" and "merging" (11). In BCCTs, the LCCA origin migrates to the right and merges with the BCT (under variable forms, a BCT-LCCA common trunk, or common origin, or LCCA as a BCT branch). In the BCCT pattern, the aortic sac fails to give off the right and left horns, and consequently, the LCCA origin migrates to the right and merges with the BCT trunk (12). The exact BCCT embryological path development remains unclear.

AA variants may increase the blood pressure across the abnormal vessel, which in turn increases stress in the branching vessels and descending thoracic aorta, leading to development of aneurysms (13). Aneurysms usually develop in areas of arterial bifurcations, bends and junctions which are the most common sites for atheromatous plaque formations and, consequently, atherosclerotic lesions usually predispose to aneurysms (5, 13).

The high-riding BCT and clinical landmarks: In high-riding BCTs, although the tracheal rings are used to evaluate the BCT's position, these features are quite difficult to identify precisely, preoperatively (10). The SN was proposed as an alternative and easily identified clinical landmark by Cai et al. (10). The authors (10) also identified high-riding BCTs, 2 cm above the SN, in 2.2% of patients and recorded an intraoperative risk, 51-times higher compared to the relative risk in patients with BCTs 2 cm lower. In a high-riding BCT, measures should be taken to prevent intraoperative injury. In lower anterior neck surgery, especially mediastinoscopy, caution should be taken in maximal neck extension, as this maneuver tends to elevate the BCT, especially in young patients. Thus, even with a typical anatomy, this vessel is at risk for injury during mediastinoscopy. It should be routine to palpate below the SN to ascertain the BCT level, before obtaining access to the pretracheal fascia (7). Anterior neck surgery should be modified in patients with a high-riding BCT and coexisting tortuous common carotid arteries that protrude or are positioned higher than normal (8, 14). Urgent cases of surgical intervention should be evaluated by Doppler ultrasonography to prevent an unexpected fatal complication (15).

The BCCT variant: The BCCT has been observed with a prevalence ranging from 14.4% (2) to 36% (1) in the general population. The highest BCCT prevalence was identified in African and the lowest in Asian populations (1, 2). A higher regional shear stress in the BCCT variant (tendency for thrombus formation) and flow

alterations, associated with endothelial injury and vascular stiffness were reported by Shalhub et al. (16). A significantly higher proportion of cerebrovascular events during carotid angioplasty and stenting were reported in cases of BCCT variants (17). These cases were also reported as the greatest predictors of difficult access in older patients (17). During thyroid and neck dissection surgeries, the BCT may be dissected out first and protected. In tracheotomy, it may be necessary to avoid low levels of exposure. Causes of BCT ectopia may be kinking, coiling and tortuosity, and these entities are more prevalent in elderly women with hypertension (18). The co-occurrence of common carotid artery tortuosity with the BCT's common origin with the LCCA may suggest congenital etiological factors, since AA maldevelopment may result in carotid artery anomalies (18). Magnetic resonance imaging and angiography (MRI and MRA) remain the gold standard for identifying the BCT's origin, course and branching pattern, showing more details of the carotid arteries. Since most BCT variant cases are clinically silent, a high index of suspicion makes the preoperative diagnosis of utmost importance to avoid complications (15, 19). BCCTs may cause vascular insufficiencies and become major risk factors for neurological symptoms. Syperek et al. (20) identified a BCCT in 25.7% of stroke patients. Aneurysmatic BCCTs had a higher intimal and adventitial thickness compared to the typical anatomy aneurysmatic AAs (20). The BCCT's higher shear stress might be caused by the altered branching angle of the supra-aortic vessels (16). There is a possibility that in cardioembolic stroke patients, the altered hemodynamics cause the redirection of the embolus towards the carotid arteries, leading to cerebral infarctions (20).

Limitations of Report

No clinical details were known from the subjects' medical record. No morphometric measurements were taken to justify the altered geometry of the variant vessels.

Conclusions

In two elderly body donors an ectopic (left-sided) BCT with a high-riding course was identified, coexisting with aneurysms, and trachea compression. Coiling and tortuosity of the branching vessels was identified. In a unique case, the BCT coexisted with a fused LCCA, a BCCT. Further evidence-based information, based on larger clinical studies, is needed to identify potential risk factors and surgical complications.

What Is Already Known on This Topic:
Typically the BCT is located across the anterior tracheal wall, between the 6th and 13th tracheal rings, with the most common locations at the 5th and 6th tracheal rings. The BCT can migrate left of the trachea and follow a high-level course. A high-riding BCT is of paramount clinical importance, as it may complicate tracheotomy, thyroid surgery and mediastinoscopy, leading to death. BCT injury leads to massive bleeding during neck dissection, when the vessel crosses the anterior tracheal wall.

What This Study Adds:
The current cadaveric report describes a rare ectopic BCT with a high-riding course, compressing on the trachea, coexisting with aneurysms. Coiling and tortuosity of the branching vessels were identified. The elongated BCT may be a BCCT, after the fusion of the LCCA with the BCT.

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Conflict of Interest: The authors declare that they have no conflict of interest.

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Original Article
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COVID-19 Seroprevalence in Children during Pandemic Waves in Sarajevo, a Single Center Experience

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Abstract

Objective. The aim of this study was to present data on the prevalence of seropositive children tested in the laboratory of the Eurofarm polyclinic in Sarajevo, from September 2020 to May 2021. **Material and Methods.** Peripheral blood samples were collected and anti-SARS-CoV-2 antibodies were detected using an electrochemiluminescence immunoassay. **Results.** In the total of 762 tested children, 187 were positive (24.5%), based on cut-off value. Of all the positive cases 42.8% were female while 57.2% were male. There were 10.1% of positive children in the first age group (0-5 years), 44.4% in the second group (6-13 years), and 45.5% in the third group (14-18 years). There was no statistically significant difference in seroprevalence between age groups and gender. The lowest seroprevalence (3.6%) was observed in October 2020 after the first pandemic wave, and the highest seroprevalence (60.3%) was observed in April 2021, corresponding to the third pandemic wave. **Conclusion.** The results of our study showed that the seroprevalence in children was low, especially during the first year of the pandemic. In the second year of the pandemic, there was an evident statistically significant increase in the number of seropositive children. Similar data have been shown in studies for adults.

Key Words: Seroprevalence ▪ COVID-19 ▪ SARS-CoV-2 ▪ Antibodies ▪ Children.

Introduction

During the coronavirus disease 2019 (COVID-19) pandemic, children were less represented and had a milder form of the disease compared to adults. In the United States, children <18 years accounted for approximately 13% (1), in China less than 1% of cases in children <10 years (2). Most of the children with acute COVID-19 had a mild form of the disease, and a smaller number required hospitalization (3). A strong innate immune response, immune training with previously given vaccines (4, 5), as well as previous exposure to respiratory viruses, less comorbidity and different angiotensin converting enzyme-2 (ACE2) receptors expression in children may have led to the lower incidence and mostly mild forms of COVID-19 in children (6). Post-infectious manifestations, such as multisystem inflammatory syndrome associated with SARS-CoV-2 (MIS-C) and long COVID, were a cause for concern. Serological tests are important in the diagnosis of these complex manifestations (7). During the pandemic, a large number of children had asymptomatic infection (8). A systematic review article and meta-analyses show that the percentage of asymptomatic children was 15-42% (9). Recent research in Bosnia and Herzegovina (B&H) showed that the percentage of asymptomatic children was 35.7% (10). The gold standard for the diagnosis of acute infection is the polymerase chain reaction (PCR) test. Serological tests are helpful for assessing the total number of

infected, including asymptomatic cases, monitoring the course of the pandemic, and planning public health measures (11-13). One of the measures to monitor the number of infected people in a certain location and in a certain period of time is the COVID-19 serology surveillance strategy. Positive serology test results are a sign of a past or recent infection. Antibodies can be detected 1-3 weeks after infection. Available serological antibody tests usually reveal antibodies to the nucleocapsid or spike proteins of the virus (11, 14). Seroprevalence studies provide valuable information for vaccination strategies because they help quantify what proportion of the population has been exposed to the virus, help achieve herd immunity, and help identify populations that may be at greater risk of infection (15).

The aim of this study was to present the results of seroprevalence in children tested in the laboratory of the Eurofarm polyclinic in Sarajevo, from September 2020 to May 2021, which can be useful for planning public health measures, and help in assessing herd immunity for COVID-19.

Materials and Methods

Participants

A total of 762 children were tested in the period between September 2020 and May 2021. The participants were divided into three groups: group one (children from 0-5 years of age) consisting of 73 individuals (9.6%), group two (children from 6-13 years of age) consisting of 373 individuals (48.9%) and group three (children from 14-18 years of age) consisting of 316 individuals (41.5%). A total of 368 participants were female (48.3%), while 394 (51.7%) were male.

Materials

Peripheral blood samples were collected by venipuncture in Vacusera vacutainers with CAT serum (BD Vacutainer1, Germany). Samples were transferred for centrifuge at 500×g for 10 minutes and analyzed the same day.

Methods

Anti-SARS-CoV-2 antibodies were quantified using an electrochemiluminescence immunoassay (ECLIA). The ELISA (enzyme-linked immunosorbent assay) method was used for testing the control group. The results were interpreted according to the protocols of the American Society for Microbiology (16). Prior to testing and analysis, 60 control samples, selected randomly from the tested cohort, were used for verification and correlation between the ECLIA and ELISA methods. Verification shown that 58/60 tests gave the same results. During the ECLIA tests, 18 were positive, while the ELISA tests gave 16 positive results, and the difference was not significant (χ^2=0.164 P=0.685). For the ECLIA test the serum samples were analyzed using CE.IVD Roche CobasElecsys1 Anti SARS-CoV-2, an electrochemiluminescence immunoassay, for the qualitative detection of total pan-Ig, IgG, IgM, and IgA (Roche Diagnostics, Rotkreuz, Switzerland). The test was performed according to the manufacturer's instructions (Roche Diagnostics, Rotkreuz, Switzerland). The assay uses a recombinant protein representing the nucleocapsid (N) antigen in a double-antigen sandwich assay format, which favors detection of high affinity antibodies against SARS-CoV-2. The results are generated by an interpolating ECLIA signal, with a threshold produced during calibration. The result is given either as reactive or non-reactive in the form of a cutoff index (COI; signal sample/cut-off). If the COI is <1.0 then the result is considered non-reactive (negative for anti-SARS-CoV-2 antibodies); if the COI is ≥1.0 then the result is considered reactive (positive for anti-SARS-CoV-2 antibodies). Elecsys1Anti-SARS-CoV-2 exhibits a high overall clinical specificity of 99.8%, and sensitivity from 60.2-99.5%, depending on the number of days that have passed from the primary infection, where for up to 6 days the sensitivity is 60.2%, after 14 days the sensitivity is 99.5% (17). The ELISA was performed using a commercial ELISA kit based on the recombinant spike glycoprotein (S) and nucleocapsid protein (N) antigens of SARS-CoV-2 (ELISA COVID-19

IgG; Vircell Microbiologists, Granada, Spain). The results were expressed through the antibody index: AI=(sample optical density (OD)/cut-off serum mean OD) × 10, and interpreted as follows: IgG <4, negative; 4–6, borderline; >6, positive. The results were interpreted according to the protocols of the American Society for Microbiology (16).

Ethics Statement

The research was conducted at the Eurofarm Centre Laboratory, from September 2020 to May 2021. All the parents of the tested children signed written informed consent (prepared in accordance with the principles of the Declaration of Helsinki), and this was reviewed and approved by the local ethics committee, No: 7-EC-12/22.

Statistical Analysis

Standard descriptive statistical methods were used in the data analysis. The Chi-square (χ^2) test was used to determine a statistically significant difference by age and gender. The exact test of goodness-of-fit was used to compare the seroprevalence by month (September 2020 – May 2021). Data analysis was performed in MS Excel, as described by McDonald in 2014 (18) and IBM SPSS Statistics for Windows v27 (IBM, Armonk, NY). The level of statistical significance was P<0.05. The Bonferroni correction for 36 comparison (seroprevalence by month) was used to reduce type I error, where statistical significance was P<0.0014.

Results

Individuals were considered to have tested positive for anti-SARS-CoV-2 antibodies if COI levels were ≥1.0. On the basis of this cut-off level, a total of 187 individuals tested positive (N=762), which accounts for 24.5%. On the basis of the sensitivity and specificity of the ECLIA test, we calculated the true prevalence, where the result was 0.2948 (29.5%). We used the following formula (19): True prevalence=(Apparent prevalence+Specificity-1)/(Sensitivity+Specificity -1). The apparent prevalence was 0.2454 (24.5%), the specificity of ECLIA was 99.8% and median sensitivity 79% (60.2%-99.5%). True prevalence=(0.24+0.99-1)/(0.79+0.99-1)=0.23/0.78=0.2948 (29.5 %). In the first age group (0-5 years) there were 19 (10.1%) positive cases, while 83 (44.4%) in the second age group (6-13 years) and 85 (45.5%) in the third age group (14-18 years) (Table 1).

The chi-square test revealed the following association between the age groups: there was no significant difference in the number of positive cases between the first and second age groups (χ^2=0.493; P=0.482), or between the first and third age groups (χ^2=0.023; P=0.879). Also, there was no significant difference in the number of positive cases between the second and third age groups (χ^2= 2.003; P=0.157). A total of 80 anti-SARS-CoV-2 antibody positive individuals were female (42.8%) while 107 (57.2%) were male, and this difference in frequency was not found to be significant (χ^2=3.016; P=0.082). The seroprevalence throughout the nine-month time frame, with error bars indicating the 95% confidence interval, is shown in Figure 1.

The exact test of goodness-of-fit showed a significant increase in positive cases in November-December 2020 and February-April 2021 (Figure 1; Table 2). The lowest seroprevalence (3.6%) was observed in October 2020, and the exact test of goodness-of-fit showed that the number of cases was significantly lower when compared to March and April 2021 (Figure 1; Table 2). The highest seroprevalence (60.3%) was observed in April 2021, and the exact test of goodness-of-fit showed that the number of cases was significantly higher when compared to September and October 2020 (Figure 1; Table 2).

Table 1. The Frequency and Number of Total Tested and Positive Cases by Age Groups

Participants	Total tested N (%)	Positive cases N (%)
0-5 years	73 (9.6)	19 (10.1)
6-13 years	373 (48.9)	83 (44.4)
14-18 years	316 (41.5)	85 (45.5)

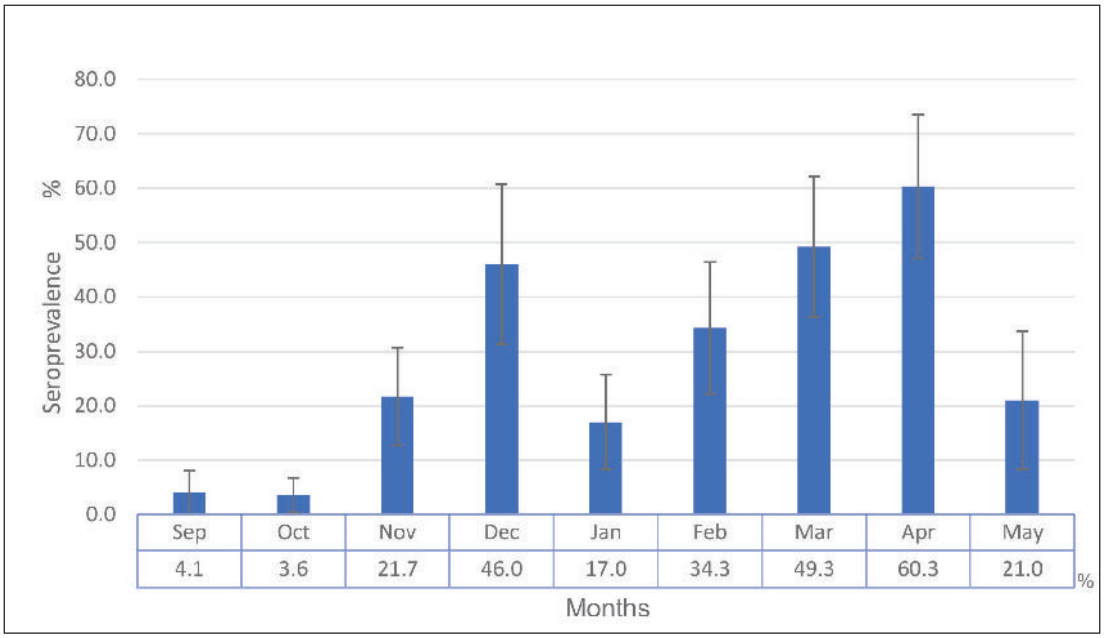

Figure 1. Seroprevalence throughout the nine-month time frame, with error bars indicating the 95% confidence interval.

Table 2. Comparison of Seroprevalence by Months (September 2020-May 2021) Based on Exact Test of Goodness-of-fit

SM* (%)	Sep (4.1)	Oct (3.6)	Nov (21.7)	Dec (46.0)	Jan (17.0)	Feb (34.3)	Mar (49.3)	Apr (60.3)	May (21.0)
	P values†								
Sep (4.1)	-	0.501	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001
Oct (3.6)	0.501	-	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
Nov (21.7)	<0.001	<0.001	-	0.002	0.314	0.052	<0.001	<0.001	0.439
Dec (46.0)	<0.001	<0.001	0.002	-	<0.001	0.109	0.419	0.103	0.001
Jan (17.0)	0.004	0.001	0.314	<0.001	-	0.012	<0.001	<0.001	0.314
Feb (34.3)	<0.001	<0.001	0.052	0.109	0.012	-	0.062	0.005	0.029
Mar (49.3)	<0.001	<0.001	<0.001	0.419	<0.001	0.062	-	0.169	<0.001
Apr (60.3)	<0.001†	<0.001	<0.001	0.103	<0.001	0.005	0.169	-	<0.001
May (21.0)	<0.001†	<0.001	0.439	<0.001	0.314	0.029	<0.001	<0.001	-

*Seroprevalence by months; †Significant level (P<0.0014). The Bonferroni correction was used to reduce type I error in 36 comparisons of seroprevalence by months.

Discussion

In this study, among the pediatric population tested in the Eurofarm laboratory in Sarajevo the number of positive cases throughout the nine-month period showed a significant increase in the number of positive cases in November-December 2020 and February-April 2021. These data coincide with the two major pandemic waves, one after the relaxation of public health measures in the autumn of 2020, and the next in February 2021 (20, 21). Similar trends were observed in studies of adults (22). The seroprevalence in Bosnia and Herzegovina among adults for the period from April to July 2020 was 3.77%, and one year later for the same population it was 29.9% (23). In Bosnia and Herzegovina, the first confirmed cases of COVID-19 were found at the beginning of March 2020, in Sarajevo Canton on 20th March. On that day, there were three positive cases (24). By mid-May 2020, in both entities, more than 2,200 cases

of COVID-19 were recorded, where children <18 years were represented by 9.5% cases (25). This was the period of the so-called "zero" wave of the pandemic. The first pandemic wave was from the middle of July to the beginning of September 2020. In our study, the lowest seroprevalence (3.6%) was registered in October 2020 after first pandemic wave. A systematic review and meta-analysis by Rostami et al. showed that SARS-CoV-2 seroprevalence in the general population in 23 countries after first six months of the pandemic varied from 0.37% to 22.1%, with a pooled estimate of 3.38%. This prevalence suggests, however, that about 96% of the world's population are still susceptible to infection (12). Most published studies provided data on the seroprevalence of COVID-19 for the adult population. Only a few studies have reported seroprevalence data in children. A study from Seattle Children's Hospital of 1076 children (ages 0-15) who sought medical care during March and April 2020, showed that about 1% of the children had antibodies to SARS-COV-2 (26). Data from Spain for the period April-May 2020 among children aged 0-19 years, showed that their seroprevalence was 3.8% (27). In Croatia. in the first pandemic wave in May 2020, the seroprevalence among the pediatric population was 2.9%, while for the period October-November 2020 it was higher, at 8.4% (28). In Sarajevo Canton the second major pandemic wave lasted from the middle of October to the end of December 2020. During this wave, the seroprevalence in our study increased from 21.7% in November to 46.0% in December. The increase in the number of cases corresponds to the time it takes for a person to create antibodies after infection. In our study, the children were divided into three age groups. In the 0-5 years group seroprevalence was 10.1%, in the 6-13 years group 44.4% and the 14-18 years group 45.5%, without any statistically significant difference. Generally, the incidence of COVID-19 in children increased with increasing age (1, 29). In a study from India, the highest number of seropositive children were aged 10-17 years (30), and in Italy, seropositivity was highest at the age of 12-17 years (31). In our study, in the total of anti-SARS-CoV-2 Ig-positive individuals 42.8% were female while 57.2% were male, without a statistically significant difference. Other similar studies also did not point out differences in seroprevalence in relation to gender (28, 30, 31). On the basis of current data, the seroprevalence in children was generally low during the first year of the pandemic (26, 27). Strict public health prevention measures were in force. Most employers enabled work from home, population movement was limited, closure of schools and kindergartens resulted in children being less exposed (32). Initial studies indicated that children were asymptomatic carriers (33). Recent data suggest that most of the children were infected by transmission from a sick parent or household member (10, 34). Studies dated from 2021 showed an increase in seroprevalence in children. In Italy, in July 2020 the seroprevalence in children was 1%, while in January 2021 it increased to 9.5% (31). In our study, the highest seroprevalence (60.3%) was observed in April 2021, corresponding to the third pandemic wave which lasted from February to April 2021. Our results show that in the second year of the pandemic, the seroprevalence among children was significantly higher. According to a recent study from India SARS-CoV-2 seropositivity rate among children was high and comparable to that of the adult population. The seroprevalence was 55.7% in the <18 years age group, and 63.5% in the >18 years age group (30). Data from United States, as of February 2022, reported that approximately 75% of children and adolescents had serological evidence of previous infection with SARS-CoV-2 (35). From previous serostudies, children and adults had a similar risk of SARS-CoV-2 infection, but SARS-CoV-2 infections among children were mostly asymptomatic compared to adults.

Limitations of the Study

During the pandemic, serological testing in Sarajevo Canton was available in hospitals and private laboratories. In this research, we present data on seroprevalence among children in Sarajevo, tested in only one laboratory (the Eurofarm polyclinic) from September 2020 to May 2021. The

data were analyzed almost a year later. The main limitation of this study was that we collected blood samples from all the patients who came for serological testing, without collecting data regarding the reasons for testing. We do not have data on whether these were healthy children or children with comorbidities, whether they had previously been infected with COVID-19, or if they had any symptoms. During the study period, vaccines for COVID-19 were not available for children so they could not affect the antibody level. This report did not include the seroprevalence for two more recent pandemic waves in Sarajevo, the fourth wave from August to October 2021 and the fifth from late December 2021 to early February 2022. This research did not include children tested in hospitals and other laboratories. Despite all the limitations, this study indicates the importance of serological testing in children.

Conclusion

The results of our study show that the seroprevalence in children was low, especially during the first year of the pandemic. In the second year of the pandemic, there was an evident statistically significant increase in the number of seropositive children. Similar data have been shown in studies for adults.

What Is Already Known on This Topic:
During the pandemic, a large number of children had asymptomatic infection. Serological tests are helpful for assessing the total number of infected, including asymptomatic cases, monitoring the course of the pandemic, and planning public health measures. Seroprevalence studies provide valuable information for vaccination strategies because they help quantify what proportion of the population has been exposed to the virus, help achieve herd immunity, and help identify populations that may be at greater risk of infection.

What This Study Adds:
This is the first study about seroprevalence in children in B&H. The data we have presented can be helpful in planning public health measures in our country, and can help in assessing herd immunity for COVID-19. Also, this report can be useful for other databases on the seroprevalence of COVID-19 in children that are monitored worldwide.

Authors' Contributions: Conception and design: SMD and MLK; Acquisition, analysis and interpretation of data: SMD, MLK and JPM; Drafting the article: SMD; Revising it critically for important intellectual content: SMD, MLK, JPM and AJ; Approved final version of the manuscript: AJ, JPM, MLK and SMD.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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Opinion Letter
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When to Attend a Webinar?

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Abstract

Objective. To determine the most appropriate delay to start a webinar. **Methods.** This cross-sectional study was conducted on weekly general staff scientific webinars held by the Institute of Human Virology (IHV), University of Maryland School of Medicine, Baltimore, USA. 35 observations were made at arbitrary chosen times of three consecutive IHV webinars. After standardizing the number of participants, a 4th-degree polynomial fit was applied to the data. A cost function was defined as the sum of the time wasted for those who attended the webinar early and the lost for those who attend with delay. The cost function was minimized to compute the most appropriate delay to start the webinar. **Results.** The model could explain almost 95% of the observed variance in the number of participants. Normally, half of the participants attended the meeting at the webinar set starting time. The cost was a minimum if the webinar was delayed for about 3 minutes. **Conclusion.** It seems that the most appropriate time for starting the IHV general staff meetings is around 3 minutes after the webinar set starting time.

Key Words: Webinar ▪ Conference ▪ Scientific meeting ▪ Time ▪ Cost.

Introduction

Sharing information is an integral part of the scientific enterprise. Scientific meetings, in any form — congress, conference, seminar, *etc.* — are important forums for meeting of and sharing data among researchers (1). With advancements made in telecommunication, particularly, the Internet, and availability of the necessary infrastructures, many of these meetings have been switched to online webinars (2). This has especially become more prevailing after the emergence of the coronavirus disease 2019 (COVID-19).

Affiliated to University of Maryland School of Medicine, Baltimore, USA, Institute of Human Virology (IHV) holds weekly general staff seminars on various aspects of virology, molecular biology, epidemiology, and medicine. After the COVID-19 pandemic, the seminars were mostly run in the form of a webinar using Zoom™ teleconference software (Zoom Video Communications, Inc., San Jose, CA, USA). The webinars are typically scheduled for Monday at 11:00 AM, US Eastern time and last for an hour. The circle of the audience is limited to about 60 scientists, mostly IHV staff members, with different specialties. In most instances, the webinar starts with a delay to ensure that enough participants have joined it.

This study was conducted to determine when is the right time to start the webinar?

Methods

I counted at various times (conveniently chosen from a couple of minutes before the webinar set starting time [11:00 AM] to the end of the webinar), the number of participants taking part in three consecutive IHV general staff seminars, held on August 22 and 29, and September 12, 2022. The Zoom™ can provide the time and the number of participants. The number of participants was different among the studied webinars. Therefore, it was standardized.

Statistical Analysis

R software version 4.2.0 (*R* Project for Statistical Computing) was used for data analysis. For each webinar, the mean number of participants presenting between the 25th and 75th percentiles (the interquartile range) of the time measured since the beginning of the presentation, was calculated. The number of participants in each webinar was then transformed and presented as a percentage of this mean (Figure 1). A nonlinear curve fitting function (*nlsLM*() from *minpack.ml* package for *R*) was used for fitting a 4th-degree polynomial equation (Eq 1) to the standardized data (3). The function works based on the Levenberg-Marquardt nonlinear least-squares algorithm (4):

$$f(t) = \sum_{i=0}^{4} a_i t^i \qquad \text{(Eq 1)}$$

where $f(t)$ represents the standardized fraction of participants attended at time t; a_i are coefficients.

In a perfect world, all webinar participants should attend the session exactly at the set starting time (11:00 AM, from now on, it is referred to as time zero, Figure 1) and leave after the end of the webinar (gray line segments in Figure 1). However, some of the participants chose to attend the webinar a couple of minutes before the set starting time (Figure 1). Although they chose to attend earlier, their time would be wasted if we would not start the webinar on time. The area under the curve between the set webinar starting time and the time when the webinar really began (the pink area in Figure 1) reflected this waste of time. Another problem was the waste of learning opportunity; a number of participants would have learned something if they had not attended the meeting with delay (after the presentation had started). This waste of resources can be represented graphically by the light orange area in Figure 1. But, the weights of these two types of lost are not similar. The following parametric equation can then be an appropriate cost function:

$$cost\,(t) = \int_0^t f(x)dx + w\left[(t_m - t)f_m - \int_t^{t_m} f(x)dx\right] \qquad \text{(Eq 2)}$$

where t is the time when the webinar really starts; f_m, the maximum standardized fraction of participants derived from Equation 1; t_m, the time when the number of participants has reached its maximum value (corresponding to f_m, Figure 1); w, the weight of the cost attributed to the late attendance compared with the early attendance (here, it was arbitrary chosen to be equal to 2); and $f(x)$ the function of the fitted curve (Eq 1). Using basic calculus, the cost function was then minimized to find the most appropriate delay to start the presentation for different values of the weight (Eq 2).

Results

We studied a total of 35 measurements made during three consecutive webinars. The webinars began with a mean delay of 3.7 (range 3–4) minutes. The 4th-degree polynomial model could explain almost 95% of the observed variance in the number of participants (Table 1).

The maximum number of participants reached after 11.4 minutes after the start of the presentation (15.1 minutes after the webinar set starting time). Thereafter, the number of participants declined followed by a plateau until the end of the presentation. Normally, 47% of the participants attended the meeting at the webinar set starting time (Figure 1).

Plugging in the values obtained in Equation 2, for a weight of 2, the cost was a minimum if the webinar would have been delayed for 2.8 minutes (red dashed line in Figure 1), when 72% of participants had attended. The most appropriate delay to start presentation increased with increasing the weight (Figure 2).

Table 1. Coefficients of 4th-degree Polynomial (Eq 1) Fitted to the Data

Parameter	Coefficient (95% CI*)	P value
a_0	4.75 (4.36 to 5.13) × 10^1	<0.001
a_1	1.04 (0.91 to 1.18) × 10^1	<0.001
a_2	-6.00 (-7.16 to -4.82) × 10^{-1}	<0.001
a_3	1.33 (0.98 to 1.67) × 10^{-2}	<0.001
a_4	-1.01 (-1.33 to -0.70) × 10^{-4}	<0.001

*95% confidence interval; N=35; $r^2 = 0.944$.

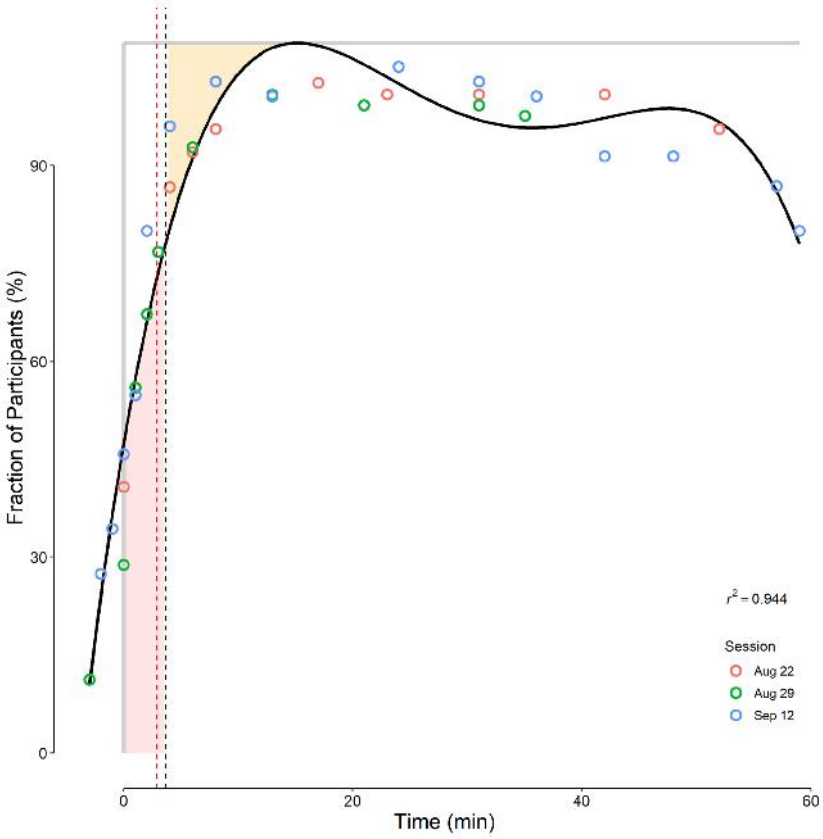

Figure 1. Standardized fraction of participants attended IHV staff webinars at different times. The gray line segments represent an ideal pattern of attendance. The solid curve is a 4th-degree polynomial curve fitting to the data points. The vertical black dashed line represents the mean delay of presentation after the set starting time for the webinar. The vertical red line is the optimum time for starting the presentation, assuming a weight of 2. The shaded areas reflect the costs incurred for participants — the pink area, for waste of their time due to delayed presentation; and the light orange area, for the lost educational opportunity due to delayed attendance.

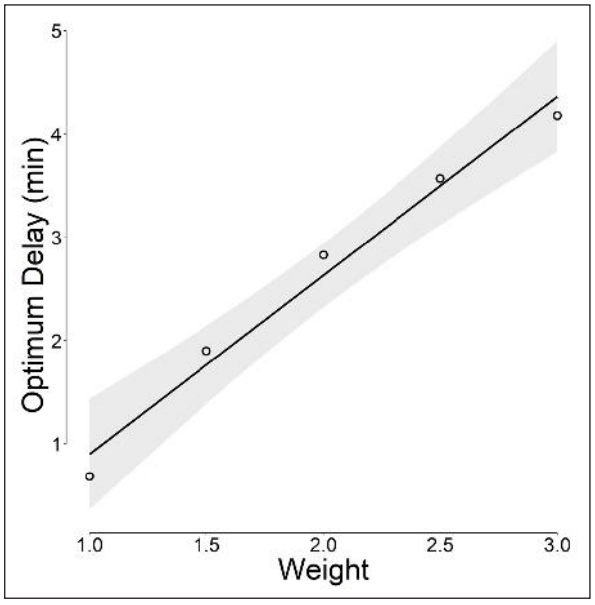

Figure 2. The optimum delay to start the presentation for different values of the weight (Eq 2). The shaded area represents the 95% confidence interval of the regression line.

Discussion

It seems that for IHV staff meetings, a delay of almost 3 minutes would minimize the total costs attributable to the waste of time for those attended early and lose of learning opportunity for those who attended late. The mean delay for the three webinars studied was 3.7 minutes, close enough to what was obtained analytically. The higher the weight, the higher the most appropriate delay to start the presentation (Figure 2).

The derived optimum delay of almost 3 minutes (for a presumed weight of 2) does just work for the IHV staff meetings and cannot be applied to other webinars. The audience of the IHV staff meeting is heterogenous in terms of their specialty and level of experience. It seems that it takes around 11 minutes for a typical audience to decide whether the presentation is interesting and stay in, or prefer to leave the webinar. This is the cause of the sharp decline in the number of participants after 11 minutes of the presentation and the plateau thereafter (Figure 1), I believe. Although starting the webinar after a certain delay would increase the effectiveness of the meeting, this action would inclusively give a signal to the audience that the meeting will be held with a known fixed delay, which in turn is equivalent to the shifting of the webinar set starting time. Knowing about this fixed delay, the audience may then adjust to the new set time, change their behavior and attend the conference with more delay. This adaptive process will ultimately result in regression to the *status quo*.

Limitations of Study

One of the limitations of this study was the low number of webinars studied. However, considering the audience in each webinar is almost invariant, their behavior can be considered almost constant (Figure 1). Therefore, the 35 observations made could provide enough study power. While the methodology can be applied to other meetings, the values reported herein cannot be generalized to other scientific meetings or group of audience.

Conclusion

In conclusion, it seems that being acquainted with most of the audience and being familiar with the structure of the IHV and its meetings, the IHV webinar organizers could heuristically, manage the necessary delay of 3–4 minutes to minimize the cost incurred.

Conflict of Interest: The author declares that he has no conflict of interest.

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Short Narrative Review
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The Facial Artery as a Branch of the External Carotid Artery in Thomas Turner's (1793-1873) Treatise on the Arterial System (1825) and a Comparison with Modern Anatomy

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Abstract
The aim of the present paper is to contribute to the understanding of the history of the anatomical study of the facial artery. The facial artery plays a fundamental role in the study of the anatomy of the face, and in maxillofacial and vascular surgery. The debate on the understating of this vessel is essential in the educational process, with special focus on the historical development of topographical and descriptive ideas about it. The comparison between the study of facial artery in the work of Thomas Turner (1793-1873) and modern anatomical concepts serves as an excellent educational paradigm. The documentary research method was used this short historical survey. **Conclusion.** Thomas Turner laid the scientific foundation for the accurate anatomical study of the facial artery.

Key Words: Facial Artery Types ▪ Education ▪ History of Medicine.

Introduction

Since ancient times facial surgery has presented challenges in reconstructive and aesthetic surgery. Preoperative knowledge of individual variations of the anatomy of the facial artery and its branches would benefit surgeons. Its course, as depicted classically in the anatomy textbooks, shows it ascending along the side of the mouth and nose, and terminating at the medial palpebral commissure, where it joins the dorsal nasal branch of the ophthalmic artery (1). Various anatomy and surgery treatises had included the facial artery as one of the most significant arteries of the human body. One such scientist was Thomas Turner, whose work entitled *A Practical Treatise On the Arterial System* published in 1825 (2). This historical article gives documentary research to present Turner's opinion concerning the facial artery and to discuss it in comparison with the modern concept of surgical anatomy.

The aim of the present article is to contribute to an understanding of the history of the anatomical study of the facial artery.

Thomas Turner

Thomas Turner (1793-1873) (Figure 1) was born in Truro in Cornwall, England. At a young age he apprenticed under the British surgeon Nehemiah Duck (1782-1841) at Saint Peter's Hospital in Bristol.

Soon after his apprenticeship, he left for London where during the autumn of 1815 he enrolled as a student under the British surgeon and anatomist Astley Paston Cooper (1768-1841), practicing at the united borough hospitals of Guy's and St. Thomas. In 1816, Turner was admitted both as a member of the London College of Surgeons and as a licentiate of the London Society of Apothecaries. An educational trip to Paris followed and in 1817

Figure 1. Thomas Turner from sketches of the lives and work of the honorary medical staff of Manchester Infirmary, from its foundation in 1752 to 1830 when it became the Royal Infirmary, University of Manchester Press, 1904.[1]

he was appointed house surgeon at Manchester Infirmary. During 1822 Turner proceeded to give lectures at the Literary and Philosophical Society of Manchester on the anatomy, physiology and pathology of the human body. Soon he made plans to open a school. His suggestion was well received by the local authorities and scientific societies, until in October 1824 a suitable building was engaged and a Medical School opened in Pine Street. The School was successful and, although London College was reluctant, recognition by the Edinburgh's College of Surgeons forced official recognition in 1827. In 1843 Turner was appointed honorary professor of physiology at the Manchester Royal Institution. In 1873 he died in his beloved Manchester. Apart from being a great anatomist and a prolific writer (Table 1), he gained his place in history as the man who developed medical education outside its then traditional base of London (2, 3).

The Arterial System

In his work, Turner noted that the arterial system is composed of a series of vessels that are like tubes in a cylindrical form (Figure 2). Although they branch, their tubular shape is maintained due to the elasticity of their walls. This elasticity is provided by one of their tunics or coats. This is a property characterizing only arteries. Three coats exist as an external elastic coat, with a middle muscular layer and an internal membrane. According to his opinion, the circulatory system is divided into two trunks, the pulmonary artery and the aorta. The pulmonary artery supplies the lungs, and is responsible for local circulation after respiration, while the aorta supplies blood to the whole human body. Concerning the arteries' topographic anatomy, he believed that they terminate in six ways: i) in anastomoses, ii) in veins, iii) in cells (like in the spleen), iv) in exhalent orifices (like in the dura mater or serous membranes), v) in the excretory

Table 1. Thomas Turner's Written Work

Title and year of publication
1. Outlines of a System of Medico-Chirurgical Education, London and Manchester, 1824; 2nd edit. 1826
2. An Address to the Inhabitants of Lancashire & C., on the Present State of the Medical Profession, London, 1825
3. A Practical Treatise on the Arterial System, London, 1825
4. Outlines of a Course of Lectures on the Laws of Animal Life, Manchester, 1825
5. Outlines of a Course of Lectures on the Anatomy, Physiology, and Pathology of the Human Body, Manchester, 1833
6. Anatomico-Chirurgical Observations on Dislocations of the Astragalus, Worcester, 1843

[1]Thomas Turner [cited 2023 Jan 31]. Available from: https://en.wikipedia.org/wiki/Thomas_Turner_%28surgeon%29.

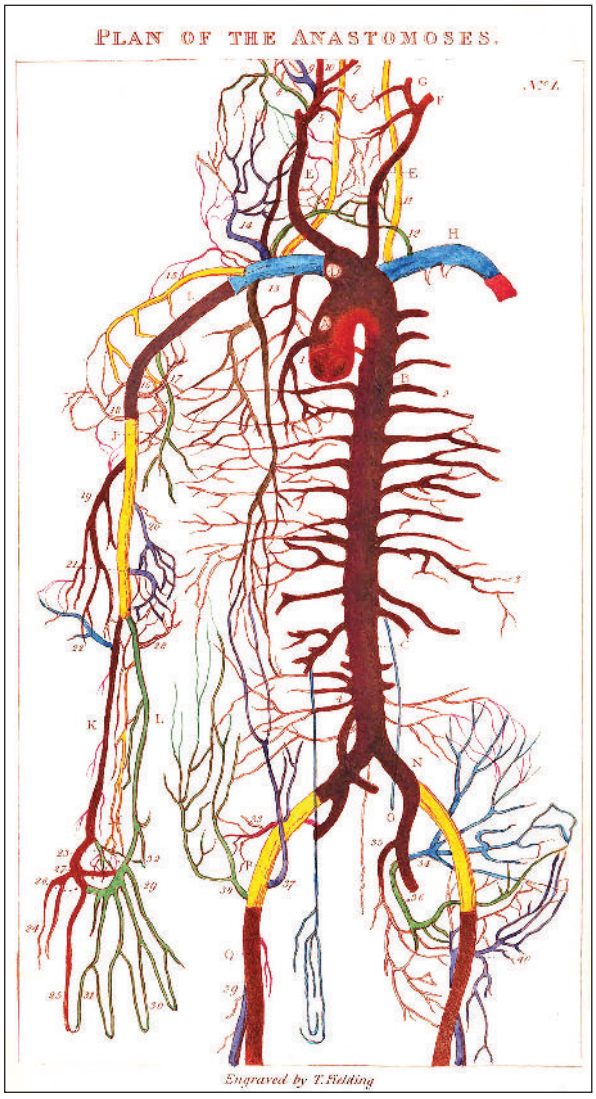


Figure 2. The circulatory system in Thomas Turner's A Practical Treatise on the Arterial System, London 1825.[2]

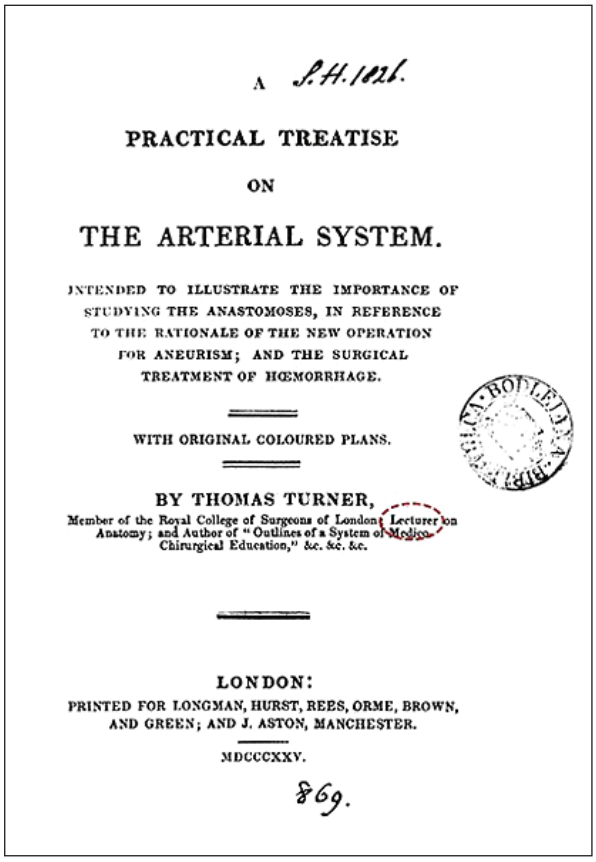
ducts of glands, and vi) in colorless or serous branches. The arterial branches of the head and neck run for the most part in a straight direction, while smaller branches are more or less tortuous in their course (4).

The Facial Artery

Turner described the arterial system by starting with the more central branches, moving to the smaller peripheral ones. In his book, the right common carotid artery, which is shorter than the left, ascends in the neck area by the side of the trachea and opposite to the upper part of the thyroid cartilage. It is divided into internal and external branches. The external carotid artery, in the area of its origin, lies on the inner side of the internal carotid, passing later on in its course outwards towards the angle of the jaw, first concealed by the digastricus and stylohyoideus muscles, and secondly by the parotid gland. Numerous branches arise throughout its course, namely: i) the superior thyroideal, ii) lingual, iii) external maxillary (or facial), iv) ascending pharyngeal, v) occipital, vi) posterior aural, vii) temporal and viii) internal maxillary. All the branches communicate and anastomose with the branches on the opposite side of the cranium. The external maxillary or facial artery supplies blood and nutrients to the muscles and glands under the lower jaw, to the chin, to the lower lip, to the ala of the nose, to the corner of the eye and the forehead (5, 6).

Surprisingly, Turner gave no more details concerning all the branches. However, the knowledge of the era, as presented in the case of Blandin in 1834, also mentioned the superficial malar arteries as vessels with their origin in the external maxillary artery. Blandin furthermore indicated that the zygomaticus major muscle proceeds towards the commissure, outside of which it unites with both the levator and the depressor anguli oris muscles, directly covering the facial artery. It seems that Turner's book was limited to being an educational tool, a purpose mentioned on its cover page, when Turner mentions his position as lecturer (Figure 3). Moreover, the term external maxillary began to disappear and the nomenclature defined the artery under the name "facial" (4, 5).

[2]Thomas Turner. A Practical Treatise on the Arterial System. Longman, Hurst, Rees, Orme, Brown, and Green : [printed for] J. Aston, London, Manchester, MDCCCXXV. [1825].



A PRACTICAL TREATISE
ON
THE ARTERIAL SYSTEM.

INTENDED TO ILLUSTRATE THE IMPORTANCE OF STUDYING THE ANASTOMOSES, IN REFERENCE TO THE RATIONALE OF THE NEW OPERATION FOR ANEURISM; AND THE SURGICAL TREATMENT OF HŒMORRHAGE.

WITH ORIGINAL COLOURED PLANS.

BY THOMAS TURNER,
Member of the Royal College of Surgeons of London; Lecturer on Anatomy; and Author of "Outlines of a System of Medico-Chirurgical Education," &c. &c. &c.

LONDON:
PRINTED FOR LONGMAN, HURST, REES, ORME, BROWN, AND GREEN; AND J. ASTON, MANCHESTER.
MDCCCXXV.

Figure 3. The front page of Turner's book, A Practical Treatise on the Arterial System, 1825, with the term Lecturer marked with a red circle.[3]

Discussion

As anatomy demonstrates nowadays, the facial artery arises in the area of the carotid triangle, originating from the external carotid artery over the lingual artery and down from the ramus of the mandible. Its root is obliquely below the digastric and stylohyoid muscles, and it curves upwards over the body of the mandible at the antero-inferior angle of the masseter. It then passes forward and upward across the cheek, along the side of the nose, ending at the medial commissure of the eye, where it changes into the angular artery. The external carotid artery is presented in modern textbooks as having eight branches: i) the superior thyroid, ii) the ascending pharyngeal, iii) the lingual, iv) the facial, v) the occipital, vi) the posterior auricular, vii) the maxillary and viii) the superficial temporal arteries. Some very rare branches of the artery are: the superior laryngeal, tonsillar, sternocleidomastoid and sublingual. The inferior and superior labial branches of the facial artery are sometimes underdeveloped or missing, and are replaced by an enlarged contralateral vessel (6, 7).

The facial artery is a superficial vessel of the face, covered by the fat of the cheek. It has two major branches, the cervical one, consisting of: the ascending palatine artery, the tonsillar branch, the submental artery, and the glandular branches; and the facial one, consisting of: the inferior labial artery, the superior labial artery, the lateral nasal branch to the nasalis muscle, and the angular artery, which is the terminal branch. The muscles supplied with arterial blood by the facial artery are: the buccinator, levator anguli oris, levator labii superioris, levator labii superioris alaeque nasi, levator veli palatini, masseter, mentalis, mylohyoid, nasalis, palatoglossus, palatopharyngeus, platysma, procerus, risorius, styloglossus and the transverse portion of the nasalis. Although the nomenclature represents its unexpected proximity (Table 2), the presentation of local anatomy depicts some deficits concerning the topographical description of the various entities and vessel courses in Turner's work (8, 9).

Turner failed to recognize that the facial artery may be absent in favor of a nasal branch of the ophthalmic artery at the medial side of the orbit, the transverse facial, or the maxillary artery, depending on the variation. He also neglected some points of origin, as the facial artery may arise from a common trunk with the lingual artery (4). Recent studies, such as the one presented by Midy et al. in 1986, in which they surveyed the application of the anatomic vascular bases of the facial flaps, described three types of proximal facial artery. The facial artery, according to their conclusion, represents a global description of vascularization concerning the face area and the principal anastomoses, being a paramount key factor in facial flaps and the plastic surgery of the face. Occasionally, the facial artery arises above its usual position, then descends beneath the angle

[3]Thomas Turner. A Practical Treatise on the Arterial System. Longman, Hurst, Rees, Orme, Brown, and Green : [printed for] J. Aston, London, Manchester, MDCCCXXV. [1825].

Table 2. Nomenclature of the Branches of the External Carotid Artery

Branches in Turner's work	Branches in modern anatomy books
Superior thyroideal	Superior thyroid
Lingual	Lingual
External maxillary (or facial)	Facial
Ascending pharyngeal	Ascending pharyngeal
Occipital	Occipital
Posterior aural	Posterior auricural
Temporal	Superficial temporal arteries
Internal maxillary	Maxillary

of the jaw to assume its ordinary course. The arch formed above the submandibular gland can extend some distance beneath the ramus of the jaw, lying between the lateral pterygoid and styloglossus muscles. Three more variables were described in the 1986 study. The first variation presented an artery which arose at the level of the angle of the mandible, ran around the submandibular gland to the mandible, lastly entering the face area. The second type was described arising from the external carotid artery rather distally, deep to the posterior belly of the digastric artery, continuing along the inner circumference of the mandible, finally traveling through the submandibular gland (10, 11). Nevertheless, in 2013 Furukawa et al proposed a classification of anatomical variations of the facial artery depending on its termination pattern and recognizing four different types. In the first, the facial artery terminates proximal to the superior labial artery, in the second it terminates distal to the superior labial artery near the nasolabial fold, in the third the artery extends to the lateral nasal-alar or angular branch, while in the fourth the facial artery is double with a dominant lateral angular branch (12). In 2019, Koziej et al. used Furukawa's classification, however, they recognized a fifth type of facial artery when it is hypotrophic with the dominant transverse facial artery and its course replaces the facial artery from the superior labial artery to the angular artery (13).

This deficit concerning a more accurate description of the facial artery's anatomy probably derives from the fact that Turner did not had the opportunity at the time to conduct a much more significant number of dissections, due to the shortage of corpses, to acquire better experience, focusing on vascular courses. Corpses were only available to esteemed professors by order of magistrates and hospital directors (14). Moreover, publication of vascular variations was scarce before the era of the Japanese physician and anthropologist Buntaro Adachi (1865-1945) (15), while scientific medical publications experienced logistic barriers and only Societies, Universities and the upper classes could afford them until the formation of new classes (Quakers, industrial workers etc.) (16). Above all, during Turner's time and until the mid-19th century, the development of maxillofacial surgery did not allow surgical exploration in deeper body layers, while the surgical instruments then used were not precise enough for more delicate surgical maneuvers, which prohibited a more accurate description of the facial artery and its branches. The facial artery became a fundamental vessel for facial surgeons in reconstruction and the blood supply to the area (17).

Conclusion

It is clear, after a comparison with modern research on the facial artery (18) that the work of Thomas Turner, although it did not manage to describe the route and the relationships of all arteries in the area completely, it nevertheless laid the scientific foundation for the accurate anatomical study of each artery branch, a cluster of vessels which play a significant role in the blood supply to the face and surgical anatomy.

What Is Already Known on This Topic:
A modern anatomical study on facial artery, its variations and its topographical placement is known.

What This Study Adds:
This study uses the example of the anatomical studies by Thomas Turner (1793-1873), especially in relation to the facial artery, to demonstrate how the anatomical study of the human body and especially the facial artery has developed gradually, and how the anatomical studies of the past help modern anatomical studies.

Authors' Contributions: Conception and design: EM and AS; Acquisition, analysis and interpretation of data: AS and EM; Drafting the article: MS, PG and AS; Revising it critically for important intellectual content: AS, MS; Approved final version of the manuscript: EM, AS and MS

Conflict of Interest: The authors declare that they have no conflict of interest.

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Book Review
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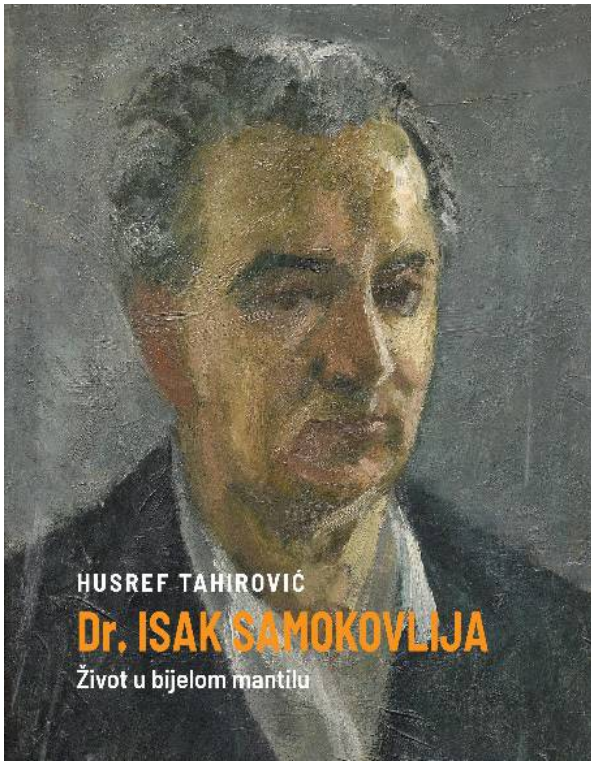
Dr. Isak Samokovlija: Life in a White Coat

Author: Husref Tahirović. Publisher: Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, 2022, VI + 214 pages, Illustrated.

ISBN: 978-9926-410-79-7. [In Bosnian].

Jovan Dimitrijević retired professor of the Faculty of Medicine in Sarajevo, Bosnia and Herzegovina



The book "Dr. Isak Samokovlija: Life in a White Coat" is by Academician Husref Tahirović and the publisher is the Academy of Sciences and Arts of Bosnia and Herzegovina, 2022. In the very title of the book, Academician Tahirović has indicated to us what the book is about, that is, it is dedicated to Isak Samokovlija and his professional work as a doctor. There is also another side to Isak Samokovlija, who was a well-known and recognized writer, a writer of set books used in schools, a writer to whom books and gatherings are dedicated, a public figure. The main character of Academician Tahirović's book is not this man. The book is about a modest and quiet county doctor who was taken by his service to various parts of the country, and in 1939 he was appointed a regular member of the Ban Health Council of the Drina Banovina. Being a district doctor in Bosnia and Herzegovina after the First World War "meant encountering the severe poverty of the population, the poor health and hygiene conditions, especially of the rural population, with the appearance of various infectious diseases that had the character of epidemics, all with a meagre number of health personnel". The county doctor was often in a situation where he saw a patient for the first and also the last time, especially in the countryside. Doctor travelled to the villages on foot, sometimes on horseback, in all weathers and seasons. During World War II, he was the manager of the clinic in the Alipašin Most Camp, a camp for refugees from Eastern Bosnia, and after World War II, he was a doctor at the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina. This is a book about a person, but also about a period of time that we get to know through the life of Dr. Isak Samokovlija.

The book was conceived and written in two parts. The first part consists of a biography, and the second part consists of health education texts by Dr. Samokovlija. The first part, the biography, has chapters on "Family, Growing Up, and Education", "The Peaceful Period Between the Two Wars", "The

Period of the Second World War, 1941–1945", "The Post-War Period, 1945–1955" and "Notes" at the end.

Isak Samokovlija was born into a modest family in Goražde in 1889. He was lucky enough to receive a scholarship to study medicine in Vienna, through the "La Benevolencija" cultural and educational society. He participated in the First World War, first as a medical student, then as a qualified doctor. There was a period between the two wars, a "peaceful period", and then another war. In World War II, he was faced with being a Jew and a doctor. Being a Jew meant immediate danger to his life and the lives of his loved ones; being a doctor saved him from that fate. The authorities of the Independent State of Croatia allowed him and a few other Jewish doctors to stay in Bosnia and work on suppressing endemic syphilis, with their headquarters in Banja Luka. However, due to his circumstances, he did not do this but was instead appointed manager of the clinic in the Alipašin Most Camp near Sarajevo, a camp for refugees from Eastern Bosnia. At the end of the war, he was again mobilized into the Home Guard, but managed to move to free territory. The next chapter talks about Dr. Isak Samokovlija's work in the new state until his death in 1955. He was appointed head of the Department for Health Education at the Ministry of Public Health of the People's Republic of Bosnia and Herzegovina. He no longer visited the sick in the city or villages with a medical bag, on foot or on horseback. He now did the same work as a traditional healer, but in a different way. With the help of a group of colleagues and enthusiasts, he founded the first Bosnian medical journal, entitled Život i Zdravlje (Life and Health, Journal of Health Education). The journal was founded in 1946, had a circulation of 6,000 copies, and was published four times a year. Doctor Samokovlija was the editor of the journal, and the editorial board included other well-known doctors. He left the Ministry of Public Health in 1949, when his work on the journal Život i Zdravlje also ended. He became the editor of the literary journal *Brazde* and then the editor of the publishing company *Svjetlost*. He died in 1955.

The last part of this text is entitled "Notes". This part is interesting because the reader can learn more about the various sources used for the biography, and also become acquainted with the short biographies of the doctors who met or collaborated with Dr. Isak Samokovlija during his life.

The second part of the book refers to the health education work of Dr. Isak Samokovlija. As we have already mentioned, this work took place through the journal Život i Zdravlje. In addition to editing that journal, in which there were also articles by prominent doctors of the time, Dr. Samokovlija also contributed a total of twenty-nine articles on health education. The texts dealt with the current health situation in Bosnia and Herzegovina after World War II. "Those were years in which difficult living conditions were caused by poverty, poor nutrition, the people's lack of general and health education, and poor hygiene and sanitary conditions. In addition, the adversities that arose as a result of treatment using irrational folk medicine procedures came to the surface...". The topics covered by Dr. Samokovlija can be divided into those related to infectious diseases, their causes, ways of spreading, treatment and prevention, then topics from general health culture, and finally articles where superstition and quackery are discussed. Of the infectious diseases, the most space was given to the fight against malaria, followed by smallpox and tuberculosis, and less to syphilis. Academician Tahirović divided these articles into texts using imaginary characters, sketches, scholarly articles and poetry. Already in the first issue of the journal, where Dr. Isak Samokovlija states the reasons for starting the journal, he says that: "Our articles and writings will not be embellished or written in a difficult and incomprehensible style. They should be characterised by simplicity and clarity." All the contributors adhered to this, but Dr. Samokovlija's articles stood out from the others, because they were written by a man who was also a writer. His professional medical education left its mark on the content of the text, and his literary talent and experience shaped its form and style. Sometimes it was in the form of an imaginary gathering, where the speaker communicated directly with the listeners

through dialogue (Baba Mara, health striker), sometimes he wrote brief notes, or sketches, with examples from life, usually from his medical practice. All his texts had a clear message and lesson, and they were all written in the "vernacular", or "conversational" language. The group of "scholarly articles" were longer, delving deeper into the topic, but even here he managed to maintain his simple vocabulary and immediacy in addressing the reader.

Academician Tahirović divides these texts whereby the last group is poetry. Reading these nine poems, which are simple, humorous, instructive, and beautifully and harmoniously rhymed, a comparison with another children's poet, Jovan Jovanović Zmaj, somehow comes to mind. Without going into an evaluation of the artistic value of the poems of one or the other, the comparison is that both of them combined their medical education with a literary gift, and these characteristics complement each other.

Academician Tahirović has given us a beautiful, well-thought-out, well-documented, and interesting book, written in fluent, professional, and likable language. The book is about a person from our past, about whom most people only know one side, the literary side. The other side has been in the shadow of the first, and known to a much smaller number of people. Now that other side has been revealed to us too, and we have gained a complete insight into the personality of Dr. Isak Samokovlija. We are given a glimpse of him, but we are also given a glimpse of a period of time, and the book is an expression of respect and recognition to Dr. Isak Samokovlija, as well as to all his other colleagues who worked to improve public health in this region during those times.

Academician Tahirović has been dealing with topics from the history of medicine for some time. This book is another fine and valuable contribution to that field.

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