Mieczyslaw Pokorski *Editor*

Advancements and Innovations in Health Sciences

Springer

Advances in Experimental Medicine and Biology

Clinical and Experimental Biomedicine

Volume 1211

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Advancements and Innovations in Health Sciences

Springer

Editor
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Opole Medical School
Opole, Poland

Contents

The Internet as a Source of Health Information and Services

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Abstract

The Internet is increasingly used for health-related purposes and evolves with the ever-changing needs of patients. The aim of this study was to assess the level of reliance on the Internet as a health information source, to examine which online communication activities are the most common for health purposes, and to determine the attitudes and needs of patients in this area and the factors affecting its use. A total of 1000 adults were selected from the Polish population by random sampling. The survey was administered by the Computer-Assisted Telephone Interview (CATI). The study concluded that 76.9% of the participants used the Internet for health purposes, among whom 72.6% of active and 27.4% of passive users were distinguished. The role of the Internet as a source of health information has increased, which corresponds to a growing interest in online health services. The majority of individuals searching for health information in the Internet lived in urban areas, had a high level of education, and was professionally active. We conclude that the increased interest in the use of the Internet related to health determines the direction in which e-health should be developed in the future.

1   Introduction

The future of health care services is increasingly associated with the rapid development of information and communication technologies (ICT). New forms of telecare bring considerable benefits in the field of using health systems, patient–doctor relations, and individuals' health (Santana et al. 2011). A significant increase in the number of chronically ill people, due to population aging and severe constraints imposed on health budgets in response to the shortage of health care staff, requires a fundamental change in the care process (Peeters et al. 2013). Online health communication results in lower management costs as, instead of being referred to hospitals, more and more patients receive support in their homes. Telecare works well for both acute and chronic illnesses as it allows health professionals to make diagnoses, treat, and provide specialist consultation to distant communities (Burke and Hall 2015). It increases the level of convenience, and thus the quality of

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healthcare, and it ensures the independence of patients who wish to stay at home. On the one hand, the Internet reduces barriers and facilitates the availability of medical services, but on the other hand, it affects the doctor–patient relationship and may influence treatment. Nowadays, patients are well-informed about a wide range of health-related topics and more and more willing to take advantage of the opportunities created by the ICT development.

Many studies confirm that the Internet is an increasingly popular source of health information for health care providers and consumers alike. The Pew Internet and American Life Project Survey, conducted in 2012, has shown that 72% of US adults use the Internet for health purposes and one-third believes it is a good diagnostic tool (Fox and Duggan 2013). In the Polish population, the use of the Internet for health-related purposes significantly increased from 41.7% in 2005 to 66.7% in 2012 (Bujnowska–Fedak 2015). For many years, it has been mainly used to seek information. However, in the last two decades, there was a shift in the role of the patient from a passive recipient of health information to an active Internet user (Lustria et al. 2011). Data generated from the US Health Information National Trends Surveys (HINTS) of 2003–2013 have shown a growing trend in using online health services, including ordering medications, sending emails to doctors, and accessing personal medical information through online patient portals. According to the HINTS, 7% of Internet users reported communicating online with a health care provider in the past 12 months as of 2003. Then, the proportion of the American population that used the Internet for communication was gradually growing. There were 10% of Internet users in 2005, 14% in 2008, 19% in 2011, and 30% in 2013 (Tarver et al. 2018; Beckjord et al. 2007). Two surveys conducted in seven European countries in 2005 and 2007 have shown a higher interest in using the Internet to communicate with health professionals. Kummervold et al. (2008) have reported a growth in the use of interactive Internet health services from 15.3% in 2005 to 22.7% in

2007. In the Polish population, the use of such services also is on the rise. It was 15.5% of the population in 2005, 22.9% in 2007, and 38.2% in 2012 (Bujnowska–Fedak 2015).

Even though the ways and methods in which people obtain health information from the Internet have already been studied in the past, there are still few data on the current use of the Internet for health communication and on the factors affecting it. Therefore, the aim of this study was to assess the level of trust with regard to the Internet as a health information source, to examine which online communication activities are the most common for health purposes, and to determine the attitudes and needs of patients in this area and the factors affecting its use.

2   Methods

2.1   Study Design

This survey-type study expands on the previous one that has presented the needs and expectations of the lay public in Poland, potential recipients of health services, concerning the role of mobile communication devices in medical care (Waligóra and Bujnowska–Fedak 2019). The source of research material for both studies was the same and consisted of a nationwide random sample of 1000 Polish adults (F/M: 558/442). The median age was 53 years (min-max: 18–88). The study was conducted in December 2017 and January 2018. The use of the Internet for health information and services, and the determination of patients' attitudes and activities in this area were considered an independent research ramification of the e-health services. Therefore, it was herein described as a separate entity.

The questionnaire was carried out through the Computer-Assisted Telephone Interviews (CATI). Geographical distribution of participants was controlled on the basis of voivodeship and town size, to ensure the representativeness of the study group. The selection of participants was planned in such a way as to reach people with the required sociodemographic characteristics.

Both landline and mobile telephones were included in the survey, with a ratio of 37–63%, respectively. An average response rate was 5.2%. A "nonresponse" group included people who failed to answer the phone, did not want to participate in the interview, or were too sick to participate. The vast majority of "nonresponses" were people who refused to participate in the survey without giving any reason. In such a case, the household was replaced by another one with the same characteristics (region, town size, etc.). Sampling continued until at least 1000 complete interviews were carried out.

2.2 Questionnaire

The questionnaire was developed based on the currently available literature and our earlier studies (Bujnowska–Fedak and Mastalerz–Migas 2015; Bujnowska–Fedak and Pirogowicz 2014; Andreassen et al. 2007). It included 23 questions covering demographic and health status characteristics and also questions related to online information-seeking behavior and interactive use of e-health services. Firstly, participants were asked how often they used the Internet and how often they used it for health-related purposes. The response categories were the following: "everyday" (1), "at least once a week" (2), "once or several times a month" (3), and "less than once a month" (4), "I never use the Internet" (5), and "I never use the Internet but I ask others to do it for me" (6). The importance attributed to the Internet (1) as a source of medical information compared with other sources such as TV/radio (2), books/health and encyclopedias/booklets (3), courses/lectures/seminars (4), newspaper/magazines (5), family/friends/colleagues (6), pharmacy (7), and direct personal contact with medical professionals (8) was also assessed. The responses to this question were given according to the five-point Likert scale from "not important" to "very important" with the neutral response in the middle. The frequency of various activities related to online communication was assessed by asking the participants how often they used the Internet to communicate with doctors, participate in forums or self-help groups, and buy medications or medical components. The response categories were: "everyday" (1), "at least once a week" (2), "once or several times a month" (3), "less than once a month"(4), and "I never use the Internet" (5). The frequency of the following activities was measured among the Internet users who contacted their family doctors online: obtaining a prescription, making an appointment, asking a particular health question, and getting to know the content of health professionals' websites. The questionnaire also contained questions related to the sociodemographic characteristics and health conditions (e.g., respondent's age, gender, education, or place of residence).

2.3 Data Analysis

The entire group of participants was divided into four different groups: the total sample, which represented the general population, and subsamples of the participants who reported that they used the Internet for health-related purposes (HI-users), further divided into active (HI-active users) and passive users (HI-passive users). A descriptive analysis, followed by a statistical analysis, was carried out to identify significant associations between the participant independent variables and their opinion on the use of the Internet for health-related communication purposes. The correctness of the distribution of quantitative variables was checked using the Shapiro-Wilk test. None of the variables had a normal distribution. For this reason, only the following nonparametric ones were used for further analysis: Fisher's exact test of independence, Chi-squared test for equal proportions (homogeneity test), and Wilcoxon multiple comparison test for unrelated samples. The significance level of 0.05 was assumed in all the tests. The statistical package R software v3.5.1 was used in the calculations.

3   Results

3.1   Characteristics of Participants

There were 769 out of the 1000 people who declared they used the Internet for health-related purposes. 61.3% of the participants lived in the city and 38.7% in rural areas. As far as the employment status is concerned, 59.4% of the participants performed paid or unpaid work, 31.7% were retired or chronically ill/disabled, and 4.4% were university students. More details on the study population are provided in Table 1.

3.2   Sources of Information About Health and Illness

Forty-eight percent of the participants declared that the Internet was an important source of health information. In fact, it turned out to be more popular than other media such as television and newspapers. The most important source of information, however, was direct contact with health professionals. In the ranking of the most popular sources of medical information, the Internet took the third position, following direct contact with health professionals and family and friends, among active users, and the fourth position following pharmacies, among passive users (Table 2).

3.3   Internet Use for Health Purposes

The Internet was used by 89% of the entire study group, 77% of which used it for health-related purposes (HI users). Unlike non-HI users, HI users were younger ($p < 0.001$) and more often females ($p = 0.049$). People with higher or secondary education were more willing to use it than those with basic/vocational education ($p < 0.00001$). Furthermore, the participants who were university students and professionally active used the Internet more frequently than retirees and disabled people ($p < 0.00001$). A significantly greater percentage of HI users than that of non-HI users lived in the city ($p = 0.026$)

or with someone else ($p = 0.008$). What is more, healthy people more often used the Internet for health purposes (87) than those in poor health (50) ($p < 0.00001$) (Table 3).

Differences between the groups that used the Internet in an active and passive way were also revealed. Active users were younger ($p < 0.001$) and better educated ($p < 0.00001$) than passive ones. The significant majority of active users were employees or students rather than retirees or the unemployed ($p < 0.00001$). Active users of the Internet more often lived in big cities than in rural areas ($p = 0.028$). E-health services were more often used actively by those who were in better health than those who assessed their health state as poor ($p = 0.001$). The interactive use of medical services is showed in Table 4.

3.4   Attitudes Toward E-Health Services and Factors Affecting Their Use

Interacting with unknown health professionals was the most frequently used e-health service. It concerned 47% of the HI users (Fig. 1). Statistically significant factors affecting the interaction with unknown health professionals included: age and employment status (Table 5). As far as age is concerned, the largest percentage was observed in the youngest age group (18–35 years old) – 51.6% ($p = 0.043$). Further, participants who performed paid or unpaid work and those who were still under education were more likely to interact with unknown health professionals than retirees ($p = 0.001$). There was no statistically significant difference depending on gender, place of residence, or type and mobile use. Thirty-seven percent of the HI users participated in forums or self-help groups; the most willing participants were young and middle-aged people ($p = 0.037$). Almost one-third of the HI users reported the purchase of medicines and medical components. It concerned more often younger people ($p < 0.00001$) with secondary or higher education ($p < 0.00001$), who were professionally active ($p < 0.00001$) and healthy ($p = 0.003$).

Table 1 Characteristics of the study group

Characteristics	All participants ($n = 1,000$) n (%)
Gender	
Men	442 (44.2)
Women	558 (55.8)
Median age (min-max) (years)	53 (18–88)
Age groups	
18–35	302 (30.2)
36–59	429 (42.9)
60+	269 (26.9)
Education	
Basic/vocational	339 (33.9)
Secondary	373 (37.3)
College/university	288 (28.8)
Employment status	
Education underway	44 (4.4)
Paid or voluntary work/other	594 (59.4)
Unemployed	45 (4.5)
Retired/sick or disabled	317 (31.7)
Residency type	
Alone	615 (61.5)
With family/other	385 (38.5)
Residency place	
Urban	613 (61.3)
Rural	387 (38.7)
Health status	
Good/very good	575 (57.5)
Fair	350 (35.0)
Bad/very bad	75 (7.5)
Mobile use	
Yes	957 (95.7)
No	43 (4.3)

Table 2 Ranking of various sources of health information

Source of health information	All participants	Rank	Health Internet users (HI users)					
			All users	Rank	Active users	Rank	Passive users	Rank
Internet	3.49 ± 1.54	4	3.69 ± 1.27	3	3.76 ± 1.22	3	3.51 ± 1.39	4
TV/radio	3.15 ± 1.42	6	3.05 ± 1.38	7	3.03 ± 1.37	7	3.10 ± 1.40	5
Books/medical encyclopedias/leaflets	3.48 ± 1.40	5	3.56 ± 1.33	4	3.59 ± 1.32	4	3.51 ± 1.34	4
Courses and lectures	3.00 ± 1.68	8	3.13 ± 1.63	6	3.21 ± 1.60	6	2.91 ± 1.71	7
Newspapers, magazines	3.00 ± 1.37	7	2.94 ± 1.31	8	2.92 ± 1.30	8	3.00 ± 1.33	6
Family, friends, and colleagues	3.87 ± 1.23	2	3.81 ± 1.20	2	3.79 ± 1.18	2	3.87 ± 1.25	2
Pharmacies	3.56 ± 1.37	3	3.55 ± 1.32	5	3.56 ± 1.28	5	3.52 ± 1.41	3
Face-to-face contact with health professionals	4.36 ± 1.07	1	4.40 ± 1.01	1	4.44 ± 0.97	1	4.31 ± 1.10	1

Data are mean ± SD scores on a 5-point scale of ranking, where 1 stands for "unimportant" and 5 for very "important"

Table 3 Characteristics of users and nonusers of e-health services

| Category of users | Internet health-related services | | |
| | HI users | Non-HI users | |
	n (%)	n (%)	p
All	769 (76.9)	121 (12.1)	
Gender			
Men	337 (43.8)	65 (53.7)	0.049
Women	432 (56.2)	56 (46.3)	
Median age (min-max) (years)	51 (18–84)	53 (18–88)	<0.0001
Age groups			
18–35	274 (35.6)	20 (16.5)	<0.0001
36–59	362 (47.1)	49 (40.5)	
60+	133 (17.3)	52 (43.0)	
Education			
Basic/vocational	210 (27.3)	56 (46.3)	<0.0001
Secondary	297 (38.6)	47 (38.8)	
College/university	262 (34.1)	18 (14.9)	
Employment status			
Education underway	41 (5.3)	3 (2.5)	<0.0001
Paid or voluntary work/other	522 (67.9)	55 (45.5)	
Retired/ill or disabled	166 (21.6)	60 (49.5)	
Unemployed	40 (5.2)	3 (2.5)	
Residency type			
Alone	89 (11.5)	25 (20.7)	0.008
With family/other	680 (88.5)	96 (79.3)	
Residency place			
Urban	496 (64.5)	65 (53.7)	0.026
Rural	273 (35.5)	56 (46.3)	
Health status			
Good/very good	495 (64.4)	52 (43.0)	<0.0001
Fair	237 (30.8)	49 (40.5)	
Bad/very bad	37 (4.8)	29 (16.5)	
Mobile phone use			
Yes	754 (98.0)	109 (90.1)	<0.0001
No	15 (2.0)	12 (9.9)	

P-value shows significant differences between the two groups, using Fisher's independence test or Wilcoxon's test

3.5 Patterns in the Use of the Internet in Communication with General Practitioners

Fifteen percent of the HI users declared that they had contacted their general practitioners via the Internet. Citizens who were more willing to do it were younger ($p < 0.0001$), lived in the city ($p < 0.0001$), were better educated ($p < 0.0001$), and were university students or employees ($p < 0.0001$). The vast majority of citizens contacted their family doctors via the Internet to make an appointment (79%). Other services included: becoming acquainted with the doctor's website (48%), studying their medical records (34%), asking a particular health question (29%), and obtaining a prescription (27%) (Fig. 2). Young or middle-aged participants were most likely to make an appointment online ($p = 0.033$). People in bad or very bad health condition were most frequently interested in

Table 4 Interactive use of e-health services

Category of users	Active users n (%)	Passive users n (%)	p
All	558 (55.8)	211 (21.1)	
Gender			
Men	243 (43.5)	94 (44.5)	
Women	315 (56.5)	117 (55.5)	
Median age (years) (min-max)	49 (18–80)	51 (18–84)	<0.001
Age groups (years)			
18–35	230 (41.2)	44 (20.9)	<0.00001
36–59	253 (45.3)	109 (51.7)	
60+	75 (13.5)	58 (27.4)	
Education			
Basic/vocational	134 (24.0)	76 (36.0)	<0.00001
Secondary	210 (37.6)	87 (41.3)	
College/University	214 (38.4)	48 (22.7)	
Employment status			
Education underway	32 (5.7)	9 (4.2)	<0.00001
Paid or voluntary work/other	403 (72.2)	119 (56.4)	
Retired/sick or disabled	96 (17.3)	70 (33.2)	
Unemployed	27 (4.8)	13 (6.2)	
Residence place			
Urban	373 (66.8)	123 (58.3)	0.028
Rural	185 (33.2)	88 (41.7)	
Residency type			
Alone	66 (11.8)	22 (10.5)	0.703
With family/other	492 (88.2)	189 (89.5)	
Health status			
Good/very good	385 (69.0)	113 (53.8)	0.001
Fair	149 (26.7)	85 (40.0)	
Bad/very bad	24 (4.3)	13 (6.2)	
Mobile phone use			
Yes	548 (98.2)	206 (97.6)	0.570
No	10 (1.8)	5 (2.4)	

P-value shows significant differences between the two groups, using Fisher's independence test or Wilcoxon's test

obtaining a prescription ($p = 0.032$). A positive correlation between asking a particular health question and living in rural areas was observed ($p = 0.025$). There was no relationship between the above-mentioned activities and other sociodemographic variables; more details are presented in Table 6.

4 Discussion

This study showed that the Internet plays an increasingly significant role, and almost half of the participants considered it an important source of information. It was positioned just behind family/friends and doctors, who remained the biggest authorities and verifiers of medical information.
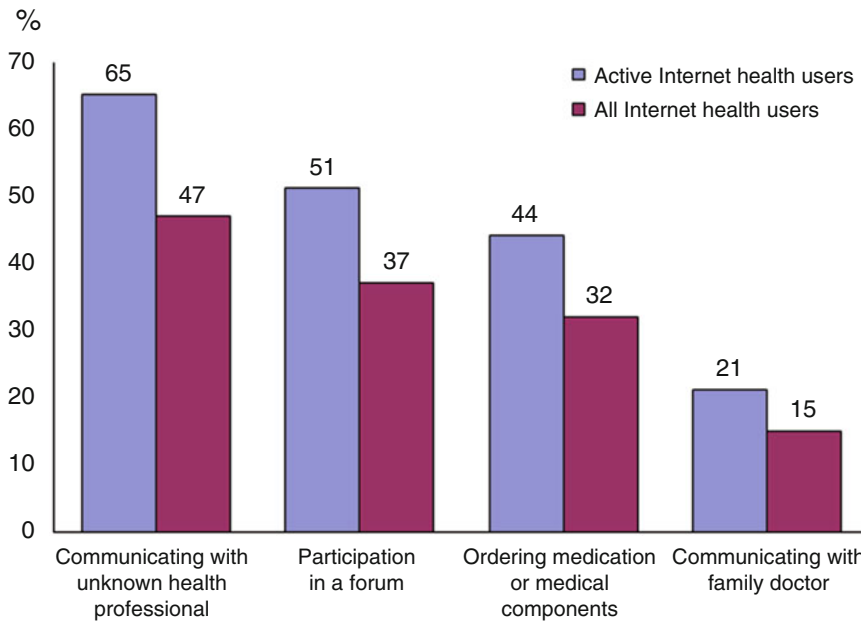
Fig. 1 The most frequent health-related online activities performed by Internet users

These findings are consistent with the results of other studies (Bidmon and Terlutter 2015; Kummervold et al. 2008) and with the US National Trends Survey of 2005–2013 (Tarver et al. 2018; Beckjord et al. 2007). The present study confirmed that although more confidence was placed in the Internet than in the "traditional" media, such as TV and radio, a lot of people still trust doctors, family, and friends during the last 10 years (Thai et al. 2018). In our previous research conducted in 2005 and 2007, TV and radio were indicated as more important sources of health information than the Internet. This situation had changed in 2012 when the Internet began ranking higher than the other media (Bujnowska–Fedak 2015). The growth of importance of the Internet may be caused by increasing difficulties in arranging an appointment with a doctor. In the face of population aging, the number of people in need of medical care rapidly grows. At the same time, there is no increase in employment in medical care. Furthermore, Internet absorbed other established media, and we now have a lot of e-books, online radio stations, or TV channels for content distribution. In the future, the Internet is likely to be considered not as a single source of information, but as a network of services that are potential sources of health information.

In this study, over three-quarters of the Polish society reported using the Internet for health purposes. There were slightly more women (56%), which also is in line with reports by other authors (Fox 2011a; Kummervold et al. 2008). Unlike men, women are generally more interested in issues related to health (Ek 2015). Evidence shows that due to the presumed gender role and social constructions of masculinity, men are unwilling or lack the motivation to engage in the exchange of health-related information (Wellstead 2011; Rice 2006). On the other hand, men demonstrate greater digital skills. Therefore, the evidence of gender differences in the use of the Internet for health purposes is divergent. The study conducted by Bidmon and Terlutter (2015) has shown that women and men differ in terms of how often they use different channels related to health. Women more frequently consult with friends and pharmacists, and they participate in forums and blogs related to health. Men, in turn, more frequently use applications to search for health-related information and contact their GPs

Table 5 Type of e-health services and factors affecting their use

Category of users	Communication with family doctors (YES answers)	p	Communication with unknown health professionals (YES answers)	p	Participation in forums (YES answers)	p	Ordering medications or medical components (YES answers)	p
Gender								
Men; n (%)	42 (12.5)	0.084	160 (47.0)	1	115 (33.9)	0.154	98 (28.9)	0.161
Women; n (%)	74 (22.3)		204 (47.2)		169 (38.9)		147 (33.9)	
Age group (years)								
18–35; n (%)	54 (19.7)	<0.0001	143 (51.6)	0.043	114 (41.1)	0.037	117 (42.2)	<0.00001
36–59; n (%)	57 (15.7)		170 (46.8)		133 (36.6)		112 (30.9)	
60+; n (%)	6 (4.5)		52 (38.5)		38 (28.1)		17 (12.6)	
Education								
Basic/vocational; n (%)	20 (9.5)	<0.001	101 (47.9)	0.962	76 (36.0)	0.870	41 (19.4)	<0.00001
Secondary; n (%)	39 (13.1)		139 (46.6)		114 (37.9)		84 (28.2)	
College/university; n (%)	57 (21.8)		124 (47.0)		95 (36.0)		120 (45.5)	
Employment status								
Education underway; n (%)	9 (21.2)	<0.0001	20 (48.8)	0.001	15 (36.6)	0.751	15 (36.6)	<0.00001
Paid/voluntary work/other; n (%)	90 (17.2)		259 (49.4)		199 (38.0)		191 (36.5)	
Unemployment	11 (27.5)		26 (65.0)		14 (35.0)		7 (17.5)	
Retired/sick or disabled; n (%)	6 (3.6)		59 (35.1)		56 (33.3)		32 (19.0)	
Residence place								
Urban; n (%)	92 (18.5)	<0.0001	238 (47.7)	0.652	188 (37.7)	0.484	166 (33.3)	0.226
Rural; n (%)	24 (8.8)		126 (46.0)		96 (35.0)		79 (28.8)	
Residency type								
Alone; n (%)	15 (17.0)	0.639	36 (40.0)	0.177	32 (35.6)	0.817	30 (33.3)	0.719
With family/other; n (%)	101 (14.9)		328 (48.1)		252 (37.0)		215 (31.5)	

(continued)

Table 5 (continued)

Category of users	Communication with family doctors (YES answers)	p	Communication with unknown health professionals (YES answers)	p	Participation in forums (YES answers)	p	Ordering medications or medical components (YES answers)	p
Health status								
Good/very good; n (%)	83 (16.8)	0.110	245 (49.3)	0.214	193 (38.8)	0.270	178 (35.8)	0.003
Fair; n (%)	26 (11.2)		99 (42.5)		77 (33.0)		57 (24.5)	
Bad/very bad; n (%)	7 (18.9)		17 (44.7)		12 (31.6)		8 (21.1)	
Mobile phone use								
Yes; n (%)	113 (15.0)	0.481	354 (46.7)	0.190	276 (36.4)	0.187	242 (31.9)	0.411
No; n (%)	3 (20.0)		10 (66.7)		8 (53.3)		3 (20.0)	

P-value denotes significance of differences between or among the percentages of each category users of a given type of communication pattern (Fisher's independence test)
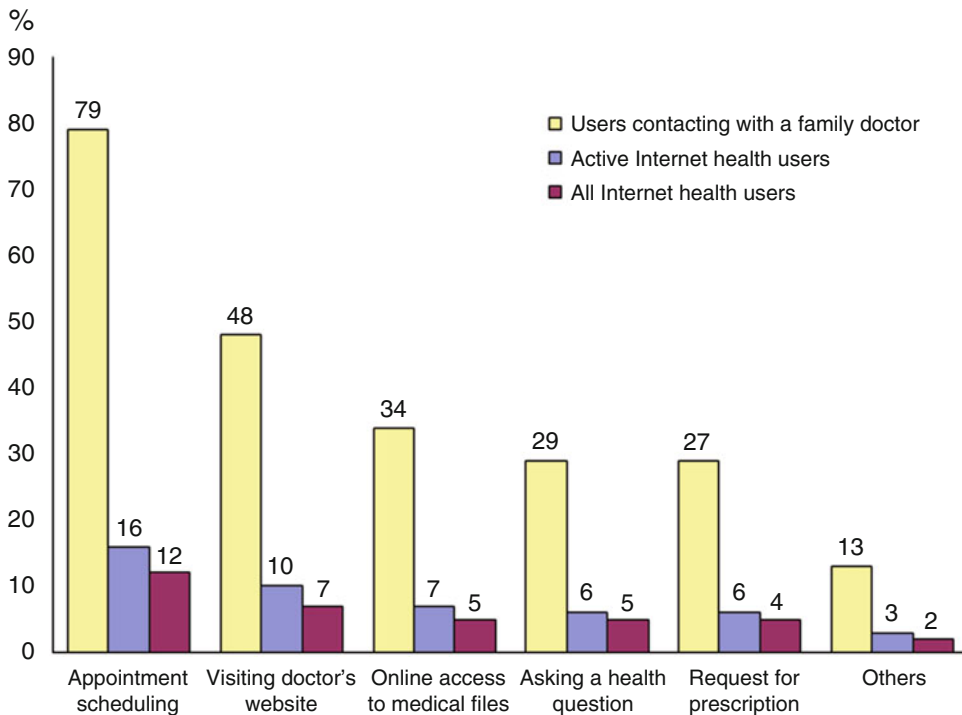
Fig. 2 The most frequent reasons to communicate online with a family doctor

online. They are also more willing to communicate with doctors and undergo treatment via the Internet than women. The present study, however, showed that women more frequently used online communication channels than men, even though the respective gender differences did not meet statistical significance.

Young people, who accessed the online network on an everyday basis, were the most active users of the Internet for health purposes (Asibey et al. 2017; Kummervold et al. 2008). The reason for that could be that they were more willing to try new technologies (Chiu 2011). The elderly, on the other hand, had lower computer skills and the frequency of using the Internet for health purposes decreased with age (Fox and Duggan 2013; Zajac et al. 2012; Livingston et al. 2008). However, as reported by Blusi et al. (2016), the frequency with which older people used the online network for medical issues increased after training.

This study showed a positive correlation between using the Internet for health purposes and living in urban areas, and being educated and professionally active or still at school. Such a relationship has also been found in other studies (Fox and Duggan 2013; Livingston et al. 2008; Andreassen et al. 2007). People who live in metropolitan areas more often graduate from universities, obtain higher income, and have easier access to computers with Internet connection. These findings are related to the digital divide (Romano et al. 2015; Connolly and Crosby 2014; Cresci et al. 2010), which must be perceived in terms of differences not only in access to technology but also in the retrieval, understanding, and use of information (Zach et al. 2012). Jensen et al. (2010) have shown that despite having access to the Internet, the people do not know how to open an Internet browser and retrieve information online, and they often cannot overcome this barrier. It has also been shown that accessing the Internet is associated with health outcomes. Those who have better digital skills tend to have better self-management of health and greater interaction with doctors via the

Table 6 Patterns of communication with family physicians using e-health services

Category of users	Online registration (YES answers)	p	Visiting doctor's website (YES answers)	p	Online access to patient file (YES answers)	p	Asking a health question (YES answers)	p	Recipe request or renewal (YES answers)	p	Others (YES answers)	p
Gender												
Men; n (%)	29 (69.0)	0.110	17 (40.5)	0.248	18 (42.9)	0.162	13 (31.0)	1	7 (16.7)	0.053	5 (11.9)	1
Women; n (%)	61 (82.4)		39 (52.7)		22 (29.7)		22 (29.7)		26 (35.1)		10 (13.5)	
Age group (years)												
18–35; n (%)	41 (77.4)	0.033	24 (54.3)	0.527	19 (35.8)	0.806	17 (32.1)	0.795	14 (26.4)	0.512	8 (15.1)	0.576
36–59; n (%)	47 (82.5)		30 (52.6)		20 (35.1)		17 (29.8)		16 (28.1)		6 (10.5)	
60+; n (%)	2 (33.3)		2 (33.3)		1 (16.7)		1 (16.7)		3 (50.0)		1 (16.7)	
Education												
Basic/vocational; n (%)	15 (75.0)	0.956	11 (55.0)	0.334	3 (15.0)	0.135	6 (30.0)	1	8 (40.0)	0.295	4 (20.0)	0.578
Secondary; n (%)	31 (79.5)		15 (38.5)		15 (38.5)		12 (30.8)		12 (30.8)		4 (10.3)	
College/university; n (%)	44 (77.2)		30 (52.6)		22 (38.6)		17 (29.8)		13 (22.2)		7 (12.3)	
Employment status												
Education underway; n (%)	6 (66.7)	0.956	3 (33.3)	0.106	6 (40.0)	0.116	3 (33.3)	0.482	3 (33.3)	0.949	0 (0.0)	0.578
Paid or voluntary work/other; n (%)	72 (80.0)		47 (52.2)		31 (33.7)		29 (32.2)		26 (28.9)		14 (15.6)	
Unemployed; n (%)	8 (72.7)		2 (18.2)		2 (18.2)		1 (9.1)		3 (27.3)		1 (9.1)	
Retired/sick or disabled; n (%)	4 (66.7)		4 (66.7)		1 (16.7)		2 (33.3)		1 (16.7)		0 (0.0)	

Residence place

Urban; n (%)	72 (78.3)	0.785	42 (45.7)	0.360	32 (34.8)	0.772	23 (25.0)	0.025	26 (28.3)	1	12 (13.0)	0.409
Rural; n (%)	18 (75.0)		14 (58.3)		8 (33.3)		12 (50.0)		7 (29.2)		3 (12.5)	

Health status

Good/very good; n (%)	65 (78.3)	0.735	39 (47.0)	0.474	27 (32.5)	0.461	24 (28.9)	0.311	23 (27.7)	0.032	11 (13.3)	1
Fair; n (%)	19 (73.1)		12 (46.2)		9 (34.6)		7 (26.9)		5 (19.2)		3 (11.5)	
Bad/very bad; n (%)	6 (85.7)		5 (71.4)		4 (57.1)		4 (57.1)		5 (71.4)		1 (14.3)	

Mobile phone use

Yes; n (%)	89 (78.8)	0.126	54 (47.8)	0.609	39 (34.5)	1	34 (30.1)	1	32 (28.3)	1	14 (12.4)	0.342
No; n (%)	1 (33.3)		2 (66.7)		1 (33.3)		1 (33.3)		1 (33.3)		1 (33.3)	

P-value denotes significance of differences between or among the percentages of each category users of a given type of communication pattern (Fisher's independence test)

Internet (Neter and Brainin 2012). Thus, e-health literacy improves individuals' health.

Another criterion that positively influenced the use of the Internet for medical purposes is good health. This correlation has also been found by other authors (Cresci et al. 2010; Andreassen et al. 2007). People in poor health may not be mentally or physically capable of using the online network. Moreover, we asked the participants about their feelings related to health, so it cannot be excluded that those who suffered from chronic illnesses or disabilities might believe that they were in good health. In addition, it was not clear whether people used the Internet on their own or on behalf of someone else. In fact, many caregivers could search the World Wide Web to find solutions to their patients' problems.

According to the present findings, 55.8% of the population used the Internet actively, which is encouraging. Communicating with unknown health professionals turned out to be the most frequent online activity. This is not surprising due to difficulties in making an appointment with a specialist in Poland. The similar tendency was observed in other European countries (Kummervold et al. 2008; Andreassen et al. 2007). It also indicates that e-health has considerable potential to improve access to health services. High levels of participation in forums or self-help groups were observed in the present study. According to the research carried out by Klemm et al. (1999), men more often use forums to ask questions, whereas women to share their experiences, with an increasing rate of 14.9% in 2005, 20.4% in 2007, and 24.1% in 2012, and 37% in 2018, which may indicate that young people place more confidence in social media and the Internet than in health professionals (Bujnowska–Fedak 2015; Fox 2011b). The role of a doctor as the only authority in the field of health has changed over the years. Nowadays, people more often search for health-related information before they visit the doctor's office, and they participate in making decisions about their health which may affect their relationship with the doctor. Generally, communicating with a GP via the Internet is not a common practice (15% in the group of the HI-users), but it increased in the

years 2005–2012 (Bujnowska–Fedak and Mastalerz–Migas 2015; Kummervold et al. 2008). Making an appointment with a doctor is still the most frequent online activity. In 2007, it constituted 3.2% of cases among the general population and 5.4% of cases among health-related Internet users (Santana et al. 2010). In comparison, it increased to 12% among health-related Internet users in the present study. This growth may be largely attributed to an increased interest in the use of online communication tools and opportunities currently offered by e-health services.

This study has a few limitations. The response rate was low, which might hinder the representativeness of the survey. A very large category of unrealized interviews were those in which no one answered the phone. This issue could, in part, have been related to the date of the audit (December–January), which was a time of Christmas and winter school holidays. Increased resistance to answering unwanted phone calls during a downtime has been observed in other studies (O'Toole et al. 2008). Reasons for a decline may include the unlisted telephone numbers, privacy (do not call lists), or distrust. Due to the swift nature of a phone conversation in CATI interviews, participants could hardly be able to give thought to the answer or to determine the appropriate point on the scale. In addition, specific e-health services were not tested separately, so that we do not exactly know what the participants had in mind when they evaluated the reliability of each source.

5 Conclusion

The development of online communication technologies has changed the way in which patients use the Internet for medical purposes. With new possibilities, patients' needs can be met not only through the passive search for information but also through the active participation in e-health services. The role of the Internet as a source of health information has increased, which corresponds to growing interest in online health services. The most common activity in

online health communication is interacting with unknown health professionals. Hence, it can be expected that e-health services might complement deficiencies in ordinary medical care. Given the prominence and importance of online communication for health purposes, the study offers insights into the understanding of the relations concerning the active use of the World Wide Web and a considerable potential to practitioners and researchers all over the world. Future strategies related to e-health development should take such relations into consideration in order to assess what type of e-applications would meet the expectations of the population at large to the greatest extent. There is certainly a need to continue and expand studies to this end.

Conflicts of Interest The authors declare no conflicts of interest in relation to this chapter.

Ethical Approval All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study protocol was approved by the Bioethics Committee of Wroclaw Medical University in Wroclaw, Poland (permit ST. C290.17.040/2017).

Informed Consent All of the participants were provided with comprehensive information concerning the objectives and scope of the survey and gave their informed consent.

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Genetic Predisposition for Osteoporosis and Fractures in Postmenopausal Women

Tomasz Mitek, Łukasz Nagraba, Jarosław Deszczyński, Magda Stolarczyk, Ernest Kuchar, and Artur Stolarczyk

Abstract

Osteoporosis is a disease with complex etiology where the genetic factors may account for as much as 50–85% of the risk of its development in postmenopausal women. The polymorphism of estrogen receptor genes (*ESR1*, *ESR2*) seems essential among the genetic factors. The goal of this study was to analyze polymorphisms of selected genes in a population of postmenopausal women treated for osteoporosis and to evaluate the influence of genetic and nongenetic factors on the estimated 10-year risk of fracture. The study group consisted of 214 women hospitalized for treatment of postmenopausal osteoporosis. We investigated the presence of *ESR1*, *ESR2*, *LRP5*, and *WNT16* genetic polymorphisms and the risk of fracture in each woman. The main finding was that of significant differences in the polymorphisms of the *WNT16* rs2908004 genetic variant, notably, the less frequent presence of TC allele in women with a greater risk of osteoporotic fractures. We conclude that the polymorphism of the *WNT16* gene seems highly relevant in the pathogenesis of osteoporosis, which makes it a promising object for further research on the genetic background of fracture risk.

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1 Introduction

Osteoporosis with a prevalence of 20–40% in postmenopausal women is a common problem. The disease is characterized by a generalized reduction in bone mineral density (BMD), low bone mass, microarchitectural deterioration of bone tissue, compromised bone strength, and consequently an increased fracture risk. Osteoporotic fractures lead to a significant decrease in quality of life, with increased morbidity, mortality, and disability. Osteoporosis is defined to exist when BMD values fall more than 2.5 standard deviations below the young adult mean. Osteoporosis can occur as part of the aging process in conjunction with decreasing sex hormones or secondarily due to metabolic disorders, nutritional deficiencies, or medication's adverse effects. Medications such as hormonal therapies, glucocorticosteroids, lithium, thiazolidinediones (glitazones), and long-term

anticoagulation or proton pump inhibitors may contribute to osteoporosis. The twin and family studies have shown that osteoporosis has a strong genetic component. According to the available data, genetic predisposition may account for 50–85% of the risk of osteoporosis (Stewart and Ralston 2000). However, the underlying genetic mechanisms, conducive to osteoporosis, i.e., gene variants leading to phenotypic changes, are not full clear. These mechanisms, notably, concern the polymorphisms that predispose to decreases in bone density and bone matrix quality and consequently to increased propensity for osteoporotic fractures.

The incidence of postmenopausal osteoporosis is long since believed to be related to the polymorphism of the estrogen receptor genes *ESR1* and *ESR2*, as estrogens play a significant role in the regulation of bone homeostasis and prevention of bone mass loss (Becherini et al. 2000). Studies have described the influence of *ESR1* polymorphism on the propensity for osteoporotic fractures and, indirectly, on the deterioration of bone tissue parameters such as BMD, bone surface of the lumbar vertebrae, or the pace of losing bone density in the femur neck (Albagha et al. 2005; Colin et al. 2003; van Meurs et al. 2003). The equal importance in the pathogenesis of osteoporosis is attributed to protein polymorphisms in the transmembrane Wnt signaling pathway (Monroe et al. 2012). One of the primary genes that influence bone mass is *LRP5* that plays a role in shaping differentiation of osteoblasts. In a cross-sectional genetic research, a relationship has been described between some variants of *LRP5* and the mineral density and bone mass of the lumbar spine, which may have a direct bearing on the risk of osteoporosis and fractures (Ferrari et al. 2004; Mizuguchi et al. 2004; Boyden et al. 2002). Variants of a specific component of this signaling pathway, *WNT16* protein, have to do with increased propensity for forearm fractures in the European population (Zheng et al. 2012). Other studies point to the possible influence of *WNT16* gene polymorphisms on the mineral density of the femur neck and frequency of hip joint fractures (García-Ibarbia et al. 2013; Koller et al. 2013).

In view of yet unsettled but increasing evidence of genetic predisposition for osteoporotic fractures above outlined, in this study we set out to define polymorphisms of selected genes in the population of postmenopausal women treated for osteoporosis and to evaluate the influence of both genetic and nongenetic factors on the estimated 10-year risk of a fracture.

2 Methods

2.1 Patients and Study Procedures

The study group consisted of 214 women aged 49–90 years (mean age 71 years). The women were treated for postmenopausal osteoporosis in the Clinic for Orthopedics and Rehabilitation of the Second Department of Medicine at the Medical University of Warsaw in 2011–2012. Exclusion criteria were as follows: age over 90 years, active neoplastic disease, chronic steroid use, parathyroid overactivity, unbalanced thyroidal disease, kidney failure, and malabsorption syndrome. Osteoporosis was diagnosed following the WHO guidelines by measuring bone mineral density (BMD) in the region of the femur neck, expressed as a T-score (Kanis 2007).

Patients were examined with a standard questionnaire developed by the authors. The questions concerned physical activity, osteoporosis and its treatment, fractures in the past, underlying chronic conditions, and the use of medications. The FRAX questionnaire was used to estimate a 10-year risk of fractures (Kanis et al. 2009). The anthropometric evaluation of the patients included weight and height, from which the body mass index (BMI) was calculated (Table 1).

Peripheral blood taken at EDTA (10 mL) constituted the material for genetic research.

Table 1 Patients' characteristics ($n = 214$)

Parameter	Median	Tercile 1	Tercile 3
Age; years	71.0	67.0	75.0
BMI; kg/m^2	25.0	22.5	27.6
T-score	−2.80	−3.19	−2.44
Z-score	−0.53	−1.00	−0.13

BMI body mass index, *BMD* bone mineral density

Polymorphisms of the genes encoding the following proteins were investigated: estrogen receptor 1 (ESR1), estrogen receptor 2 (ESR2), low-density lipoprotein receptor-related protein 5 (LRP5), and wingless-type MMTV integration site family member 16 (WNT16). DNA has been isolated by a standard method using guanidine isothiocyanate (Mozioğlu et al. 2014). The genetic methods to detect single nucleotide polymorphisms consisted of allele-specific polymerase chain reaction (AS-PCR), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and sequencing various exons of the genes using the TaqMan® SNP Genotyping Assay (Thermo Fisher Scientific Ltd.; Seoul, Korea).

2.2   Data Collection and Analysis

Qualitative variables were evaluated with chi-squared test. For continuous variable, the nonparametric Mann-Whitney U or Kruskal-Wallis test was used. Logistic regression was used for the evaluation of associations between polymorphisms and the occurrence of osteoporosis. Initially, the presence of possible associations

was identified by a preparatory comparative analysis. Next, one-factor regression model was employed, controlled for age. The odd ratios (OR) with 95% confidence intervals (95%CI) were computed for (i) occurrence of FRAX index values above the median, relative to those below the median, and (ii) presence of at least one fracture. Moreover, for sensitivity analysis, similar computations were made for FRAX values over the third tercile, relative to those in the first tercile. A p-value of <0.05 defined statistically significant changes.

3   Results

We found that the rs3736228 polymorphism in the *LRP5* gene predominated in the population investigated. This single nucleotide polymorphism (SNP) was present in 54.7% of women (Table 2). The distribution of other polymorphisms is shown in Tables 3, 4, 5, and 6.

A comparative analysis of two groups of postmenopausal patients, i.e., patients with a fracture risk FRAX score lower or equal to the median and greater than the median, showed significantly differences in frequency of the *WNT16*

Table 2 Polymorphism rs3736228 in the *LRP5* gene in postmenopausal women

	CC	TC	TT
Women; n (%)	117 (54.7%)	88 (41.1%)	9 (4.2%)

Table 3 Polymorphism rs2234693 in the *ESR1* gene in postmenopausal women

	CC	TC	TT
Women; n (%)	18 (8.4%)	96 (44.9%)	100 (46.7%)

Table 4 Polymorphisms rs4986938 in the *ESR2* gene in postmenopausal women

	AA	GA	GG
Women; n (%)	31 (14.5%)	103 (48.1%)	80 (37.4%)

Table 5 Polymorphisms rs2908004 in the *WNT16* gene in postmenopausal women

	CC	TC	TT
Women; n (%)	56 (29.0%)	109 (50.9%)	43 (20.1%)

Table 6 Polymorphism rs2707466 in the *WNT16* gene in postmenopausal women

	CC	TC	TT
Women; *n* (%)	57 (26.6%)	115 (53.7%)	42 (19.6%)

rs2908004 genetic variant (Table 7). A smaller proportion of TC allele in this variant was rather typical in the group with the greater fracture risk. The polymorphisms of the *LRP5* (rs3736228), *ESR1* (rs2234693), and *ESR2* (rs4986938) genes and of the other *WNT16* genetic variant, rs2707466, failed to discriminate between the two groups. To compare the polymorphisms among the patients with increased fracture risk, we subdivided the group of postmenopausal women with the FRAX score greater than the median into three terciles. Next, we compared the presence of the various polymorphisms between the extreme terciles. A significant difference was found only in case of the TC allele in the *WNT16* rs2908004 variant, which was less frequent in the third tercile of the women with the greater fracture risk (Table 8).

4 Discussion

Osteoporosis is a disease of multiple origins. Hence it is difficult to estimate the influence of various genetic risk factors. Moreover, the way in which certain genes might be responsible for reduced resistance of bones and for the occurrence of osteoporotic fractures is not fully understood at the present state of knowledge (Del Real et al. 2018; Ralston and Uitterlinden 2010; Zmuda et al. 1999). Genome-wide association studies have allowed for a partial verification of the value of the so far proposed "candidate genes" (Richards et al. 2009). Among the presumed genes, there are *SPP1*, *ESR1*, *ITGA1*, *OPG*, *LRP4*, *LRP5*, *RANKL*, *SOST*, and *RANK*. From among the SNP of the mentioned genes, *SOST*, *SPP1*, *LRP5*, *RANK*, and *RANKL* have a statistically relevant relation with fracture risk. The *ESR1* gene has been related to osteoporosis, right from the moment of its detection. Becherini et al. (2000) have shown a significant relation between the allelic variants of TA repetition in the *ESR1* gene and

bone mineral density and an almost threefold increase in fracture risk among patients with a low number of TA repetitions. A Rotterdam study, performed in postmenopausal women, has also suggested a dose-dependent relation between the haplotype allele with a low number of TA repetitions, on one side, and low BMD and increased fracture risk in the lumbar spine, on the other side (van Meurs et al. 2003). Other studies describe a relationship between the pace of bone mass loss and bone turnover and the *ESR1*-related genotype (Albagha et al. 2005; Sowers et al. 2004). A relationship between some SNPs of the *ESR2* gene and BMD in the lumbar spine has also been observed in population research (Ogawa et al. 2000). In particular, in the cancellous bone, being an essential component of the vertebrae, an unusually high expression of the *ESR2* gene has been reported. The research published so far shows a significant relationship between the *ESR2* polymorphism of repeated CA (D14S1026) and differences in BMD after menopause (Bord et al. 2001). As this relationship may also be present before the last menstruation, the *ESR2* polymorphism seems influential in shaping behavioral and bone fabric changes also before menopause (Ichikawa et al. 2005).

The *LRP5* gene comes to expression within the osteoblasts during their differentiation. The gene enables cell signal transmission over the Wnt pathway. This signaling pathway influences various aspects of osteoblast differentiation. The available, reliable scientific proof suggests that the Wnt signaling pathway influences bone homeostasis, both during the fetal stadium and after birth. By a cross-sectional analysis, Ferrari et al. (2004) have noticed significant differences in MBD of the lumbar spine and of the bone surface, dependent on the presence of substitution in the ninth exon of the *LRP57* gene. Also, the presence of *LRP5* allele with a majority of specific polymorphisms (e.g., 2220C-T) in patients with postmenopausal osteoporosis supports the hypothesis of a causal relation

Table 7 Frequency of gene polymorphisms in postmenopausal women with a lower and higher risk of osteoporotic fracture according to the FRAX score

	FRAX ≤ median (n = 109)	FRAX > median (n = 105)	
	n (%)	n (%)	p-value
LRP5 – rs3736228			
CC	55 (50.4)	62 (59.1)	0.40
TC	49 (45.0)	39 (37.1)	
TT	5 (4.6)	4 (3.8)	
ESR1 – rs2234693			
CC	9 (8.3)	9 (8.6)	0.69
TC	52 (47.7)	44 (41.9)	
TT	48 (44.0)	52 (49.5)	
ESR2 – rs4986938			
AA	16 (14.7)	15 (14.3)	0.61
GA	49 (45.0)	54 (51.4)	
GG	44 (40.3)	36 (34.3)	
WNT16 – rs2908004			
CC	25 (22.9)	37 (35.2)	0.03
TC	65 (59.6)	44 (41.9)	
TT	19 (17.4)	24 (22.9)	
WNT16 – rs2707466			
CC	25 (22.9)	32 (30.5)	0.13
TC	66 (60.6)	49 (46.7)	
TT	18 (16.5)	24 (22.9)	
Age, median (T1; T3)	67 (62; 73)	75 (72; 78)	<0.001
BMI, median (T1; T3)	25.0 (22.5; 27.6)	25.3 (22.5; 27.5)	0.81
< 18.5	3 (2.7)	5 (4.8)	0.71
18.5–24.9	51 (46.8)	46 (43.8)	
≥ 25.0	55 (50.5)	54 (51.4)	
No of fractures, median (T1; T3)	0 (0; 1)	1 (0; 2)	<0.001
0	59 (54.1)	27 (25.7)	<0.001
1	35 (32.1)	44 (41.9)	
2 or more	15 (13.8)	34 (32.4)	
Osteoporotic fractures	35 (32.1)	69 (65.7)	<0.001
Working	7 (6.4)	3 (2.9)	0.28
Sports	24 (22.0)	20 (19.1)	0.59
Calcium *plus* vitamin D	91 (83.5)	94 (89.5)	0.20
Anti-resorptive drugs	73 (67.0)	78 (74.3)	0.20
Calcium and/or anti-resorptive drugs	99 (90.8)	99 (94.3)	0.34
Good compliance	88 (94.6)	90 (96.8)	0.40
T-score, median (T1; T3)	−2.50 (−2.81; −1.95)	−3.13 (−3.46; −2.80)	<0.001
Z-score, median (T1; T3)	−0.31 (−0.80; 0.12)	−0.79 (−1.10; −0.34)	<0.001
BMD, median (T1; T3)	0.69 (0.66; 0.78)	0.62 (0.58; 0.66)	<0.001

LPR5 low-density lipoprotein receptor-related protein 5, *ESR* estrogen receptor genes, *WNT16* wingless-type MMTV integration site family member 16, *BMI* body mass index, *BMD* bone mineral density, *T1-T3* first and third tercile

of this gene with BMD and the risk of osteoporosis. Another component of the Wnt pathway, which may be a possible genetic factor of osteoporosis, is *WNT16*. A large-scale meta-analysis has shown a significantly increased risk of osteoporotic fracture of a forearm in the European population having the *WNT16* gene with rs2908004 variant (Zheng et al. 2012). Further, this

Table 8 Frequency of gene polymorphisms in postmenopausal women with a higher risk of osteoporotic fracture according to the FRAX score > median; subdivided into three terciles (T); statistical comparison between T1 vs. T3

	FRAX T1 ($n = 71$)	FRAX T2 ($n = 72$)	FRAX T3 ($n = 71$)	T1 vs. T3
	n (%)	n (%)	n (%)	p- value
LRP5 – rs3736228				
CC	38 (53.5)	36 (50.0)	43 (60.6)	0.69
TC	29 (40.9)	34 (47.2)	25 (35.2)	
TT	4 (5.6)	2 (2.8)	3 (4.2)	
ESR1 – rs2234693				
CC	6 (8.5)	5 (6.9)	7 (9.9)	0.31
TC	35 (49.3)	35 (48.6)	26 (36.6)	
TT	30 (42.2)	32 (44.5)	38 (53.5)	
ESR2 – rs4986938				
AA	13 (18.3)	10 (13.9)	8 (11.3)	0.49
GA	31 (43.7)	36 (50.0)	36 (50.7)	
GG	27 (38.0)	26 (36.1)	27 (38.0)	
WNT16 – rs2908004				
CC	15 (21.1)	23 (31.9)	24 (33.8)	0.04
TC	46 (64.8)	32 (44.4)	31 (43.7)	
TT	10 (14.1)	17 (23.6)	16 (22.5)	
WNT16 – rs2707466				
CC	12 (16.9)	24 (33.3)	21 (29.6)	0.16
TC	46 (64.8)	33 (45.8)	36 (50.7)	
TT	13 (13.3)	15 (20.8)	14 (19.7)	

LPR5 low-density lipoprotein receptor-related protein 5, *ESR* estrogen receptor genes, *WNT16* wingless-type MMTV integration site family member 16

polymorphism has a relationship with BMD in the femur neck and with the frequency of fractures in the hip region among patients aged 49–80 (García-Ibarbia et al. 2013). The polymorphism rs2707466, on its turn, has been related to the cortical thickness of the radial and tibial bones (Lorentzon et al. 2005). A later meta-analysis has confirmed a significant influence of *WNT16* polymorphisms on bone mass (Koller et al. 2013). Postmenopausal osteoporosis is a multifactorial disease. The absence of a clear Mendelian inheritance pattern makes it extremely difficult, to determine a priori the number of genes involved and their effects. A potential pitfall of the present study was related to the possibility of a biased population stratification, in particular, that the sample size was relatively small. A critical issue also is a multifactorial, age-related nature of osteoporosis, in which genetic determinants are modulated by hormonal, environmental, and nutritional factors such as diet and physical exercise. In this study we did not exclude women with diabetes or being on diuretics. Since it has been estimated that approximately 50% of all women will have osteoporosis by the age of 80, the advanced age of the women studied (mean 71 years) could influence the results as well.

In conclusion, we believe we have shown that the less frequent TC allele in the *WNT16* rs2908004 variant associates with a lower risk of osteoporotic fractures in postmenopausal women. The role of *ESR1*, *ESR2*, and *LRP5* polymorphisms seems rather doubtful for the distinguishing between a lower and greater osteoporotic fracture risk. Therefore, polymorphisms in the *WNT16* gene are a promising object for further research on the genetic background of fracture risk, which may lead to improved effectiveness in prevention and treatment of osteoporosis.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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The "Optimum Volume" of Acrylic Cement Filling for Treating Vertebral Compression Fractures: A Morphometric Study of Thoracolumbar Vertebrae

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Abstract

Percutaneous vertebroplasty is a treatment option in vertebral compression fractures (VCF). The aim of the study was to propose the mathematical calculation of the "optimum volume" of acrylic cement filling of the vertebral body, depending on the severity of a fracture. Two hundred computed tomography (CT) scans of vertebral columns in healthy adult Caucasians were analyzed. Vertebral body width (VBW), vertebral body depth (VBD), vertebral body height (VBH), and vertebral body volume (VBV) were measured. The "optimum volume" of cement injections in mild (25% collapse) and moderate (40% collapse) VCF were calculated. We found that moving caudally from Th_{11} to L_2, the mean values of the examined parameters

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increased: VBH from 22.6 to 26.0 mm, VBW from 34.0 to 39.5 mm, VBD from 28.1 to 30.9 mm, and VBV from 17.1 to 24.8 cm^3. The calculated hypothetical "optimum volume" of cement injection increased from 7.4 to 10.0 cm^3 in mild VCF and from 5.9 to 7.8 cm^3 in moderate VCF, with some variability depending on the vertebral level and gender. These values are akin to those present in other past studies. We conclude that morphometric measurements, based on CT images, are a reliable source of practical anatomical savvy, which may be of help in spine surgery.

1 Introduction

Vertebral compression fractures (VCF) are a significant global socioeconomic problem owing to the rapid growth of the aging population. The most common causes are osteoporosis, bone metastasis, trauma, and multiple myeloma. VCF can also result from endocrine diseases, e.g., diabetes mellitus, endogenous hypercortisolism, or primary hyperparathyroidism; from prolonged glucocorticoid use, drugs inhibiting the secretion of sex hormones, nonmalignant hemopathies, and systemic mastocytosis leading to increased osteoclastogenesis; and from alcoholism and anorexia nervosa (Che et al. 2018; Xie et al. 2015). In the USA alone, there are approximately 1.5 million cases of new VCF annually, and a fracture occurs worldwide every 22 s (Beall et al. 2018). The prevalence rate of VCF in females aged 50 is about 10%, which increases to 25–50% by the age of 80 (Morosano et al. 2011). It is estimated that 25% of postmenopausal women are affected by osteoporotic VCF during their lifetime. Approximately 9–29% of patients with vertebral column metastases will have a

pathological fracture, with 90% of them requiring surgical treatment. The cost of VCF treatment, including medical complications and postsurgical rehabilitation, amounts to about $ 17 billion per year (Beall et al. 2018).

The first percutaneous vertebroplasty, consisting of injection of the polymethylmethacrylate (PMMA) cement into the vertebral body by a percutaneous transpedicular approach, was performed in 1984 for hemangioma treatment (Deramond et al. 1998; Galibert et al. 1987). This procedure is used to achieve pain relief, stabilize the anterior and middle columns, reinforce the fractured bone, improve the activity of patients, reduce mortality, and enhance quality of life. Several articles regarding the volume of artificial bone cement filling in VCF due to tumors have been since published (Rabei et al. 2019; Mansoorinasab and Abdolhoseinpour 2018; Blasco et al. 2012). However, the question of the optimum volume of PMMA remains open. The differences concerning the cement volume reach as much 750%, with the estimations in various trials from 2 cm^3 to 15 cm^3, depending on the level of the spinal column (Sun et al. 2018; Fu et al. 2016; Chew et al. 2011; Jin et al. 2011; Buchbinder et al. 2009; Pflugmacher et al. 2006; Molloy et al. 2005; Belkoff et al. 2001).

The thoracolumbar level is particularly susceptible to VCF. This is due to a change in the curvature from thoracic kyphosis to lumbar lordosis, structural and mechanical stress, and a higher dynamic motility of this junction (Yang et al. 2018, 2019). That is the main reason why concentrated on the Th$_{12}$–L$_2$ level in the present study. The goal was twofold. Firstly, we sought to evluate the morphology of the thoracolumbar vertebral bodies in a Caucasian population, using computed tomography (CT) images, for comparison with data from other past studies. Secondly, we defined the "optimum volume" in vivo of artificial cement injection into the vertebral body, which would be required to treat VCF depending on their severity, on the basis of morphometric measurements. The accurate knowledge of vertebral column morphology is

essential for surgeons performing percutaneous vertebroplasty or kyphoplasty. To the best of our knowledge, there are no studies that report on the physiological distribution of vertebral body volumes in a Caucasian population, which would provide a calculation of the "optimum volume" of artificial cement to be injected.

2 Methods

2.1 Study Population

The study group consisted of 102 men and 98 women, with no spinal disorders, diagnosed in the Radiological Department of the Medical University of Gdansk in Poland. The mean age was 45.4 ± 6.5 years for males (range: 21–62 years) and 48.3 ± 4.1 years for females (range: 29–65 years). CT was used due to its high sensitivity, repeatability, availability, low cost, and noninvasiveness and ease for morphometric measurements (Dzierżanowski et al. 2014, 2019). The patients with degenerative disease of the spine (osteophytes and hypertrophy), scoliosis, diseases destroying the bone structure of bone (tumors and osteoporosis), and fractures were excluded in order to have a group of "healthy" spines for further evaluation.

2.2 Imaging and Vertebrae Measurements

All radiological examinations were performed using a 64-row multidetector CT scanner (General Electric LightSpeed VCT; GE Healthcare Technologies, Milwaukee, WI). Noncontrast computed tomography protocols were performed with the helical technique using the following parameters: 120 kV; smart mA (100–450); rotation time – 0.6 s; pitch/speed (mm/rotation) – 1.375:1, 55.00 mm; gantry angle – 0; noise index – 16.0; adaptive statistical iterative reconstruction – 20; dose reduction – 20%; detector width – 0.625 mm; display field of view – 18 cm; slice thickness/spacing – 2.5 mm × 2.5 mm (recon: 0.625 mm); CT dose index per volume – 11.3 mGy; and dose length product – 656 mGy∗cm.

Raw data were reconstructed using the image postprocessing, using a separate workstation equipped with a professional software package to generate three-dimensional (3D) images of thoracic and lumbar vertebrae (Advantage Workstation 4.4; GE Healthcare Technologies, Milwaukee, WI). The following measurements were made (Fig. 1 and Fig. 2a, b):

- Vertebral body depth **(VBD)** – the distance between the anterior and posterior walls of the vertebral body, measured in the transverse plane
- Vertebral body width **(VBW)** – the distance between the lateral walls of the vertebral body, measured in the transverse and coronal plane
- Vertebral body height **(VBH)** – the distance between the superior and inferior endplates, measured in the sagittal plane in the middle part of the vertebral body
- Vertebral body volume **(VBV)** – assessed from the parameters above outlined

We collected 800 measurement figures for further statistical analysis regarding the differences
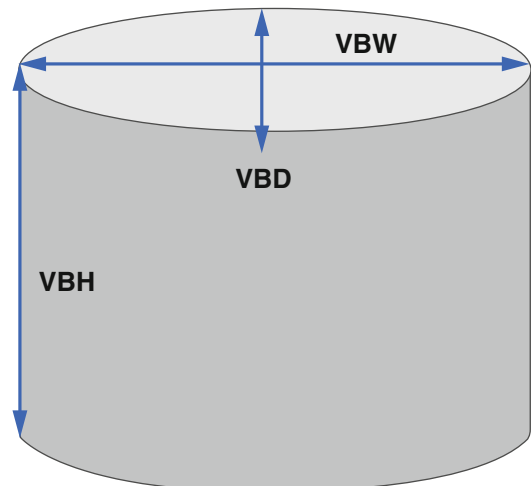

Fig. 1 Measurements of the vertebral body taken: *VBD* vertebral body depth, *VBH* vertebral body height, and *VBW* vertebral body width
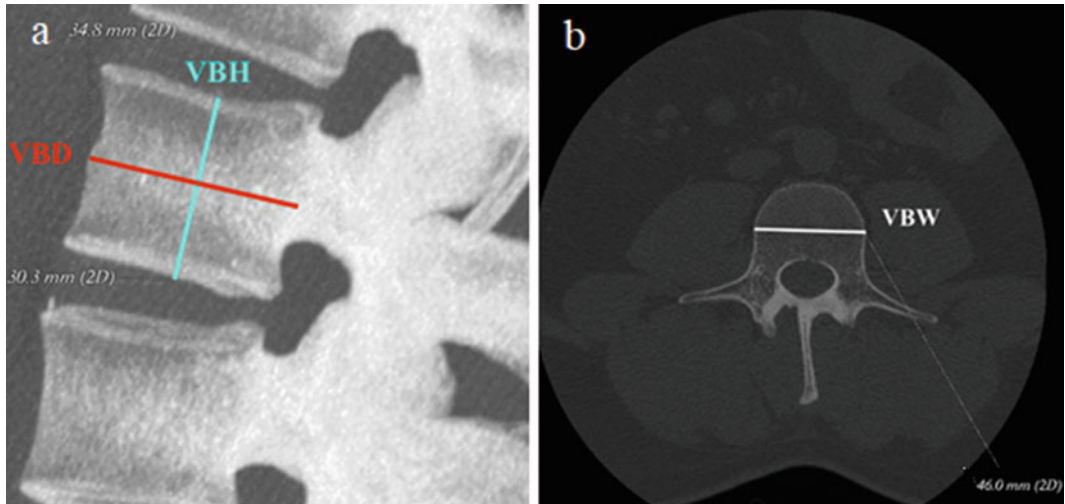
Fig. 2 Sagittal sections of CT scans: (**a**) demonstration of the measurement of vertebral body height (VBH) and vertebral body depth (VBD) and (**b**) vertebral body width (VBW)

between the tested levels and gender. All measurements were performed by two radiologists with 20 years of clinical experience. The reading accuracy of the numerical values was 0.1 mm.

The measurements were performed in the middle part of the vertebral body to avoid spuriously increased values due to the outgrowth of osteophytes, which could especially affect the VBW in sagittal CT views. An analogical approach also was applied for VBH and VBW due to the possibility of osteophytes growing into the spinal canal, outside the anterior walls of vertebrae (VBD) and outside the lateral walls of vertebrae (VBW) in transverse views.

For simplification of the measurement of VBV, we assumed a figure similar to an elliptical cylinder like that presented in Fig. 1. Knowing the height and the field of the base of the figure (F_b), we calculated the volume V using the equation:

$$V = F_b \cdot VBH,$$

where $F_b = \pi \cdot \frac{1}{2} VBW \cdot \frac{1}{2} VBD$

The results were compared with those for other than Caucasian populations, based on the literature available in international databases, such as

Embase and PubMed, using the keywords "lumbar spine morphometry", "thoracic spine morphometry", and "vertebroplasty". Only original articles in English language published from 2002 to 2018 and utilizing measurements on cadavers, radiological imaging, and finite element models based on the adult human spine were taken into consideration. Case reports without literature reviews, abstracts, and research involving children were excluded from the study.

2.3 Calculation of the Volume of Cement Injection into the Vertebral Body

To describe the severity of compression fracture, we used Genant et al's. (2009) classification of mild (20–25%) or moderate (26–40%) vertebral body collapse. For simplification, we took the two upper limits of the estimated values: 25% for mild compression fracture (a reduction of the previous volume of vertebra by 25%) and 40% for moderate compression fracture (a reduction of the previous volume of vertebra by 40%). To reduce the potential risk of cement leakage outside the vertebra, we reduced the parameters VBD, VBH, and VBW by two standard deviations (SD). There were VBD minus one SD in the anterior and one

Fig. 3 Formula for the volume of an elliptical cylinder. The optimum distribution of the cement inside the vertebra (darker color). *SD* standard deviation
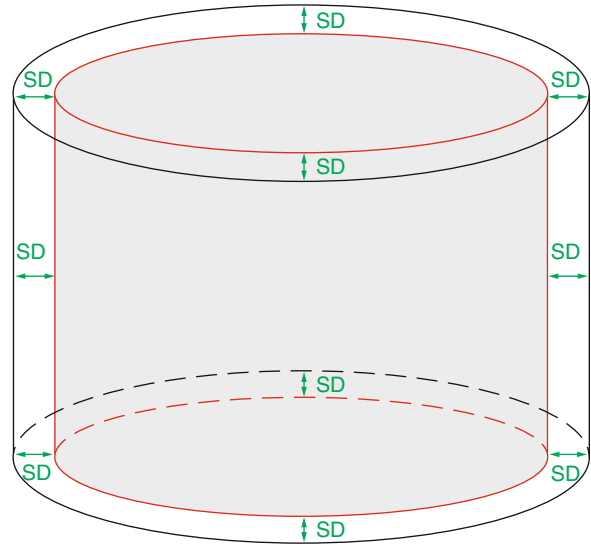
SD in the posterior projections, VBH minus one SD in the superior and one SD in the inferior projections, and VBW minus SD on both sides in the lateral projection (Fig. 3). Then, the result of VBV was reduced by 25% or 40% to calculate the optimum quantity (V) of bone cement (PMMA) to be injected. In case of the mild 25% collapse, the estimation was:

$$
V_{PMMA} = \pi \frac{1}{2} (VBW - 2SD_{VBW}) \frac{1}{2} \\
(VBD - 2SD_{VBD}) (VBH - 2SD_{VBH}) \, 0.75
$$

and in the moderate 40% collapse it was:

$$
V_{PMMA} = \pi \frac{1}{2} (VBW - 2SD_{VBW}) \frac{1}{2} (VBD - 2SD_{VBD}) \\
(VBH - 2SD_{VBH}) \, 0.6
$$

2.4 Statistical Analyses

Data were presented as means ±SD and ranges. The Shapiro-Wilk and Levene tests were used to assess normality of data distribution and equality of variances, respectively. A *t*-test and Mann-Whitney test were used for relevant comparisons. The volume of vertebrae was compared between spine levels by means of one-way ANOVA. A p-value ≤ 0.05 defined statistical significance. Commercial statistical packages of Statistica v12.0 (StatSoft; Tulsa Inc., OK) and GraphPad Prism v6.05 (GraphPad Company; La Jolla, CA) were used for the analysis.

3 Results

The mean results for all tested elements for both genders are presented in Fig. 4a, b, c, and d and in Table 1.

3.1 Morphometry of Vertebral Bodies

Moving caudally from Th_{11} to L_2, the mean VBD increased from 28.7 to 30.9 mm in men and from 28.1 to 29.4 mm in women. The values for the whole group for both genders had the following distribution: Th_{11} 23.9–33.7 mm ($\pm$ 2.8 SD), Th_{12} 23.9–36.2 mm ($\pm$ 3.3 SD), L_1 24.0–37.7 mm ($\pm$ 3.3 SD), and L_2 23.9–37.0 mm ($\pm$ 3.6 SD).
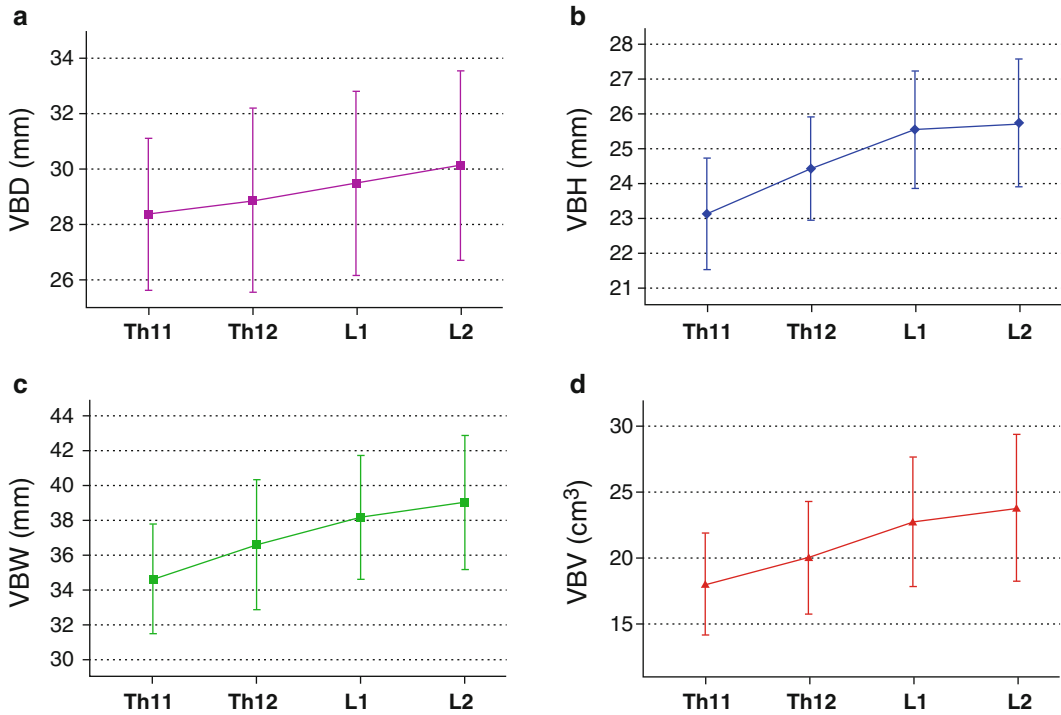
Fig. 4 Diagrams show the results of measurements, moving caudally from Th_{11} to L_2 for the whole study group (both genders): (**a**) change in vertebral body depth (VBD) (anteroposterior diameter); (**b**) change in vertebral body height (VBH); (**c**) change in vertebral body width (VBW) (transverse diameter); and (**d**) change in vertebral body volume (VBV). Values are means ±SD

The mean VBH increased in men from 23.7 to 26.0 mm and from 22.6 to 25.5 mm in women. The values for the whole group for both genders had the following distribution: Th_{11} 19.7–25.6 mm ($\pm$ 1.6 SD), Th_{12} 21.3–27.4 mm ($\pm$ 1.5 SD), L_1 22.5–28.9 mm ($\pm$ 1.7 SD), and L_2 21.9–30.5 mm ($\pm$ 1.8 SD).

The mean VBW increased from 35.1 to 39.5 mm in men and from 34.0 to 38.4 mm in women. The values for the whole group for both genders had the following distribution: Th_{11} 28.6–41.8 mm ($\pm$ 3.2 SD), Th_{12} 29.3–45.0 mm ($\pm$ 3.8 SD), L_1 30.0–45.8 mm ($\pm$ 3.6 SD), and L_2 31.0–48.6 mm ($\pm$ 3.9 SD).

The mean VBV increased from 19.0 to 24.8 cm^3 in men and from 17.1 to 23.0 cm^3 in females. The values for the whole group of both genders had the following distribution: Th_{11} 18.0 cm^3 ($\pm$ 3.9 SD), Th_{12} 20.1 cm^3 ($\pm$ 4.3 SD), L_1 22.8 cm^3 ($\pm$ 5.0 SD), and L_2 23.8 cm^3 ($\pm$ 5.6 SD).

3.2 Optimum Volumes of Cement Distribution

For mild (25% collapse) compression fractures, the calculated hypothetical optimum volumes of cement injected increased from 8.0 to 10.0 cm^3 in males and from 7.4 to 9.3 cm^3 in females going caudally from Th_{11} to L_2. The detailed data are shown in Table 2. For moderate (40% collapse) fractures, the optimum volumes of cement injected were from 6.4 to 7.8 cm^3 in males and from 5.9 to 7.4 cm^3 in females going likewise caudally.

4 Discussion

4.1 Morphometry of Vertebral Body

4.1.1 Vertebral Body Height (VBH)

The findings of this study were that VBH increased caudally going down from Th_{11} to L_2.

Table 1 Anatomical estimations of the vertebral bodies from Th_{12} through L_2

Dimension	Th_{11}		Th_{12}		L_1		L_2	
	Male	Female	Male	Female	Male	Female	Male	Female
VBD (mm)	28.7 ± 2.6	28.1 ± 2.8	29.4 ± 3.1	28.4 ± 3.5	30.4 ± 3.8	28.6 ± 2.6	30.9 ± 3.7	29.4 ± 3.1
VBH (mm)	23.7 ± 1.4	22.6 ± 1.5	24.7 ± 1.3	24.1 ± 1.6	25.9 ± 1.4	25.2 ± 1.9	26.0 ± 1.1	25.5 ± 2.3
VBW (mm)	35.1 ± 3.5	34.0 ± 2.8	37.4 ± 3.8	35.6 ± 3.5	38.9 ± 3.8	37.4 ± 3.3	39.5 ± 4.6	38.4 ± 3.0
VBV (cm^3)	19.0 ± 4.0	17.1 ± 3.6	20.9 ± 4.4	19.3 ± 4.0	23.8 ± 5.3	21.7 ± 4.5	24.8 ± 6.0	23.0 ± 5.2

VBD vertebral body depth, *VBH* vertebral body height, *VBW* vertebral body width, *VBV* vertebral body volume. Values are means $\pm$SD

Table 2 Optimum volumes (cm^3) of the cement distribution in the whole study group (both genders) in case of a mild 25% collapse versus moderate 40% collapse due to compression fractures

% of collapse	Gender	TH_{11}	Th_{12}	L_1	L_2
25%	Male	8.0 ± 1.7	9.0 ± 1.9	9.7 ± 2.2	10.0 ± 2.4
	Female	7.4 ± 1.5	7.5 ± 1.5	8.5 ± 1.8	9.3 ± 2.1
40%	Male	6.4 ± 1.3	7.2 ± 1.5	7.8 ± 1.7	7.8 ± 1.9
	Female	5.9 ± 1.2	6.0 ± 1.2	6.8 ± 1.4	7.4 ± 1.7

There were significant correlations between VBH at all vertebral levels studied, except between L_1 and L_2, which also have been shown in our previous study (Dzierzanowski et al. 2019). Likewise, increasing trends in VBH have been described in other studies: for Th_{11} 19.5–32.1 mm, Th_{12} 21.1–28.2 mm, L_1: 22.9–28.2 mm, and L_2 23.1–39.5 mm (Kaur et al. 2016; Mahato 2011; Tan et al. 2004). Some investigators have evaluated only the posterior VBH (posterior wall of a vertebral body) in the sagittal plane (Kunkel et al. 2010; Wolf et al. 2001) or the anterior and posterior VBH (Alam et al. 2014; Kunkel et al. 2010; Abuzayed et al. 2010). Only did Štern et al. (2012), Diacinti et al. (2011), and us in the present study perform the measurement in the middle part of a vertebra. We postulate that only this kind of a measurement can give the most reliable value.

4.1.2 Vertebral Body Width (VBW)

In this study, VBW increased in both gender groups from Th_{11} to L_2 (Table 1). It increased significantly from Th_{12} to L_1, with an insignificant difference between L_1 and L_2. The only significant inter-gender difference in VBW was present at Th_{12} level. In other studies, VBW has increased as follows: Th_{11} 31.6–42.8 mm, Th_{12} 29.2–44.2 mm, L_1 27.3–48.9 mm, and L_2 29.9–51.7 mm (Alam et al. 2014; Karabekir et al. 2011; Kaur et al. 2016; Abuzayed et al. 2010; Tan et al. 2004; Wolf et al. 2001). As in case of VBH, we removed osteophytes from the calculations and the measurement was performed in the middle part of a vertebra. Such an approach may be a reason of discrepancies between the present and previous findings.

4.1.3 Vertebral Body Depth (VBD) in the Anteroposterior Diameter

The anteroposterior diameter was the parameter in the present study in which the Th_{11}–L_2 vertebrae showed the biggest anatomical similarity. We attempted to exclude osteophytes from the estimations, by appropriately choosing the place for the anatomical measurements conducted. The VBD increased in both males and females caudally from Th_{11} to L_2 (Table 1). In other studies, the increase has been observed between Th_{11} and L_1, with a decrease for L_2 in proportion to Th_{11} and L_1. The changes were as follows: Th_{11} 25.4–30.5 mm, Th_{12} 26.7–37.6 mm, L_1 27.7–39.5 mm, and L_2 25.4–36.7 mm (Alam et al. 2014; Karabekir et al. 2011; Abuzayed et al. 2010; Tan et al. 2004; Wolf et al. 2001).

4.1.4 Vertebral Body Volume (VBV)

In this study, VBV increased in both males and females moving caudally from Th_{11} to L_2 (17.1–23.0 cm^3) (Table 2). The increase was smaller than that reported by Limthongkul et al. (2010) who have shown the increases in vertebral body volume, except for L_1 whose volume decreased, based on the measurements of 40 CT exams performed in patients with no diagnosed spinal pathology. According to that study, the mean VBV increased from 23.8 to 41.5 cm^3 in men and from 23.6 to 27.4 cm^3 in women, going caudally from Th_{12} to L_2. The smaller values noticed in the present study can be attributed to different points of measurement. We also found insignificant inter-gender differences for L_2 level in terms of VBV. Overall, we report a volume similarity in all L_2 bodies studied, irrespective of gender.

4.2   Overview of Study Findings

This study evaluated and quantified morphometric parameters of lower thoracic and upper lumbar vertebral bodies in a Caucasian population and presented a simple mathematical model for the estimation of the optimally "safe" volume of PMMA cement distribution in the vertebral bodies.

Although the vertebral cement augmentation techniques have been well described for the last three decades and are widely used in clinical practice by neurosurgeons, radiologists, orthopaedists, and anesthesiologists (pain treatment), the issue of complications and distant outcomes still require discussion (Rabei et al. 2019). Compression fractures can potentially lead to chronic pain and long-term disability. Percutaneous vertebroplasty offers localized and minimally invasive therapy to reduce pain, reconstructs biomechanical properties, prevents progressive collapse of a fractured vertebral body, improves functional outcomes (quality of life), and reduces mortality in the geriatric population (Mansoorinasab and Abdolhoseinpour 2018; Yang et al. 2019; Clark et al. 2016; Limthongkul et al. 2010; Klazen et al. 2007). Most of those recent studies, however, are from 2016 to 2019 and were not available at the time when the American Academy of Orthopaedic Surgeons issued the guidelines disavowing percutaneous vertebroplasty (Beall et al. 2018; Esses et al. 2011). Moreover, the studies on which those guidelines were predominantly based did not include standardization of the volume of PMMA cement (Buchbinder et al. 2009; Kallmes et al. 2009).

Not all the studies have provided similar results, and some of them have reported insignificant differences compared with nonsurgical management over the long term (Beall et al. 2018; Bozzo and Bhandari 2018; Buchbinder et al. 2009). For instance, the randomized clinical trial VERTOS IV shows a lack of a significant pain relief after percutaneous vertebroplasty, compared to a sham procedure, during a 12-month follow-up in patients with acute osteoporotic compression fracture (Firanescu et al. 2018). The sham procedure consisted of a focal infiltration with 1% lidocaine followed by 0.25% bupivacaine into each pedicle and simulated cement injection with verbal and physical cues (maximum placebo effect). The volume of PMMA cement used in this investigation ranged from 1 to 11 cm^3. That study raises questions concerning the cement distribution and its influence on the final outcome.

The general consensus is that the most serious complications are connected to cement leaks. PMMA cement can overflow into the epidural space, neural foramina (all consequences of mechanical spinal cord compression and thermal damage of the neural structures during hardening of the artificial cement in an exothermic polymerization reaction), epidural veins, paravertebral veins (with subsequent pulmonary cement embolism and intra-cardiac cement embolism), paravertebral soft tissues, and toward the disk space (causing adjacent vertebral collapse and disk degeneration). The incidence of all types of cement displacement has been estimated at 80% in older investigations. Recent studies show the incidence of cement displacement at more than 20% intraoperatively and postoperatively in radiographs or CT scans made during control examination (Yu et al. 2019; Fadili Hassani et al. 2018; Mansoorinasab and Abdolhoseinpour 2018; Wang et al. 2018; Fu et al. 2016; Jin et al. 2011; Uppin et al. 2003). Fortunately, less than 12% of such cases have a clinical meaning, with only 2% representing serious complications (Scheuter et al. 2018; Chew et al. 2011; Calmels et al. 2007).

Some authors report about 14–38% incidence of re-collapse in the cement-augmented vertebrae after percutaneous vertebroplasty within 1–2 years (Hou et al. 2018; Yu et al. 2019; Han et al. 2014) and a 12% incidence of secondary fracture (Uppin et al. 2003). Herein we demonstrate such an example of cement leakage and additional compression fractures above the augmented vertebra in an elderly female patient, which is depicted in Fig. 5. The experimental biomechanical studies explain the propensity for
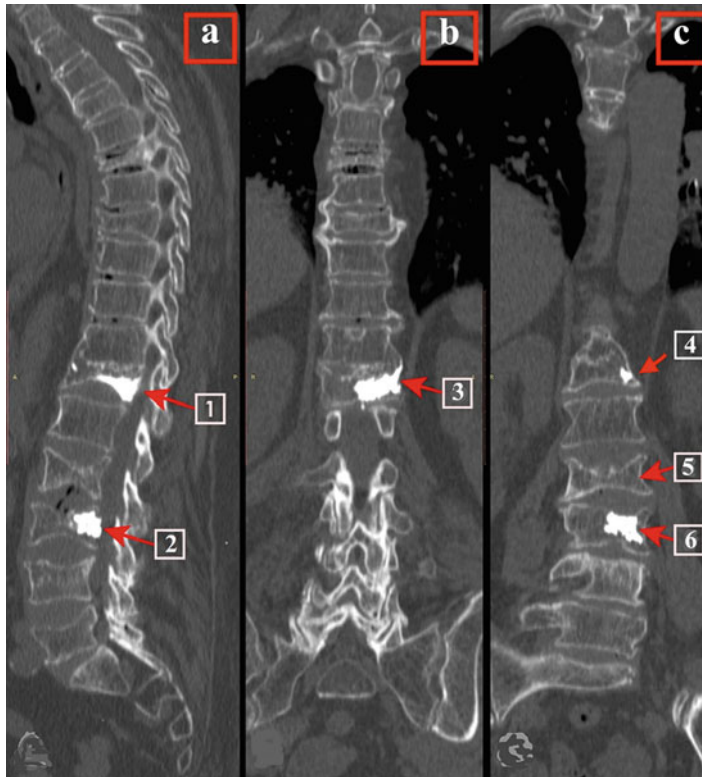
Fig. 5 CT scans of a 72-year-old woman, taken 2 years after she had been treated with percutaneous vertebroplasty due to compression fracture: (**a**) saggital plane – arrows 1 and 2 show cement injected into Th_{12} and L_3 vertebrae; (**b**) coronal plane – arrow 3 shows extra-vertebral leakage of the cement from Th_{12} to the adjacent soft tissue; and (**c**) coronal plane of a different layer – arrow 4 shows cement inside Th_{12}, arrows 5 points to another compression fracture above the originally treated L_3, and arrow 6 shows a unilateral (left side) inadequate distribution of the cement inside L_3 cuase by an insufficient volume of PPMA injected

new fractures by increased stiffness and strength in the augmented bone, which is transferred to the adjacent vertebrae and, in consequence, may cause fractures. Additionally, percutaneous vertebroplasty may lead to an anterior load shift, causing re-fracture of the cemented vertebrae (Lin et al. 2017; Lin et al. 2008). However, other investigators do not confirm this hypothesis, as they do not find an increase in the number of new fractures after vertebroplasty (Yang et al. 2018, 2019; Zhang et al. 2017; Xie et al. 2015; Han et al. 2014; Aquarius et al. 2013).

In our opinion, the essential factor to reduce complications is the use of a "safe" and adequate volume of PMMA cement and pressure of injection. Too low a cement volume and its unsatisfactory spatial distribution are a source of poor results. On the other hand, injection of too much PMMA can be a reason for complications and poor results. Jin et al. (2011) have shown that the injection of a volume over 9.2 cm^3 causes subsequent adjacent fractures. A study of the experimental model of cement leakage has shown a reduced incidence of this complication from 50% to less than 10% when the cement viscosity is increased from low to medium (Baroud et al. 2006). A high viscosity cement leads to more efficient vertebral stabilization and a reduced risk of leakage and complications. High-pressure injection of PMMA increased cement leakage by 10–40% (Limthongkul et al. 2010). The fracture severity grade, particularly severe and very severe (more than 67% of the primary height), based on the Genant et al. (2009)

classification, associates with cement displacement. Likewise, bilateral cement injections are connected with a greater risk of PMMA displacement. One of the possible reasons might be a larger bone cement volume compared to a unilateral approach. Interestingly, patients with spinal metastases have a greater incidence of cement leakage compared to those with osteoporotic fractures (Zhu et al. 2016).

Generally, there are no recommendations of the cement volume depending on the level of spinal column, gender, and the severity of compression fracture, which would be mathematically estimated on the basis of anatomical studies, except for a study of Jin et al. (2011) and a few others (Sun et al. 2018; Kwon et al. 2016). Usually, the surgeon subjectively estimates the volume of cement to be injected into a vertebral body during treatment of compression fracture. For that reason, the volume range is wide and variable. Hiwatashi et al. (2003) have used 14 cm^3 of cement, Buchbinder et al. (2009) 3 cm^3, Fu et al. (2016) 7.9 cm^3 for the thoracolumbar region and 11.2 cm^3 for the lumbar region, Jin et al. (2011) 3.35 cm^3 for Th_{11}–L_1 levels, and Martinčič et al. (2014) have recommended the average of 4–6 cm^3 for the thoracolumbar region, which corresponds to about 15% of the VBV. There is no consensus on the cement volume for either pain relief or the restoration of strength and stiffness. Belkoff et al. (2001) have reported that strength may be restored with the injection of 2 cm^3 of cement. Molloy et al. (2003) have suggested that cement volumes of the order of 30% of the VBV should suffice and show that a volume of 6 cm^3 is needed to restore strength. On the other side, 7 cm^3 is needed to restore vertebral stiffness (Molloy et al. 2005), although in the lumbar region it gets hardly restored even with 8 cm^3 of PMMA (Molloy et al. 2003). Different volumes are necessary to achieve adequate pain relief with PMMA injections in patients with metastases. Usually, 1.5 cm^3 of cement suffices to reduce pain considerably (Gangi et al. 2003). Pflugmacher et al. (2006) have used cement in the mean volume of 1.8 cm^3, while Yang et al. (2018, 2019) used a 5 cm^3 volume. Chew et al. (2011) have postulated in a systematic review on percutaneous vertebroplasty in malignancy that, on average, injections of 4 cm^3 or more of PMMA cement associate with a higher complication rate than those of less than 4 cm^3. These authors postulate that filling of the vertebral body should be stopped when it is done halfway, as later cement extravasation may occur.

In this study, we presented a mathematical model for the measurement of the maximum "safe" volume of PMMA cement distribution. On the basis of the estimations made, we postulate a 7.4–10.0 cm^3 volume of injected cement in the mild (25% collapse) compression fractures and 5.9–7.8 cm^3 in the moderate (40% collapse) fractures, depending on the gender and spine level. These values are akin to those proposed formerly by Fu et al. (2016) and Hiwatashi et al. (2003). This amount of cement is two- to threefold greater than those proposed in some other investigations (Yang et al. 2018, 2019; Buchbinder et al. 2009). We believe that a regular augmentation of the vertebral body with the preservation of the margins of the natural bone (minus the standard deviation width on both sides) is the only way to successfully treat osteoporotic compression fractures. There is a difference between the optimum volume of cement "injection" and "distribution". An inadequately small quantity of cement can be a reason of poor outcome during the long-term follow-up. Some investigators failed to find differences between the conservative and percutaneous vertebroplasty treatments after 1 year of observation (Bozzo and Bhandari 2018; Kallmes et al. 2009; Buchbinder et al. 2009). They usually postulate using a small 1–3 cm^3 volume of PMMA, approximately. This volume can have an analgesic effect, but it is questionable whether it restores strength and stiffness or prevents secondary and new fractures of the neighboring spinal structures. Molloy et al. (2005) have investigated the same concept. They show in ex vivo biomechanical studies on cadavers that the strength is restored at a cement volume over 2 cm^3 in the thoracic region, 4 cm^3 in the thoracolumbar region, and 6 cm^3 in the lumbar region, but the procedure fails to restore stiffness for which twice as much cement is needed. Jin et al. (2011) have demonstrated a "safe range" of volumetric values

during percutaneous vertebroplasty based on the following formula: volume fraction (VF) = (calculated cement volume/calculated fractured body volume) × 100. The cut-off level, i.e., the minimum VF% to relieve pain and minimize complications after percutaneous vertebroplasty is 11.65%; thus the authors recommend 3.35 cm^3 of cement for Th_{11}–L_1 level. Kwon et al. (2016) have proposed a greater VF% (approx. 28%), akin to the 32% of Mousavi et al. (2003) and the 20% of Sun et al. (2018), which corresponds to 4–6 cm^3 of cement. All the differences above outline may be explicable by the fact that cement volume and distribution are not tantamount. In this in vitro study, we made a presumption of a regular distribution of artificial acrylic cement, leading to a complete shading of the fractured vertebra in the intra-operative radiological examination. In this kind of vertebral body augmentation, final outcome of percutaneous vertebroplasty was the best.

5 Conclusion

All vertebral body parameters increased going down from Th_{11} to L_2 in both genders. There were significant differences in the vertebral body parameteyrs between the successive spine levels, except between L_1 and L_2, which confirms earlier observations regarding the anatomical similarity between the two latter vertebrae. We further found significant inter-gender differences in the vertebral body dimensions concerning all the spine levels measured, except the volume of L_2. The vertebral body height, depth, and width were greater in males than those in females. The morphometric parameters of the lower thoracic and upper lumbar vertebral bodies in the Caucasians population investigated in this study are akin to those described in other previous studies on the subject.

The "optimum volume" of acrylic cement augmentation of vertebral bodies is approx. 5.9–10.0 cm^3, depending on the gender and spine level. This volume is larger than those in many previous studies. We submit that the cement volume ought to strictly associate with cement distribution. The mathematical model for obtaining morphometric data to set the maximum "safe" volume of PMMA cement we herein presented provides repeatable and accurate measurements. It may have a clinical bearing for neurosurgeons and orthopaedists engaged in vertebroplasty of osteoporotic compression fractures application due to its accessibility.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Independent Ethics Committee of the Medical University of Gdansk, Poland.

Informed Consent Written informed consent was obtained from all individual participants included in the study.

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Integrated Rehabilitation Approach with Manual and Mechanic-Acoustic Vibration Therapies for Urinary Incontinence

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Abstract

This study aims to verify whether an integrated rehabilitation protocol comprising neuromuscular manual therapy and focused mechanical-acoustic vibrations can significantly reduce pelvic floor dysfunctions in women affected by stress, urge, or mixed urinary incontinence. Sixty-two women were treated with a combination of neuromuscular manual therapy and mechanical-acoustic vibrations at the level of superficial pelvic floor muscle groups. The results were analyzed before the beginning and after the end of the study protocol with the myometric measuring device MyotonPRO, the Pelvic Floor Disability Index (PFDI-20),

and the Pelvic Floor Impact Questionnaire (PFIQ-7). Two patients withdrew from the study after the first visit. The 60 remaining patients showed significant improvements of myometric parameters, with the percentage variations ranging from +8.5% to +20.7% for the muscle logarithmic decrement, from −11.2 to −13.9% for muscle frequency, and from −4.8% to −12.3% for muscle stiffness. There has been a reduction of 56% in the perceived disability induced by urinary incontinence, measured with the PFDI-20, and 43% reduction in the impact of the problem on daily living, measured with the PFIQ-7. We conclude that a combination of neuromuscular manual therapy and mechanical-acoustic vibrations effectively reduces pelvic symptoms in patients affected by urinary incontinence, with minimal invasiveness.

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1   Introduction

Urinary incontinence is defined as "the complaint of any involuntary leakage of urine" and represents a major disabling clinical problem for the general population, especially the elderly. It is characterized by a heterogeneous pathology and commonly divided into stress incontinence, urge incontinence, and mixed incontinence, depending on symptoms (Leong and Mok 2015). In particular, mixed urinary incontinence results from a combination of urodynamic stress incontinence and detrusor muscle overactivity. The primary factors predisposing individuals to stress incontinence are pregnancy, childbirth, obesity, and all conditions that result in a loss of contractile capacity of the urethral sphincter muscle, which physiologically maintains continence (Carneiro et al. 2010).

Since the pelvic floor represents an unfamiliar area of the body to which little or no attention is given, it would be desirable to introduce an educational program for the general population, considering that pelvic floor alterations are quite a frequent pathological condition. It is estimated that urinary incontinence affects approximately 30% of the general population, with a higher prevalence in Americans (37%) compared to Europeans (26%). The epidemiological data vary greatly, depending on the definition of incontinence, which also reveals an uncertain role of the age factor in the pathogenesis (Bump and Norton 1998). Moreover, dysfunction of the pelvic floor is a pathology that is extremely limiting for the bodily and psychosocial domains of patient functioning, greatly affecting quality of life. The psycho-emotive factors have a strong impact on the pathological state of the pelvic floor connected with the function of continence and urination, which is characterized by an intrinsic intimacy that exacerbates the degree of perception by those affected (Davis and Kumar 2003).

Continence and urination are the result of a coordinated action of smooth and striated muscles of the low urinary tract and pelvic floor. The neural control of these muscles consists of the autonomic and somatic integration, which is the basis of a "distrectual" coordination that gains a specific functional meaning, greater than that of any other anatomic region (Enhorning 1976). Many a study demonstrates that the striated muscles of the urethral wall contract together with contraction of pelvic floor muscles (Bø and Stain 1994). A significant increase of resting urethral pressure after the exercise of pelvic floor muscles (Bø et al. 1990; Benvenuti et al. 1987) can thus be explicable not only by increased tone of the striated inter-wall muscle but also by increased tone of pelvic floor muscles. The striated muscle of the urethral wall, much like pelvic floor muscles, also appears contracted during the contractive activity of the gluteus muscle and the abductor of the hip. In this way, these external muscles facilitate the synergistic contraction of inter-pelvic muscles. This synergy is important because the involvement of the gluteus and the abductor of the hip during daily activities stimulates the urethral wall (Bø and Stain 1994).

Guidelines for pelvic floor muscle exercise affirm that a correct isolation of involved muscles is necessary in the activity of abdominal muscles to obtain more effective results (Neumann and Gill 2002). The isolated contraction of pelvic floor muscles counteracts an increase in the inter-abdominal pressure, which can cause or increase symptoms of stress incontinence. The pressure in the abdominal cavity is determined by a combined action of the pelvic floor, the abdominal wall, and the diaphragm muscle (Sapsford et al. 2001). Furthermore, it has been demonstrated that pelvic dysfunction and lumbar-sacral vertebral mechanical restriction can alter neural function, blood supply, and fluid drainage, generating local inflammation associated with pain sensitization (Genders et al. 2003; Proctor et al. 2001), which implies that all the musculoskeletal structures of this region share the same neural as do pelvic organs.

Neuromuscular manual therapy is a global and advanced system of manipulations of soft tissues, aimed at the re-establishment of homeostatic status of the musculoskeletal and central nervous systems. It represents one of the most advanced and validated approaches to treating dysfunctions affecting the muscle system and their eventual

visceral consequences (Granger 2011). Another effective therapy in treating muscle system dysfunctions is vibration therapy, in particular focused mechanic-acoustic vibrations, which shows an ability to increase muscle metabolic activity, inducing a greater release of anabolic hormones and reduced production of catabolic factors, while also producing a mechanical stimulation of specific mechanoreceptors, increasing the recruitment activity of muscle fibers, and stimulating higher centers of motor control (Pietrangelo et al. 2009). Local application of focused mechanical-acoustic vibrations shows efficacy in normalizing myofascial dysfunction of hypotonic and hypertonic natures, depending on the specific working frequency selected (Saggini 2017). In particular, a frequency of 120 Hz is effective in reducing pathologic muscle hypertone, while 300 Hz increases muscle tone and function in hypotonia (Saggini et al. 2016).

The knowledge of muscle system therapy becomes fundamental in pursuing new rehabilitative methods for pelvic floor dysfunctions, which would complement the training of pelvic muscles to ameliorate urinary incontinence. It is a reasonable assumption that an integrated therapeutic approach consisting of pelvic floor and bladder muscle training would produce better results than bladder training alone. Yet, evidence on the best training approach is inconclusive (Hay–Smith et al. 2011). Therefore, this study seeks to define the efficacy of an integrated physiotherapy program, incorporating the neuromuscular manual therapy and focused mechanical-acoustic vibrations, as a feasible, tolerable, and effective management instrument for urinary incontinence.

2   Methods

The study sample consisted of 62 female patients with the mean age of 59 ± 6 years. Two of the patients withdrew from the study procedures after the first visit; thus data of the 60 patients were subject to analysis. Inclusion criteria were the age between 50 and 70 years old and the diagnosis of stress, urge, or mixed urinary incontinence. All included patients underwent a medical gynecological examination and received a therapeutic advice. Exclusion criteria were the performing of other therapeutic protocols and the presence of urogenital infections, neurological pathologies, and spinal lesions of any grade. Patients were examined before the beginning (time 0 – T0) and after the end (time 1 – T1) of the study protocol. The evaluation methods consisted of a MyotonPRO device for the assessment of muscle rheological parameters (Myoton AS, Estonia, and Myoton Ltd., London) and of clinical inventories for the assessment of pelvic floor disability: Pelvic Floor Disability Index (PFDI-20) and Pelvic Floor Impact Questionnaire (PFIQ-7) (Barber et al. 2005).

The MyotonPRO is a handheld, noninvasive device that evaluates mechanical characteristics of a selected muscle, which is useful for the assessment of muscle function and metabolic efficiency (Pruyn et al. 2016; Peipsi et al. 2012). The device provides information on the following parameters:

- Muscle elasticity/plasticity – capacity of a muscle to recover its initial shape after a mechanical modification, expressed by numbers to two decimal places (D, logarithmic decrement).
- Muscle tone – basal involuntary mechanical tension of a muscle, expressed in hertz to one decimal place (F, oscillation frequency).
- Muscle resistance – ability of a muscle to counteract an external mechanical deformation through the activation of antagonistic muscles. It is strictly correlated with muscle tone and is expressed in N/m to the ones place (S, dynamic stiffness).

The measurements with the MyotonPRO were performed on the most superficial muscles surrounding the pelvic floor. The muscles evaluated were the abdominal rectus, hip adductors, latissimus dorsi, and the left and right gluteal muscles. These muscles, hips, and spine appear strictly correlated with pelvic floor function, at both neurological and biomechanical levels (Faubion et al. 2012). A needle-shaped pressure sensor was applied to the central part of

a muscle selected. The measurements were done in triplicate, with the acceptable disparity of results set at 2%.

The PFDI-20 evaluates the general disability level of the pelvic floor, considering symptoms from the preceding 3 months. It is composed of three subscales: Urinary Distress Inventory 6 (UDI-6), Colorectal-Anal Distress Inventory 8 (CRADI-8), and Pelvic Organ Prolapse Distress Inventory 6 (POPDI-6). The score for each item varies from 0 (no symptoms) to 4 points (extremely disabling symptoms). The mean score for each subscale is multiplied by a factor of 25 (100/4) to obtain the score from 0 to 100 points. The final score of PFDI-20 derives from the sum of the subscale scores (between 0 and 300 points).

The PFIQ-7 evaluates the impact of urogenital problems on daily activities, considering symptoms from the preceding 3 months. It is composed of 7 items, each one relative to a specific urogenital symptom. The score for each item varies from 0 (no symptoms) to 3 points (extremely disabling symptoms). Each symptom is assessed regarding its presence at the level of bladder/urine (the Urinary Impact Questionnaire (UIQ-7)), intestine/rectus (the Colorectal-Anal Impact Questionnaire (CRAIQ-7)), and vagina/pelvis (the Pelvic Organ Prolapse Impact Questionnaire (POPIQ)). The mean score for each subscale is multiplied by a factor of 33.3 (100/3) to obtain the score from 0 to 100 points. The final score of PFIQ-7 derives from the sum of the subscale scores (between 0 and 300 points). The score of each patient was approximated to the nearest integer.

The experimental therapeutic protocol, consisting of the integrated application of two innovative techniques of pelvic floor rehabilitation, was aimed at the rebalancing of myometric parameters of the muscles above outlined, which are involved in the control of urinary function and intra-abdominal pressure. Each patient underwent focused mechanic-acoustic vibrations treatment at a frequency of 300 Hz for 15 min, with applicators placed at the level of the abdomen, perineal area, lumbar area, and gluteal muscles. This treatment was conducted with the ViSS One system that uses air flow produced by a compressor to induce adjustable vibrations inside plastic applicators placed on the specific muscles (Saggini 2017; Saggini et al. 2016; Pietrangelo et al. 2009). Subsequently, patients underwent neuromuscular manual therapy to treat the diaphragm, iliopsoas, square of loins, and piriformis muscles, as well as the sacrotuberous and sacroischiatic ligaments. Each therapy session took about 1 h. Tissues were first warmed with a superficial massage and then treated with direct strokes such as stripping, deep transversal and longitudinal friction, and pincer pressure (Granger 2011). These techniques apply a progressive and firm pressure along the area of myofascial dysfunction in transversal, parallel, and compressive directions with respect to muscles fibers. The whole therapeutic protocol consisted of 8 sessions, performed twice a week, spaced at least 48 h apart, for a total duration of 4 weeks.

Statistical elaboration consisted of comparing data collected at time T0 and T1 for each parameter, using the Student's t-test. A p-value <0.05 defined statistically significant differences. The analysis was made using commercial NCSS 9 Statistical Software (NCSS LLC; Kaysville, UT),

3 Results

The analysis of MyotonPRO data showed improvements in all parameters (Table 1). A significant reduction in muscle F and S, i.e., in muscle tone and rigidity, was observable on both left and right sides of all the treated muscles. In addition, there was an increase in muscle D, i.e., in muscle plasticity, of all the muscles in question. These changes also are clearly apparent when presented as the mean percentage difference from T0 to T1 for each myometric parameter of each treated muscle (Fig. 1). In many instances, there was a greater difference in each parameter, between the left and right hemisomes at T0 (white lines) than at T1 (black lines) indicating a symmetrization and rebalancing of muscle condition (Fig. 2).

Table 1 Evaluation of muscle parameters in 60 female patients with incontinence before treatment (T0) and after (T1) combined neuromuscular manual and focused mechanic-acoustic vibration treatment

Variable	Mean ± SD	95% CI	p-value
AM-D_T0-L	1.51 ± 0.37	1.41–1.60	0.0009
AM-D_T1-L	1.73 ± 0.39	1.62–1.82	
AM-D_T0-R	1.46 ± 0.31	1.38–1.54	0.00001
AM-D_T1-R	1.72 ± 0.46	1.60–1.84	
AM-F_T0-L	12.0 ± 1.6	11.6–12.6	0.00001
AM-F_T1-L	10.6 ± 1.1	10.3–11.3	
AM-F_T0-R	12.1 ± 1.9	11.7–12.6	0.00001
AM-F_T1-R	10.6 ± 1.1	10.3–10.9	
AM-S_T0-L	204 ± 32	196–212	0.00001
AM-S_T1-L	183 ± 29	175–190	
AM-S_T0-R	201 ± 39	191–211	0.00001
AM-S_T1-R	181 ± 33	173–190	
DM-D_T0-L	1.50 ± 0.49	1.37–1.63	0.00014
DM-D_T1-L	1.71 ± 0.36	1.62–1.81	
DM-D_T0-R	1.40 ± 0.38	1.30–1.50	0.00001
DM-D_T1-R	1.69 ± 0.48	1.56–1.82	
DM-F_T0-L	13.8 ± 2.2	13.3–14.4	0.00001
DM-F_T1-L	12.0 ± 2.4	11.4–12.6	
DM-F_T0-R	13.9 ± 2.1	13.4–14.5	0.00001
DM-F_T1-R	12.0 ± 2.1	11.4–12.5	
DM-S_T0-L	272 ± 65	256–289	0.00002
DM-S_T1-L	243 ± 56	229–257	
DM-S_T0-R	266 ± 54	252–280	0.00001
DM-S_T1-R	240 ± 49	228–253	
GM-D_T0-L	1.76 ± 0.40	1.65–1.86	0.0003
GM-D_T1-L	1.91 ± 0.38	1.81–2.01	
GM-D_T0-R	1.65 ± 0.31	1.57–1.73	0.00001
GM-D_T1-R	1.85 ± 0.34	1.76–1.94	
GM-F_T0-L	12.4 ± 1.7	12.0–12.9	0.00001
GM-F_T1-L	11.0 ± 1.3	10.7–11.4	
GM-F_T0-R	12.3 ± 1.8	11.8–12.8	0.00001
GM-F_T1-R	10.9 ± 1.3	10.6–11.3	
GM-S_T0-L	235 ± 33	226–243	0.00001
GM-S_T1-L	217 ± 30	209–224	
GM-S_T0-R	222 ± 30	214–230	0.00001
GM-S_T1-R	211 ± 29	204–219	
RAM-D_T0-L	1.92 ± 0.37	1.82–2.01	0.00300
RAM-D_T1-L	2.17 ± 0.66	2.00–2.34	
RAM-D_T0-R	1.98 ± 0.54	1.84–2.13	0.00023
RAM-D_T1-R	2.22 ± 0.64	2.05–2.39	
RAM-F_T0-L	12.5 ± 1.4	12.2–12.9	0.00001
RAM-F_T1-L	11.0 ± 1.2	10.7–11.3	
RAM-F_T0-R	12.8 ± 1.6	12.4–13.2	0.00001
RAM-F_T1-R	11.2 ± 1.4	10.9–11.6	
RAM-S_T0-L	228 ± 33	219–236	0.00001
RAM-S_T1-L	215 ± 29	207–222	
RAM-S_T0-R	234 ± 34	225–243	0.00001
RAM-S_T1-R	215 ± 32	207–223	

Acronyms of muscles: *AM* adductor muscle, *RAM* rectus abdominis muscle, *DM* dorsal muscle, *GM* gluteal muscle
MyotonPRO parameters: *D* logarithmic decrement, *F* frequency, *S* stiffness (see Methods for parameters' units)
Body side: *L* left and *R* right. 95%CI, 955 confidence intervals; P-values refer to differences between T0 and T1
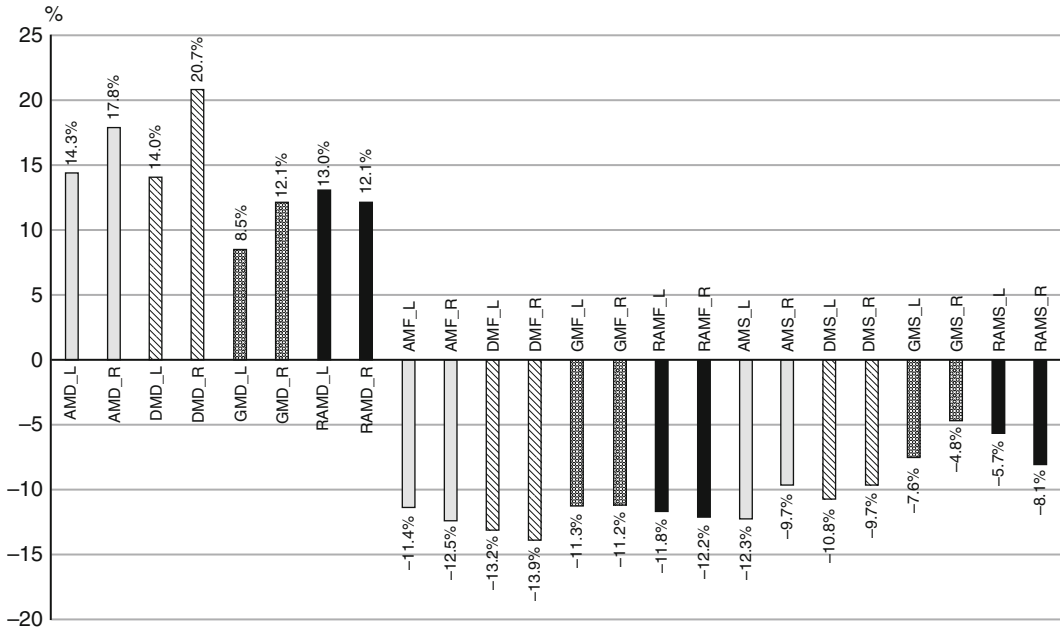
Fig. 1 Percentage differences in parameters between baseline (T0) and after (T1) combined neuromuscular manual and focused mechanic-acoustic vibration treatment in 60 female patients with incontinence. Acronyms of muscles: *AM* adductor muscle, *RAM* rectus abdominis muscle, *DM* dorsal muscle, *GM* gluteal muscle. MyotonPRO parameters: *D* logarithmic decrement, *F* frequency, *S* stiffness. Body side: *L* left and *R* right side
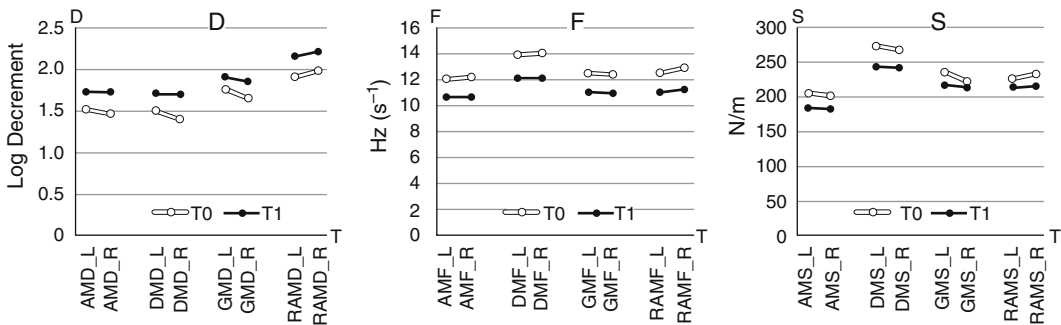

Fig. 2 Differences between the left and right hemisome parameters at time T0 (before treatment) (white lines) and T1 (after combined neuromuscular manual and focused mechanic-acoustic vibration treatment) (black lines) in 60 female patients with incontinence. Parameters: *S* stiffness, *D* logarithmic decrement, and *F* frequency. Acronyms of muscles: *AM* adductor muscle, *RAM* rectus abdominis muscle, *DM* dorsal muscle, and *GM* gluteal muscle. Body side: *L* left and *R* right side

The PFDI-20 and PFIQ-7 questionnaire results showed a significant reduction of scores between time T0 and T1; an average drop in the scores amounted to 43% for PFDI-20 and 56% for the PFIQ-7 off the baseline T0 level (Table 2, Fig. 3).

4   Discussion

The findings of this study demonstrate that a peripheral neurosensory stimulation is an effective and safe treatment for women who suffer

from various types of urinary incontinence. The beneficial effects we noticed could stem from the optimization of the treatment protocol we employed in the study. The treatment spanned the time of only 4 weeks, which, as other studies show, suffices to enhance neural functional adaptation and muscle hypertrophy (Dannecker et al. 2005; Bø 2003). Another benefit stemmed plausibly from the integration of neuromuscular therapy and mechanic-acoustic vibrations, which is conducive to gaining muscle strength and coordination greater than that achievable with the maximum voluntary contractions used in standard programs for incontinence treatment.

Table 2 Scores of the Pelvic Floor Disability Index (PFDI)-20 and the Pelvic Floor Impact Questionnaire (PFIQ)-7 at time T0 (before treatment) and T1 (after combined neuromuscular manual and focused mechanic-acoustic vibration treatment) in 60 female patients with incontinence

Questionnaire	Mean $\pm$ SD	95% CI	p-value
PFDI20_T0	78.1 $\pm$ 30.9	70.1–86.0	0.00001
PFDI20_T1	44.4 $\pm$ 20.2	39.2–49.6	
PFIQ7_T0	119.6 $\pm$ 38.4	109.7–129.5	0.00001
PFIQ7_T1	52.5 $\pm$ 27.4	45.5–59.6	

95%CI, 95% confidence intervals; p-values refer to differences between T0 and T1

Conceivably, a combination treatment working on pelvic floor muscles via different pathways provides better gains than the application of a single therapy such as pelvic floor training (Wallace et al. 2004). It has been reported that approximately 30% of women are unable to correctly contract pelvic floor muscles while performing only isolated pelvic floor contractions (Bø 2003). In contradistinction, manual treatment of the psoas and diaphragm muscles facilitates the correct contraction of pelvic floor muscles, exploiting the convergence and reciprocal inhibition of visceral and cutaneous afferents in the lumbar spinal cord (Selzer and Spencer 1969).

Concerning the myometric parameters, we noticed significant reductions in muscle tone (F) and resistance (S) and an increase in muscle plasticity (D) of all the muscles treated (Figs. 1 and 2). High values of basal muscle tone are associated with decreased muscle metabolic and mechanical efficiency because of increased intramuscular pressures. Low levels of muscle tone are usually associated with relaxation (O'Sullivan 2006). Therefore, a reduction of muscle tone is a good indicator of general relaxation of the pelvic floor muscles, which improves the control over muscle function in individuals affected by
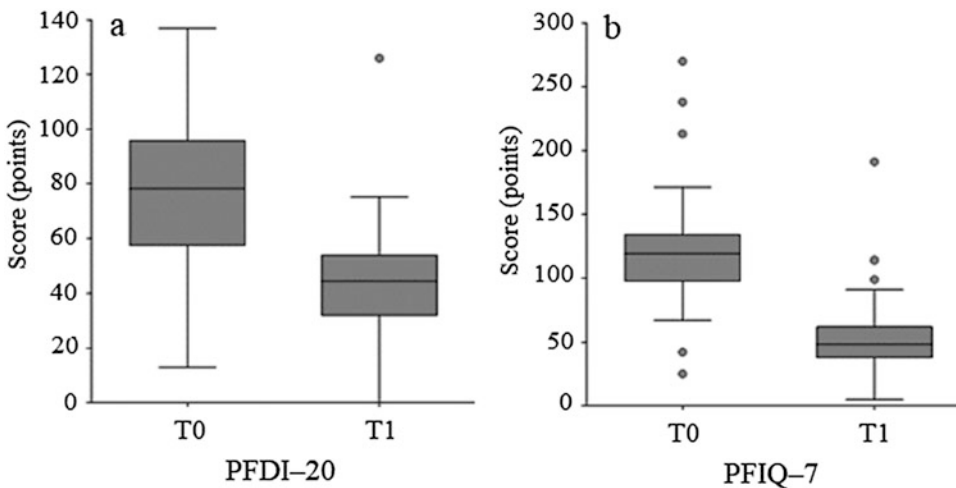

Fig. 3 Box and whisker plots presenting reductions in the scores of the Pelvic Floor Disability Index (PFDI-20) and the Pelvic Floor Impact Questionnaire (PFIQ-7) from time T0 (before treatment) to T1 (after combined neuromuscular manual and focused mechanic-acoustic vibration treatment) in 60 female patients with incontinence. Boxes span the interquartile ranges, horizontal lines inside the box show the median score, whiskers show the bottom and top 25% of scores, and the dots mark the outliers

hypertonic pelvic floor disorders, such as stress-related incontinence and other tone-/pressure-related problems (Butrick 2009; McGuire et al. 1993).

Increased muscle resistance is typically indicative of a low grade of coordination between agonist and antagonist muscles, associated with muscle rigidity. A low value of muscle resistance, on the other side, is usually associated with unstable muscle and tendinous control, because of excessive compliance of elastic tissues (Benazzo and Bisciotti 2007; Proske and Morgan 1987). In case of pelvic floor dysfunction, a small but significant reduction in muscle resistance helps improve the compliance of the pelvic floor in high pressure-induced conditions (McGuire 1993) while preserving a functional grade of muscle rigidity to increase both voluntary and involuntary coordination of pelvic floor components (Sapsford and Hodges 2001). Changes in muscle tone and resistance above outlined can be attributed to neuromuscular manual therapy. This therapy, aside from the muscle tissue, also is capable of influencing the activity of the central nervous system due to a common ectodermic origin of the two, producing a feedback brain-elaborated reaction of afferent somatic information (Granger 2011; Selzer and Spencer 1969). This mechanism explains how neuromuscular manual therapy influences muscle homeostasis, leading to a reduction in muscle contractility and rigidity in response to cutaneous stimulation. In effect, normalization and symmetrization in muscle function of the two hemisomes is observed (Barassi et al. 2018).

An increase in muscle plasticity, a major positive outcome noticed in this study, is liable to bring about muscle strengthening, which is needed to increase the control over the pelvic floor and continence function (Neumann and Gill 2002). The strengthening effect can be mostly attributed to mechanical-acoustic vibrations that increase muscle strength at frequencies of 300 Hz through mechanic activation of class III and IV receptors and Pacinian corpuscles (Saggini 2017; Saggini et al. 2016)

and also induce metabolic improvements (Iodice et al. 2011; Pietrangelo et al. 2009). Finally, there was a significant reduction in perceived disability caused by incontinence as evidenced by a drop in the PFDI-20 and PFIQ-7 questionnaire scores. Considering a debilitating effect of pelvic floor dysfunction, this outcome is a worthwhile sign of improvement in the psychosocial sphere of patients (DeLancey 2005). The psychological improvement is rendered by both relaxing and rebalancing effects of neuromuscular therapy (Barassi et al. 2018; Granger 2011; Selzer and Spencer 1969) and strengthening and corticalization effects induced by mechanic-acoustic vibrations (Saggini 2017; Saggini et al. 2016).

This study has limitations. The most serious are a lack of a sham control group for comparison of results and a lack of an extended follow-up to observe the sustenance of improvements due to the pelvic floor rehabilitation program employed. Other limitations consist of a potential selection bias due to the convenience sampling of patients being drawn from those close to hand, the absence of assessors' blinding, and the possibility of over-reporting of improvements due to subjectivity of patients' responses, an inherent feature of a survey method (Hay–Smith et al. 2011). On the positive side, patients' compliance with the treatment employed was excellent, as evidenced by high attendance rate and the occurrence of only two dropouts, i.e., 3% of the patients. An explanation of the good compliance could lie in a short duration of the therapy protocol and a significant improvement in the initial therapy phase, which plausibly increased the patients' motivation and trust in accessing the program of pelvic floor muscle training. In contradistinction, the dropout rate in some previous studies was much higher, ranging from 12% to 41% (Fan et al. 2013; Castro et al. 2008; Hay–Smith et al. 2001).

Despite the limitation above outlined, we believed we have shown in this study that the integrated manual and vibrational rehabilitative treatment of pelvic floor muscles ameliorates the signs and symptoms of urinary incontinence in

female patients. Of note, the treatment also reduces the psychosocial impact of the incontinence, a feature that is hardly amenable to hitherto used single treatment modalities. We report herein the short-term benefits of the integrated rehabilitative treatment. Further explorations, using alternative study designs, are required to firmly establish the place of this integrated approach in achieving the long-term improvements of urinary incontinence, before it could successfully replace or supplement the supervised training of pelvic floor muscles.

Conflicts of Interest The authors declare no conflicts interest in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Written informed consent was obtained from all individual participants included in the study.

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Sulfurous Balneotherapy in Poland: A Vignette on History and Contemporary Use

Bożena Kosińska and Marek L. Grabowski

Abstract

Balneotherapy experiences a sharp increase in popularity during recent years. The present paper gives a perspective on the therapeutic use of mineral baths in the town of Solec-Zdroj in southeastern Poland, an old time spa endowed with rich natural sulfurous water resources. Historical aspects of the formation and development of the spa are presented, along with the contemporary insights into the plausible mechanisms and benefits of sulfide treatment, not only in otherwise hardly treatable chronic skin disorders but also in a variety of disease processes in organ systems. Sulfurous balneotherapy is not without potential risks, particularly for the skin, a tissue it is considered the most viable treatment for. The healing effect of sulfide waters does not increase in proportion to the sulfur content. As in every stimulus-based treatment, the stimulant strength should not go beyond the favorable hormetic boundaries of safety. Although the exact bioproperties of a high content of hydrogen sulfide in natural mineral springs are yet to be fully unraveled and understood in the context of healing capability, sulfide bath treatment is capable of growing and developing. It remains a cost-effective alternative to pharmaceutical products in a variety of disorders.

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1 Background

Mineral baths as healing treatments have been used since the Roman time. They mainly consisted of bubbled mineral waters, also containing sulfide salts, which supposedly have a beneficial effect on skin ailments (Gupta and Nicol 2004). The first Polish-language article that classified mineral waters and springs in Poland and their curing properties, entitled *Cieplice*, was published by Wojciech Oczko (1578), a court physician to kings Sigismund II Augustus, Stephen Báthory, and Sigismund III Vasa in the royal city of Cracow. The knowledge on the healing properties of sulfide-containing baths quickly propagated at the time, which was followed by another foundational description of balneotherapy by Erazm Syksta in the city of Lviv in 1617 and by Jedrzej Krupinski, who described a local pond with sulfur efflorescence, named

"gray water", in 1775 (see Dowgiałło 2015). In the nineteenth and twentieth centuries, many experimental studies were devoted to the therapeutic effect of mineral waters on the human body. The sulfide water is a water that, irrespective of its general mineralization, contains no less than 1 mg/L of total iodometric sulfur. This ratio has been statistically determined and still remains to be pharmacodynamically verified (Ponikowska and Latour 2017). The main component of sulfide water is hydrogen sulfide (H_2S), hydrosulfides of alkali metals, and other inorganic and organic sulfide-containing salts. A longer contact of sulfide water with atmospheric air causes oxidation of divalent sulfur to four- and six-valent sulfur, which leads to the loss of reducing and healing properties of sulfurous water.

The mechanism of action of sulfide baths on the body depends mainly on the penetration of sulfur through the skin layers into the tissues. The skin, in and of itself, is an organ that accumulates as much sulfur as 60 mg per 100 g tissue. Proteins of the horny outgrowths of the skin such as hair or nails bind the largest amount of sulfur, followed by epidermal proteins. Part of the systemic body sulfur associates with hydrocarbons forming chondroitin-sulfuric acid, a component of cartilage and synovial fluid, and mucoitin-sulfuric acid, a component of mucus. Low molecular weight sulfur compounds play a large role in the processes of catalysis and detoxification. Selective hydroxyl sulfation, producing sulfate esters, is used in many biologically essential molecules, among others, estradiol 17β-sulfate (Hoiberg and Mumma 1969). Sulfate esters also serve the same purpose of detoxification and breaking down phenols, such as salicylates that are metabolized by the sulfotransferase enzyme. Sulfur is at the core center of the ubiquitous molecules of glutathione and cysteine that are essential in the regulation of redox balance and also play a protective role in poisoning with heavy metals as well as other toxic compounds (Carbajo and Maraver 2017; Bashir et al. 2015). In addition, sulfur belongs to the innate structural elements of a number of vital from the biological standpoint proteins in mammals. Glutathione sulfhydryl

(GSH), the most essential antioxidant in the body, owns its tremendous scavenging power to the sulfhydryl (SH) groups it contains. The amino acid cysteine contributes sulfur for GSH synthesis. Reactive disulfide bonds also are essential for bioactivity of class IgG immunoglobulins (Schauenstein et al. 1996).

In balneotherapy, sulfide water is most often used in the form of baths. Three concentrations of sulfur are distinguished: high >100 mg H_2S/L, medium of 50–100 mg H_2S/L, and weak <50 mg H_2S/L of water. Both H_2S in the water and that produced from colloidal sulfur settling on the skin are absorbed through the undamaged skin. The polysulfides formed in the skin, easily soluble in fats, penetrate through the tissue capillaries to reach general circulation. Studies with sulfur radioisotopes have shown that sulfur absorbed through the skin from mineral waters is incorporated into protein molecules (Jordan 1964). Sulfide baths increase the level blood glutathione and antioxidant capacity (Ekmekcioglu et al. 2002). They also stimulate skin neuroreceptors, strengthen metabolic processes in the skin, and retune skin's reactivity. Weigmann and Schindewolf (1954) have demonstrated that sulfide waters reduce or eliminate itching and generally have a soothing effect. The stimulus delivered by an air-jet esthesiometer, which measures the tactile sensitivity of the skin, which normally causes pain in healthy people, gives only a sense of pruritus after bathing in sulfide waters, and in people who complain of pruritus, the ailment is ameliorated after bathing. Sulfide waters are conducive to the formation of adenylic acid and other compounds with histamine-like properties in the skin, which by expanding and dilating of the vascular plexuses cause active tissue congestion. As a result, inflammatory infiltrations are more easily and quickly absorbed, and skin nutrition is improved. The baths with the weaker sulfide concentration cause that abnormal or excessive epidermal growth returns to the physiological norm and new epidermis is cohesive and elastic, which constitutes a keratoplastic effect of such waters (Carbajo and Maraver 2017; Schempp et al.

1996). In contradistinction, sulfide water with the strong concentration has a keratolytic effect, softening and exfoliating the epidermis. Sulfur compounds react on the skin surface with urea contained in the sweat, creating a mildly toxic ammonium sulfide. Thanks to that and to the reducing properties of divalent sulfur compounds, sulfide water has a disinfecting and antiparasitic action. Sulfur has a clear ameliorative effect in scabies and is the second-line treatment after topical phenothrin lotion (Guideline 2017; Gulati and Singh 1978). Sulfide waters have long since been recognized as the anti-psoriasis treatment modality (Thurmon 1935). Other indications for treatment with sulfide waters are the following skin diseases: neurodermatitis, chronic urticaria, spontaneous pruritus, diffuse and limited systemic sclerosis, lichen planus, varicose veins syndrome, occupational skin diseases, seborrheic dermatitis, acne vulgaris, chronic streptococcal and staphylococcal skin infections, vascular skin diseases, keratosis dermatitis, and others (Leslie et al. 2004; Matz et al. 2003). There are also potential toxic or adverse effects of sulfur applied to the skin, which can intensify skin ailments. Sulfur should thus be judiciously used and/or may be contraindicated in acne-prone conditions, skin cancers, some viral infections, syphilis, tuberculosis, pemphigoid skin diseases, Duhring's disease – a skin variety of celiac disease – or discoid lupus erythematosus, which all may be general contraindications for spa treatment. It also may cause secondary dermatitis (Lee and Wu 2014; Andriasian 1969). Sulfur treatment may be particularly dangerous in infants (Lin et al. 1988). Thus, views and clinical experience on the healing value of sulfide waters and particularly on the optimum beneficial content of sulfur are, to an extent, divergent and are based on examples in a pattern rather than evidence-based medicine. The use of natural bathing spas with a high content of sulfur remains of empiric nature, and the patient should participate in the decision for this kind of treatment.

The systemic mechanisms of sulfide baths include the following bioactivities:

- Enhancement of protein metabolism, resulting in increased urinary excretion of creatinine
- Enhancement of carbohydrate metabolism, resulting in decreased blood sugar level, due to increased secretion of insulin by the pancreas
- Alterations in purine metabolism, resulting in decreased blood uric acid level and increased urinary excretion of uric acid
- Lowering the level of cholesterol in the blood
- Antiplatelet and anti-atherogenic activity
- Arthritic pain-relieving activity
- Enhancement of digestive activity of diastase and trypsin and inhibiting anti-hyaluronidase and choline esterase
- Increase activity of blood complement and immunoglobulin proteins, participating in the antigen-antibody reactions
- Antirheumatic and antithyroid effects
- Enhancement of detoxifying activity against organic and mineral poisons, and other effects

(Gálvez et al. 2018; Xiao et al. 2018; Carbajo and Maraver 2017; Zair et al. 2013; Verhagen et al. 2007; Evcik et al. 2002; Goszcz et al. 1997; Sukenik et al. 1990; Chmielewski and Madry 1971; Sokolova and Grigor'ian 1970).

2 Solec-Zdroj Balneological Spa

The town of Solec-Zdroj is located in the basin of Nida river, a left tributary of the Vistula River, in southeastern Poland. The landscape is diversified by forests, mostly coniferous ones, having a temperate climate, with small temperature fluctuations and small amounts of rainfall. This geographical region is a Landscape Park designated protected area. The first description of physicochemical elements of local underground spa waters was carried out in 1830 by Adam Maksymilian Kitajewski (1789–1837), a professor of chemistry at the University of Warsaw, one of the most outstanding Polish chemists of the time and a pioneer of

balneochemistry. He described it as "alkaline sulfate-salty water". The efficiency of this so-called old spa amounted to 45 cubic meters of 12.4 °C and alkaline pH (see Dłuska 1994). In 1837, the design of a spa was completed by Karol Godeffroy, a French nobleman. He built spas next to the mineral springs and a summer hospital for 20 beds, dedicated to Saint Edward. A summary of the number of patients and treatments performed in the period 1862–1928, displayed in Table 1, underscores the development and importance of the Solec-Zdroj spa at the time.

The spa owes its international prominence to Jozef Dietl, a pioneer of Polish balneology, who in his publication of 1858 wrote that "this spring belongs to the most effective waters of this type in all of Europe" (Dietl 1858). The sulfide seltzer obtained from the 170-meter deep wells contains sulfide, chloride, bromide, iodide, and boron. A high content of H_2S of 137 mg/L and a high mineralization make this water the most effective of the kind (Grabowski et al. 2018). According to the Institute of Spa Medicine in Poznan, Poland, the optimum content of H_2S in the water source should be in a range of 150 mg/L. The Solec-Zdroj spa meets this criterion. There are four shafts of sulfurous water, with a capacity of up to 72 m^3/day. After being extracted from the ground, the water is completely transparent, to become turbid on contact with air due to the precipitation of sulfur off it. The water has the characteristic foul odor of rotten eggs and its taste is salty. It is directly piped to the therapeutic bathtubs. The usual treatment paradigm consists of 4–6 bathing sessions a week, for a total of 10–15 baths *per* therapy, each bath of 10–20 min duration at water temperature of 35–38 °C. Patients' tolerance is generally good. Occasionally, a so-called bath reaction occurs in the form of skin erythema and mild fever. This is a temporary stimulus effect that goes away in a couple of days at the most. A rundown of skin conditions in 525 patients treated by sulfurous water in Solec-Zdroj spa, along with the effectiveness of treatment, is shown in Table 2. It appears that the greatest proportion of patients (about 70%) with improvements in the health

condition was noticed in psoriasis and allergic skin ailments, which was followed by a 20–30% improvement rate in leg varicosities and seborrheic skin disease. A deterioration or exacerbation of the existing ailment in dermatological terms, along with sporadic adverse effects, occurred in 1–6% of cases. A complete lack of effects of sulfurous balneotherapy was reported in patients with erythroderma and alopecia areata. Deterioration or lack of improvement was most frequently noticed in patients with a history of treatment with corticosteroids.

Recently, indications for sulfurous balneotherapy have been broadened by the Polish National Health Fund, so that strictly dermatologic ailments constitute just a 3% minority of patient referrals. At the moment, diseases of the osteoarticular, muscular, and connective tissues (90%) and nervous system diseases (7%) are most common indications for treatment. That is consistent with a progressive increase in the number of patients treated each year as shown in Table 3. The most numerous group of patients were people aged 56–65 (43%), and the smallest percentage was the youngest in the age bracket of 19–40 years (1.7%). Women outnumbered men by more than twofold. The patient demographic characteristics are shown in Table 4. Among the spa patients, about 75–80% had their abode in villages or small towns, mainly located in southeastern Poland. Patients often times presented with chronic and sometimes neglected dermatological changes.

It is worthwhile to report progress in the treatment efficacy of sulfurous mineral balneotherapy that occurred in the Solec-Zdroj spa over the past four decades. Comparison of the 1972–1977 and 2011–2015 cohorts of similar size, exceeding 500 patients, shows that the average rate of beneficial treatment responses increased from 70.6% to as much as 84.9% in the periods in question, respectively. The improvements were, in particular, outstanding in psoriasis and allergic skin disorders, reaching 85–90% of cases (see Table 5 vs. Table 2). Notably, lack of improvement concerned just one half of the patient fraction seen four decades earlier, with a similar

Table 1 Number of patients and sulfide baths performed in the years 1862–1928

Year	Number of patients	Average number of baths *per* patient
1862	367	28
1872	355	25
1882	468	28
1895	554	28
1913	1,400	25
1923	1,460	25
1924	1,420	25
1925	1,180	25
1926	1,240	25
1927	1,460	26
1928	1,720	25

Table 2 Number of patients with skin diseases treated in sulfurous mineral water in the Solec-Zdroj spa during 1972–1977. Treatment paradigm consisted of spa treatment for 21 days, followed by outpatient treatment for another 21 days

Diagnosis	Total n	Improvement n (%)	No improvement n (%)	Deterioration n (%)
Psoriasis	357	250 (70.0)	103 (28.9)	4 (1.1)
Allergy (chronic eczema, atopic or contact dermatitis)	103	69 (67.0)	28 (27.2)	6 (5.8)
Leg varicosities	38	31 (81.6)	5 (13.1)	2 (5.3)
Seborrheic dermatitis, acne vulgaris	26	20 (76.9)	5 (19.2)	1 (3.9)
Total	**524**	**370 (70.6)**	**141 (26.9)**	**13 (2.5)**

Table 3 Number of patients and days of stay in the Solec-Zdroj spa in recent years

Year	Number of patients	Average number of days/patient
2010	3,834	15.8
2011	4,048	15.8
2012	4,521	15.7
2013	5,074	16.3
2014	5,154	16.3
2015	5,268	15.8
Total	**23,374**	**16.0**

Table 4 Demographic characteristics of patients treated in the Solec-Zdroj spa during 2005–2015

Age group (year)	Total	Female	Male	Female/male ratio
19–40	399	246	151	1.57
41–55	4,319	3,128	1,191	2.63
56–65	10,238	7,415	2,823	2.63
65+	8,618	5,763	2,855	2.02
Total	**23,574**	**16,552**	**7,022**	**2.36**

Table 5 Number of patients with skin diseases treated in sulfurous mineral water in the Solec-Zdroj spa during 2011–2015. Treatment paradigm consisted of 6 bathing sessions a week, each 10–20 min duration for 3 weeks

Diagnosis	Total	Improvement	No improvement	Deterioration
	n	n (%)	n (%)	n (%)
Psoriasis	382	338 (88.5)	41 (10.7)	3 (0.8)
Allergy (chronic eczema, atopic or contact dermatitis)	48	40 (83.3)	7 (14.6)	1 (2.1)
Leg varicosities	27	17 (63.0)	7 (25.9)	3 (11.1)
Seborrheic dermatitis	46	34 (73.9)	8 (17.4)	4 (8.7)
Lichen planus	28	22 (78.6)	6 (21.4)	0 (0)
Total	**531**	**451 (84.9)**	**69 (13.0)**	**11 (2.1)**

meager 2–2.5% rate of deterioration that mainly occurred among the patients with dyshidrotic eczema. Beneficial effects consisted of reductions in skin inflammation and later lichenization and relief of subjective symptoms of pain, burning, pruritus, and the granulation and reepithelization of ulcerations. Minor lesions subsided or completely healed. In the group of patients with seborrheic disorders, highly favorable effects also were manifest.

3   Discussion

In recent years, the attractiveness of balneotherapy is on the rise in the geographical regions, mostly mountainous, where there are natural underground water springs. The popularity of this kind of treatment stems from its viability as adjunctive treatment of otherwise hardly treatable chronic skin disorders which, albeit most often rather mild, are a cause of a substantial health distress and hampered daily functioning of a patient and due to chronicity might also become a health hazard. Increased popularity for balneotherapy reflects the contemporary demand for holistic approach in medical practice, in particular employing the Earth's natural resources. The Solec-Zdroj spa with its rich sulfurous mineral waters is no exception in the process. This perspective on the history and development of the spa pointedly demonstrates steady progress, intensified in recent years, in medical indications to treat and treatment efficacy, which is consistent

with a worldwide trend. Advancing effects of balneotherapy also spur a continuing research into the underlying mechanisms of benefits to be gained. Cutaneous effects of sulfur are getting progressively unraveled. Sulfur, on the skin surface, becomes hydrogen sulfide and pentathiols which possess antibacterial activity (Leslie et al. 2004). The SH bond evolves to S-S which softens keratin and makes it more permeable. There is a biological plausibility of externally provided S-S bond interference in a number of physiological processes, notably immunoglobulin formation or antioxidant defenses. The exact mechanisms of the bioactivity of sulfurous waters remain, however, hardly tangible or consistent due to the very complex nature of multifarious elemental content of the water and entwined pathways of activities. Thus, the practice remains to be empirical rather than based on evidence-based medicine, and it contains an appreciable psychosomatic anti-stress component as it usually takes place in a relaxing environment.

Bathing spas with a high content of natural sulfur have been in use as ancillary treatment for cutaneous disorders for several centuries. Beneficial results of treatment have been confirmed by reductions of symptom severity and significantly prolonged periods of remissions. The most numerous groups of patients investigated have been those suffering from psoriasis. Significant improvement is noticed right after the treatment end. However, in the case of the long-term, high-grade psoriasis, the results are less satisfying. In such cases, the treatment should be extended

beyond 21 days. Recent developments suggest to expand the use of natural sulfur waters to a number of systemic diseases that manifest skin changes or even in the conditions without skin manifestation, which may be exemplified by cardiovascular disorders such as pulmonary or arterial hypertension, atherosclerosis, gastric disorders, and a plethora of inflammatory conditions (Nasermoaddeli and Kagamimori 2005). Inhalation of sulfurous vapors, which incidentally is a feature of bathing, has beneficial effects in chronic obstructive pulmonary disease due to anti-elastase activity of thiol groups, which may help curb airway inflammation (Contoli et al. 2013; Braga et al. 2010). When sulfur penetrates through the skin into the systemic circulation, there is a biological plausibility that H_2S, a gasotransmitter formed, may facilitate the hypoxic ventilatory reactivity either through its increased availability at the carotid body (Prabhakar 2012) or agonistic activity toward transient receptor potential ankyrin 1 (TRPA1) channels (Pokorski et al. 2014; Miyamoto et al. 2011). In contrast, H_2S appears to be an inhibitory neurotransmitter in the gastrointestinal tract (Zhang et al. 2016), so that its actions are multiple and varied in various signal transduction pathways.

The use of sulfurous balneotherapy is not devoid of risks. The effects of sulfur balneotherapy seem to follow the hormetic-like biphasic biological response curve. That is to say that small doses of a potentially toxic agent may cause a biologically or clinically advantageous stimulatory response, either direct or within stemming from preconditioning. The same compound in greater doses or in different underlying conditions may lead to reverse-phase toxic effects. The art thus relies in staying within the safe-effect boundary zone (Calabrese 2018). The healing effect of sulfide waters does not increase in proportion to the sulfur concentration. Waters containing large amounts of sulfur are considered potential irritants and may cause skin redness or dermatitis, particularly in persons of increased vulnerability such as the elderly or infants (Lee and Wu 2014; Lin et al. 1988). Severe adverse effects are very rare. Sulfurous spa treatment fits well into the complexity of treating dermatological diseases, which undoubtedly will promote its further development. Sulfurous mineral waters of Solec-Zdroj spa are very effective in treatment of chronic, often old, and neglected dermatological lesions. In view of consistent, positive long-term medical effects, further randomized specific clinical trials are required to get insight into the pathophysiological mechanisms of treatment and to standardize the optimum treatment paradigms.

In conclusion, the exact bioproperties of a high content of hydrogen sulfide in natural mineral waters are yet to be fully unraveled in the context of body, and particularly skin, healing capability. Despite the lingering doubts, we believe we have shown in this narrative about balneotherapy that sulfurous water baths live up to the medical expectations. Balneotherapy remains a viable and cost-effective alternative to pharmaceutical products in a variety of pathological conditions, particularly those involving the skin tissue. It gets more and more popular, driven by the patients' demand for a natural cure not being embroiled in a hassle of complex medical services and treatments. Since sulfur balneotherapy is an area of limited scientific understanding, the method should be carefully titrated to the patient age and medical needs, so that the clinical response to exposure to the sulfide stimulus would not get beyond the favorable hormetic boundaries. That is a challenging task, requiring more investigations with different exposure doses, larger sample sizes, and greater reproducibility.

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Body Composition and Endocrine Adaptations to High-Altitude Trekking in the Himalayas

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Abstract

Long-term exposure to high altitude causes adaptive changes in several blood biochemical markers along with a marked body mass reduction involving both the lean and fat components. The aim of this study was to evaluate the impact of extended physical strain, due to extensive trekking at high altitude, on body composition, selected biomarkers in the blood, and the protective role of a high-protein diet in muscle dysfunction. We found that physical strain at high altitude caused a significant reduction in body mass and body fat, with a concomitant increase in the cross-sectional area of thigh muscles and an unchanged total lean body mass. Further, we found reductions in plasma leptin and homocysteine, while myoglobin, insulin, and C-reactive protein significantly increased. Creatine kinase, lactate dehydrogenase, and leptin normalized per body fat were unchanged. These findings demonstrate that high-altitude hypoxia, involving extended physical effort, has an impact on muscle function and body composition, facilitating sarcopenia and affecting body mass and fat distribution. It also activates pro-inflammatory metabolic pathways in response to muscular distress. These changes can be mitigated by a provision of a high-protein diet.

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1   Introduction

The body responds to high-altitude hypoxia with molecular, cellular, and systemic adaptations (West 2012; Bosco et al. 2003; Cerretelli and Di Prampero 1987; Pugh 1962) across cardiovascular (Kylhammar and Rådegran 2017; Luks et al. 2017; Verratti et al. 2015), respiratory (Beretta et al. 2017), musculoskeletal (Bosco et al. 2010; Doria et al. 2011), hormonal (Verratti et al. 2017; Pelliccione et al. 2011), reproductive (Verratti et al. 2011), and metabolic (Boos et al. 2013; San et al. 2013) organ systems. Exercise performance and muscle damage and regeneration in the hypoxic condition have been extensively studied (Sumi et al. 2018; Mancinelli et al. 2016). However, the underlying mechanisms of muscle dysfunction and the significance of enhanced serum creatine kinase content following physical exercise are unsettled (Baird et al. 2012). A combination of hypoxia and physical strain may induce an inflammatory response, which is reflected by enhanced serum C-reactive protein (Li and Fang 2004; Hartmann et al. 2000). Hypoxia also affects oxidative metabolism, stimulating the process of oxygen transport and storage to ensuring adequate tissue oxygenation (Verratti et al. 2009; Di Giulio et al. 2006).

It is known that long-term exposure to high altitude causes a marked reduction in body mass, which involves both the lean and fat components (Cacciani et al. 2008; Rose et al. 1988). Exposure to hypobaric hypoxia changes the attitude toward eating by decreasing appetite and food intake (Westerterp-Plantenga et al. 1999), leading to a negative energy balance (Richalet 2010). Another cause of reduced appetite is a negative feedback system between adipocytes and the hypothalamus, which increases leptin content and energy expenditure (Paoli et al. 2014, 2015). Studies show that leptin alters the neuroendocrine regulation, inducing a loss of appetite and increased energy expenditure which lead to weight loss (Palmer and Clegg 2014; Petousi et al. 2014; Vats et al. 2007; Tschop et al. 1998). Decreased protein intake, associated with physical stress, is a central anabolic stimulus for muscles, which is conducive to sarcopenia (Yanai 2015).

In view of the phenomena above outlined, the present study seeks to define metabolic and endocrine adaptations to high-altitude hypoxia, associated with physical strain due to extensive trekking, in an attempt to get insight into the protective role of a high-protein diet on the sarcopenia related to the extended exposure to high altitude.

2   Methods

2.1   Manaslu Expedition Profile

This study describes aspects of medical research performed during the Manaslu Himalaya expedition that has addressed a wide range of physiological issues connected with the fertility, metabolism, muscular changes, and physical performance in subjects exposed to chronic high-altitude hypoxia (Verratti et al. 2017; Doria et al. 2011; Pelliccione et al. 2011; Verratti et al. 2011; Mariggiò et al. 2010). There were seven healthy male climbers, members of the expedition, mean age 39 ± 15 years and height 1.72 ± 0.10 m, who were the subjects in the study framework presented herein.

There were several successive stages of the experimental procedure performed at variable altitude. The high-altitude stage was carried out in Nepal. After arriving in Kathmandu (1,300 m above sea level), the subjects were taken to the Buri Gandaki valley for the initiation of procedures. From there, an acclimatization trek was carried out along the Buri (Budhi) Gandaki River for 13 days, arriving at the Manaslu base camp at 5,000 m. Once at the camp, the subjects stayed there for 22 days without oxygen enrichment. During this time, they climbed Camp 1 (5,900 m) and then to Camp 2 (6,400 m) without oxygen. Finally, they took an 8-day trek back from the base camp to Kathmandu via an exceptionally difficult Larke Pass (5,135 m), covering in all about 450 km by the end of the expedition (Fig. 1).
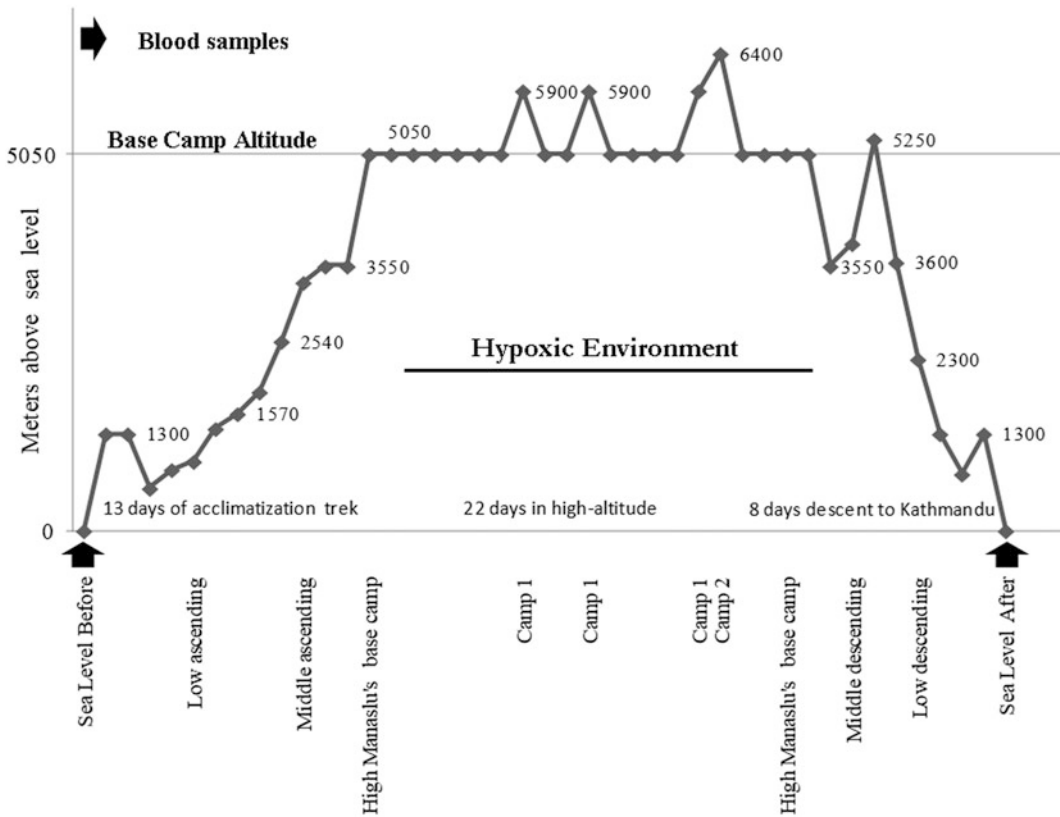
Fig. 1 Profile of the Manaslu expedition that lasted for 43 days, September 8–October 20, 2008

2.2 Anthropometry and Body Composition

The anthropometric characteristics such as body weight (BW), body mass index (BMI), waist circumference (WC), body fat (BF%), total lean body mass, and the thigh cross-sectional area (CSA) were obtained before and after the expedition in the Exercise Physiology Laboratory of Chieti-Pescara University in Italy, located at the altitude of 110 m above sea level. BF% and total lean body mass were estimated from the measurement of the subcutaneous skinfold thickness (Durnin and Womersley 1974). CSA was calculated from the equation:

CSA = (4.68 × thigh circumference in cm) − (2.09 × anterior thigh skinfold in mm) − 80.99 derived from the multiple regression analysis performed in a study of Housh et al.

(1995) for estimation of thigh muscle CSA. Thigh circumference was measured approximately at a third of the trochanter-patella distance. These measurements were based on the previously elaborated methods available in the literature.

2.3 Blood Biomarkers

Venous blood samples were drawn at sea level before (SL_B) and 2 days after (SL_A) returning to Kathmandu, which ended the expedition. Sampling was performed at 8 am, after fasting for 12 h. The serum was collected for the measurement of biomarkers, and it was frozen in liquid nitrogen until use. The samples were immediately placed on ice and centrifuged for 10 min at 3000 rpm. The following biomarkers were measured leptin, insulin, C-reactive protein,

homocysteine, and creatine kinase, myoglobin, and lactate dehydrogenase. Blood analysis was performed with the ADVIA Centaur® CP Immunoassay System (Siemens Healthcare, Milan, Italy) in the Laboratory of Clinical Pathology of Teramo Hospital in Italy.

2.4   Dietary Intervention

The subjects did not follow any individualized dietary program before the expedition, except the instruction of a dietary intake of 2,000 kcal/day, with the following macronutrients content: carbohydrates 49%, proteins 25%, and lipids 26%, which was based on the software WinFood®-Medimatica-Italy. During the expedition, the subjects were instructed to consume about 3,600 kcal/day consisting of carbohydrates 50%, protein 30%, and fat 20% (Table 1).

2.5   Statistical Elaboration

Data were presented as means ±SD. Data distribution was checked with the Kolmogorov-Smirnov test. Differences between the baseline measurements of indices and those after the expedition were compared with a two-tailed paired t-test or Wilcoxon's test. A p-value <0.05 defined statistically significant changes. The analysis was performed with a commercial Statistica v8.0 package for Windows (StatSoft; Tulsa, OK).

3   Results

We found that chronic physical strain at high altitude caused substantial reductions in body mass and fat components assessed, with a simultaneous increase in CSA (Table 2). A total lean body mass remained unchanged.

Plasma level of leptin decreased by 38% after the stay at high altitude compared with the baseline level before the expedition, from 37.2 ± 11.8 to 23.0 ± 10.1 ng/mL, respectively, $p < 0.05$. This effect lost significance after normalization for FM due to a large spread of data, although a decreasing trend in leptin was there, from 1.8 ± 1.0 to 1.3 ± 0.5 ng/mL/kg of FM. Likewise, homocysteine decreased by 39%, from 4.74 ± 1.52 before to 2.89 ± 0.57 µmol/L after the expedition, $p < 0.03$ (Fig. 2).

In contradistinction, myoglobin, insulin, and C-reactive protein showed increased significantly by 29%, 49%, and 26%, from 16.9 ± 3.4 to 21.8 ± 2.4 ng/mL, from 5.3 ± 2.1 to 7.9 ± 2.1 mcU/mL, and from 0.8 ± 0.2 to 1.0 ± 0.3 mg/dL, respectively, $p < 0.05$ for all (Fig. 3). Creatine kinase and lactate dehydrogenase decreased from 138.1 ± 60.9 to 117.1 ± 43.0 U/L and from 68.4 ± 40.8 to 47.7 ± 27.1 U/L; the decreases failed to reach statistical significance.

4   Discussion

Surprisingly, considering the reduction of FM in the climbers, it is interesting to note that insulin level increased. This increase could be secondary to FM reduction since the energy expenditure was

Table 1 Breakdown of nutritional components by percentage before and during the expedition at high altitude

Nutritional components	Before expedition	During expedition
Proteins (%)	25	30
Lipids (%)	26	20
Carbohydrates (%)	49	50
Oligosaccharides/total carbohydrates (%)	25	25
Saturated fat/total (%)	25	43
Animal protein/total (%)	77	75
Vegetable proteins/total (%)	23	25
Total fiber/1000 kcal (g)	11	13
Calories (kcal)	2009	3584

Table 2 Anthropometric measurements performed at sea level before (SL_B) and after (SL_A) chronic high-altitude physical strain

	Parameters	SL_B	SL_A	p	Effect size
Reductions	BW (kg)	79.3 ± 15.3	76.0 ± 12.2	< 0.05	0.23
	BF%	26.3 ± 4.5	23.2 ± 4.1	< 0.01	0.72
	BMI (kg/m^2)	26.7 ± 3.3	25.6 ± 2.6	< 0.05	0.93
	WC (cm)	93.2 ± 11.0	89.5 ± 9.2	< 0.01	1.27
Increase	CSA (cm^2)	158 ± 20	172 ± 17	< 0.001	0.75

BW body weight, *BF%* percentage of body fat, B*MI* body mass index, *WC* waist circumference, *CSA* cross-sectional area
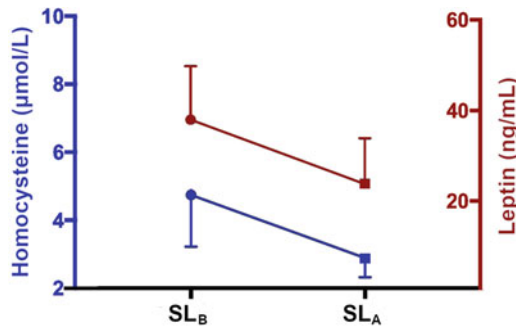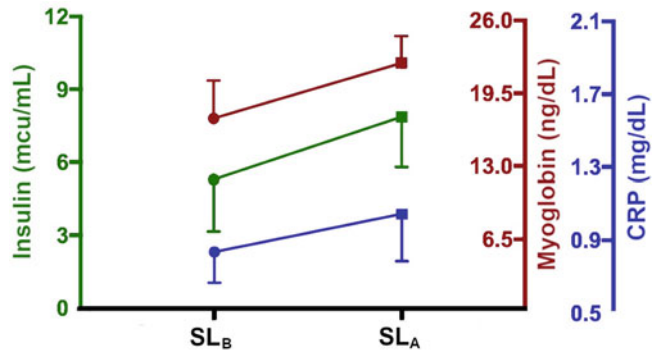

Fig. 2 Decreases in plasma level of leptin and homocysteine assessed at sea level before (SL_B) and 2 days after (SL_A) chronic high-altitude physical strain. Data are means $\pm$SD

Fig. 3 Increase in plasma level of insulin, myoglobin, and C-reactive protein (CRP) assessed at sea level before (SL_B) and 2 days after (SL_A) chronic high-altitude physical strain. Data are means $\pm$SD

not adequately compensated by the caloric intake during the trek. The FM reduction may have stimulated the release of a liposynthetic hormone, such as insulin, in an attempt to balance off a loss of fat. Larsen et al. (1997) have shown that healthy subjects exposed to hypobaric hypoxia at high altitude have hyperinsulinemia that persists for 1 week but reverts with the time of adaptation to low oxygen pressure. The literature suggests that a decrease in BW is generally associated with a decrease of leptin, while the opposite is true for BW excess (Benso et al. 2007; Broglio et al. 2006). In line with that notion, in this study we found a significant decrease in leptin content of about 38% compared with the baseline level before the expedition. A consistent decreasing trend of about 28% remained when leptin content was normalized for FM, even though it lost significance due to a large data spread.

In this study we found an increase in C-reactive protein, a marker of inflammation, while lactate dehydrogenase was unchanged. In addition, creatine kinase was unchanged, although we would have expected an increase in its content. The creatine kinase-related manifestation of muscle damage due to intensive trekking strain could possibly be masked by a short half-life of creatine kinase, amounting to about 1 day and a half. Creatine kinase increases in the first 12 h after onset of rhabdomyolysis, peaks within 3 days, returning to the baseline level in 3–5 days (Zhang 2012). Concerning homocysteine, the amino acid appreciably decreased by about 39%. Since a high-protein diet, in and by itself, does not alter fasting plasma total homocysteine content (Verhoef et al. 2005), it seems a reasonable assumption that a decrease in homocystein would reflect the exposure to chronic hypoxia. Bailey et al. (2000) have reported a decrease in homocysteine after hypoxic as opposed to normoxic physical training. An increase in the myoglobin level we demonstrate in this study is a normal adaptive response to training at high altitude. However, a persisting myoglobin enhancement may be a foretelling sign of muscle damage and dysfunction. This finding is consistent with the data reported by Nedergaard et al. (2013) and Hoppeler and Vogt (2001). In addition, high level of myoglobin is found in the populations living at high altitude, which probably belongs to the compensatory mechanisms of chronic exposure to hypoxic environment (Moore et al. 2002). A diet rich in calories, with a 30% protein content, has been highlighted as a fundamental nutritional intervention in sarcopenia (Woo 2018). On the other side, an increase in CSA of lower limbs with unchanged total lean body mass, we noticed in this study, is difficult to explain. We believe it might likely be the edema-forming redistribution of body fluids during chronic physical strain at high altitude. Trekkers are known to suffer from a moderate lower extremities edema that reverts on return to a lower altitude (Hultgren 1978).

In conclusion, the results of this study demonstrate that long strenuous trekking at high altitude is conducive to inflammatory response and exerts pro-cachexic and pro-sarcopenic effects. Degradation of muscle proteins, if sustained, may be a presage of muscular failure. These changes, in all likelihood, reflect the effects of exposure to chronic hypobaric hypoxia. A high caloric intake, in general, and a high protein diet, in particular, may at least partially counteract such untoward effects of high altitude hypoxia.

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Prevalence of Sleep Apnea in Patients with Carotid Artery Stenosis

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Abstract

Obstructive sleep apnea (OSA) is a common disease affecting about 13% of men and 6% of women, usually having severe cardiovascular sequalae. OSA is responsible for the systemic inflammatory response and oxidative stress and results in endothelial injury being a risk factor for atherosclerosis. The aim of this study was to estimate the prevalence of OSA among patients with severe carotid artery stenosis. Fifty-five patients (F/M-24/31, mean age 70 $\pm$ 7 years, body mass index 28.3 $\pm$ 6.3 kg/m^2) were enrolled into the study. The patients were qualified for elective surgical treatment of carotid artery stenosis. Polysomnography was performed in all patients the night before surgery. Thirty-six patients underwent surgical endarterectomy and nineteen patients underwent carotid artery stenting. Sleep apnea was diagnosed in 44 (80%) of all patients. The mean apnea-hypopnea index (AHI) was 14.5 $\pm$ 12.9. The OSA severity distribution was as follows: 22 mild, 16 moderate, and 6 severe cases. We found that the percentage of carotid endarterectomies was the greatest in mild and moderate OSA. It was approximately twofold greater than that in non-OSA patients. Carotid artery stenting was performed in nearly half of the patients in each of these groups. We conclude that OSA is highly prevalent in patients with carotid artery stenosis scheduled for carotid surgery. Nonetheless, the exact pathogenetic mechanisms underlying mutual interaction between OSA and vascular wall damage remain elusive. OSA is not routinely diagnosed among patients with advanced atherosclerosis. The study results might be an argument for performing polysomnography in patients with carotid artery stenosis.

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Keywords

Advanced age · Apnea-hypopnea index · Atherosclerosis · Carotid artery stenosis · Carotid endarterectomy · Obstructive sleep apnea · Polysomnography · Risk factor

1 Introduction

Obstructive sleep apnea (OSA) is a disease characterized by an air-flow limitation or obstruction in the upper airway tract during

sleep. One of the underlying mechanisms is a narrowing of the upper airway tract caused by palate collapse that results in mechanical block-ade. This condition is often correlated with snor-ing. The other cause of OSA is an impairment of the brain stem respiratory network, which leads to intermittent inhibition of inspiratory signals run-ning down to the respiratory muscles. OSA often manifests as arousals during sleep, nocturia, chronic tiredness, morning headaches, and day-time sleepiness, all of which leading to the deteri-oration of physical and psychological conditions.

OSA is a common disease affecting ca. 13% of men and 6% of women (Peppard et al. 2013). The prevalence of severe OSA, based on the STOP-BANG questionnaire, in the adult population of the city of Wroclaw in Poland is estimated at 18%. The main risk factors for OSA are obesity (Drager et al. 2013), increased neck perimeter, and male gender (Seet and Chung 2010). Sleep apnea is connected with a greater risk of arterial hypertension, diabetes type 2, coronary artery disease, stroke, and circulatory insufficiency, irrespective of the degree of obesity (Savransky et al. 2007), and with carotid atherosclerosis (Lee et al. 2008). There are reports pointing to the role of sleep apnea in the induction of inflammation and oxidative stress (Iturriaga et al. 2015; Rasche et al. 2010; Lavie 2008). Damage to the vascular endothelium resulting from the processes outlined above is a risk factor for the development of arteriosclerosis. Hypoxia induces inflammatory responses, including inflammatory cells migra-tion, and secretion of proinflammatory cytokines, such as interleukin (IL)-1, 6, and 8, and tumor necrosis factor alpha (TNF-α) (Lam et al. 2012). Further, hypoxia increases secretion of intercellu-lar adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (Lattimore et al. 2005), disorders the redox balance (Pialoux et al. 2009), intensifies low-density lipoprotein peroxidation (Adedayo et al. 2014), and activates carotid body chemoreceptor-mediated overexpression of vascular endothelial growth factor (VEGF) (Del Rio et al. 2011).

The pathogenetic background of sleep apnea-induced neurobiological alterations outlined above speaks for the plausible predominance of atherosclerosis among adverse effects of the dis-ease. Therefore, the aim of this study is to define the frequency of OSA among patients with severe carotid artery stenosis (CAS).

2 Methods

There were 55 consecutive patients (F/M – 24/31) enrolled into the study, with the mean age 70 $\pm$ 7 years and the mean body mass index (BMI) of 28.3 $\pm$ 6.3 kg/m^2. The patients were diagnosed with CAS and qualified for surgical treatment. The sample size was calculated on the premise that the prevalence of severe OSA, based on the STOP-BANG questionnaire, is 18% (based on 613 patients) and the prevalence of CAS patients is twice as much in the general population. With the sample size of 80 patients and the significance level of alfa <0.05, the power of the results is >0.99. Before surgery, polysomnography test with NOX-T3 portable respiratory sleep monitor device (ResMed, San Diego, CA) was performed in all subjects to record airflow, pulse oximetry, chest, and abdom-inal respiratory movements. Data were stored in a spreadsheet form and statistically analyzed offline. A structured medical history was taken and all the patients filled out the Stop-Bang ques-tionnaire. Endarterectomy or endovascular inter-vention was performed in line with the current clinical recommendations. Carotid endarterec-tomy is the first-line treatment for symptomatic patients with carotid artery stenosis of 50–99% and in asymptomatic patients with stenosis of 60–99%. Carotid artery stenting is performed among symptomatic patients with stenosis of 50–99% in whom open surgery is risky due to general health condition or anticipated periopera-tive difficulties or complications (Ricotta et al. 2011). Patients with moderate-to-severe OSA had polysomnography repeated after 3 months from the initial one and were qualified for contin-uous positive pressure treatment (CPAP) if required. In case of mild OSA, polysomnography was performed after 12 months.

Data were expressed as means ±SD or counts and percentages of different categories of OSA cases. Statistical differences were assessed with a *t*-test. Pearson's correlation coefficient was used as a measure of the linear correlation between BMI and AHI changes. A *p*-value <0.05 defined statistically significant differences.

3 Results

Thirty-six out of the fifty-five patients with CAS underwent surgical endarterectomy and nineteen patients were treated with the carotid artery stenting. Sleep apnea was diagnosed in 44 (80%) patients. The majority of patients with sleep disordered breathing were diagnosed as having obstructive sleep apnea. Central sleep apnea was observed in a single, statistically irrelevant case. The mean apnea-hypopnea index (AHI) was 14.5 ± 12.9 episodes/h sleep and the mean oxygen desaturation index (ODI) was 15.3 ± 12.3 episodes/h sleep. Sleep apnea severity distribution was as follows: 22 mild, 16 moderate, and 6 severe cases. There were a significant percentage of different co-morbidities noticed in the patients investigated: hypertension (84.9%), stroke (22.6%), diabetes (39.6%), and heart disease (7.7%) including atrial fibrillation (15.1%) and coronary artery disease (52.8%).

We noticed that the percentage of carotid endarterectomies was the greatest in mild and moderate OSA. It was approximately twofold greater than that in non-OSA patients. Carotid artery stenting was performed in nearly half of

the patients in each of these groups (Fig. 1). Patients were divided into the groups, according to gender, obesity, history of heart disease, history of stroke, and the type of surgical treatment. There was no statistically relevant difference between male and female subjects concerning the breathing variables during sleep, such as AHI, ODI, and the lowest and average arterial oxygen saturation levels (Fig. 2). There was, albeit rather weak, correlation between BMI and AHI ($r^2 = 0.29$; $p < 0.05$) (Figs. 3 and 4). The BMI adversely correlated with the history of heart disease, such as congestive heart failure, coronary artery disease, atrial fibrillation, and other arrhythmias ($p = 0.05$). Rather unexpectedly, the patients with a history of stroke had the breathing variables comparable to those in the patients with no history of stroke (AHI 13.9 ± 13.6 vs. 15.2 ± 7.3/h, ODI 15.2 ± 12.6 vs. 15.3 ± 11.6/h, and an average arterial oxygen saturation 92.7 ± 1.8 vs. 91.9 ± 2.9%, respectively). The same was also true for the patients with and without history of a heart disease (Table 1). The comparison according to type of carotid artery management was performed.

4 Discussion

The interrelationship between OSA, endothelium dysfunction, and atherosclerosis has been described in epidemiological studies. OSA severity has a relation with carotid artery intima media thickness and calcification of coronary arteries
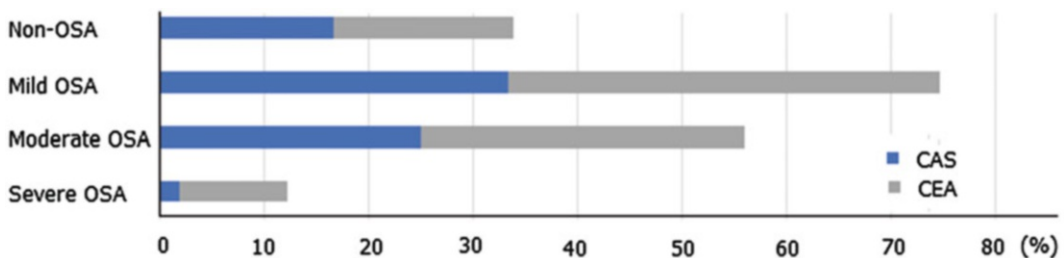

Fig. 1 Percentage of carotid artery stenting (CAS) versus carotid endarterectomy (CEA) by the severity of obstructive sleep apnea
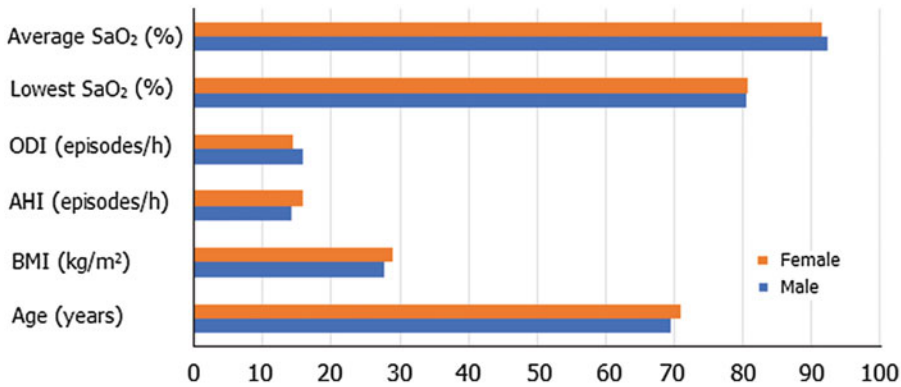
Fig. 2 Nighttime breathing and demographic data in male and female populations. SaO_2 arterial oxygen saturation; *ODI* oxygen desaturation index, *AHI* apnea-hypopnea index, *BMI* body mass index
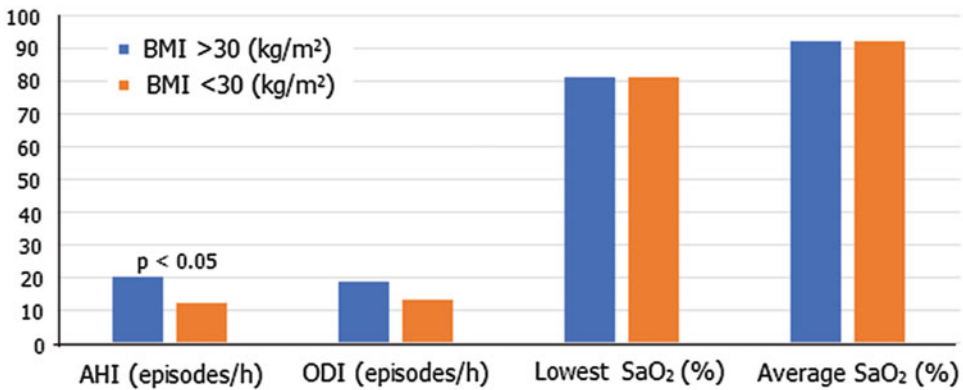

Fig. 3 Nighttime breathing parameters in obese and non-obese populations of sleep apnea patients. *AHI* apnea-hypopnea index, *ODI* oxygen desaturation index, SaO_2 arterial oxygen saturation, *BMI* body mass index

(Fox et al. 2014). Magnitude of nocturnal arterial desaturations influences carotid plaque formation (Gunnarsson et al. 2015) and cardiovascular disease risk (Gunnarsson et al. 2014). Lee et al. (2008) have assessed the influence of snoring on carotid artery atherosclerosis. The authors show that sleep apnea has a negative influence on the cardiovascular system, mostly through the elevation of arterial blood pressure, autonomic dysfunction, and changes in lipid and glucose metabolism (Drager et al. 2009). Chronic intermittent hypoxia caused by sleep apnea might be an independent risk factor for atherosclerosis as it is conducive to proinflammatory propensity

(Eisele et al. 2015). Schaefer et al. (2015) have diagnosed OSA in 98% of patients with peripheral artery disease. The authors show that the presence of plaque is associated with age and apnea-hypopnea index.

OSA is highly prevalent in patients with asymptomatic carotid artery stenosis and is associated with the severity of stenosis. This association is also valid for central apnea patients (Ehrhardt et al. 2015). Coloma Navarro et al. (2016) have evaluated the influence of OSA on the cerebral blood flow based on a transcranial Doppler examination. The authors point out a reduction in cerebral blood flow velocity and
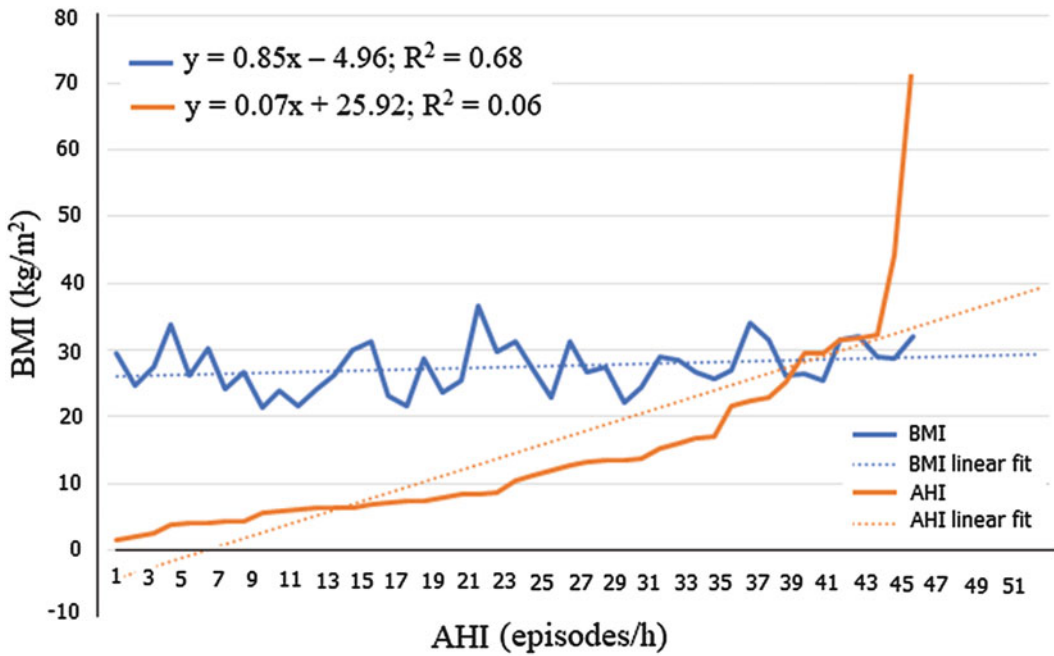
Fig. 4 Correlation between body mass index (BMI) and apnea-hypopnea index (AHI). Dotted lines depict linear regression fit of data

Table 1 Body mass index (BMI) and breathing variables during sleep among patients with heart disease (HD) and with no history of heart disease (nHD)

	BMI (kg/m^2)	AHI (/h)	ODI (/h)	Lowest SaO$_2$ (%)	Average SO$_2$ (%)
HD	26.9 ± 3.9	15.1 ± 14.0	14.6 ± 12.6	81.3 ± 6.3	92.2 ± 2.0
nHD	30.0 ± 8.2	14.6 ± 11.5	16.1 ± 12.2	80.5 ± 3.9	92.1 ± 1.8
p-value	0.05	0.44	0.34	0.29	0.41

AHI apnea-hypopnea index, *ODI* oxygen desaturation index, *SaO$_2$* arterial oxygen saturation

impaired cerebrovascular reactivity in OSA-positive patients compared to a control group. An improvement in the endothelial function after CPAP treatment has also been noticed. OSA is an independent risk factor for stroke, and CPAP treatment reduces the risk and improves recovery after stroke (King and Cuellar 2016). Beneficial effects of CPAP are due like to a decrease in sympathetic activation, as rather expected reductions in inflammatory biomarkers, insulin resistance, and advantageous changes in the lipid and glucose metabolism are not substantiated (Jullian-Desayes et al. 2015). CPAP treatment may also decrease central blood pressure and arterial stiffness (Phillips et al.

2008). Although the reversibility of structural cardiovascular remodeling induced by intermittent hypoxia has been noticed in the mouse model after resumption of normoxia (Castro-Grattoni et al. 2016), a reduction in cardiovascular-related mortality after CPAP treatment is a contentious issue (Drager et al. 2017).

The prevalence of OSA in the general adult population is estimated at anywhere between 9 and 38%, and it is associated with age and obesity. The disease is more prevalent in male gender (Senaratna et al. 2017). The present study shows a high percentage of OSA patients in a group qualified for surgical treatment of carotid artery stenosis (80% of patients with AHI >5/

h and 36% with AHI >15/h). That may result from common risk factors for OSA and atherosclerosis, as well as from advanced age of the screened population as OSA occurs more often in the elderly. On the other hand, we found that the influence of body mass was rather irrelevant, as there were only 28% of patients with BMI $\geq$30 kg/m^2; the findings were in line with an epidemiological study of Stepaniak et al. (2016). A connection of OSA with obesity is not fully clear as, at best, it seems weak in the general population. That may indicate other than obesity risk factors for OSA among patients with atherosclerosis or, conversely, OSA being a risk factor for carotid stenosis. Further studies are required to colligate these conditions. Interestingly, in this study, we noticed no significant differences in OSA indices between the patients with heart diseases (a history of atrial fibrillation and other arrhythmias, heart failure, or coronary artery disease) and those without them. The influence of obstructive apneas on the appearance of such heart diseases is yet to be firmly established as opposed to central apneas that are conducive to atrial fibrillation or heart failure (May et al. 2016).

In conclusion, sleep apnea and atherosclerosis coexist in a large number of patients. OSA is highly prevalent in patients with carotid artery stenosis scheduled for carotid surgery, which is often associated with advanced age and increased body mass. Co-morbidities are common, but they not influence sleep apnea severity. Surgical endarterectomy is performed more frequently in patients with sleep apnea of moderate severity, whereas carotid artery stenting among non-OSA and mild OSA patients. The relation between sleep apnea and atherosclerosis is widely discussed, but there is still no obvious point of intersection of the two pathogenetic pathways. OSA is not routinely diagnosed among patients with advanced atherosclerosis. The study results might be an argument for performing polysomnography in patients with carotid artery stenosis.

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Excessive Body Weight and Immunological Response in Children with Allergic Diseases

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Abstract

The prevalence of allergy and obesity is sharply on the rise in children. However, the nature of a mutual relation of the two conditions remains unclear. The aim of the study was to assess the impact of excessive body weight on the immune response in children with allergies. There were 56 children with allergies, aged 4–15 years, included into the study (41 with asthma and 15 with atopic dermatitis). Based on the body mass index, children were divided into two groups: normal weight (body mass index (BMI) <85th percentile) and excessive weight (BMI $\geq$ 85th percentile). The immunological parameters were evaluated by flow cytometry. We found that children with excessive body weight had a significantly lower percentage of CD4$^+$ lymphocytes and a higher percentage of natural killer T cells (NKT) and CD16/56$^+$ lymphocytes than those with normal weight. In the group with allergy, a significant positive association was noticed between BMI and the percentage of human leukocyte antigen (HLA)-DR-specific CD3. Further analysis was done after dividing the allergy group into the children with normal and excessive weight. There were an adverse association between BMI and the percentage of CD8$^+$ lymphocytes in those with normal weight and a positive one between BMI and the percentage of CD4$^+$ in those with excessive weight. We conclude that excessive body weight plays a major role in mediating the immunological response in children with allergy.

Keywords

Allergic diseases · Asthma · Atopic dermatitis · Body weight · Immunological response · Lymphocytes

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1 Introduction

The prevalence of allergy and obesity in children has sharply increased of late. A recent report by WHO (2016) estimates is that over 340 million children and adolescents aged 5–19 years have excessive body weight and obesity, which is an increase from 4% in 1975 to 18% in 2016. The

exact prevalence of overweight and obesity in the population of Polish children remains unknown due to variable assessment criteria and variable age groups included into the relevant studies. The Health Behavior in School-aged Children (HBSC) study conducted in 2013–2014 has reported that 2.4% of children in Poland, aged 11–15 years, are obese and 12.4% are overweight (Oblacińska 2015). Another investigation performed in the OLAF study has revealed the excessive weight in the population of Polish children, aged 6–19 years, is in a range of 14.1–17.9%, depending on the region (Grajda et al. 2011). The latest UNICEF (2013) report shows that weight gain in the Polish children progresses at the fastest pace among the European countries.

Another rising health problem in the Polish population are allergic diseases. The latest data from the ECAP study in three age groups (6–7 years old, 13–14 years old, and young adults 20–44 years old) show that allergic diseases affect about 40% of these populations. The most frequent diagnosed allergic ailments are rhinitis (24–30%), asthma (11%), and atopic dermatitis (9%) (Samoliński et al. 2014).

The available data concerning the association of obesity and allergy do not provide equivocal information. Obesity is associated with a chronic inflammation process of low intensity, known as metabolic inflammation in which adipokines, such as leptin, resistin, and visfatin, produced by fat cells are involved. Recent studies show that the formation of these factors leads to numerous metabolic irregularities and modulates the immunological response. Metabolic and immune systems are strictly related. Consumption of excessive amount of food by obese people is identified by pattern recognition receptors as a harmful, stress-inducing biological event. Fat tissue, consisting mainly of adipocytes, requires preadipocytes, macrophages, fibroblasts, and vascular inflammatory cells for its growth. The exact mechanism of inflammation progression is an area of limited understanding. It is suggested that a hypoxic milieu of adipocytes, oxidative stress, and impairment of peroxisome proliferator-activated receptors could have a crucial meaning (Góralska et al. 2015).

The role of overweight and obesity in asthma progression is proven (Granell et al. 2014; Egan et al. 2013; Flaherman and Rutherford 2006). Further, more than 26% of children suffering from asthma are obese (Lang et al. 2012). A coincidence between asthma and obesity also has to do with more severe exacerbations of asthma (Ahmadizar et al. 2016), requiring mechanical ventilation and longer hospitalization (Okubo et al. 2016) and with a worse response to inhaled corticosteroids (Forno et al. 2011). However, although asthma may associate with allergic rhinitis (Bousquet et al. 2012), there is no evidence that rhinitis coexists with obesity (Han et al. 2016). Data regarding the association between atopic dermatitis and obesity are inconsistent either (Sybilski et al. 2015; Zhang and Silverberg 2015). Therefore, this study seeks to define the influence of excessive body weight on the immune system elements in children with allergies. We addressed the issue by evaluating the phenotype of peripheral blood lymphocytes such as $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, CD16/56, natural killer T cells (NKT), human leukocyte antigen (HLA)-DR-specific CD3, and natural regulatory T lymphocytes ($CD4^+CD25highCD127^-Foxp3^+$) and the content of the pro-inflammatory interleukin (IL)-1, IL-2, IL-6, IL-17A, IL-22, tumor necrosis factor alpha (TNFα), and interferon gamma (IFNγ) and the anti-inflammatory cytokines (IL-4, IL-10, and transforming growth factor beta (TGFβ)).

2 Methods

2.1 Study Participants

The study group consisted of 56 children with allergies aged 4–15 years hospitalized at the Department of Pediatrics, Pediatric Nephrology and Allergology of the Military Institute of Medicine in Warsaw, Poland, since August 2014 till September 2016. In this group there were

Table 1 Criteria for participation in the study

Inclusion criteria	Exclusion criteria
Positive results of skin prick tests and/or increased concentration of total IgE	Age < 4 years and > 18 years
	Acute respiratory tract infection
Age > 4 years and < 18 years	Coexisting autoimmune system diseases
Consent to participate in the study	Allergen-specific immunotherapy
	Exacerbation of allergic disease

41 children with asthma and 15 children with atopic dermatitis. Atopic background was confirmed by the elevated content of total serum IgE and by positive results of skin prick tests with common alimentary and inhaled allergens. The patients were in a stable period of the disease, with no symptoms of acute respiratory tract infection. The inclusion and exclusion criteria are presented in Table 1.

We tallied the following data: disease duration, severity of symptoms, previous diagnostic procedures, and treatment. All children included into the study had a physical examination with the measurement of body height and weight. The children were divided into two groups based on the body mass index (BMI; kg/m^2), according to the WHO recommendation:

– Group I: normal body weight (BMI < 85 percentile)
– Group II: excessive body weight (BMI $\geq$ 85 percentile)

Body height was measured with the accuracy to 0.5 cm in the upright standing position without shoes and weight with use of a legalized and standardized scale with the accuracy to 100 g for children without shoes and wearing light clothes. Based on measurements of body height and weight, the body mass index (BMI) was calculated according to applicable formula.

Calculated BMI value was applied on the centile grids. Following the WHO recommendations, overweight was defined as BMI at or above the 85th percentile and below the 95th percentile and obesity as BMI at or above the 95th percentile. In further analysis, overweight and obesity are referred to as excessive body weight.

2.2 Analysis of Immunological Indices

2.2.1 Phenotype of Peripheral Blood Lymphocytes

Immunophenotyping of CD3$^+$/19$^+$, CD4$^+$/CD8$^+$, CD19$^+$, CD16/56, NKT, and HLA-DR-specific CD3 lymphocytes was performed in whole blood specimens, collected into ethylenediamine-tetraacetic acid (EDTA)-primed tubes. A 100 μl of blood was taken for analyses. The samples were incubated with 20 μl of primary antibodies against the lymphocytic antigens for 20 min at room temperature, using an IMK Plus kit (BD Biosciences, Warsaw, Poland). Next, lysis of erythrocytes was done, using FACS Lysing solution (BD Biosciences, Warsaw, Poland), and the cells were washed with phosphate buffer solution (PBS) twice and fixed in 1% solution of paraformaldehyde in PBS. Cells were analyzed in a FACS Calibur Flow Cytometer (BD Immunocytometry Systems, San Jose, CA), equipped with CellQuest Pro software (BD Biosciences, Warsaw, Poland). The results of lymphocyte phenotypes were presented as the mean percentages ±SD.

2.2.2 Natural T Regulatory (Treg) Lymphocytes

To characterize nTreg cells, whole blood samples (100 μl) were stained with the primary antibodies against CD4-PerCP, CD25-APC, and CD127-FITC (BD Biosciences, Warsaw, Poland) or with an appropriate isotype control with the addition of CD4-PerCP antibody. After a 20-min incubation, lysis of erythrocytes was performed; the cells were washed with PBS. Then, cells were fixed and permeabilized in a specific buffer and

incubated for 40 min at 2–8 °C. Then, cells were stained with FoxP3 PE or isotype IgG1 kappa PE antibody for 45 min in the dark at room temperature. Next, cells were washed twice with PBS, fixed in 300 μl of 1% paraformaldehyde in PBS, and examined by flow cytometry. Ten thousand counts of CD4 PerCP-positive cells was the upper limit of cytometric acquisition. The percentage of nTreg cells (FoxP3^{+}, CD25^{high}, and CD127^{low}) was counted in CD4-positive cells. The results were presented as the mean percentages $\pm$ SD.

2.2.3   Cytokines

After "on clot" blood collection and serum isolation (centrifugation at $2{,}000 \times g$, 20 min, 4 °C), samples were stored in aliquots at -80 °C, until the analysis was conducted. The content of selected cytokines was determined in the serum using the Human Th1/Th2/Th17 Cytokine Kit (cat. no. 560484, BD Biosciences, Warsaw, Poland), according to the manufacturer's recommendations. Briefly, 50 μl of bead pellet, 50 μl of undiluted serum, and 50 μl of a detection reagent were added to the cytometric tubes. Dilutions of cytokines tested were prepared accordingly (standard curves) (Table 2). Samples were incubated for 3 h at room temperature. Then, they were centrifuged twice ($500 \times g$, 5 min), and the bead pellet was resuspended in 300 μl of wash buffer. The samples were evaluated with flow cytometry (FACS Calibur Flow Cytometer, BD Immunocytometry Systems, San Jose, CA).

The cytokines investigated were divided into two groups:

- Pro-inflammatory: IL-1, IL-2, IL-6, IL-17A, IL-22, TNFα, and IFNγ
- Anti-inflammatory: IL-4, IL-10, and TGFβ

The results were presented as mean $\pm$ SD concentrations (pg/ml).

2.3   Statistical Analysis

Data distribution was evaluated with the Kolmogorov-Smirnov and Lilliefors tests. Statistical differences between normally distributed data were tested with Student's *t*-test. Otherwise, the Mann-Whitney U test was used. Correlation analysis was performed using Spearman's factor for variables without normal distribution or Pearson's factor for variables with normal distribution. A p-value <0.05 defined statistically significant differences. The analyses were made using a commercial STATISTICA v12 package (StatSoft, Tulsa, OK).

3   Results

Excessive body weight was present in 16 (29%) out of the 56 children participating in the study, out of whom 8 were obese and 8 were overweight. Detailed characteristics of the two groups

Table 2 Cytokine concentration and standard dilution

Tube label	Concentration (pg/ml)	Cytokine standard dilution
1	0 (negative control)	No standard dilution
2	20	1:256
3	40	1:128
4	80	1:64
5	156	1:32
6	312.5	1:16
7	625	1:8
8	1250	1:4
9	2500	1:2
10	5000	Top standard

are presented in Table 3. There were no significant differences in the absolute or percentage values of eosinophils and in total IgE antibodies between both groups (Table 4).

Children with excessive weight had a significantly lower percentage of $CD4^+$ lymphocytes (30.6 ± 6.1 vs. 36.7 ± 7.9, $p = 0.01$) and higher percentages of NKT and $CD16/56^+$ lymphocytes (3.9 ± 1.9 vs. 2.8 ± 1.9; 15.0 ± 5.7 vs. 11.6 ± 5.7, $p = 0.05$, respectively) than those with normal weight. There were no significant differences between the other immunological indices assessed (Table 5).

We used the BMI percentile value as an objective measure of nutritional status to evaluate the association between obesity and immunological indices, irrespective of children's age. In the entire group of children with atopy, there was a significant association between BMI and the percentage of CD3 anti-HLA-DR lymphocytes. There also were an inverse association between BMI and CD8 percentage in children with normal weight and positive association between BMI and CD4 percentage in children with excessive weight (Table 6; Figs. 1, 2, and 3). There was no significant association between BMI percentile and atopy indicators, such as total IgE or eosinophils, either in the entire group or subgroups with normal and excessive weight.

Table 3 Characteristics of children with normal (Group I) and excessive body weight (Group II)

	Group I	Group II
	($n = 40$)	($n = 16$)
Age (year; mean $\pm$ SD)	9 ± 4	9 ± 3
Gender (M/F)	23/17	11/5
BMI (kg/m^2; median, q_{25}-q_{75})	16 (15–18)	23 (20–25)
Height (cm; mean $\pm$ SD)	134 ± 23	144 ± 19
Weight (kg; median, q_{25}-q_{75})	25 (21–47)	48 (20–25)

4 Discussion

A more severe course of allergic diseases in children with excessive weight than that in children with normal weight has been well established (Peters et al. 2018). Children with asthma and obesity have a worse asthma control and worse quality of life (van Gent et al. 2017; Borrell et al. 2013). Excess adipose tissue has also an impact on lung function (Schatz et al. 2015). However, the influence of obesity on the immunological system in children with allergies is still poorly known.

The majority of studies that address the relationship between obesity and immune system have been conducted in non-allergic children. In contrast, in the present study, we assessed the influence of excessive weight on the immunological system's responses in children with atopy. We noticed a higher percentage of eosinophils in children with normal weight compared to overweight or obese ones. However, the difference did not reach a statistical significance. The study conducted by Wu et al. (2011) has shown that adipose tissue eosinophilia mitigates chronic inflammation and positively influences glucose metabolism. Those authors also noticed an inverse association between the amount of adipose tissue and the number of eosinophils. Hence, eosinophils may play a crucial role in shaping the adipose tissue function. The mechanisms that underlie the adipose tissue homeostasis are yet an area of limited understanding. Bolus et al. (2018) have examined whether normalization of the eosinophil count would result in a body weight reduction and improvement in carbohydrate metabolism. The study was conducted in mice, fed with a high content of fat in the chow, followed by parenteral administration of recombinant interleukin 5 (rIL-5) to normalize the

Table 4 Atopy indicators in children with normal body weight (Group I) and excessive body weight (Group II)

	Group I	Group II	
	($n = 40$)	($n = 16$)	p
Eosinophils ($\times 10^3$/μl; mean $\pm$ SD)	0.62 ± 0.42	0.42 ± 0.34	0.09
Eosinophils (%; mean $\pm$ SD)	7.68 ± 4.68	5.40 ± 4.48	0.10
Total IgE (IU/ml; median, q_{25}–q_{75})	293 (204–780)	488 (291–1153)	0.28

Table 5 Immunological indices in children with normal (Group I) and excessive (Group II) body weight

	Group I (n = 40)	Group II (n = 16)	p
Peripheral T lymphocytes (%; mean ± SD)			
CD3	65.6 ± 6.9	62.6 ± 7.5	ns
CD19	16.5 ± 4.4	17.1 ± 5.9	ns
CD4	36.7 ± 7.9	30.6 ± 6.1	0.01
CD8	27.6 ± 5.1	30.6 ± 5.9	ns
CD16/56	11.6 ± 5.7	15.0 ± 5.7	0.05
NKT	2.8 ± 1.9	3.9 ± 1.9	0.05
CD3 anti-HLA-DR	5.8 ± 3.1	7.0 ± 3.5	ns
Natural regulatory T lymphocytes (%; median, q_{25}-q_{75})			
Tregs	0.7 (0.4–0.9)	0.5 (0.4–0.9)	ns
Cytokines – pro-inflammatory (pg/ml; median, q_{25}-q_{75})			
IL-1	0.0 (0.0–0.1)	0.0 (0.0–0.8)	ns
IL-2	1.7 (0.0–2.8)	2.3 (1.2–2.9)	ns
IL-6	2.1 (1.6–3.2)	2.4 (2.1–3.6)	ns
IL-17A	5.9 (2.6–11.0)	3.3 (0.8–7.1)	ns
IL-22	8.5 (2.6–43.0)	8.4 (0.0–17.1)	ns
TNFα	1.6 (1.2–1.8)	1.5 (1.3–1.9)	ns
IFNγ	0.0 (0.0–1.4)	0.0 (0.0–1.3)	ns
Cytokines – anti-inflammatory (pg/ml)			
IL-4 (median, q_{25}-q_{75})	1.3 (0.0–1.6)	0.5 (0.0–1.5)	ns
IL-10 (median, q_{25}-q_{75})	1.9 (1.7–2.5)	2.3 (1.9–3.1)	ns
TGFβ (mean ± SD)	437.2 ± 170.1	457.9 ± 140.7	ns

NKT, natural killer T cells; *Tregs*, regulatory T cells; *IL*, interleukin; *TNFα*, tumor necrosis factor alpha; *IFNγ*, interferon gamma; *TGFβ*, transforming growth factor beta

Table 6 Associations between body weight, represented by the percentile value of body mass index (BMI), with the phenotypes of peripheral lymphocytes and cytokines in children with normal (Group I) and excessive weight (Group II)

	All children	Group I	Group II
BMI versus	r	r	r
CD3	−0.17	−0.10	0.43
CD19	0.21	0.26	−0.09
CD4	−0.14	0.15	0.45∗
CD8	−0.01	−0.34∗	−0.17
CD16/56	0.04	−0.19	−0.45
NKT	0.11	−0.09	−0.29
CD3 anti-HLA-DR	0.30∗	0.17	0.19
Tregs	0.15	0.28	−0.03
IL-2	0.05	0.04	−0.47
IL-4	−0.14	0.00	0.19
IL-6	0.25	0.26	−0.35
TNF	0.13	0.17	−0.24
IL-10	0.13	0.22	0.02
IFNγ	−0.04	−0.06	−0.01
IL-17A	−0.05	0.12	−0.39
IL-1	0.02	−0.09	−0.22
IL-22	−0.08	0.08	−0.20
TGFβ	−0.12	−0.26	0.39

r linear regression coefficient; ∗$p < 0.05$; acronyms of lymphocytes and cytokines same as in Table 5
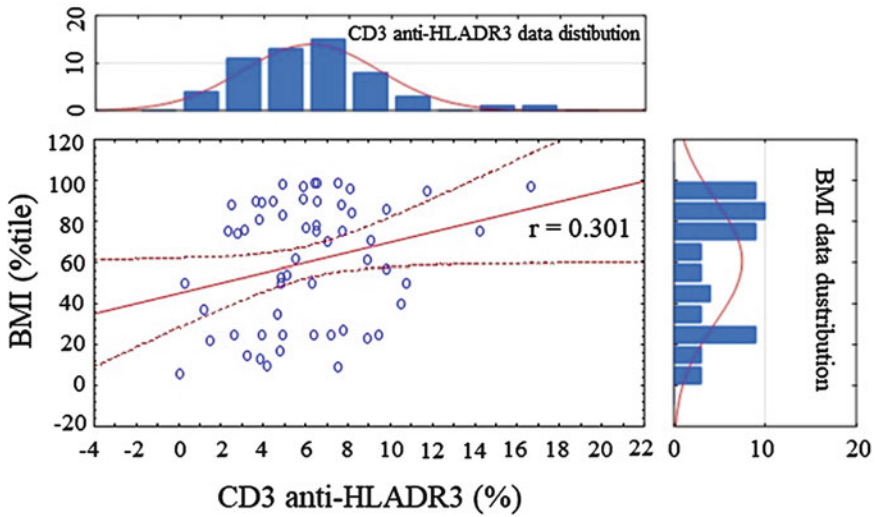
Fig. 1 Association between the percentile of body mass index (BMI) and CD3 anti-HLADR3 lymphocyte count in the entire study group of children. Solid line, linear regression line; dashed lines, 95% confidence intervals; side panels, distribution of data presented on the vertical and horizontal axes

Fig. 2 Association between the percentile of body mass index (BMI) and CD8 lymphocyte count in children with normal body weight. Solid line, linear regression line; dashed lines, 95% confidence intervals; side panels, distribution of data presented on the vertical and horizontal axes
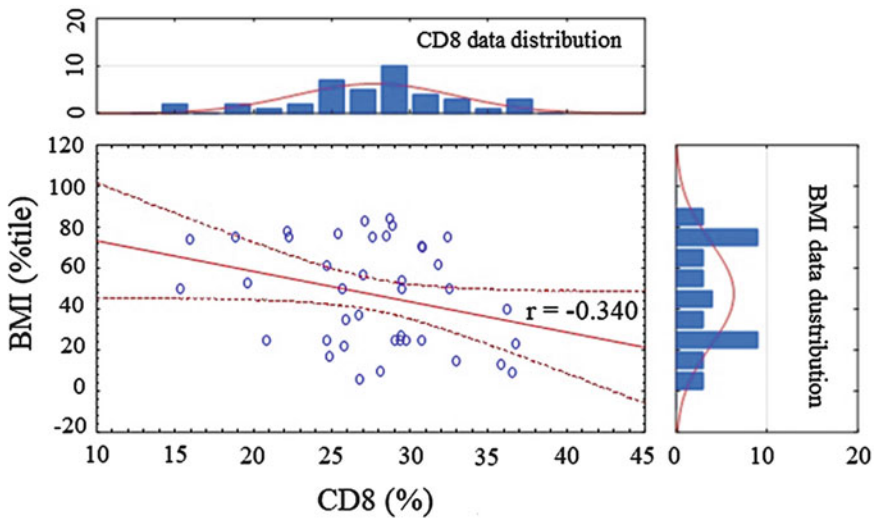
eosinophil count. There was no significant difference in the metabolic tests and no body weight reduction noticed after 8 weeks despite a three-fold increase in the eosinophil count.

Excessive body weight is associated with the inflammatory processes in which immune cells also are involved. Th2 lymphocytes, natural regulatory T lymphocytes (Tregs), and eosinophils are present in the adipose tissue of slim persons, whereas there is an inflow of macrophages, which are conducive to inflammation in obese persons (Góralska et al. 2015; Exley et al. 2014). Available data suggest that the phenotype of macrophages depends on the amount of
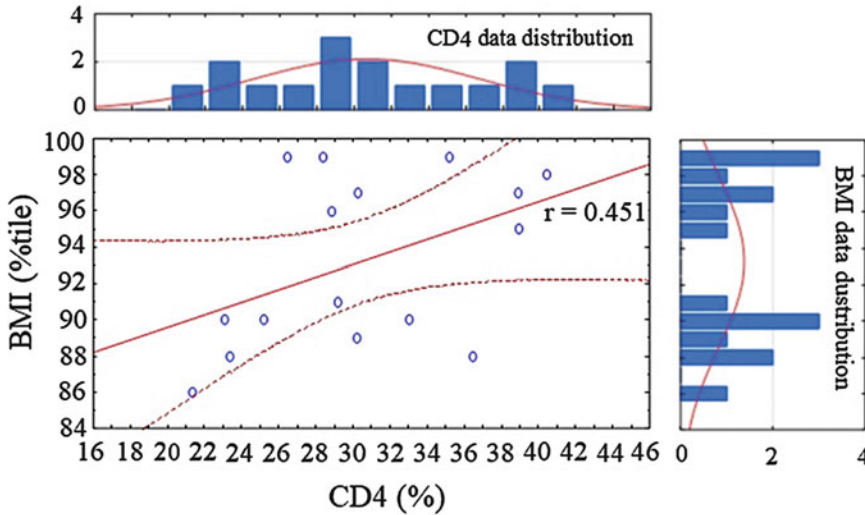
Fig. 3 Association between the percentile of body mass index (BMI) and CD4 lymphocyte count in children with excessive body weight. Solid line, linear regression line; dashed lines, 95% confidence intervals; side panels, distribution of data presented on the vertical and horizontal axes

the adipose tissue. In slim persons, mostly M2 macrophages are present, while in obesity there are M1 macrophages which, activated by Th1 cells, stimulate release of the pro-inflammatory TNFα. In contrast, M2 macrophages are stimulated by Th2 lymphocytes and inhibit the inflammatory propensity (Sharma et al. 2018; Lumeng et al. 2007). Yet, it is unknown whether the inflammatory process linked to obesity is limited to the adipose tissue or it is associated with a systemic reaction. The present study showed a higher percentage of macrophages in the population of peripheral lymphocytes in obese children than that in children with normal weight. Thus, it is possible that the inflammation of obesity leads to a systemic reaction. Similar observations have been made by Periyalil et al. (2018) who show that obesity associates with systemic inflammation and with a more severe respiratory track inflammation, irrespective of the coexisting asthma. However, visceral adipose tissue contains more macrophages in obese patients with asthma than without asthma. The number of macrophages also is associated with disease severity.

There are many other cells involved in the inflammatory process coexisting with obesity.

Recent studies reveal that the presence of CD1d on adipocyte surface activates NKT cells (Satoh and Iwabuchi 2016). A higher percentage of NKT cells is present in children with excessive weight compared to those with normal weight. NKT cells are a subpopulation of T lymphocytes. They influence many stages of the immune response through cytokine secretion (Crosby and Kronenberg 2018). Experimental studies show that these cells play a role in insulin resistance (Lynch et al. 2012; Ohmura et al. 2010). The role of NKT in the development of obesity is an area of limited understanding, but there is an adverse association between NKT cells and body weight (Sag et al. 2014). Owing to increased production of cytokines by NKT cells, typical for Th2 lymphocytes such as IL-4 and IL-10, M2 macrophages become activated and mitigate inflammation associated with obesity. In contradistinction, some other studies show that obesity development slows down when NKT cells are not present (Wu et al. 2012). Recently, Satoh and Iwabuchi (2018) have reviewed the influence of NKT cells on the development of obesity and point that their modulating influence on adipocytes depends on lipids in the diet and in particular on the intestinal microbiome.

Other studies point to the role of Treg lymphocytes in insulin resistance and obesity (Matarese et al. 2010). A reduction in Tregs results in increases in insulin and pro-inflammatory cytokines. Conversely, increased number of Tregs induces IL-10 and improves insulin action in the mouse (Feuerer et al. 2009). There is an apparent reduction in Tregs in obese persons (Agabiti-Rosei et al. 2018; Yun et al. 2010). In line with that, we noticed in the present study a decreased percentage of Tregs in children with excessive weight compared to those with normal weight, although the difference failed to reach statistical significance. This lack of significance in Treg changes might be explicable by not taking into account the influence of physical activity on the metabolism and immune responses: the element we failed to control in the study. A prospective study of Momesso dos Santos et al. (2015) has shown that regular moderate physical exercise results in inhibition of inflammation in obese children through modulating the immunological response.

This study showed that an increase in BMI is associated with the percentage of activated CD3 anti-HLA-DR. The HLA-DR molecule is a marker of T-lymphocyte activation and appears, on average, 3–5 days after binding with the antigen (Holling et al. 2004). The assessment of T-lymphocyte activation based on the presence of HLA-DR may indirectly reflect B-lymphocyte stimulation and antibody synthesis. Therefore, the findings suggest the impact of excessive body weight on T lymphocytes as with increasing BMI also increases the percentage of activated $CD3^+$ lymphocytes, which points to the stimulation of the immune system. We also noticed that children with excessive weight have a lower percentage of $CD4^+$ lymphocytes, but their number increases with increasing BMI. This finding is in line with that of Schindler et al.'s (2017) who have found an association between obesity and the occurrence of increased level of $CD4^+$: the cells that are a major source of IL-17A. That study shows the association between BMI and Th17 lymphocytes based on the investigation of 65 children of whom 15 were obese.

We conclude that excessive body weight importantly and adversely modulates the immunological response in children with allergy. The macrophages and NKT are the immune cells that play a pivotal role in the modulation of immunological responsiveness.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the Ethics Committee of the Military Institute of Medicine (permit 123/14).

Informed Consent Written informed consent was obtained from all legal guardians of individual participants included in the study.

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Activity of Lysosomal Enzymes During Protein Malnutrition and Progesterone Supplementation in the Mouse

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Abstract

This study investigated the effects of protein malnutrition and progesterone supplementation on the activities of a spectrum of lysosomal enzymes in tissue fragments of mouse liver and kidney. The working hypothesis was that the known anti-stress action of progesterone could have to do with the inhibition of lysosomes which are engaged in apoptotic and oxidative stress-induced responses. The study investigated the effects of exogenous progesterone in chronically (3 weeks) protein-malnourished (10% protein) mice on the activities of lysosomal hydrolases in liver and kidney tissues. Progesterone was injected intraperitoneally in a dose of 2 µg/g body mass dissolved in a vehicle volume of 10 µL/g body mass during the final 3 days of exposure to either low 10% or standard 16% protein content in the chow. After euthanizing the animals, tissue fragments of liver and kidney assayed for the content of lysosomal enzymes. The results demonstrated the stimulating effect of protein malnutrition on lysosomal activities. We further found, contrary to our hypothesis, that progesterone supplementation during both standard and low-protein conditions enhanced lysosomal activities, particularly acting in concert with protein malnutrition in kidney tissue. The effects were selective concerning both lysosomal enzymes and tissues and of highly variable magnitude. Nonetheless, we believe we have shown that progesterone assists protein malnutrition in stimulation of lysosomal enzymes, which suggests the possibility of the hormone's engagement in cleansing the cellular milieu in disorders consisting of accumulation of toxic molecules.

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1   Introduction

Lysosomal enzymes are involved in the physiological degradation and recycling of cellular components, and thus in the maintenance of cellular homeostasis, which determines the adaptability of cells in response to changes in the surrounding environment. Beside the involvement in autophagy, these enzymes also are at play in the progression of apoptosis, notably in cellular responses to oxidative stress-induced apoptotic responses (Stanisławska et al. 2018; Brunk et al. 2001). Dysfunction of apoptosis may worsen the course of a spate of disorders, namely of atherosclerotic or neurodegenerative background, often having to do with enhanced oxidative stress. In such conditions enhancement of lysosomal hydrolytic activity might seem advantageous. However, there is an obvious dichotomy concerning the issue of lysosomal function, as in other pathologies, for instance, in diabetes where the enzymes are released from ruptured cell lysosomes, their degradative proteolytic effects may factor in microvascular diabetic complications (Witek et al. 2018).

Progesterone is produced by luteal cells of the corpus luteum in female ovaries and it enables the implantation of an embryo in the uterine mucosa and its maintenance during the beginning weeks of pregnancy. An adequate content of progesterone also is important for the proper course of a menstrual cycle and for the exfoliation of endometrium (Wu et al. 2018; Wessel et al. 2014). Progesterone also is a precursor of steroid hormones (Clark et al. 2018). Thus, progesterone's physiological action goes much beyond the preparation for pregnancy and its maintenance (De Ziegler et al. 2018). Progesterone is considered a hormone of adaptation and resistance to stress, including oxidative stress, and it has an anti-inflammatory function (Aksoy et al. 2014; Nadal et al. 2009). During stress, progesterone mitigates the production of adrenal cortisol and thus plays a protective role against the harmful effects of excess adrenocortical hormones (Miller 2018). Reproductive physiology in female mice is akin to that of other mammals concerning the peak secretion of progesterone in the estrus and post-estrus phase of the estrous cycle, except the cycle repeats itself every 5 days or so (Sato et al. 2016).

In this study we reasoned that the action of progesterone could have to do with the inhibition of lysosomes which are engaged in apoptosis and oxidative stress. We further hypothesized that any such action of progesterone could better come to light on the background of enhanced hydrolytic enzyme activity. Protein malnutrition is one condition that is reportedly reported to increase lysosomal enzyme activity, cellular catabolic rate, apoptosis, and oxidative stress (Mahadik et al. 2006; Iyengar and Vakil 1985). Therefore, we sought to define the effects of progesterone supplementation on the activities of a spectrum of lysosomal enzymes in the liver and renal tissues on the background of chronic protein-malnourished mice (10% protein), compared with standard protein nutrition (16% protein in chow). Contrary to our hypothesis, progesterone supplementation acted in concert with protein malnutrition to enhance the activities of lysosomal enzymes.

2   Methods

2.1   Animals and Study Protocol

The study was conducted in 60 Swiss female mice, weighing of 25.0 ± 1.3 g, aged 6 weeks. The animals came from the breeding farm of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzębiec, Poland. They were kept at 12-h light/dark cycle at a temperature of 20–22 °C with water ad libitum. The animals were divided into two groups of 30 each, differing in the content of protein provided in the chow: standard 16% protein content and low 10% protein content; the former providing 14.04 MJ/kg and the latter providing 13.47 MJ/kg of daily energy intake ($p > 0.05$). After 21 days, the two groups were further divided into the progesterone and control subgroups of 15 mice each. The former subgroup received i.p. injections of progesterone (Jelfa SA,

Jelenia Gora, Poland) in a dose of 2 μg/g body mass dissolved in a volume of 10 μL/g body mass of *oleum pro injectione*. The injections for the latter group consisted of *oleum pro injectione* alone, given in like manner. The scheme of injections was the same in both groups and consisted of a single injection daily, made at 8:00 am for three consecutive days.

At the time of weaning away from the mothers at the age of 6 weeks, the mice showed diestrous behavior. The quiescent period of the reproductive cycle was confirmed by the measurement of plasma progesterone at baseline before the commencement of its administration. The level of progesterone was 14.5 ± 2.7 ng/mL and 15.8 ± 3.4 ng/mL in the 16% protein and 10% protein groups, respectively; $p > 0.05$. The dose of exogenous progesterone was chosen on the inferential basis, supported by a couple of pilot trials, so that it would cause a 50–60% increase in blood progesterone content. The presumptive reasoning was that this kind of progesterone excess would be sufficient to induce changes in the activity of the lysosomal enzymes investigated; yet not interfering with the course of systemic physiological processes.

2.2　Lysosomal Enzymes

Six hours after the last injection of progesterone on Day 3 of the experimental paradigm, the mice of all groups were decapitated, and the kidneys and liver were immediately removed. The organs were superfused with chilled 0.9% NaCl and tissue fragments were homogenized in 0.1 mM phosphate buffer of pH 6.0 at a concentration of

0.5 g of solute in a final volume of 5 mL of buffer solution (10% m/v) at 200 RPM (Potter Elvehjem; Thomas Scientific; Swedesboro, NJ). Homogenates were further subjected to fractionated centrifugation, according to the method of Beaufay (1972), to obtain a lysosomal tissue fraction. Then, activities of the following lysosomal hydrolases were determined: acid phosphatase, β-N-acetyl-hexosaminidase, β-galactosidase, β-glucuronidase, lysosomal esterase, lysosomal lipase, alanine aminopeptidase, and leucine aminopeptidase. The exact method use for the determination of each lysosomal hydrolase, including the substrates used is depicted in Table 1.

Extinction measurements were made at 520 nm in a Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific; Waltham, MA). The protein content was determined by a modified method of Kirschke and Wiederanders (1984). The activity of lysosomal enzymes was expressed in nmol/mg protein/h. All substrates were produced by SERVA Feinbiochemica (Heidelberg, Germany).

Data were presented as means $\pm$SD. Differences in the mean values of enzyme activity were assessed using a paired or unpaired two-tailed *t*-test as required. A p-values <0.05 defined statistically significant differences.

3　Results

Blood plasma content of progesterone increased after the 3 days of progesterone administration from 14.5 ± 2.7 to 23.4 ± 0.8 ng/mL and from 15.8 ± 3.4 to 26.6 ± 1.2 ng/mL in the standard

Table 1 Lysosomal enzymes investigated

Enzyme	Symbol and EC number	Method
Acid phosphatase	AcP (EC 3.1.3.2)	Barrett and Heath (1977)
β-N-acetyl-hexosaminidase	Hex (EC 3.2.1.52)	
β-galactosidase	β-Gal (EC 3.2.1.23)	
β-glucuronidase	β-GlcUr (EC 3.2.1)	
Lysosomal esterase	EL (EC 3.1.1.2)	
Lysosomal lipase	LL (EC 3.1.1.13)	
Alanine aminopeptidase	AlaAP (EC 3.4.11.2)	McDonald and Barrett (1986)
Leucine aminopeptidase	LeuAP (EC 3.4.11.1)	

Table 2 Activity of lysosomal enzymes (nmol/mg protein/h) in the mouse liver after 3-day-long progesterone administration on the background of standard 16% nutritional protein and protein restriction to 10%

Enzyme	16% Protein		10% Protein	
	Control	Progesterone	Control	Progesterone
AcP	4.65 ± 1.17	6.79 ± 1.99**	43.00 ± 4.07†††	41.70 ± 5.57
Hex	3.56 ± 1.01	5.72 ± 1.89***	24.70 ± 3.04†††	24.40 ± 3.53
β-Gal	9.01 ± 2.05	14.7 ± 5.32***	3.13 ± 0.48†††	4.16 ± 0.99*
β-GlcUr	0.38 ± 0.10	0.52 ± 0.01*	0.41 ± 0.06	0.52 ± 0.06*
EL	3.35 ± 0.80	2.97 ± 0.74	2.17 ± 0.20††	3.41 ± 0.42***
LL	3.49 ± 0.72	5.36 ± 1.03***	3.04 ± 0.46	4.19 ± 0.71*
AAP	4.98 ± 1.23	6.54 ± 1.04*	13.90 ± 1.07†††	23.10 ± 3.58***
LAP	3.22 ± 0.87	4.64 ± 1.09**	26.40 ± 2.91†††	30.80 ± 1.95

Data are means ±SD. *AcP* acid phosphatase, *Hex* β-N-acetyl-hexosaminidase, *β-Gal* β-galactosidase, *β-GlcUr,* β-glucuronidase, *EL* lysosomal esterase, *LL* lysosomal lipase, *AAP* alanine aminopeptidase, *LAP* leucine aminopeptidase, *Cath D* cathepsin D

*$p \leq 0.05$; **$p \leq 0.01$; and ***$p \leq 0.001$ for differences between progesterone and control in either protein content; ††$p < 0.01$ and †††$p < 0.001$ for differences in the control level, i.e., the effect of protein restriction alone

16% protein and 10% protein malnourished groups, respectively. The increases of plasma progesterone were significant ($p < 0.001$) in either group, but the differences between the two groups were not ($p > 0.05$).

Changes in the activities of lysosomal enzymes in both liver and renal tissues in response to chronic protein malnutrition and the effects of progesterone administration on these activities are presented in Tables 2 and 3. Protein restriction caused distinct several-fold increases in the content of acid phosphatase, hexosaminidase, alanine aminopeptidase, and leucine aminopeptidase and decreased β-galactosidase in both tissues, with the activity of the other enzymes being rather mildly affected.

Administration of progesterone on the background of the standard 16% protein content in the chow caused significant increases in all enzymes in both tissues, except lysosomal esterase in the liver and β-galactosidase, β-glucuronidase, and alanine aminopeptidase in the kidney, whose content fluctuated around the baseline level. Likewise, there were strong increases in the enzyme activities after progesterone administration on the background of nutritional protein restriction to 10%. The content of all enzymes significantly increased in both tissues, except acid phosphatase and β-N-acetyl-hexosaminidase in the liver and alanine aminopeptidase in the kidney. None of the enzymes in either liver or kidney and at either

nutritional protein level was significantly inhibited by progesterone administration.

4   Discussion

The findings of this study were that the plasma content of the majority of lysosomal enzymes investigated increased in the mouse tissue fragments of both liver and kidney during protein malnutrition. However, stimulation of hydrolytic activity was rather selective and of highly variable magnitude. Notably, there were many-fold increases in acid phosphatase, β-N-acetyl-hexosaminidase, and leucine aminopeptidase activities; the major proteolytic enzymes responsible for breaking down toxic molecules and participating in peptide recycling. On the other side, β-galactosidase was inhibited by about 75% and 50% in the liver and kidney tissues, respectively. There was an apparent tissue selectivity of the enzyme responses as β-glucuronidase and lysosomal lipase tended changed inversely in the liver and renal tissues.

Administration of progesterone caused further significant increases in the activities of enzymes on the background of protein restriction-induced stimulation. The increases remained enzyme and tissue selective and highly variable, amounting to about 22% for alanine aminopeptidase in both tissues, 63% and 182% for β-galactosidase in

the liver and kidney, or about 50% and 100% for lysosomal esterases and lipases in the liver and kidney, respectively. Progesterone administration also increased lysosomal enzyme activities during the standard protein nutrition. The findings give a consistent impression that protein restriction with progesterone on top of it potentiated the increases in lysosomal enzymes, particularly concerning the kidney. However, a substantial spread of the enzyme data in the protein conditions used in this study makes a meaningful direct comparison unfeasible. Nonetheless, it seems a reasonable assumption that enhanced activity of lysosomal enzymes noticed would be an expression of intensified engagement in cleansing the liver and renal cellular milieu from biological debris to revert to normal homeostatic function.

The present findings are in line with previous studies showing an activation of lysosomal enzymes during protein malnutrition. Iyengar and Vakil (1985) have observed stimulation of selective lysosomal enzymes during restriction of protein intake in the rat. Aside from the stimulation, the authors report an inhibition of β-glucuronidase activity by 60% in 4% protein-fed rats. In the present study, we noticed about 33% inhibition of the enzyme's activity in 10% protein-fed mice in the kidney, but not the liver. Likewise, Tutel'ian et al. (1987) have shown an increase in lysosomal enzyme activities in the rat liver during restriction of protein intake. Glew et al. (1982) have shown a threefold increases in serum hexosaminidase and 0.5-fold increase in beta-glucuronidase activities on 3% casein diet for 2–4 weeks in the rat kidney, but decreases in acid phosphatase. The authors ascribe such changes to profoundly altered tissue blood perfusion during protein malnutrition, leading to secondary release of lysosomal enzymes. Muñoz-Martínez et al. (1982) have found a strong increase in beta-glucuronidase in the rat liver during protein intake restriction to 1%. In contrast, acid phosphatase remained unmodified. Muñoz et al. (1981) have also found increases in beta-glucuronidase and acid phosphatase in the rat spleen and thymus after 4 weeks of protein intake restriction to 4%, although there were decreases in some other hydrolytic enzymes.

Yet, the effects of protein intake restriction on lysosomal enzyme activity remains a contentious issue. Other studies show an inhibition of lysosomal enzyme during protein restriction (Volgarev et al. 1984). It appears that the mechanisms regulating catabolic activity of lysosomes are unclear. Divergent literature data are likely due to different protocols of protein malnutrition, varying from 1% through 4%, 8%, and 14% protein content in the chow, different periods of exposure, different animal species, and different tissues being investigated, let alone the methodological differences of assays used in the past studies. The lack of experimental standardization makes it hard to draw definite conclusions on the issue.

In this study, interestingly, the majority of lysosomal hydrolases increased their activity under the influence of exogenous progesterone. That raises a suggestion that progesterone, one of the main female fertility regulating hormones, acts to intensify the catabolic rate of liver and kidney cells. These effects seem intensified by protein intake restriction. The observed increases in the activity of glycosidases (β-Gal and β-GlcUr) could be associated with increased glucose production and a breakdown of own proteins in the framework of renal cell response to decreased protein intake (Kilicalp et al. 2005). How exactly progesterone would cause an increase in the activities of lysosomal enzymes is however open to conjecture.

An incorrectly balanced level of protein intake causes negative consequences in the area of cellular protein degradation. Alleman et al. (2000) have reported that a downward shift in protein intake, from 16% to 10%, in chickens increases the content of triiodothyronine, thyroxine, and insulin-like growth factor-1 in the blood, which, in turn, increases the amino acid transport to muscles and the intensity of ongoing proteolytic processes. As a consequence, alanine aminopeptidase activity may increase as well. In the present study, alanine aminopeptidase increased by about 180% and 55% in the mouse liver and kidney, respectively, in response to protein intake restriction to 10%. The increases could be a natural adaptive cellular reaction since protein

Table 3 Activity of lysosomal enzymes (nmol/mg protein/h) in the mouse kidney after 3-day-long progesterone administration on the background of standard 16% nutritional protein and protein restriction to 10%

Enzyme	16% Protein		10% Protein	
	Control	Progesterone	Control	Progesterone
AcP	14.40 ± 2.59	$19.70 \pm 4.46*$	$30.50 \pm 2.98^{†††}$	$53.70 \pm 5.03***$
Hex	2.83 ± 0.41	$3.96 \pm 0.94*$	$28.60 \pm 2.58^{†††}$	$43.10 \pm 3.71***$
β-Gal	7.30 ± 0.81	7.97 ± 1.62	$3.58 \pm 0.29^{†††}$	$10.10 \pm 0.69***$
β-GlcUr	0.40 ± 0.10	0.35 ± 0.07	$0.27 \pm 0.04^{†††}$	$0.62 \pm 0.06***$
EL	3.72 ± 0.93	$5.02 \pm 1.32*$	$2.69 \pm 0.30^{†}$	$5.29 \pm 1.05***$
LL	0.48 ± 0.05	$0.62 \pm 0.09*$	0.53 ± 0.03	$1.09 \pm 0.06***$
AAP	120.60 ± 13.3	107.10 ± 9.29	$187.70 \pm 13.30^{†††}$	229.10 ± 25.20
LAP	42.00 ± 6.92	$64.90 \pm 16.50***$	$126.60 \pm 7.71^{†††}$	$171.90 \pm 14.10*$

Data are means $\pm$SD. $*p \leq 0.05$ and $***p \leq 0.001$ for differences between progesterone and control in either protein condition; $^{†}p < 0.05$; $^{††}p < 0.01$, and $^{†††}p < 0.001$ for differences in the control level, i.e., the effect of protein restriction alone. See Table 2 for the acronyms of lysosomal enzymes

malnourished animals show a positive nitrogen balance for a period of time, while lowering the level of urea.

Lysosomal esterase and lipase were little altered with protein restriction in this study. However, a combination of progesterone with either standard or lowered protein content strongly enhanced the activity of both enzymes (Tables 2 and 3), which are mostly engaged in the degradation of fatty acid esters and cholesterol. These findings are in line with those of Yoshizawa et al. (1997), who have reported that protein malnutrition causes increases in free fatty acids and glycerol in the blood and in the percentage of fat in the liver and kidneys, which may increase the need for the action of lipases. An enhancement of the activity of both enzymes under the influence of progesterone implies that the hormone accelerates tissue lipid turnover.

In clinical diagnostics and treatment, lysosomal enzymes are increasingly used to assess the extent of genetic lysosomal storage disorders, such as Fabry's or Gaucher's diseases, and others in which there is an insufficient degradative processing of sphingolipid molecules that accumulated in cells, mostly in the brain (Giugliani et al. 2018). The enzyme replacement therapy faces hurdles since recombinant enzymes cannot sufficiently penetrate through the blood-brain barrier. The findings of the present study and other past studies showing the enhancement

of lysosomal enzymes during low-protein nutrition offer a simple approach to enzyme activation. Likewise, exogenous supplementation of progesterone seems a similar effective route to increase the activity of lysosomal enzymes. Whether this would also be an effective measure to increase lysosomal enzyme activity in brain tissue is unknown. The low-protein-progesterone supplementation method would require trial investigations and tailoring to the specific clinical entities and individual patients, as well as the explorations addressing individual enzymes, all of which requires alternative study designs.

In conclusion, the findings of this study hint on the potential interaction of low protein nutrition and modest supplementation of progesterone in enhancing lysosomal enzyme activity. To the extent that lysosomes serve as the cell's detoxifying and molecular waste recycling centers, the enhancement of their function might have a bearing in disorders that consist of excessive intra-cellular accumulation of pathological molecules.

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Infections Caused by Influenza Viruses Among Children in Poland During the 2017/18 Epidemic Season

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Abstract

Influenza is an infectious disease that is a threat to both children and adults. The most effective way to prevent infections among children is seasonal vaccination in every epidemic season, which is recommended from the age of 6 months onward. This study is a report of the prevalence of influenza infection in the population of children up to the age of 14 years and of the type of influenza virus involved during the 2017/18 epidemic season in Poland. We found that influenza A and B viruses co-dominated in the season. Among the influenza A viruses, A/H1N1/ pdm09 subtype was a more frequent source of infection than A/H3N2/ subtype. In addition, the prevalence of infection was re-analyzed in children stratified into the age groups of 0–4, 5–9, and 10–14 years old. We found a relation between the age of a child and the type of influenza virus causing infection. The youngest children under 4 years were the most vulnerable to both influenza and influenza-like infections; the former caused mostly by influenza A and the latter by RSV. In contradistinction, influenza B dominated in the oldest children aged 10–14 and RSV infections were not present in this age group. The characteristics of influenza viruses may however vary on the seasonal basis.

Keywords

Children · Infection · Influenza · Molecular biology · Respiratory tract · Virology

1   Introduction

The most characteristic symptom of influenza is a sudden onset of fever ($\geq$38 °C), sore throat, muscle pain, cough, and general weakness (Brydak 2008). The diagnosis is particularly difficult among children in whom verbal communication is not yet developed (Wrotek et al. 2018).

The most effective way to prevent the infections among children is seasonal flu vaccination administered every season (Committee on Infectious Diseases 2017). According to the Advisory Committee on Immunization Practices in the USA, a trivalent vaccine is recommended from the age of 6 months for the 2017/18 epidemic season (Grohskopf et al. 2017). Unfortunately, the highest percentage of hospitalizations and deaths is recorded in infants <6 months of age

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(Zawłocka et al. 2016). To protect newborns who are at high risk of complications after influenza infection, a cocoon vaccination strategy is recommended. People from the immediate infant environment, such as parents, grandparents, or siblings, are vaccinated against influenza, which protects the child against the illness in a secondary manner (Nitsch-Osuch 2017). The quadrivalent vaccines, which have been already developed, are recommended from the age of 3 years due to the lack of available research in younger children. This study seeks to define the prevalence of influenza infection in the population of children up to the age of 14 years and of the type of prevailing influenza virus causing infection among children during the 2017/18 epidemic season in Poland.

2   Methods

The study group included children up to 14 years of age, with an additional division into three successive age groups of 0–4, 5–9, and 10–14 years old. The material for the study were nasal and throat swabs taken during the 2017/18 epidemic season and analyzed in 16 Voivodship Sanitary and Epidemiological Stations and in the Department of Influenza Research, National Influenza Center in the National Institute of Public Health – National Institute of Hygiene (NIPH-NIH) in Warsaw, Poland. During the epidemic season, 1286 samples were tested for influenza and reported in the Sentinel and Non-Sentinel Influenza Surveillance System.

The ribonucleic acid (RNA) was isolated from the nasal and pharyngeal swabs. A Maxwell 16 Viral Total Nucleic Acid Purification Kit was used (Promega Corporation; Madison, WI), according to the instructions provided by the manufacturer. From 200 μl of clinical samples, suspended in 1 ml of physiological saline, 50 μl of RNA resuspended in RNase-free water was obtained. Molecular tests were performed to confirm the presence of influenza A and B viruses and to determine the viral subtypes. The Light Cycler 2.0 System was used (Roche Diagnostics; Rotkreuz, Switzerland). The primers and probes were obtained from the International Reagent

Resource (IRR) run by the Centers for Disease Control and Prevention (CDC). The reaction was carried out according to the manufacturer's instructions. RNA was subjected to reverse transcription (at 50 °C for 30 min). The obtained DNA was subjected to the initiating denaturation process (1 cycle at 95 °C for 2 min), followed by 45 cycles of amplification: denaturation at 95 °C for 15 s, annealing at 55 °C for 10 s, and elongation at 72 °C for 20 s. Positive control was the viral RNA obtained from the strains used for the 2017/2018 vaccine (A/Michigan/45/2015 (H1N1)pdm09, A/HongKong/4801/2014 (H3N2), and B/Brisbane/60/2008/). Negative control was the water free from RNase.

Using the RT-PCR reactions, the presence of the following respiratory viruses was confirmed: influenza A virus, influenza B virus, human respiratory syncytial virus A and B, human adenovirus, human metapneumovirus, human coronavirus 229E/NL63, human coronavirus OC43, human parainfluenza 1, 2, 3, and 4 viruses, human rhinovirus A/B/C, human enterovirus, and human bocavirus 1/2/3/4. The RV15 OneStep ACE Detection Kit (Seeplex; Seoul, South Korea) was used for these reactions, according to the manufacturer's instructions. After the reaction, the product was separated on a 2% agarose gel using electrophoresis.

Discrete data were presented as counts and proportions. Differences between the proportions of infected people were compared with a *t*-test. A value of zero was assumed for the noninfected person and a value of one for the infected one, and the arithmetic mean was used to calculate the percentage of the infected persons. A *p*-value <0.01 defined statistically significant differences. The prevalence of influenza in the age groups was determined with 95% confidence intervals (95%CI).

3   Results

In the 2017/18 epidemic season, there were 1286 samples tested from children up to 14 years of age. We noticed the co-dominance of influenza A and B viruses in the whole of children's population; 53.9% and 46.1%, respectively. Among the

influenza A viruses, subtype A/H1N1/pdm09 was confirmed in 18.5% of all positive samples and it prevailed over subtype A/H3N2/ (Fig. 1).

In the children's population stratified into the age groups of 0–4, 5–9, and 10–14 years, influenza A virus dominated in 0–4 year olds, accounting for 28.8%. The infections caused by influenza B virus were noticed in 14.4% in this group. A different situation was noticed in the age groups of 5–9 and 10–14 years, where influenza B virus dominate, accounting for 34.1% and 31.5% of infections, respectively. In all three age groups, the unsubtyped infections of influenza A dominated, accounting for 17.8%, 17.2%, and 10.7% of infections, respectively, whereas subtype A/H3N2/was present in the lowest proportions; 0.4%, 1.2%, and 0.7%, respectively (Fig. 2).

The results were also analyzed taking into account only the type of influenza virus and the patient's age. There were a significantly lower percentage of children infected with influenza A virus in the age group 10–14 years than in the groups 0–4 and 5–9 years ($p < 0.001$ and $p < 0.01$, respectively). The difference in the number of influenza A virus infections between the age groups 0–4 and 5–9 years was insignificant ($p = 0.21$). In addition, there was a significantly higher percentage of children infected with influenza B virus in the age groups 5–9 and

10–14 years than in the group 0–4 years ($p < 0.001$). The difference in the number of influenza B virus infections between the age groups 5–9 and 10–14 was insignificant ($p = 0.28$).

A comparative analysis of the 2017/18 and 2016/17 epidemic seasons was also performed for the age group 0–4 years. In the former season, there was a clear dominance of influenza A (111 infections) and subtype A/H3N2/ (74 infections). In contradistinction, in the latter season, co-dominance of influenza A and B viruses (209 and 104 infections, respectively) was recorded. There was a clear difference in the dominant subtype of the influenza A virus. In the former season, it was subtype A/H3N2/, while in the latter season it was A/H1N1/pdm09. In the 2016/17 season, there were only two infections of influenza B virus and none of A/H1N1/pdm09 virus infection (Fig. 3).

The influenza-like infections were reported in the 0–4 and 5–9 age groups and accounted for 81.2% and 18.8%, respectively (Fig. 4). Among children aged 10–14 years, no influenza-like virus was reported. Influenza-like viruses were dominated by RSV (30 infections). The highest number of infections with this virus was recorded in the age group 0–4 years. In this group, there also were four PIV-3 infections and single infections with other influenza-like viruses.

Fig. 1 Proportions of influenza infections in children aged 0–14 years in the 2017/18 epidemic season
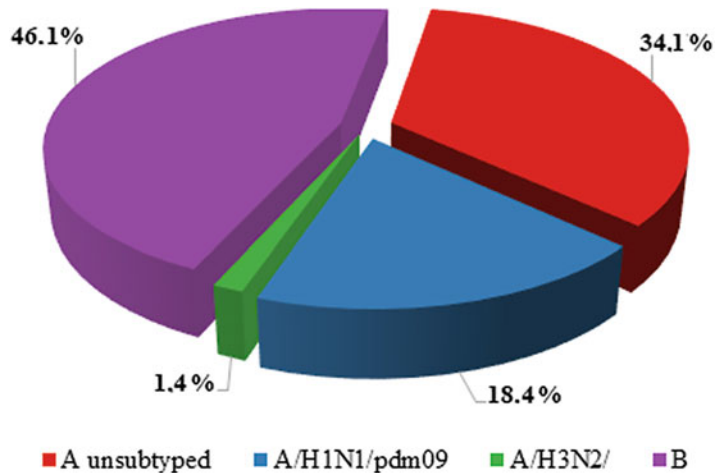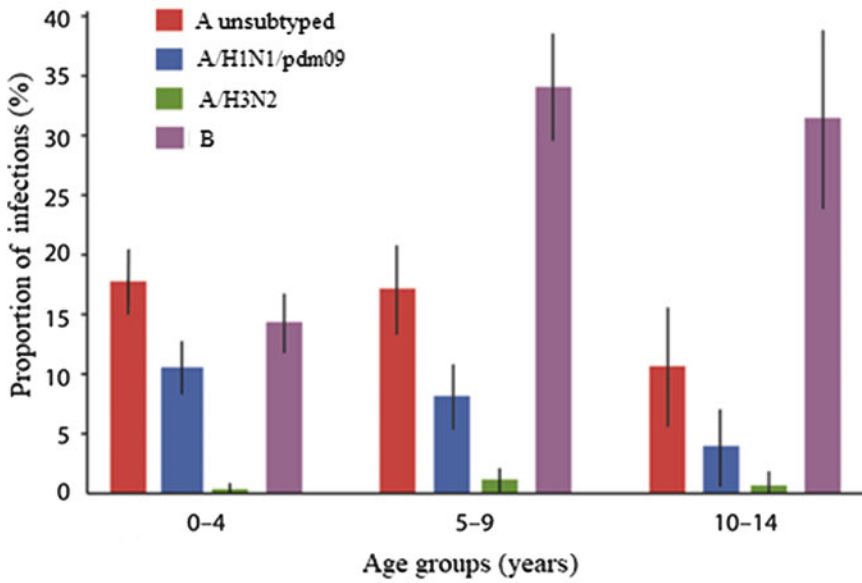
Fig. 2 Proportions of influenza virus infections in children stratified into three successive age groups in the 2017/18 epidemic season. Data are means ±SD
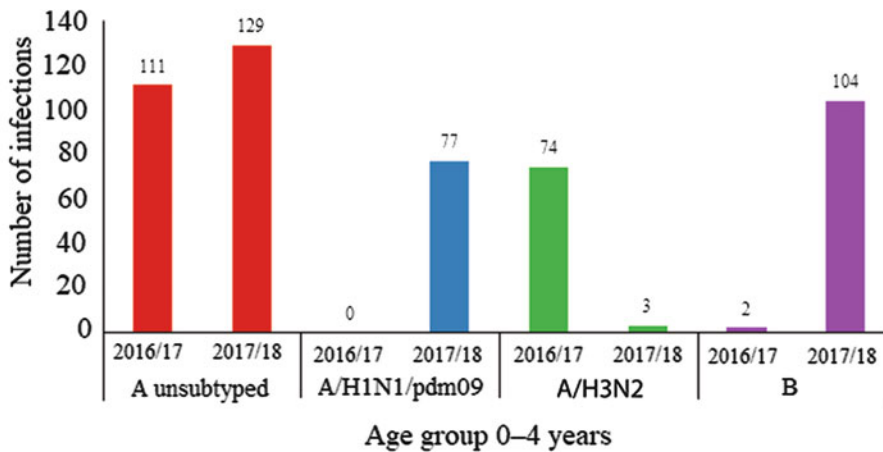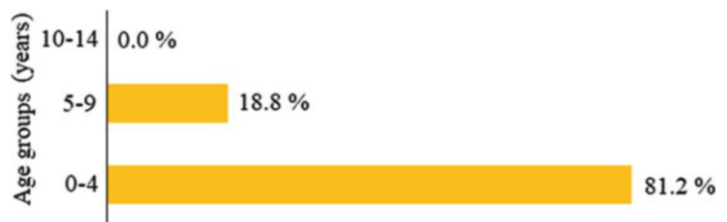

Fig. 3 Number of confirmed influenza infections in the age group 0–4 years in the 2016/17 and 2017/18 epidemic seasons

Fig. 4 Proportions of confirmations of influenza-like infections in children stratified into three successive age groups in the 2017/18 epidemic season

4   Discussion

In the 2017/18 epidemic season, influenza A and B viruses co-dominated in the population of children under the age of 14 years, with a margin of influenza B over A. These results are akin to those noticed in other European countries, e.g., Denmark or Spain (Flu News Europe 2018). Among the influenza A viruses, infections with subtype A/H1N1/pdm09 dominated over A/H3N2/. This season had entirely different characteristics from the preceding 2016/17 season in Poland when no A/H1N1/pdm09 infections had been reported among children (Cieślak et al. 2018).

An interesting tendency appeared in the analysis of the prevailing viral contagion by children's age. In the older children of 5–9 and 10–14 years, there was a significantly higher incidence of influenza B infection than in the youngest children of 0–4 years. That is in line with the past results from other European research centers. In one of them, results from 12 European countries have been analyzed with reference to the 2012/13 season and the dominance of influenza B virus is reported in children aged 5–14 years (Beauté et al. 2015). In another study, the relation between influenza virus type and subtypes and the age of children was determined. That analysis covers 29 countries around the world in the years 1999–2014. It has been confirmed that infections with influenza B virus most often occur in the 5–17 age bracket (Caini et al. 2018).

The present report demonstrates the lowest percentage of influenza A in the oldest children aged 10–14. Further, infection with subtype A/H3N2/ constituted the lowest percentage of influenza A in each age group. This last trend seems to have been in line with the notion put forward by Adlhoch et al. (2018) suggesting that subtype A/H3N2/ may be the most common in older persons, particularly aged over 65 rather than in children. This report also demonstrates that unsubtyped influenza A was the most prevalent across all the age groups of children. This finding, however, may contain a spurious component due to possibly ineffective subtyping of influenza virus or occasional lack of

subtyping it at all. Despite these limitations, we believe we have demonstrated in this report that there is a relation between the child's age and the type of influenza virus causing infection. In the 2017/18 epidemic season, the youngest children under 4 years were the most vulnerable to both influenza and influenza-like infections, the former caused mostly by influenza A and the latter by RSV. In contradistinction, influenza B dominated in the oldest children aged 10–14 and RSV infections were not present in this age group. However, characteristics of influenza viruses are highly changeable season to season. These data may thus not exactly apply to each and every epidemic season.

Conflicts of Interest The authors declare no conflict of interests in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by an institutional Ethics Committee.

Informed Consent Informed consent was obtained from all individual participants included in the study before collection of nasopharyngeal samples.

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The Influence of National Guidelines on the Management of Community-Acquired Pneumonia in Children. Do Pediatricians Follow the Recommendations?

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Abstract

This is a retrospective study whose main objective was to analyze the influence of the Polish Guidelines for the Management of Respiratory Tract Infections of 2010 (PGMRTI) on in-hospital treatment of children with community-acquired pneumonia (CAP). Files from four Warsaw hospitals were reviewed to identify children with uncomplicated CAP, treated before (2008–2009) (pre-PGMRTI) and after (2011–2012) (post-PGMRTI) publication of the guidelines. Predefined data on the management were compared. A cohort of 2,359 children (1,081 pre-PGMRTI and 1,278 post-PGMRTI) was included. We found that co-amoxiclav was the most common first-line therapy in children >3 months of age (34.6% and 40.4% pre- and post-PGMRTI, respectively), followed by cefuroxime (31.8% and 20.9% pre- and post-PGMRTI, respectively; $p < 0.0001$) and macrolides (17.4% and 24.5% pre- and post-PGMRTI, respectively; $p < 0.0001$). Amoxicillin was rarely used (5.4% and 4.9%, pre- and post-PGMRTI, respectively). The study revealed an overuse of inhaled bronchodilators, corticosteroids, and mucoactive drugs. Blood diagnostic tests were applied to a significant percentage of patients: blood cultures (41.2% and 44.5%

pre-and post-PGMRTI, respectively) and serology for atypical pathogens (27.9% and 44.9% pre-and post-PGMRTI, respectively; $p < 0.0001$). The number of follow-up chest X-rays increased (30.5% and 53.8% pre- and post-PGMRTI, respectively; $p < 0.0001$). In conclusion, the study demonstrates an unsatisfactory influence of the guidelines on in-hospital management of CAP in children. Despite an explicit recommendation for the use of amoxicillin, it was still underused. Other methods of education and guideline dissemination are needed to optimize the prescribing of antibiotics.

1 Introduction

Community-acquired pneumonia (CAP) is one of the most common infections in children with the annual incidence of 36–46 per 1000 children younger than 5 years of age and about 16 per 1000 in an older age group (Black et al. 2002; Heath 2000). The diagnosis of CAP is based on clinical signs and symptoms as well as laboratory and radiological findings, which are not specific enough to reliably suggest the etiology of infection (Dean and Florin 2018; Principi and Esposito 2018; Korppi 2004; Virkki et al. 2002; Nohynek et al. 1995). In most cases, the causative microorganism of CAP is unknown. This is due to the difficulty of obtaining adequate material from the lower respiratory tract and a low yield of positive blood cultures. Therefore, treatment of CAP is usually empirical. The current epidemiological data about the most common pathogens causing pneumonia and their patterns of resistance allow clinicians to choose optimal treatment. Since *S. pneumoniae* remains the most important pathogen in childhood CAP, the first-line therapy in most countries are narrow-spectrum penicillins, such as amoxicillin or ampicillin (Principi and Esposito 2018; Bradley et al. 2011; Harris et al. 2011).

Antimicrobial resistance is a growing problem and its prevention is a global challenge. Inappropriate antibiotic use significantly contributes to antibiotic resistance, resulting in reduced efficacy of drugs (WHO 2015; Cunha 2004). One strategy to optimize the use of antimicrobial agents is to develop and promote national guidelines for the management of infectious diseases and the rational use of antibiotics according to local antimicrobial resistance profiles. The Polish Guidelines for the Management of Respiratory Tract Infections (PGMRTI) were published in 2010. The document presents guidelines for all types of respiratory tract infections, including childhood CAP, and supports every aspect of the management, including diagnostic procedures and treatment (Hryniewicz et al. 2016). Despite clear recommendations, clinical practice seems to vary (Deptuła et al. 2018; Ross et al. 2014; Bowen and Thomson 2013). Therefore, the aim of this study was to evaluate the influence of the PGMRTI publication on the management of children hospitalized for CAP in Warsaw, Poland. The primary goal of the study was to evaluate changes in the pattern of antibiotic use before and after the publication of the guidelines. A secondary goal was to compare the differences in symptomatic therapy and in the performance of diagnostic procedures in the same period of time.

2 Methods

This is a retrospective study in which databases of four main pediatric hospitals in Warsaw, Poland were searched for medical files of children diagnosed with uncomplicated CAP before the publication of the guidelines in 2008–2009 (pre-PGMRTI group) and after the publication of the guidelines in 2011–2012 (post-PGMRTI group). The inclusion criteria were as follows: uncomplicated CAP requiring hospital admission and age between 0 and 18 years. The diagnosis of CAP was based on clinical features: fever (>38 °C), dyspnea, cough, and abnormal auscultatory findings such as crackles and bronchial sounds. Pneumonia was confirmed in chest X-rays in most of the cases. Children with CAP who could potentially require nonstandard treatment, like patients with complicated CAP (para-

pneumonic effusion requiring thoracentesis or pleural drainage, pleural empyema, and necrotizing pneumonia) and with chronic conditions like immunodeficiency, aspiration, and cystic fibrosis were excluded.

Medical files were reviewed for data on the diagnostic procedures and treatment. In particular, the rate of prescribing the recommended first-line antibiotics (amoxicillin or ampicillin) was analyzed. The results were compared in the pre- and post-PGMRTI groups. The comparison was made in two age subgroups: 1/0–18 years of age, i.e., all children included, and 2/from 3 months to 18 years of age, i.e., when amoxicillin is recommended as the first-line treatment according to PGMRTI. Additionally, data on symptomatic treatment, such as mucoactive drugs, bronchodilators, inhaled and systemic corticosteroids, as well as the diagnostic tests, including microbiological investigations such as blood cultures, nasopharyngeal swabs (RSV and influenza virus), serology (*M. pneumoniae* and *C. pneumoniae*), sputum culture, and imaging (initial and follow-up chest X-rays) were collected and compared in both study periods.

Continuous variables were expressed as means $\pm$SD and compared between groups using the Mann-Whitney U test. A Chi-squared test and Fisher's exact test were used to assess proportions of children with different diagnostic procedures and treatments in the two groups pre- and post-PGMRTI. A p-value <0.05 defined statistically significant differences. The analysis was performed using a commercial Statistica v12.0 package (StatSoft, Tulsa, OK).

3 Results

Two thousand three hundred and fifty-nine children with CAP were enrolled into the analysis.

There were 1,081 and 1,278 children in the pre- and post-PGMRTI groups, respectively. Children in the pre-PGMRTI group were younger than those in the post-PGMRTI group; 2.7 ± 3.0 vs. 4.0 ± 3.8 years of age, respectively ($p < 0.001$). Demographic details are presented in Table 1.

3.1 Antibiotic Treatment

3.1.1 Children Aged 3 Months to 18 Years

Two thousand and fifty-seven children were aged ≥ 3 months; 906 children in the pre-PGMRTI group and 1,151 in the post-PGMRTI group. The most common antibiotic used in the respective groups was co-amoxiclav – 34.6% and 40.4% ($p = 0.3$), followed by cefuroxime – 31.8% and 20.9% ($p < 0.0001$), macrolides – 17.4% and 24.5% ($p < 0.0001$), third-generation cephalosporins – 8.2% and 5.3% ($p = 0.009$), and amoxicillin – 5.4% and 4.9% ($p = 0.300$). A combination of two antibiotics, i.e., amoxicillin and a macrolide, was prescribed in 45 children (5%) pre-PGMRTI and in 158 children (13.7%) post-PGMRTI. Five children (0.5%) in the pre-PGMRTI group and 30 (2.6%) children in the post-PGMRTI group were treated without antibiotics (Fig. 1).

3.1.2 Children Aged Less than 3 Months

There were 175 (16.2%) and 127 (9.9%) children younger than 3 months of age in the pre-PGMRTI and post-PGMRTI groups, respectively. The most common antibiotics used in the respective groups were co-amoxiclav – 46.3% and 59.1% ($p = 0.036$), followed by cefuroxime – 26.9% and 11.8% ($p = 0.001$).

Table 1 Demographics of study population

	pre-PGMRTI	post-PGMRTI	
Total; n	1081	1278	p
Mean age $\pm$ SD; years	2.7 ± 3.0	4.0 ± 3.8	<0.001
0 to 3 months of age; n (%)	175 (16.2)	127 (9.9)	<0.001
4 months to 18 years of age; n (%)	906 (83.9)	1,151 (90.0)	<0.001

PGMRTI Polish Guidelines for the Management of Respiratory Tract Infections of 2010
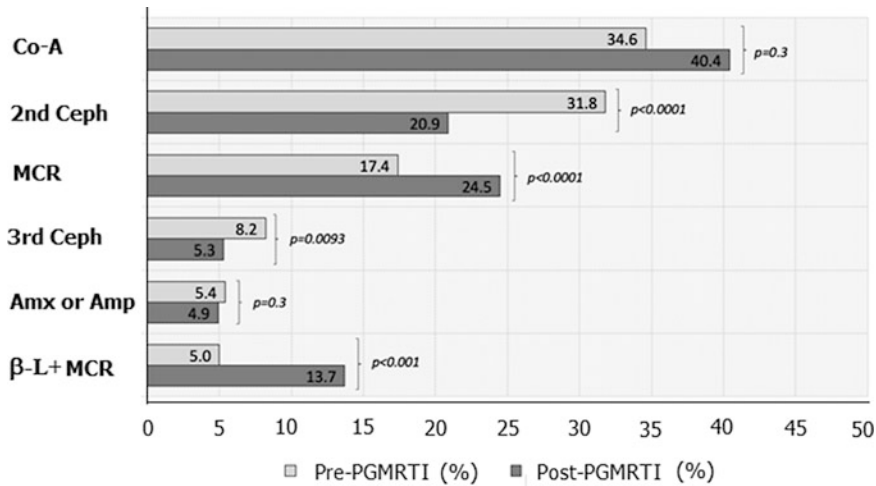
Fig. 1 First-line antibiotics used in children ≥3 months. *Co-A* co-amoxiclav, *2nd Ceph* second-generation cephalosporins, *3rd Ceph* third-generation cephalosporins, *Amx or Amp* amoxicillin or ampicillin, *β-L + MCR* beta-lactam antibiotic and macrolide, *PGMRTI* Polish Guidelines for the Management of Respiratory Tract Infections of 2010
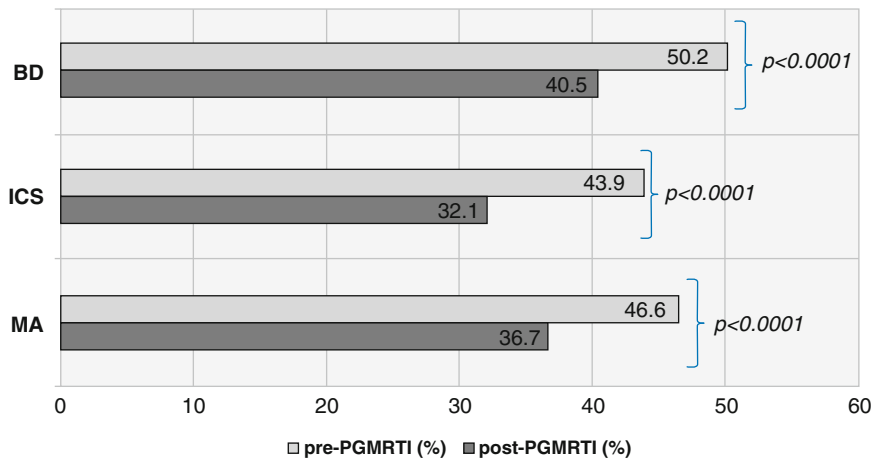

Fig. 2 Adjunctive treatment in children with community-acquired pneumonia (CAP). *BD* bronchodilators, *MA* mucoactive drugs, *ICS* inhaled corticosteroids, *PGMRTI* Polish Guidelines for the Management of Respiratory Tract Infections of 2010

3.2   Additional Treatment

3.2.1   Mucoactive Drugs

Data concerning the use of various adjunctive drugs in children with CAP are displayed in Fig. 2. We found that more children were treated with mucoactive drugs in the pre-PGMRTI group (46.6% vs. 36.7% post-PGMRTI; $p < 0.0001$). Ambroxol was the most common choice both before and after the publication of the guidelines. Acetylcysteine was less frequently prescribed in the pre-PGMRTI group (1.1% vs. 3.7% post PGMRTI; $p = 0.012$).

3.2.2 Inhaled Bronchodilators

A combination of fenoterol with ipratropium bromide or salbutamol alone predominated among inhaled bronchodilators. The former was prescribed more often in the pre-PGMRTI group (40.1% vs. 23.7% post-PGMRTI; $p < 0.0001$). Salbutamol was a less common choice in the pre-PGMRTI group (20.1% vs. 29.0% post-PGMRTI; $p < 0.0001$).

3.2.3 Corticosteroids

Inhaled corticosteroids were prescribed in a significant number of children with CAP. It was a more common practice in the pre-PGMRTI group (43.9% vs. 32.1% post-PGMRTI; $p < 0.0001$). We noticed a similar occurrence concerning systemic corticosteroids (pre-PGMRTI 13.3% vs. 10.2% post-PGMRTI; $p = 0.020$). Children treated with corticosteroids were significantly younger in both study periods; a median age amounted to 26.1 months in the pre-PGMRTI group and to 36.7 months in the post-PGMRTI group. Likewise, the median age of children untreated with corticosteroids was 38.4 months and 53.5 months in the two groups, respectively ($p < 0.0001$). The number of children with asthma in the study group was 122 (5.2%). Asthma was less common among children in the pre-PGMRTI group (3.5% vs. 6.5% post-PGMRTI; $p = 0.001$).

3.3 Diagnostic Procedures

3.3.1 Microbiological Investigations

The most common microbiological investigations performed in children with CAP were blood culture, serology for *M. pneumoniae* and *C. pneumoniae*, and nasopharyngeal swabs for viral detection. No significant differences between the two study periods were found in the percentage of children who had blood cultures and nasopharyngeal swabs performed (pre-PGMRTI vs. post-PGMRTI – 41.2% vs. 44.5% and 2.4% vs. 3.2%, respectively; $p = 0.100$). Serology for atypical pathogens was less often performed in pre-PGMRTI group (27.9% vs. 44.9% post-PGMRTI; $p < 0.0001$). None of the children had a sputum culture performed.

3.3.2 Radiology

CAP was confirmed in chest X-rays of 2277 children; 2080 images were taken at admission and 197 in the outpatient settings. The diagnosis was based solely on clinical signs and symptoms in another 82 children. Six hundred and fifty children had follow-up chest X-rays in accord with the guidelines. There were fewer children who had follow-up X-rays pre-PGMRTI than post-PGMRTI (30.5% vs. 53.8%, respectively; $p < 0.0001$).

4 Discussion

This study demonstrates that the antibiotics most frequently ordered for CAP treatment, both before and after the publication of national guidelines, were co-amoxiclav, macrolides, and the second-generation cephalosporins. Amoxicillin was rarely chosen as the first-line treatment of CAP. The assessment of compliance with the guidelines regarding antibiotic selection in respiratory tract infections has been conducted in numerous countries. Such studies have usually demonstrated that clinical practice diverges from the guidelines. Studies published so far indicate that amoxicillin, recommended as the first-line treatment, is not the most frequently used antibiotic in children requiring hospitalization for CAP. The audit evaluating the implementation of the guidelines, conducted in 2011/2012 in the UK, has demonstrated that amoxicillin constituted 24.2% of all first-line antibiotics ordered for CAP. Admittedly, the use of this antibiotic has increased from 10.8% in the period before the publication of the UK guidelines to 21.1% afterwards, but this increase was considered insufficient (Bowen and Thomson 2013). A significant increase in the use of the preferred first-line antibiotics (amoxicillin, ampicillin, and penicillin) has been noted in the USA from 2011 on, after the publication of the guidelines for pneumonia treatment. The analysis of data from 38 hospitals performed by Ross et al. (2014) has revealed that the first-line drugs were used in 29% of children

with CAP before and in 43% of children after the publication of the guidelines. The greatest consistency of therapy with the guidelines has been noted in hospitals having a well operating system of monitoring of prescribed antibiotics (Williams et al. 2017). The present study showed that the publication of the guidelines did not increase the proportion of children treated with amoxicillin; the proportion in fact is significantly lower than those in the studies above outlined (approximately 5%). This finding also differs from another Polish study on the issue conducted in 667 patients, both children and adults, hospitalized for pneumonia in Poland, of whom 22.8% were treated in accord with the guidelines, while 51.9% received different, albeit also potentially effective treatment (Deptuła et al. 2018).

In the present study, the most frequently prescribed first-line antibiotic for CAP in children was co-amoxiclav both before and after the publication of the guidelines, with a tendency to increase from 34.6% to 40.4% after the guidelines, which failed to achieve statistical significance. Co-amoxiclav has also been a predominant selection (34.2%), without a decline in use after the publication of the guidelines, in a British study (Bowen and Thomson 2013). The cause of such frequent selection of this particular antibiotic by prescribing physicians is open to conjecture. We also noticed that the use of the second-generation cephalosporins, the drugs that were not part of the guidelines, significantly declined after their publication. In light of growing bacterial resistance to macrolides (Schroeder and Stephens 2016; Semczuk et al. 2004), a significant increase in the number of children with CAP treated with these drugs we found is alarming (17.4% pre-guidelines vs. 24.5% post-guidelines; Fig. 1). One of the factors that could bear on the matter was a somehow older age of children in the post-guideline group, which plausibly raised the suspicion of pneumonia with atypical etiology in more cases. However, macrolides topped the list of antibiotics used for CAP in a British study after the publication of the guidelines, when the compared groups did not vary in age (Bowen and Thomson 2013). Halting the upward trend in macrolide use in CAP is particularly important

in Poland where the proportion of *S. pneumoniae* resistant to these drugs was on the rise and it reached 35.4% in 2015 (Skoczyńska et al. 2007; Semczuk et al. 2004), which could end up with frequent treatment failures. Popularity of macrolides may result from the willingness to administer a drug that has a broad spectrum that could be effective against both atypical and typical bacteria. There is a consistent impression that the sole publication of the guidelines does not suffice to change the practice of antibiotic prescribing. More significant changes require the implementation of efficient monitoring systems of antibiotic prescribing (Dona et al. 2018; Davey et al. 2017; Kreitmeyr et al. 2017; Williams et al. 2017; Smith et al. 2012).

In the present study, nearly one half of CAP patients received mucoactive drugs in period before the publication of the guidelines. Ambroxol was the most popular choice. However, a reduction in the use of these products has been noted after the publication of the guidelines that underlines the lack of unequivocal evidence supporting the efficacy mucoactive drugs in CAP. Nonetheless, these drugs were still ordered in approximately 40% of children. On the other side, there was a relatively high number of children with CAP treated with bronchodilators and inhaled corticosteroids. That was rather unexpected as the proportion of children with CAP who had had asthma constituted only 3.5% of the pre-guidelines group and 6.5% of the post-guidelines. After the publication of the guidelines, which did not recommend this form of treatment, the number of children treated with bronchodilators and inhaled corticosteroids dropped significantly, even though this group included more children with asthma. In a British Thoracic Society study, the proportion of children treated with bronchodilators in the course of CAP has been as high as 35–43% in three consecutive audits of treatment guidelines covering 2009–2012. The authors of that study submit that the high proportion is liable to result from a growing number of children with wheezing during CAP (Bowen and Thomson 2013). In the present study, children treated with inhaled corticosteroids were significantly younger than those untreated. Thus, it might be possible that pneumonia was

accompanied by airway obstruction in younger children. However, there were no similar age differences concerning the use of bronchodilators. The publication of the guidelines had no influence on the frequency of microbiological tests in the groups studied. The most common test was blood culture, performed in over 40% of children, which, according to the guidelines, should be ordered only in children hospitalized for severe pneumonia. The percentage of blood cultures we noticed was comparable to the 52.1% of a British study (Bowen and Thomson 2013). Attention should be paid to a growing number of serological tests for atypical infections despite the lack of guidelines for their routine performance.

In the present study, the frequency of routine follow-up chest X-rays, for reasons other than those recommended in the guidelines, decreased from 69.5% before to 46.2% after the publication of the guidelines. Nevertheless, the proportion of children who had such examinations was markedly higher than that in other countries. In the audit of the British guidelines, chest X-rays were performed less frequently with each passing year, starting from 15% in 2009/2010 to 14% in 2010/2011, and 11% in 2011/2012 (Bowen and Thomson 2013). We were unable to determine the cause of such frequent radiographic follow-up examinations in the present study. The reason for such unrecommended examinations could stem from the physicians' desire to ascertain the regression of changes.

A limitation of this study was that it involved children with CAP at various stages of treatment. Clinical course of CAP could then be more severe and could require the use of antibiotics of a broader spectrum of action. Moreover, the history of previous antibiotic treatments in the children investigated was unknown, which could have contributed to the therapeutic decisions. Also, clinical picture of CAP was not analyzed in individual patient. A decision about antibiotic treatment, macrolide versus beta-lactam, is in practice frequently made on the basis of clinical picture, even though it cannot determine the etiology of CAP. Finally, the study included children treated in only four hospitals in Warsaw; the results cannot be generalized for other regions of the country.

In conclusion, this study demonstrates an unsatisfactory compliance with the published guidelines on the management of community-acquired pneumonia in children. Other methods of education and guidelines dissemination are needed to optimize the antibiotic prescribing. In other countries, an increase in the proportion of CAP patients treated in accord with the guidelines was achieved by the efficient implementation of strict hospital control over the drugs, particularly antibiotics, prescribed.

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Antibiotic Treatment in Patients with Bronchiolitis

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Abstract

This study seeks to define the indications and the economic impact of the use of antibiotics in infants hospitalized due to bronchiolitis during 2010–2017. There were 459 children with bronchiolitis, median age of 2.2 months, 390 infections with respiratory syncytial virus (RSV), and 69 were non-RSV. Twenty two percent of all these children (102/459) required a workup toward urinary tract co-infections (UTI). A control group, consisting of 8,456 children without bronchiolitis, was created to assess UTI frequency in the general population. We found that 16.0% (73/459) children with bronchiolitis received antibiotics; 63 (13.7%) due to respiratory infection and 9 due to UTI. A time-trend analysis showed a decreasing use of antibiotics, from 57.0% in 2010 to 13.7% in 2017, with the lowest value of 6.4% noticed in 2014. Children treated with antibiotics required a 4-day longer hospitalization than those untreated (p < 0.01), but there were no other clinically relevant differences. After excluding the first 2 years with the highest antibiotic ordering, antibiotics, on average, were used in 9.8% of children with bronchiolitis. Frequency of UTI accompanying bronchiolitis was comparable to that in the control group (8.9% vs. 10.9%, respectively). Specificity of urine culture was 71%, with 100% sensitivity assumed, while the positive predicted value of only 41%. The unnecessary costs of urine cultures, if performed in each patient, would have been €2,236, and with additional laboratory tests in each case of a false positive result it would have reached €5,448. We conclude that antibiotics should be used for bronchiolitis only in justified cases, and their use should not exceed 10% of patients. Since UTI is no more frequent in bronchiolitis than in the general children's population, urine cultures should not be performed routinely.

Keywords

Antibiotics · Bronchiolitis · Children · Infection · Respiratory syncytial virus · Urinary tract infection · Urine culture

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1 Introduction

Bronchiolitis is one of the major pediatric health concerns in the youngest group of patients. It is defined as an acute disease in children under 2 years of age, starting with rhinorrhea or other signs of upper respiratory tract infection, leading to increased respiratory effort and wheezing. The main etiological factor is the respiratory syncytial virus (RSV), but it may be also related to other

contagions, such as human metapneumovirus (hMPV), rhinovirus, or parainfluenza virus (Baraldi et al. 2014). The natural course of the disease may vary from transient episodes of apnea to respiratory distress, causing globally between 66,000 and 199,000 deaths per year, mainly in low-income countries (Ralston et al. 2014; Nair et al. 2010). The majority of studies focus on the RSV versus non-RSV classification of bronchiolitis, with the former distinguished by a more severe course (Stollar et al. 2014). Children with RSV infection tend to require longer hospitalization (Ramagopal et al. 2016). There are just a few therapeutic options and the management recommendations underscore the maintaining of adequate hydration and feeding and oxygen supplementation when arterial oxygen saturation drops below 90%. A number of treatments, which had been tried in the past, turned out not to be more effective than placebo. That concerns the trials with inhaled corticosteroids, bronchodilators (albuterol, salbutamol), or nebulized epinephrine (Ralston et al. 2014). The attempts with antimicrobial treatment, e.g., ribavirin, are not a recommended option either. On the other hand, the use of antibiotics is somehow controversial. In case of viral infections, which bronchiolitis is in the vast majority of cases, antibiotics are generally not recommended, but a possibility of antibiotic treatment is left open in case of proven or sometimes even suspected bacterial coinfection (Friedman et al. 2014; Ralston et al. 2014). There are studies pointing to increased bacterial colonization in the nasopharynx during RSV infection, which is conducive to bacterial coinfection (McGillivary et al. 2009; Murphy et al. 2009; Hament et al. 2004) or even to an invasive pneumococcal disease during the peak of an RSV season (Weinberger et al. 2015; Weinberger et al. 2013; Techasaensiri et al. 2010; Ampofo et al. 2008; Talbot et al. 2005; Madhi et al. 2004). Nonetheless, a Cochrane review points out that there is no sufficient evidence to support antibiotic treatment of children's bronchiolitis, except for the high risk conditions, for instance, patients with respiratory failure (Farley et al. 2014)

The present study seeks to define the use of antibiotics in children hospitalized due to bronchiolitis, along with the economic cost of such treatment. In addition, we aimed to define the frequency of upper respiratory tract coinfections (UTI) in children with bronchiolitis and the clinical implications of UTI diagnosis.

2   Methods

This retrospective study was conducted in a single pediatric center in Warsaw, Poland. There were 459 children, median age of 2.2 months, eligible for inclusion into the study. Nearly all of them (452/459) were under 12 months of age. They were hospitalized due to bronchiolitis diagnosed according to the International Classification of Diseases (ICD-10: J21.0 = acute bronchiolitis due to RSV and J21.9 = acute bronchiolitis, unspecified) between January 2010 and June 2017. A conclusive diagnosis was made in the majority of cases on the basis of results obtained from a rapid RSV diagnostic test using swabs taken from the nasopharynx and in some cases on the basis of a polymerase chain reaction (PCR). In 390 (85%) children, the underlying pathogen was RSV, while in the remaining 69 (15%) children, the pathogen remained unspecified.

Both laboratory and clinical findings were reviewed to compare the patients treated with antibiotics with those who did not obtain antibiotics. The lab tests included a white blood cell count (WBC), percentage of neutrophils and lymphocytes, C-reactive protein (CRP) and procalcitonin (PCT) content, sodium and potassium level, urine analysis, and arterialized blood acid-base and gas content. The clinical assessment consisted of heart, breath rate (at admission), and the length of hospital stay. The urine culture result was considered positive when there were at least 10^5 cfu/mL (colony forming units per milliliter) in the midstream specimen of urine, or at least 10^3 cfu/mL in a catheterized specimen. According to the Polish guidelines, UTI is diagnosed when there is a positive urine culture, along with pyuria or the

presence of nitrites and esterases in urine, or clinical signs and symptoms. Pyuria is defined as at least 5 leukocytes visible in a 400-fold magnified microscopic field or at least 10 leukocytes in a centrifuged urine sediment. Clinically, the infection should encompass some of the following symptoms: fever, reluctance to food, vomiting, lack of body weight gain, anxiety and cry during micturition, excessive drowsiness, and changes in color, transparency, or smell of urine in children aged 2–12 months. In older children of 2–6 years of age, diarrhea, lack of appetite, irritability, and abdominal pain may also be observed (Zurowska 2012).

To assess the true frequency of UTI in children with bronchiolitis, a comparative group of children with UTI, but without bronchiolitis, was created. This group consisted of 8456 children, hospitalized at the Pediatric Department of the Bielanski Hospital in Warsaw, Poland in 2013–2017.

Total treatment cost was calculated as a sum of urine culture costs and the additional costs of lab tests that are routinely performed in patients suspected of UTI, i.e., white blood cell count, CRP, and procalcitonin. Due to the use of outsourcing lab services by the Bielanski Hospital in Warsaw, the mean cost of each procedure was estimated on the basis of the most recent public tender. To evaluate the percentage of costs generated by the urine cultures, calculations were referenced to the total sum of reimbursement paid by the National Health Fund in Poland. The reimbursement is based on the general patient-diagnosis policy rather than on clinical specifics such as the length of hospital stay, therapeutic or diagnostic methods, or medication costs. The percentage of costs generated by false positive urine culture results was calculated in a similar manner. The costs were presented in euro (€) with a mean exchange rate of 4.3 Polish zloty to €1, as of August 24, 2017.

Quantitative data were expressed as means ±SD or medians with interquartile ranges, depending on the data distribution that was checked with the Shapiro-Wilk test. A two-tailed unpaired t-test and the Mann-Whitney U test were applied as required. A p-value <0.05 defined statistically significant differences. The analysis was performed using a commercial Statistica v13 software (StatSoft; Tulsa, OK).

3 Results

A total of 73 (15.9%) out of the 459 children were treated with antibiotics. The main reason for the implementation of antibiotic regimen was a severe respiratory condition, lack of improvement, or a suspicion of complications. Such events were reported in 63 out of the 73 children who obtained antibiotics. The remaining nine children had antibiotic therapy due to the accompanying UTI, and one child had it prophylactically due to urinary tract catheterization.

The proportion of children treated with antibiotics substantially varied during the period analyzed. The greatest proportion was observed in the early years of 2010 and 2011, where it reached 57% and 39%, respectively. In the following years, the proportion decreased significantly and reached its lowest value in 2014 (6.4%). The mean proportion of children treated with antibiotics, primarily due to a respiratory disease, was 13.7% (63/459 children) in 2017. However, after excluding the two initial years of 2010 and 2011, with the greatest percentage in the antibiotic use and also a couple of years when a relatively small number of patients were hospitalized, this proportion dropped to 9.8% (Fig. 1).

Children who received antibiotics had a higher level of CRP and a higher percentage of neutrophils compared to those treated without antibiotics (2.13 mg/L vs. 0.90 mg/L; $p = 0.04$ and 24% vs.19%; $p < 0.01$, respectively), and a lower percentage of lymphocytes (57% vs. 62%; $p = 0.01$). There were no other appreciable differences between the antibiotic and no antibiotic groups (Table 1). The results above outlined had only statistical significance, but no clinical implications. Nevertheless, patients treated with antibiotics required a longer hospital stay than those untreated with antibiotics (12 vs. 8 days, $p < 0.01$).

Fig. 1 Percentage of patients with bronchiolitis treated with antibiotics; time-trend analysis
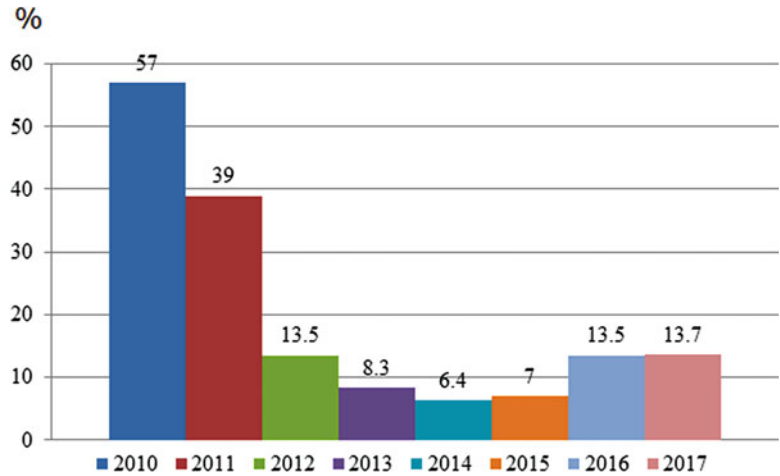

Table 1 Clinical and laboratory characteristics of children treated and untreated with antibiotics

	Antibiotic treatment			No antibiotic treatment			
	Median	Lower quartile	Upper quartile	Median	Lower quartile	Upper quartile	p
Age (months)	103.1	40.0	165.6	69.1	43.0	109.5	0.0850
Length of stay (days)	12	9	15	8	7	10	<0.0001
WBC ($*10^3$cells/µL)	10.50	8.30	13.90	10.00	8.40	13.00	0.6409
Neu (%)	23.95	17.50	42.20	19.00	12.30	29.45	0.0003
Lym (%)	57.05	46.80	67.50	62.40	52.70	70.45	0.0108
CRP (mg/L)	2.13	0.41	5.10	0.90	0.26	3.89	0.0379
PCT (ng/dL)	0.10	0.08	0.17	0.10	0.07	0.12	0.4268
Na (mmol/L)	136.6	135.8	138.5	137.3	136.0	138.4	0.3536
K (mmol/L)	5.1	4.7	5.4	5.2	4.9	5.5	0.1297
pH	7.40	7.37	7.42	7.40	7.38	7.43	0.5546
PCO_2 (mmHg)	37.5	33.9	44.8	37.1	33.8	42.1	0.6669
BR (per minute)	54	40	62	56	45	63	0.6068
HR (per minute)	143	133	160	140	132	154	0.6204
SpO_2 (%)	96.5	94.0	98.0	96.0	94.0	97.0	0.2820

WBC white blood cell count, *Neu* neutrophils, *Lym* lymphocytes, *CRP* C-reactive protein, *PCT* procalcitonin content, *Na* sodium, *K* potassium, PCO_2 partial pressure of arterial CO_2, *BR* breath rate, *HR* heart rate, SpO_2 oxygen saturation of capillary blood (pulsoximeter)

In 22% (102/459) of children hospitalized due to bronchiolitis, the tests toward the possibility of a coexisting UTI were recommended. The diagnosis of UTI was confirmed in 8.9% of those children (9/102). There were differences in frequency of UTI in RSV bronchiolitis, consisting of 390 cases, versus non-RSV bronchiolitis, consisting of 69 cases. The occurrence of UTI was 1.8% (7/390) in the former and 2.9% (2/69)

in the latter group. The incidence of UTI accompanying bronchiolitis turned out to be lower than expected. For comparison, in the control group of children, hospitalized for reasons other than bronchiolitis, the incidence of UTI was 10.9% (924 out of the 8,456 children).

In total, 157 urine cultures were performed to confirm or exclude the diagnosis of UTI. There were 16.6% (26/157) cultures with true positive

Table 2 Results of urine cultures ($n = 157$) performed to diagnose urinary tract infection (UTI) in study patients

Positive	True *n* (%)	False *n* (%)	Sensitivity	PPV
	26 (16.5)	38 (24)	100% (assumed)	41%
Negative	True *n* (%)	False *n* (%)	Specificity	NPV
	93 (59)	0 (assumed)	71%	100% (assumed)

PPV positive predictive value, *NPV* negative predictive value

Table 3 Real and hypothetical costs generated by urine cultures due to urinary tract infection (UTI)

	Cost (€)	Unnecessary cost € (%)	% Hospital costs
Real costs of urine cultures in the study group	916	765 (83.5)	0.26
Hypothetical costs of urine cultures if performed in every patient	2,679	2,236 (83.5)	0.63
Hypothetical total costs, including additional urine and lab tests, if performed in every patient	5,448		1.54

results, while 24% (38/157) were false positive. There also were 59% (93/157) true negative urine cultures, with the zero false negative results assumed as none of these children presented signs or symptoms of UTI during the hospitalization and none were readmitted to the Pediatric Ward due to UTI suspicion or diagnosis, although no other extended follow-up was performed. With the assumed 100% sensitivity and, consequently, a negative predictive value (NPV) of urine cultures, the specificity was 71%, while the positive predictive value (PPV) reached only 41% (Table 2).

A total cost of urine cultures, performed in relation to UTI, was €916, including €765 of unnecessary costs. Hypothetically, if each and every patient with bronchiolitis had had a urine culture performed, the total cost would have been €2679, with the unnecessary cost of €2236. Given the additional laboratory tests, such as WBC, CRP, PCT, and also repeated urinalysis and urine culture, in each false positive result of UTI, the costs would have reached €5448. If the National Health Fund reimbursed for the whole group of 459 children hospitalized due to bronchiolitis, then it would have to lay out €353,679. In terms of proportions, unnecessary urine cultures performed in every patient would have the 0.63% chunk of the reimbursement. In total, repercussions of unnecessary cultures and further diagnostics would consume 1.54% of the reimbursement (Table 3).

4 Discussion

The frequency of antibiotic use in case of bronchiolitis depends on a number of factors, such as inpatient versus outpatient, geographical location, and local guidelines, and on the time analyzed. In hospitalized children, without the use of mechanical ventilation, antibiotics are used in 34% (Vogel et al. 2003) to 45% (Christakis et al. 2005), while in one study 99% of children obtained antibiotics (Kabir et al. 2003). The frequency found in the present study was rather low (13.7%) compared to the other studies. Moreover, we noticed a substantial decrease in antibiotic use in children with bronchiolitis over the passage of years, which is a likely result of the expanding knowledge on the overuse of antibiotics in children with bronchiolitis. A vast majority of studies focusing on antibiotic treatment were conducted before the Cochrane review published in 2014, not recommending to use antibiotics unless the disease is complicated by bacterial pneumonia or respiratory failure (Farley et al. 2014).

A growing number of studies, showing the lack of necessity of antibiotic therapy, influence the clinical practice. On the other hand, scientific data in and of itself is not enough, and the implementation of clinical guidelines and educational programs shows a much higher efficacy in reducing the unnecessary procedures or treatments. A

study by Breakell et al. (2018), performed in a pediatric unit in England, has shown a significant decrease in the antibiotic use for bronchiolitis; a decrease from 22% to 6% between 2014–15 and 2015–16. Likewise, there was a reduction in the use of inhaled bronchodilators and chest radiographs. The greater percentage of antibiotic use reported in that study was higher than that noticed in the initial period under consideration in the present study; yet a decrease to 6% was a better achievements then the 13.7% noticed in 2017 of the present study. It remains to be established what frequency of antibiotic treatment would be a desirable optimum. It seems a reasonable proportion should not exceed 10%. Previously published data, including the achievable benchmarks of care by Parikh et al. (2014), show that no more than 19% of patients with bronchiolitis should be treated with antibiotics. The authors underline that antibiotics cannot be completely omitted in these patients due to bacterial coinfections (like UTI or otitis media) that do occur. However, the use of antibiotics should remain as low as possible. In the present study, we noticed a slight increase in the use of antibiotics after the initial decrease. A kind of rebound in the frequency of antibiotic use has also been noticed in a study by Genies et al. (2018), in which the educational program leads to a decrease in using nonevidence-based therapies, but the frequency of antibiotic use increases. Yet this frequency does not exceed the acceptable rate of 19%.

Many authors stress that the risk of bacteremia in the course of bronchiolitis or RSV infection is low. Previous studies have shown the risk of serious bacterial infections to be null (Luginbuhl et al. 2008), 0.2% (Greenes and Harper 1999), 1.1% (Levine et al. 2004; Titus and Wright 2003), or 1.2% (Hall et al. 1988). On the other hand, a recent study by Cebey-López et al. (2016) has shown a much higher 10.6% frequency of bacteremia in RSV infections, which the authors find to be more reliable thanks to precise molecular testing techniques used in the study. The authors emphasize that even with this high, previously unreported, frequency of bacteremia, antibiotics are still being overused, especially in children with an uncomplicated RSV infection. The authors conclude that antibiotics should be considered only in the most severe cases.

One of the most common complications of bronchiolitis is acute otitis media. The prevalence can be as high as 56%, with *Streptococcus pneumoniae* and *Haemophilus influenzae* being the most frequent bacterial pathogens. The RSV has been found in 56% cases of otitis media; in the majority of cases, it is present alongside with bacterial pathogens (Gomaa et al. 2012). Earlier studies also show a similar frequency of otitis media, ranging from 53% (Shazberg et al. 2000) to 62% (Andrade et al. 1998). Recent clinical recommendations attempt to restrict antibiotic overuse, suggesting a watchful waiting strategy in case of acute otitis media. The exceptions are children under 6 months of age or under 2 years of age with bilateral otitis media. Nonetheless, even unilateral acute otitis media, which is highly prevalent, may acceptably justify the use of antibiotics due to the infancy age of the majority of children hospitalized with bronchiolitis.

Another indication for antibiotic treatment may be mechanical ventilation, as patients who are undergoing this procedure are at a greater risk of a secondary bacterial infection. In intensive care unit (ICU) patients, the proportion of positive endotracheal aspirate cultures ranges from 21% (Thorburn et al. 2006) to 26% (Kneyber et al. 2005), and the antibiotic use reaches 95% in the latter study. A recently published Spanish multicenter study has shown that 56.5% patients hospitalized in one of 16 ICUs in Spain obtained antibiotic treatment (Flores-González et al. 2017). In the present study, the hospital setting was a general pediatric ward, where there were no ventilated patients, as such patients were routinely transferred to ICU. However, a decreasing tendency of antibiotic use also is noted in ICUs.

Globally, UTI diagnostic criteria widely vary. The gold standard is the urine analysis and culture of a centrifuged specimen. There are studies that point to a substantial clinical utility of signs and symptoms observed, such as pain or crying when urinating or smelly urine, which may be of higher

specificity with the same sensitivity (Hollingworth et al. 2017). In the present study, we chose the Polish guidelines that combine both clinical tests and symptoms. We used these guidelines despite the controversial assumption of no false negative results. We noticed that a risk of UTI in children with bronchiolitis was lower than that in the general population. A report by Elkhunovich and Wang (2015) has shown increased risk of UTI in febrile children with bronchiolitis, with 6.7% of positive urine cultures and 4.5% of positive both urinalysis and urine culture. The authors conclude that UTI testing should be considered in infants between 2 and 12 months of age with bronchiolitis and fever. Clinical tools need to be further evaluated regarding their effectiveness in diagnosing and managing UTI. In addition, risk factors such as a history of UTI, family history, urinary tract malformations or defects, and the presence of fever need consideration, also in the context of economic costs of urine testing.

In conclusion, this study demonstrates that the use of antibiotics did not exceed 10% in children hospitalized with bronchiolitis in recent years. In our opinion, this figure is reasonably low to spare the unnecessary use of antibiotics and, on the other side, it is high enough to the patients' safety. Antibiotics should be justifiably used only in severe bronchiolitis with complications. Children with bronchiolitis were not at high risk of UTI that was noticed less frequently than in the general population. Thus, there is no reason to routinely perform urine cultures in children hospitalized with bronchiolitis.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by an institutional Ethics Committee.

Informed Consent This was a retrospective study; thus, the requirement of obtaining consent from individual participants was waived.

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