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Nosocomial Infections in Patients Hospitalized with Respiratory Syncytial Virus: A Practice Review

August Wrotek, Małgorzata Czajkowska, and Teresa Jackowska

Abstract

Viral testing is not always recommended in children with bronchiolitis due to doubts concerning its prognostic use. In this retrospective study, we investigated how the RSV testing would influence the frequency of nosocomial infections (NI). The files of 305 children, hospitalized due to the respiratory syncytial virus (RSV) infection in the period 2010–2014, were reviewed in the study. We found ten cases of NI. The RSV preventive measures did not vary in the consecutive years investigated, but the number of viral tests substantially varied. In 2010, 2012, and 2014, when *ca.* 2 tests *per* RSV(+) patient were performed, the risk of NI *per* patient was 1.3%, while in 2011 and 2013, when the RSV testing was less frequent, the accumulated risk *per* patient was 5.2%. There was a strong adverse relationship between the number of tests performed and the number of NI ($rho = -0.975$). The children with NI, when compared to those without NI, required a longer hospital stay, generating higher hospital costs regarding treatment, productivity loss, and indirect costs. The expenditure for RSV testing in the years of a low NI risk was higher than that in the high-risk years. Conversely, the expenditure related to NI management was lower in the years of a low NI risk. Each euro spent on RSV testing saved over 26 € from the NI management expenditure. We conclude that RSV testing is needed in the hospital setting to isolate the infected children and to prevent nosocomial RSV spread. This strategy is health advantageous and requires less resources than NI treatment.

Keywords

Bronchiolitis · Community-acquired disease · Health costs · Healthcare · Nosocomial infection · Respiratory syncytial virus · Treatment efficacy

1 Introduction

Lower respiratory tract infections are the main single cause of death in children under the age of 5 (Liu et al. 2012). One of the most important etiological agents found in respiratory infections is the respiratory syncytial virus (RSV), which is the cause, alongside influenza, of approximately 6.6% of deaths in children younger than 5 (GBD 2015 LRI Collaborators 2017). There are few studies focusing on the percentage of lower

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respiratory tract infections caused by RSV, but some data suggest 20% of acute respiratory infections are caused by RSV (Nair et al. 2010). Among hospitalized children, the percentage is even higher, reaching 26% of the children. The majority of infections affect the youngest population of children; the hospitalization rate is estimated to be 5.2/1000 children under 2 months of age, while in the first month of life, the rate is fivefold greater, reaching 25.9/1000. Children aged 2 months or less comprise almost half (44%) of the hospitalized RSV patients in pediatric wards (Hall et al. 2013). Most patients with an RSV infection are diagnosed with bronchiolitis; many have pneumonia and less so bronchitis. The guidelines for bronchiolitis do not put emphasis on the importance of viral testing that has little influence on the patient management (Ralston et al. 2014). On the other hand, when the measures protecting viral spread are not implemented, the risk of an RSV transmission may be as high as 45% (Hall et al. 1975). The etiology of bronchiolitis is mainly regarded in terms of RSV vs. non-RSV etiology, and this ratio varies each year (American Academy of Pediatrics 2006). Moreover, RSV is related to a more severe course of bronchiolitis (Stollar et al. 2014). Thus, special attention should be put on the preventive measures against RSV spread in hospitalized children. Especially, as the majority of pediatric wards consist of non-single rooms, the issue of avoidance of nosocomial infections (NI) by patient isolation and undertaking disinfection measures should be prioritized. The implementation of control programs for nosocomial infection decreases RSV spread by 37–50% (Macartney et al. 2000; Karanfil et al. 1999; Leclair et al. 1987). The grates bed occupancy in most cases is seen during the peak infection season, which in the temperate climate correlates with the greatest RSV morbidity. Among the control measures of viral spread, RSV testing performed in each patient suspected of the infection, particularly during the peak season, is highly effective. Studies focusing on the management of hospital beds point to the advantage of RSV testing (Mills et al. 2011). There are, however, studies contradicting the prognostic value of such testing (Stollar et al. 2014), focusing instead on the differentiation of viral from non-viral infections. The American Academy of Pediatrics does not recommend routine viral testing, except for patients receiving palivizumab prophylaxis (Ralston et al. 2014), while the Polish recommendations point out that children with bronchiolitis should have viral testing performed for the epidemiological reasons and also to reduce antimicrobial therapy. Thus, in this study we set out to investigate the influence of RSV testing on the prevention of nosocomial RSV infections in a single pediatric ward and on the hospitalization costs incurred.

2 Methods

This retrospective review focused on medical files of 305 children with an RSV infection who were hospitalized at the Department of Pediatrics in the Bielanski Hospital in Warsaw, Poland, from January 2010 to December 2014 (5 consecutive years). There were ten cases of nosocomial RSV infection (NI) among this cohort of patients. The yearly number of RSV infections varied: 30 cases (1 NI) in 2010, 57 cases (6 NI) in 2011, 49 (0 NI) in 2012, 98 (2 NI) in 2013, and 71 (1 NI) in 2014 (Table 1).

A nosocomial infection was defined as signs and symptoms typical of an RSV infection of the lower respiratory tract, i.e., bronchiolitis, pneumonia, or bronchitis, with the confirmation of RSV presence in the nasopharyngeal swabs using a rapid RSV diagnostic test or polymerase chain reaction (PCR). When there were other respiratory symptoms prior to the suspected nosocomial infection, only the cases with initially negative followed by positive viral testing were treated as healthcare-acquired. The time criteria to define the NI were as follows: onset of signs and symptoms 48 h or later after hospital admission or up to 3 days after hospital discharge. Concerning the post-hospitalization NI, only the patients who were readmitted to the pediatric ward or the hospital emergency ward were enrolled as patients with an NI. There was no active follow-up of the

Table 1 Infections with respiratory syncytial virus (RSV) in hospitalized children

Year	Nosocomial RSV (*n*)	RSV hospitalizations (*n*)	Length of hospitalization (days)	Mean length of hospitalization (days)	Risk of NI *per* patient (%)	Risk of NI *per* hospitalization day (%)	RSV tests (*n*)	RSV tests *per* patient (*n*)	RSV tests *per* hospitalization day (*n*)
2010	1	30	335	11.2	3.3	0.30	61	2.0	0.2
2011	6	57	562	9.9	10.5	1.07	43	0.8	0.1
2012	0	49	424	8.7	0	0	103	2.1	0.2
2013	2	98	814	8.3	2.0	0.25	130	1.3	0.2
2014	1	71	561	7.9	1.4	0.18	136	1.9	0.2

NI nosocomial infection

patients at large conducted due to the retrospective nature of the investigation.

Nosocomial preventive measures did not change throughout the 5-year long period analyzed. The strategy consisted of alcohol-based hand disinfection before and after each contact with the patient or patient's environment, general guidelines for medical staff concerning hygiene (e.g., no long sleeves or jewelry), and parental information in the form of leaflets on preventive measures provided on admission. Likewise, the patient grouping and isolation systems remained unchanged. Patients with bronchiolitis shared a room of three or four; and certain patients were isolated due to individual medical indications, mainly a severe condition or comorbidities requiring isolation.

To assess the influence of RSV testing on NI frequency, the number of NI was correlated with the number of patients who had been diagnosed with RSV each year, the number of hospitalization days (total) of patients with RSV (to verify the length of stay as a factor influencing NI risk), risk *per* RSV patient (calculated by dividing the number of RSV NI by the number of RSV patients), and risk *per* hospitalization day (calculated by dividing the number of hospitalization days of NI patients by the total number of RSV hospitalization days). The number of NI was also correlated with the absolute number of RSV tests performed.

The cost-of-illness evaluation concerned the socioeconomic direct and indirect costs of NI and the patient's indirect costs such as the loss of income due to hospitalization. The socioeconomic costs were calculated as a sum of hospitalization cost and loss of productivity assessed from the mean of local wages. To calculate the cost of hospital treatment, patient-*per*-day hospitalization cost was used as the base. This way of calculation is not in line with the Polish public health insurance system, in which hospitals are being reimbursed on the patient-*per*-diagnosis base. According to this system, a fixed sum of money is paid to the hospital for each hospitalization with the same diagnosis lasting for a fixed time period. In the authors' opinion, the patient-*per*-day cost reflects the real costs more accurately than the fixed reimbursement policy. The patient-*per*-day cost at the Bielanski Hospital in Warsaw is made public by the hospital management and varies for each hospital ward. This is also the charge that is paid by patients who do not have any health insurance. It reflects real hospital expenses, with no surcharge for profit, as the hospital is a public one.

The length of hospital stay was taken as the absence from work. A loss of productivity was then calculated by multiplying the number of days spent in the hospital by the mean daily wages calculated by dividing the gross domestic product (GDP) each year according to the data of the Central Statistical Office of Poland, by 250, i.e., the mean number of working days *per* year. The number of days absent from work was also multiplied by the mean income loss to calculate the patient's indirect costs. Taking into account the different economic situations in the specific regions of Poland, the mean monthly salary for the Masovian Voivodeship was multiplied by 12 (number of months) and then divided by 250 (mean number of work days *per* year in Poland). The Polish social care system issues monthly paychecks of 80% of the current salary for parents/legal tutors who take care of a sick child from up to 60 days annually. In the evaluation, each day of hospitalization was assumed to be a day off from work. The remaining 20% of the mean daily wages was added to the patient's indirect costs. Other direct and indirect costs from the patient's perspective, e.g., cost of drugs, transportation, or ambulatory care visits, were not taken into account.

Data were expressed as means ±SD or medians with interquartile ranges in case of skewed distribution as checked with the Shapiro-Wilk test. The NI risk was presented both *per* patient and *per* day of hospitalization. A correlation between the number of RSV tests and NI risk was calculated with the nonparametric Spearman rank correlation test. A p-value <0.05 defined statistically significant differences. The analysis was performed with a commercial Statistica v13 package (StatSoft; Tulsa, OK).

3 Results

The number of RSV tests varied in the consecutive years. The lowest was 0.8 tests *per* RSV(+) patient in 2011, and the highest was 2.1 tests *per* RSV(+) patient in 2012. Conversely, the number of NI was lowest in 2012 (0 NI) and highest in 2011 (6 NI), meaning a 0% risk *per* patient in 2012 and a 10.5% risk in 2011 (6 NI out of the 57 RSV hospitalizations) (Table 1).

There was a strong correlation between the number of tests *per* RSV(+) patient and the number of NI, with Spearman's *rho* of 0.975, which also was as high for a correlation between the number of tests *per* hospitalization days and the number of NI ($p < 0.001$). There was no correlation noticed between the number of NI and the number of RSV(+) patients, the total number of hospitalization days, the risk *per* patient, the risk *per* hospitalization days, or the number of RSV tests performed (Table 2).

Patients with nosocomial infections were older than those with community-acquired infection (156 vs. 81 days of age, $p = 0.017$) and required a longer hospital stay (12 days vs. 8 days, $p < 0.01$). Also, they had a higher white blood cell count (12,050 vs. 9500 $\times$ 10^3 cells *per* μL, $p = 0.034$), higher platelet count (458 vs. 390 $\times$ 10^3 cells *per* μL, $p = 0.043$), higher neutrophil percentage (41 vs. 20%, $p < 0.01$), and a lower lymphocyte percentage (46 vs. 62%, $p = 0.023$). Nevertheless, no other statistically significant differences between the groups were seen (Table 3).

The total cost of hospitalization in nosocomial RSV infections was much higher than that in community-acquired RSV infections (3130 € vs. 2087 €, respectively). All of the component parts of the total cost were respectively higher in the nosocomial RSV infections: hospital treatment (1429 € vs. 953 €), productivity loss (1132 € vs. 755 €), and the patient's indirect costs (569 € vs. 380 €) ($p < 0.01$ for all) (Table 4).

Interestingly, when the number of tests was about 2 *per* RSV(+) patient, the risk of NI decreased. For the 2010, 2012, and 2014 years with approximately 2 tests *per* patient done, the accumulated risk of NI *per* patient was 1.3% (2 out of the 150 patients), while in the 2011 and 2013 years with approximately 1 test *per* patient done, the accumulated risk *per* patient was 5.2% (8 out of the 155 patients). Thus, relative risk was about fourfold lower with two tests *per* patients. For the determination of hospitalization costs, the patients were then stratified into low or high risk of NI groups, taking into account the respective years above outlined (Table 5).

With each single NI avoided, 3130 € could have been saved. When 88 additional RSV tests were performed, the risk of NI would decrease by 3.9% (from 5.2% to 1.3%). The costs of RSV testing in the group of low NI risk were higher (1028 € vs. 574 € *per* 100 patients), which was related to a greater number of tests performed (88 tests more *per* 100 patients), but the costs of NI were definitely lower than those in the group of high NI risk (4173 € vs. 16,155 €, respectively). Thus, with each euro more spent on testing, over 26 € could be saved from the costs related to NI. That also implies the savings of 12 € in hospitalization costs, 9 € in productivity loss, and 5 € in the patient's indirect costs.

Table 2 Spearman's rank correlation coefficient between the number of nosocomial infections (NI) with respiratory syncytial virus (RSV) and the number of tests *per* patient/*per* hospitalization days, the number of patients, the number of hospitalization days, the risk *per* patient/*per* hospitalization days, and the number of tests performed

Number of tests *per* patient	−0.975 ($p < 0.001$)
Number of tests *per* hospitalization days	−0.975 ($p < 0.001$)
Number of RSV(+) patients	ns
Days of hospitalization	ns
Risk *per* patient	ns
Risk *per* hospitalization days	ns
Number of tests performed	ns

ns nonsignificant

Table 3 Baseline characteristics of children with community-acquired and nosocomial infections with respiratory syncytial virus (RSV)

	Nosocomial RSV		Community-acquired RSV		
Parameters	Median	IQR	Median	IQR	*p*
Age (days)	156	225–88	81	141–44	<0.020
Hospitalization (days)	12	17–10	8	10–7	<0.001
WBC ($\times 10^3/\mu L$)	12,050	13,100–10,300	9500	12,400–8000	<0.034
Hb (g/dL)	11.5	12.6–11.2	11.6	12.6–10.9	ns
Plt ($\times 10^3/\mu L$)	458	542–401	390	467–324	<0.050
Neu (%)	40.6	49.7–29.4	19.9	30.2–12.3	<0.010
Lym (%)	46.0	61.6–30.0	62.2	71.0–51.8	<0.023
Na^+ (mmol/L)	137.0	138.9–136.0	137.0	138.2–135.5	ns
K^+ (mmol/L)	5.0	5.4–4.9	5.1	5.5–4.7	ns
pH	7.41	7.43–7.39	7.40	7.42–7.38	ns
PCO_2 (mmHg)	34.3	40.0–31.1	36.5	41.4–33.4	ns
SaO_2 (%)	91.0	93.9–88.6	90.3	92.3–87.1	ns
CRP (mg/L)	1.60	12.85–0.36	0.84	3.56–0.27	ns
PCT (ng/dL)	0.15	0.19–0.11	0.09	0.12–0.07	ns
BR (*per* min)	60	60–50	60	67–50	ns
HR (*per* min)	150	160–120	143	160–136	ns

IQR interquartile range, difference between 75th and 25th percentiles; *WBC* white blood cells, *Hb* hemoglobin, *Plt* platelets, *Neu* neutrophils, *Lym* lymphocytes, *Na* sodium ions, *K* potassium ions, PCO_2 partial pressure of arterial carbon dioxide, SaO_2 arterial oxygen saturation, *CRP* C-reactive protein, *PCT* procalcitonin, *BR* breathing rate, *HR* heart rate, *ns* nonsignificant

Table 4 Costs (€) of nosocomial versus community-acquired respiratory syncytial virus (RSV) infections (calculation as of August 2017)

	Nosocomial RSV		Community-acquired RSV		
	Median	IQR	Median	IQR	*p*
Hospital treatment	1429	2024–1191	953	1191–834	<0.01
Productivity loss	1132	1604–943	755	943–660	<0.01
Patient's indirect costs	569	807–474	380	474–332	<0.01
Total €	3130	4435–2609	2087	2609–1826	<0.01

Costs in euro were rounded off to the nearest unit; *IQR* interquartile range, difference between 75th and 25th percentiles

Table 5 Children stratified by nosocomial respiratory syncytial virus (RSV) infection risk (low versus high), costs of nosocomial infections (NI), and RSV tests in either group

	Low-risk years (2010, 2012, 2014)	High-risk years (2011, 2013)
Number of patients *per* year	30 + 49 + 71 = 150	57 + 98 = 155
Number of NI *per* year	1 + 0 + 1 = 2	6 + 2 = 8
Risk of NI (%)	1.3	5.2
Total cost of NI management	2 × 3130 euro = 6260 euro	8 × 330 euro = 2640 euro
Total cost of NI *per* 100 patients	**4173 euro**	**16,155 euro**
Number of RSV tests *per* year	61 + 103 + 136 = 300	43 + 130 = 173
Number of RSV tests *per* 100 patients	200	112
Total cost of RSV tests done	300 × 5.14 euro = 1542 euro	173 × 5.14 euro = 889 euro
Total cost of RSV tests *per* 100 patients	**1028 euro**	**574 euro**
Gain *per* each euro spent on RSV tests	**26 euro**	**–**

Costs in euro were rounded off to the nearest unit

4 Discussion

This study assessed the role of viral testing for the prevention of nosocomial RSV infections in children hospitalized due to bronchiolitis and for the hospital expenses incurred in the pediatric ward. Bronchiolitis is most often caused by RSV. The estimates show that it develops in 60–75% of RSV-positive hospitalized patients. Coinfections also are common, amounting to *ca* 30% (Mansbach et al. 2008, 2012). Bacterial colonization in the nasopharyngeal RSV infections has been reported in pediatric patients (Suárez-Arrabal et al. 2015) and in animal models (McGillivary et al. 2009; Murphy et al. 2009; Hament et al. 2004). Moreover, RSV may associate with an invasive pneumococcal disease (Weinberger et al. 2013, 2015; Techasaensiri et al. 2010; Ampofo et al. 2008; Talbot et al. 2005; Madhi et al. 2004). The infections can act in a two-way fashion. Patients with an RSV can transmit the virus to other patients, while patients with a lower respiratory tract infection caused by other microorganisms (e.g., pneumococci) may induce a suprainfection in patients with bronchiolitis. To avoid coinfections or suprainfections in children hospitalized with non-RSV bronchiolitis, additional protective measures should be implemented. Such measures particularly include viral testing to distinguish patients who already have the community-acquired RSV from those without an RSV infection.

We found 10 cases of nosocomial infections (NI) among the 305 medical files of children suffering from RSV-related bronchiolitis. The number of viral tests performed each year varied, on average, from about one to two *per* RSV(+) patient, and so is the risk of NI *per* patient, being 5.2% and 1.3%, respectively, i.e., the more tests, the lower the risk of NI. Children with NI, when compared to those without NI, required a longer hospital stay, generating higher total management costs, including the costs for hospital treatment, productivity loss, and the patient's indirect costs. The costs of RSV testing in the years with a low incidence of NI were higher than those in the years with a high incidence of NI, but vice versa the costs of NI management were lower in the former. Thus, each euro spent on RSV testing yielded the savings exceeding 26 € from the expenditure related to NI management.

The RSV is present in the patients' surroundings, not only the closest proximity, i.e., bed or crib railings, but also toys and table tops. The basic principle of preventing the RSV from spreading is hand hygiene, but it should be remembered that the virus survives even better on artificial surfaces than on hands. Hall et al. (1980) have shown that the RSV survives on the skin for about 20 min, on the rubber surface of gloves for an hour and a half, and on the desktop for as long as up to 6 h. The virus can be transmitted through the hands of caregivers or parents to other patients. Educational programs on the matter directed to caregivers appear to effectively decrease the risk of NI (Macartney et al. 2000). The programs, however, are limited by the time required to carry them out and by the need to involve medical personnel which usually is in short supply. In addition, our experience is that parents who fulfill all instructions given by healthcare professionals still cannot separate their children from other patients in the room; the infection risk arises even when only one person present in the patients' room fails to follow the preventive measures. The most efficient way of coping with such problems is to isolate RSV from non-RSV patients. Considering that about 80% majority of children hospitalized due to RSV infection are previously healthy (Hall et al. 2013), all preventive measures against virus spread should be implemented for each patient, not only for the patients in the risk groups. Special attention should be given to children under 3 months of age in whom the infection is the most severe (Dadlez et al. 2017).

A retrospective nature of the study hampered the possibility to follow patients throughout the hospitalization time or to set the exact source of NI transmission. For instance, a patient could contact another person infected with RSV in the emergency room but developed the symptoms of NI later on in the course of hospitalization. That, in fact, would mean a healthcare-acquired

infection. Yet RSV testing does not unravel the route of infection transmission, as it is impractical to perform tests in each and every patient entering the emergency room or earlier in the hospital waiting area. Nor is the adherence of children's parents to preventive measures, and thus the risk level, immediately known. There also could be a risk of RSV transmission outside the hospital environment. In this study, a patient with respiratory infection was taken as having NI only when the initial viral testing for RSV was negative and when repeated testing after at least 48 h showed positive. In addition, the limiting factors were the same each year over the 5-year long period investigated, making the yearly comparisons of NI equitable.

Another limitation was that the majority of NI were confirmed with a rapid RSV test rather than the gold standard molecular biology methods. Yet rapid RSV tests' sensitivity and specificity are in a high range of 90–100% (Mesquita et al. 2017; Peters et al. 2017). The risk of false-positive (i.e., patients without an RSV being diagnosed with NI) or false-negative results (i.e., patients with NI being undiagnosed as such or patients who were initially misdiagnosed with a non-RSV infection) was low enough to be neglected. The epidemiological impact was only an additional aspect in this study, which aimed to stress convenience and reliability of RSV rapid tests in the general pediatric practice are repeatedly stressed of late (Prendergast and Papenburg 2013; Mills et al. 2011).

The calculation of hospital costs usually raises some doubts and uncertainties due to local particularities, such as differences in the laboratory test price, hospitalization treatment costs, or wages, all of which should be taken into consideration. We attempted to calculate the true costs pertaining to the capital city of Warsaw in Poland. No additional costs, like the hospital's legal liability for nosocomial infections and health complications thereof or payouts of compensations sentenced, were considered confounding factors that could unpredictably skew the analysis and therefore were omitted.

In closing, we believe we have shown that with a higher frequency of RSV testing, the number of nosocomial infections decreases. RSV testing generates costs, but these costs are a fraction of those otherwise needed for the management of increased number of NI. Therefore, although viral tests may not have a direct prognostic value for an individual patient, they have a substantial economic significance. RSV testing is needed in the hospital setting to isolate the infected children and to prevent nosocomial RSV spread. This strategy is both health-advantageous and cost-effective in nosocomial infection treatment.

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

Ethical Approval This retrospective review of medical files does not contain any experiments with human participants or animals performed by any of the authors.

Informed Consent There are no identifiable participants included in this retrospective article. Therefore, there was no requirement to obtain individual informed consent.

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Regional Activity and Spread of Influenza Viruses in Poland in the Context of Neighboring Countries in the Epidemic Season 2017–2018: An Epidemiological Review

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Abstract

This article reviews the epidemiological situation in Poland during the 2017–2018 influenza season in the context of viral spread from the neighboring countries. There were 5793 specimens tested for the presence of influenza virus. The specimens were collected from patients with suspected upper airway infection. The presence of influenza virus was confirmed in 2454 specimens. The data were used to determine the extent of morbidity and the possible direction of spread of influenza virus. It was found that virus type B predominated in 13 out of the 16 Polish provinces, type A predominated in just 1 province, and both types predominated equally in another 2 provinces. Data on influenza type B virus did not enable the drawing of a clear-cut conclusion on the way of its spread. Presumptively, the route of type B virus spread originated in the Ukraine and moved westward, with the transmission enhanced, to some extent, by migration of Ukrainian citizens. Virus type A, on the other side, spread from the Southwest Europe eastward. Reviewing the epidemiological situation plays an important role in gaining more knowledge on influenza morbidity and its differentiation from other similar infections, which helps counteract future infections.

Keywords

Epidemic season · Influenza virus · Respiratory infection · Respiratory tract · Virus spread

1 Introduction

To date, four types of influenza viruses are known, namely, A, B, C, and D. The influenza virus belongs to the *Orthomyxoviridae* family, and its genetic material is RNA. Types A and B are responsible for seasonal epidemics. Influenza A virus is divided into subtypes, and the currently circulating subtypes among people are A(H1N1) pdm09 and A(H3N2). The influenza virus type B is not differentiated into subtypes, but into two

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lineages: Yamagata and Victoria. Influenza infection may have a mild course, but all too often runs a severe course, and may be lethal. Hospitalization and mortality mainly concern risk groups, which include the elderly, pregnant women, and people with a weakened immune system. Worldwide, the annual epidemics result in 3–5 million infections and 290–650 thousand deaths (WHO 2019; Paules and Subbarao 2017).

Influenza viruses can be very easily transmitted among the people, especially in crowded places such as nurseries, kindergartens, schools, clinics, shopping centers, and public transport. When a sick person sneezes or coughs, droplets with viruses suspended in them can spread to a distance of approximately 1 m. In addition, the influenza virus can also be transmitted by handshake (Killingley and Nguyen-Van-Tam 2013; Brankston et al. 2007). Viruses are constantly circulating in the form of aerosols or with particles of dust, but their concentration is too low to cause disease in a healthy person. A previous study reported that the number of influenza type A viruses in the aerosol is in a range of between 1.95 and 3.0×10^3, which is sufficient to infect a healthy person. The risk of infection depends on the concentration of infectable molecules and the individual condition of the immune system (Nikitin et al. 2014).

In countries with moderate climate, seasonal influenza epidemics occur mainly in winter. In the Northern Hemisphere, including Europe, the number of infections increases between November and April every season. Influenza infection is responsible for 15–70 thousand deaths yearly in the European economic area alone. However, influenza viruses show substantial and changeable regional variations in occurrence and circulation (ECDC 2018; Cox and Subbarao 2000).

The aim of this study was to define the spread of influenza viruses in each voivodeship of Poland in the 2017–2018 season, including also data from the neighboring countries. The patients were divided into seven progressive age groups, starting from neonates.

2 Methods

2.1 Patients and Samples

Over 5 thousand samples from people afflicted with acute upper airway infections were tested for influenza viruses across all of the Polish provinces in the 2017–2018 season, which began as of Week 47 in 2017 and extended into Week 18 of the following 2018, according to the International Organization for Standardization (ISO) week system in the Gregorian calendar year. The samples consisted of nasopharyngeal swabs and bronchial lavage. Some of the specimens were cultured on the Madin-Darby canine kidney epithelial cells (MDCK line) (Hossain et al. 2010). Patients were divided into seven progressive age groups: 0–4, 5–9, 10–14, 15–25, 26–44, 45–64, and >65 years, according to the age scheme proposed previously (Hallmann-Szelińska et al. 2019; Szymański et al. 2019).

2.2 Molecular Biology Tests

Viral RNA was isolated from a 200 μL volume of clinical sample suspended in phosphate-buffered saline (PBS). The RNA was isolated using the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Corporation; Madison, WI). The reaction was carried out in accordance with the manufacturer's instruction.

The real-time polymerase chain reaction (PCR) was carried out using the LightCycler 2.0 System (Roche Diagnostics; Rotkreuz, Switzerland). The primers and probes were acquired from the International Reagent Resource run by the Centers for Disease Control and Prevention (CDC) (Manassas, VA). The reaction was carried out in accordance with the manufacturer's instructions. In brief, RNA was subjected to reverse transcription (50 °C for 30 min). The obtained DNA was subjected to the initial denaturation process (1 cycle at 95 °C for 2 min), followed by 45 cycles of amplification consisting

of 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 vaccine strains for the 2017–2018 season (A/Michigan/45/2015 (H1N1)pdm09, A/Hong Kong/4801/2014 (H3N2), and B/Brisbane/60/2008/). Negative control consisted of RNase-free water.

2.3 Virus Isolation in Cell Culture

The MDCK cell line was used for viral replication in culture. The cells were incubated with the Dubelco Modified Medium with the addition of penicillin, streptomycin, amphotericin, (antibiotic-antimycotic solution 100X, Sigma-Aldrich; St. Louis, MO) and fetal bovine serum (FBS) to a final concentration of 10%. The cells were allowed to grow in tubes with a beveled bottom of 2.5 cm^2 area, in 5% CO_2 atmosphere at 37 °C. The passage of the currently used cell line did not exceed 3 months (or 25 passages) as recommended by WHO (2011). After achieving a 100% confluence, the monolayer was rinsed with FBS-free 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer. Then, the cells were infected with a virus containing 100 µL of a sample volume collected from the patient, followed by incubation in 5% CO_2 atmosphere at 35 °C for 30 min. Subsequently, the viral growth media were added to the tube, consisting of 2.5 mM HEPES buffer and 2.5 µg/mL 1-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK)-treated trypsin, followed by incubation at 35 °C in 5% CO_2. The liquid from cultures was collected after 1 week and tested for the presence of virus by adding 0.75% turkey blood cells suspension in sterile PBS in V-shaped 96-well plates.

2.4 Epidemiological Mapping

The maps presenting regional influenza virus circulation in Poland were created using a mapping application provided free of charge by the European Center for Disease Prevention and Control (ECDC 2019).

3 Results and Discussion

In total, there were 5793 specimens tested for the presence of influenza virus in all of Poland in the 2017–2018 season. The specimens were collected from patients with suspected upper airway infection. The presence of influenza virus was confirmed in 2454 out of these specimens. The first detection of influenza viruses in the season was recorded in the province of Lesser Poland in Week 47 of 2017. The last detections were in the provinces of Mazovia, Silesia, and Greater Poland in Week 18 of 2018. Almost half of the 5 thousand patients with suspected influenza infection, whom the nasopharyngeal specimens were collected from, came from Western Pomerania, one of the smaller in area provinces in Poland. A list of the Polish provinces, with a decreasing order of suspected and later confirmed cases of the infection, is presented in Fig. 1. There were 5 out of the 16 provinces in which the number of suspected infections was below 100 each. In 2 of such provinces, the number of influenza virus confirmation was 20 or less (Fig. 1 inset). The "heat" map of the intensity of influenza incidence shows that the greatest number of confirmed infections was recorded in the central and western parts of the country, the provinces of Western Pomerania, Greater Poland, Lubuskie, Lodz, and Lower Silesia (Fig. 2). Influenza peaked variably between Weeks 7 and 11 of 2018 in the majority of provinces, with Week 10 being the "hottest" in terms of the number of infections. There usually was one peak lasting for about 1–2 weeks, after which the infection was subsiding, but it rebounded in some provinces after a while, albeit with somehow smaller intensity. The outliers of the infection peaking tine were the provinces of Subcarpathia, Mazovia, and Lubelskie where the peak occurred at Weeks 5–6.

Concerning the predominating virus type in the 2017–2018 season, influenza B virus was clearly preponderant in the country. In Western

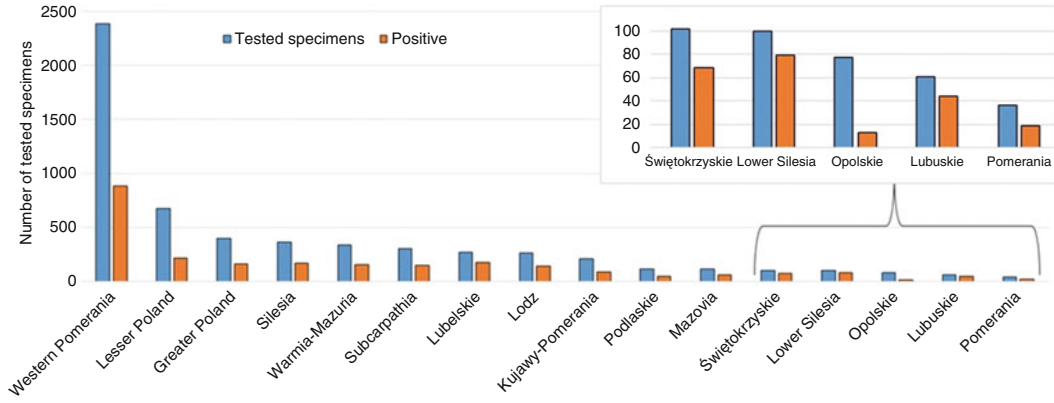

Fig. 1 Number of tested and confirmed for the presence of influenza virus specimens in different provinces of Poland in the 2017–2018 epidemic season. The blown-up inset in the upper right-hand part of the figure shows 5 provinces with fewer than 100 specimens tested

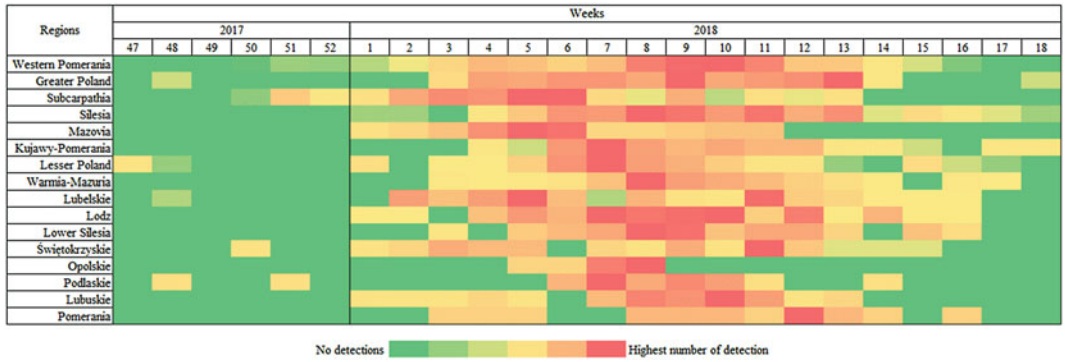

Fig. 2 "Heat" map with laboratory confirmed A and B influenza infections in different provinces of in Poland in the 2017–2018 epidemic season

Pomerania and Greater Poland, both type A and B viruses co-predominated, whereas the Pomerania province was the only one where type A predominated (Fig. 3). Type B virus also predominated in the neighboring countries, except Belarus where types A and B predominated equally. Interestingly, taking a look at the specifics of the temporal and regional intensification of infection with either type B or type A virus, there is a consistent impression that when one intensifies the other subsides and vice versa. This may be exemplified by the peak of type B infection in Weeks 10–11 of 2018 in the provinces of Lubuskie and Swiętokrzyskie where the infections with type A were few or none during that time (Fig. 4) or by the evident peak of type B infection in Greater Poland at Weeks 12–15, the time when type A infections were very rare in this province (Fig. 5). Despite this tendency, however, there was a degree of variability and overlap of both viral infections. There also was a plausibility of across eastern border transmission of influenza A virus from the Ukraine to the southern province of Subcarpathia, having likely to do with human migration over to Poland.

The evaluation of influenza infection by the age groups shows that type A virus predominated in the youngest children up to 4 years of age, while type B virus was present in 45–64 years and >65 years old patients in the northern provinces of Western Pomerania, Pomerania, and Podlaskie. In the central provinces of

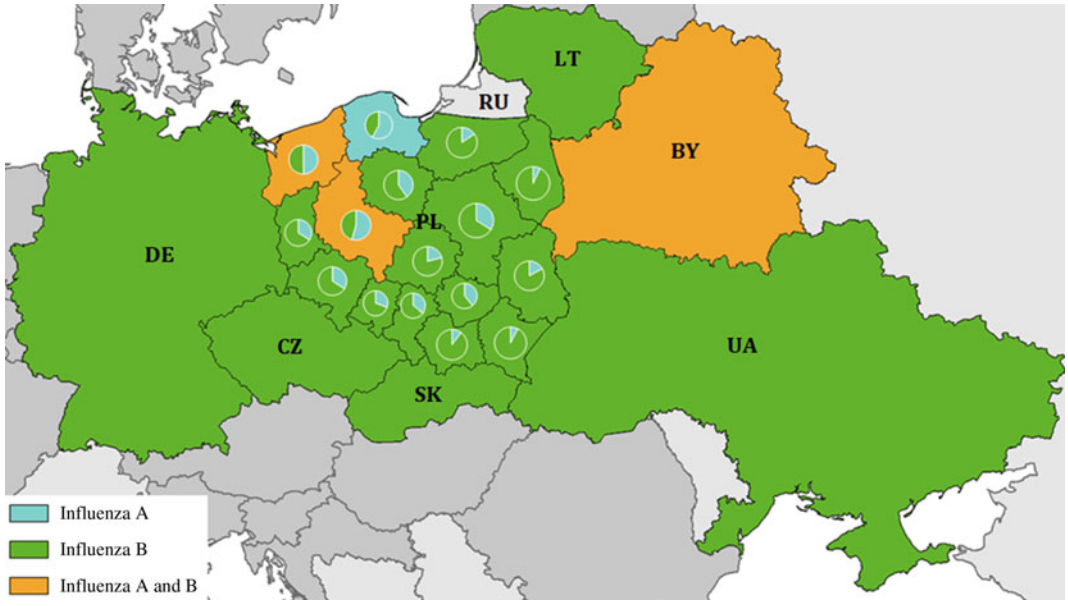

Fig. 3 Predominating influenza viruses in Poland and neighboring countries in 2017–2018 epidemic season. *BY* Belarus, *CZ* Czechia, *DE* Germany, *LT* Lithuania, *PL* Poland, *RU* Russia, *SK* Slovakia, *UA* Ukraine

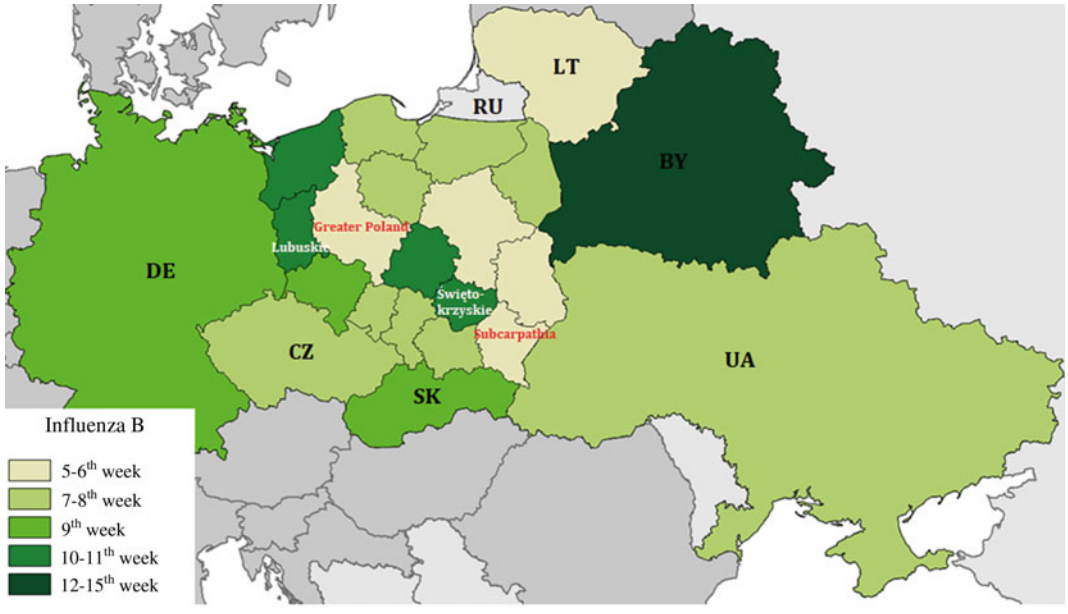

Fig. 4 Incidence of the predominant influenza type B infections by weeks in Poland and neighboring countries in the 2017–2018 epidemic season. *BY* Belarus, *CZ* Czechia, *DE* Germany, *LT* Lithuania, *RU* Russia, *SK* Slovakia, *UA* Ukraine

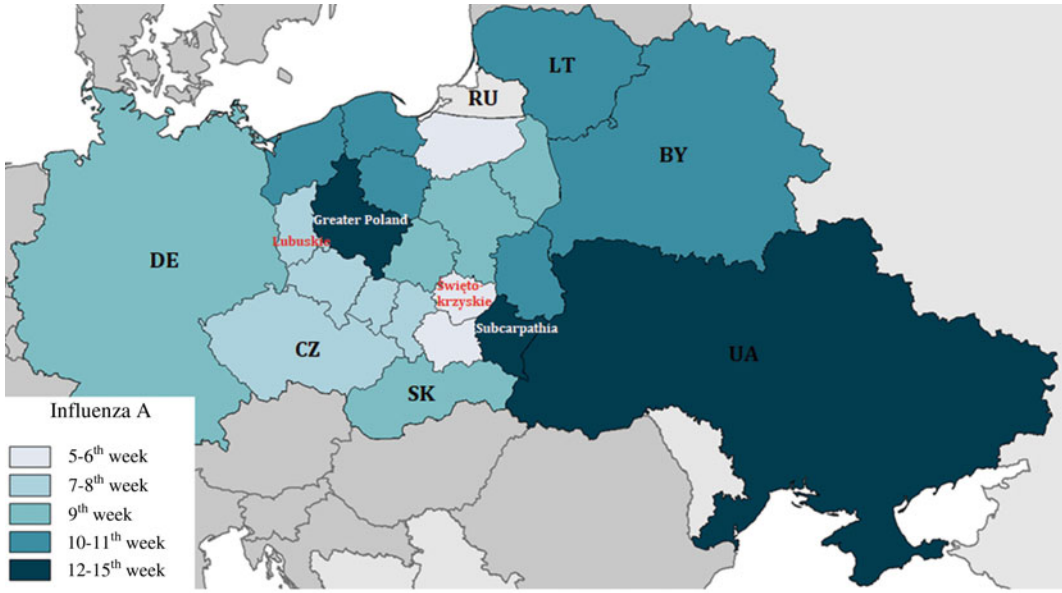

Fig. 5 Incidence of influenza type A by weeks in Poland and neighboring countries in the 2017–2018 epidemic season. *BY* Belarus, *CZ* Czechia, *DE* Germany, *LT* Lithuania, *RU* Russia, *SK* Slovakia, *UA* Ukraine

Lubuskie, Greater Poland, Kuyavian-Pomerania, Lodz, and Mazovia, type A virus was detected most frequently in children up to 4 years of age and in 26–44 years and >65 years old patients. Type B was here detected mainly in adults aged 45–64. In the southern provinces, type A virus was detected in children up to 4 years (Silesia) and up to 9 years of age (Świętokrzyskie and Subcarpathia) and in adults aged 26–44 (Opolskie) and 45–64 years (Lower Silesian and Lubelskie). Type B virus was here detected mainly in the age group 45–64 years and >65 years (Fig. 6). These findings, in the main, confirm that the most vulnerable groups for influenza infection are those below 4 years and above 65 years of age.

We compared the epidemiology of influenza virus circulation in Poland during the 2017–2018 epidemic season with that of the neighboring Germany, Czechia, Slovakia, Ukraine, Belarus, and Lithuania. The Kaliningrad enclave of Russia was not considered due to the lack of available data. In the main, type B virus also predominated in those countries, except for Belarus where there was a mixed predomination of both types A and B (Fig. 3) (WHO 2019). Among the neighboring countries, the peak of type B was observed at Weeks 7–8 in Ukraine and Czechia, followed by Slovakia and Germany at Week 9, and Belarus where the virus detection peaked at Weeks 12–15 afterward (WHO 2019). Although type B virus seemingly migrated in all directions, there is a possibility that the spread originated in the Ukraine and followed westward through Poland. This presumption is somehow strengthened by our finding of a borderline significance of the association between the percentage of confirmed cases of influenza and the proportion of migrants from the Ukraine in the whole populations living in each of the Polish provinces (Pearson's $r = 0.472$; $p = 0.06$) (Table 1). The proportion of migrants stood for 22% of the variation in the percentage of infections among the provinces. The corollary is that the 78% majority of this variation depended on other uncontrolled in this overview factors.

With regard to type A virus, we noticed a possibly opposite eastward direction of its spread, starting from the southwest neighboring countries of Germany, Czechia, and Slovakia. The peak of

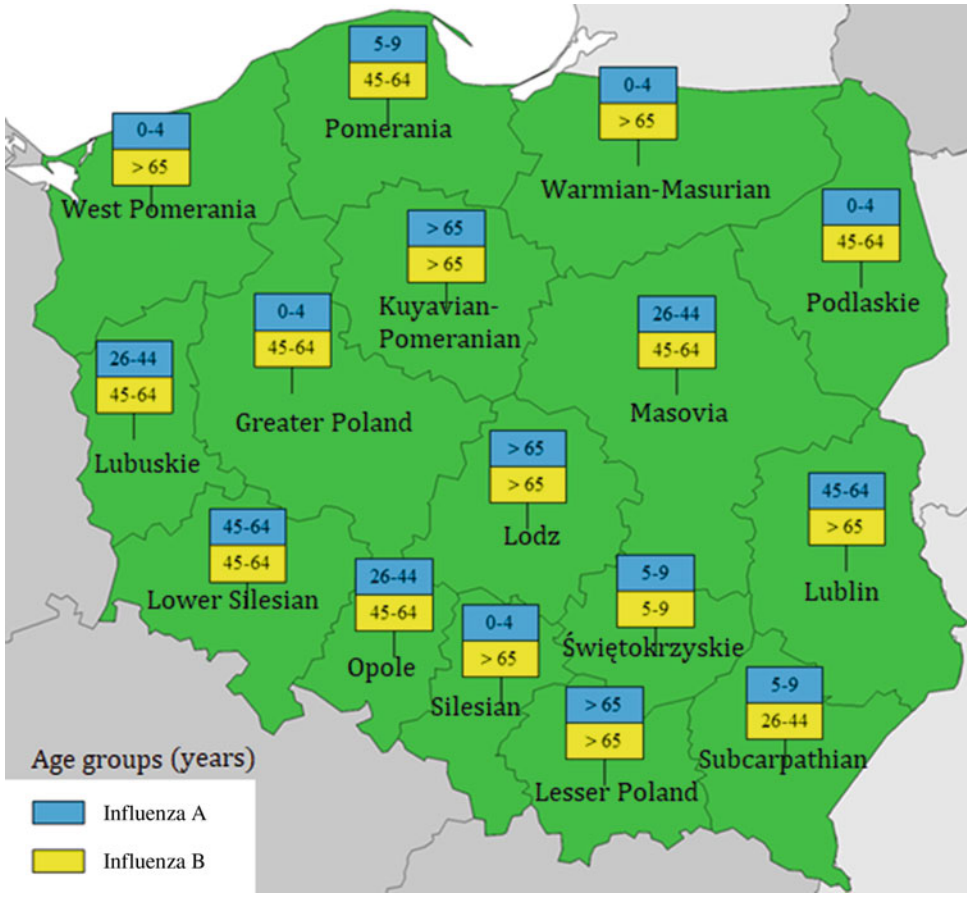

Fig. 6 Predomination of influenza A and B viruses by age groups in the provinces of Poland in 2017–2018 epidemic season

type A detection in those countries was in Weeks 7–9 of 2018, which was grossly akin to what occurred in the Polish provinces bordering these countries (Lubuskie, Lower Silesia, Opole, Silesia, and Lesser Poland). However, in the Eastern Polish provinces (Subcarpathia, Lubelskie, and Podlaskie) and also in the countries bordering Poland from the east (Ukraine, Belarus, and Lithuania), the peak was observed later on between Weeks 10 and 15. These findings from the 2017 to 2018 season are consistent with those from other European countries, where it was established that influenza virus could circulate from west to east (Adlhoch et al. 2018).

In conclusion, this review of the epidemiological situation in Poland during the 2017–2018 influenza season shows that virus type B predominated in 13 out of the 16 Polish provinces as it also did in the majority of other European countries. Data on type B virus did not enable the drawing of a clear-cut conclusion on the way of its spread. Presumptively, the route of type B virus spread originated in the Ukraine and moved westward, with the transmission enhanced, to some extent, by migration of Ukrainian citizens. Virus type A, on the other side, spread from the Southwest Europe eastward. Reviewing the epidemiological situation is essential in gaining knowledge on influenza morbidity and its differentiation from other similar infections, which helps undertake adequate countermeasures against future epidemics.

Table 1 Percentage of influenza-infected patients and the proportion of Ukrainian citizens migrated to and living and working in the Polish provinces

Province	Population	Proportion of Ukrainian citizens in population	Work permits for Ukrainians	Samples tested in a province	Laboratory-confirmed cases of influenza	Infected individuals (%)
Lower Silesia	2,902,547	0.072	209,63	100	79	79.0
Kuyavian-Pomeranian	2,082,944	0.032	65,636	208	87	41.8
Lubelskie	2,126,317	0.038	81,350	266	176	66.2
Lubuskie	1,016,832	0.075	75,799	61	44	72.1
Lodz	2,476,315	0.057	141,276	261	142	54.4
Lesser Poland	3,391,380	0.038	127,231	671	213	31.7
Mazovia	5,384,617	0.071	384,388	112	58	51.8
Opolskie	990,069	0.036	35,830	77	13	16.9
Subcarpathia	2,129,138	0.008	17,205	300	144	48.0
Podlaskie	1,184,548	0.012	14,715	113	43	38.1
Pomerania	2,324,251	0.052	120,182	36	19	52.8
Silesia	4,548,180	0.032	145,893	365	166	45.5
Świętokrzyskie	1,247,732	0.030	37,273	102	69	67.7
Warmia-Masuria	1,433,945	0.014	19,412	338	156	46.2
Greater Poland	3,489,210	0.049	172,424	400	161	40.3
Western Pomerania	1,705,533	0.039	66,514	2383	884	37.1

Number of inhabitants in each province was based on the current data for the Central Statistical Office in Poland

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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 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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Bacteremia in Children Hospitalized Due to Respiratory Syncytial Virus Infection

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Abstract

The frequency of bacteremia in children hospitalized due to respiratory syncytial virus infection (RSV) rarely exceeds 1%, but a recent study reported a 10% risk of bacteremia. In this study, we set out to verify the frequency, usefulness, and costs of blood cultures in RSV infections. We addressed the issue by reviewing medical files of 512 children, aged 8 days–121 months, who were hospitalized during January 2010 and June 2017. The RSV-related diagnoses included bronchiolitis (390 patients), RSV pneumonia (65 patients), and bronchitis (57 patients). There were 212 blood cultures performed in 185 patients (36%). In 10 cultures (5.4%), the following pathogens were identified: *Staphylococcus haemolyticus*, 4; *Staphylococcus epidermidis*, 1; *Staphylococcus hominis*, 1; *Corynebacterium*, 1 *Streptococcus parasanguinis*, 1; *Rothia mucilaginosa*, 1; *Micrococcus luteus*, 1; and *Streptococcus hominis*, 1 case. However, all of these pathogens were identified as a contamination of samples only. Therefore, both positive blood cultures turned out in fact negative, and the patients having either result of blood culturing showed no clinically relevant differences. The total cost of blood cultures in the pediatric ward amounted to €1980. If performed in each and every patient, the costs would have reached €5490. In conclusion, the frank frequency of bacteremia in children with RSV infection, with no sepsis, seems exceedingly low, which confirms the earlier findings. Thus, blood culturing, generating high costs, is of negligible clinical value. The study provides no evidence supporting a routine blood culture in case of children hospitalized due to RSV infection.

Keywords

Bacteremia · Bronchiolitis · Children · Procalcitonin · Respiratory infection · Respiratory syncytial virus · Septicemia

1 Introduction

Respiratory syncytial virus (RSV) is one of the most important single morbidity causes in children worldwide, including Poland. The RSV infection rates vary depending on the period studied, patients' age, and the research setting, ranging from 5.2 to 14.1 per 1000 children per year in children under 2 years of age, with the greatest incidence reaching 17.4–21.7 per 1000 children under in 1 year of age (Svensson et al. 2015; Hall et al. 2013; Fjaerli et al. 2004). The total number of patients infected with RSV each year remains unknown and cannot be calculated precisely, as

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many a patient will have only mild upper airway symptoms, while the infection affects the lower respiratory tract in just 12.5% of those infected (Wu et al. 2015). When the lower respiratory tract is involved, bronchiolitis and pneumonia are the most frequent diagnoses, with a rate of 41% and 34%, respectively, among hospitalized children under 2 years of age (Hacımustafaoğlu et al. 2013). In such cases, hospital treatment is often necessary, mainly in the youngest group of patients. The estimation of the hospitalization rate is 17/1000 in children under 6 months of age, which decreases to 3/1000 in the total population of patients below 5 years of age (Hall et al. 2009).

Bronchiolitis management guidelines state that antibiotics should not be introduced, unless there is clear evidence of a bacterial coinfection (Friedman et al. 2014). Nevertheless, the use of antibiotics in bronchiolitis in children still presents a frequent dilemma to clinicians as a severe condition of a child raises anxiety when antibiotics are not used. Thus, the issue seems to require further debate, particularly that RSV bronchiolitis usually runs a more severe clinical course when compared to than non-RSV infections (Ramagopal et al. 2016; Farley et al. 2014; Stollar et al. 2014). When there is clear evidence of pneumonia, be it viral and bacterial pneumonia, the British Thoracic Society recommends the antibiotic use in each child (Harris et al. 2011).

A number of studies have persistently shown a low frequency of bacteremia during an RSV infection, amounting 0% (Luginbuhl et al. 2008), 0.2% (Greenes and Harper 1999), 0.6% (Bloomfield et al. 2004), and rarely exceeding 1–1.2% (Levine et al. 2004; Titus and Wright 2003; Hall et al. 1988). Surprisingly, a molecular PCR study by Cebey-López et al. (2016) has shown the bacteremia exceeding 10.6%. The patient's poor condition and the possibility of higher bacteremia may factor in a greater use of antibacterials. Therefore, the aim of the present study was to retrospectively evaluate the frequency of blood culturing in children hospitalized due to an RSV infection, with special attention to the frequency of bacteremia. We also assessed the costs of blood cultures and their usefulness in the patient management.

2 Methods

We retrospectively analyzed patient medical files to assess the frequency of blood culturing and its results and costs. Inclusion criteria consisted of an RSV-related hospitalization at the Department of Pediatrics of the Bielanski Hospital in Warsaw, Poland. The diagnosis of an RSV infection was based on the rapid diagnostic test and/or polymerase chain reaction in selected cases. The analysis concerned the period of time between January 2010 and June 2017, which was 90 consecutive months. Patients underwent diagnostic investigation toward an RSV infection whenever there was a justified clinical suspicion of such infection. Altogether, 512 children aged 8 days to 121 months (median age 2.8 months) were diagnosed with RSV infection. The final diagnoses included bronchiolitis (390 patients), RSV pneumonia (65 patients), and bronchitis (57 patients), corresponding to the International Classification of Diseases (ICD-10) codes of J21.0, J12.1, and J20.5, respectively. Blood cultures were taken in selected cases based on a clinician's decision. The indications culturing blood were not analyzed in this study; the emphasis was put on the number and results of blood cultures.

Groups of children with and without blood culture were compared on the basis of both laboratory and clinical indices. Laboratory tests consisted of C-reactive protein (CRP), white blood cell count (WBC), procalcitonin (PCL), percentage of neutrophils and lymphocytes, sodium and potassium content, capillary blood gas, and acid-base balance. Clinical parameters consisted of breathing rate (BR), heart rate (HR), capillary blood oxygen saturation, length of hospitalization, and the need for admission to the intensive care unit (ICU).

To assess the costs of blood cultures, calculations concerned the total cost of the cultures performed, and then the total cost of the cultures in the theoretical model in which blood

culture would have been performed in every patient. In case of falsely positive (contaminated) blood culture results, the costs related to additional mandatory investigations, such as a repeated blood culture, CRP, WBC, and procalcitonin, were included into the analysis. Since the hospital performs laboratory tests at the outsourced basis, the mean cost of each test was assessed from the available data from the bidders in the most recent tender procedure. To assess the proportion of total costs of hospitalization, the results obtained in the study were presented as a part of the amount reimbursed by the Polish National Health Fund – the major Polish insurer which reimburses a fixed amount of money based on the patient's diagnosis. For patients hospitalized due to the RSV bronchitis, bronchiolitis, and pneumonia, the up-to-date reimbursement reaches approximately €462, €771, and €925, respectively. The reimbursement, generally, is not related to the length of hospitalization, although it may be somehow decreased in case of short hospitalizations or increased in extremely long justified hospitalizations.

Data were expressed as mean ± SD when normally distributed and as medians with interquartile ranges (IQR; 25th–75th percentile) when non-normally distributed, which was checked with the Shapiro-Wilk test. A two-tailed unpaired *t*-test or Mann-Whitney U test was used as required. A p-value <0.05 defined statistically significant differences. The analysis was conducted using a commercial statistical package of Statistica v13 (StatSoft; Tulsa, OK).

3 Results

There were 212 blood cultures performed. However, the number of children having blood cultures was 185, which amounted to 36% of all the patients, as there was more than one blood culture in some children. Children, whose blood was cultured, irrespective of its result, had significantly higher WBC (10.65 vs. 9.50 × 10^3 cells/μL; $p < 0.01$), higher percentage of neutrophils (25.6% vs. 19.7%; $p < 0.01$), lower percentage of lymphocytes (57.9% vs. 62.8%; $p < 0.01$), and a higher CRP content (2.19 vs. 0.86 mg/L; $p < 0.01$). In addition, these children had a lower breathing rate; they required 1 day longer hospitalization than those without blood cultures (9 days vs. 8 days, respectively, $p < 0.01$). Although being significant, these differences were of no clinical bearing (Table 1).

Table 1 Clinical and laboratory characteristics of children with RSV-related respiratory infection, with vs. without blood culturing

	Blood culture		No blood culture		
Parameter	Median	Q1–Q3	Median	Lower quartile	p
Age (months)	93	42–152	80	46–142	0.670
Length of stay (days)	9	8–12	8	6–10	<0.0001
WBC (×10^3 cells/μL)	10.65	8.75–13.45	9.50	8.00–12.30	0.001
Neu (%)	25.6	14.9–36.2	19.7	12.3–31.5	<0.001
Lym (%)	57.8	48.0–68.1	62.8	51.9–71.0	<0.003
CRP (mg/L)	2.19	0.41–9.10	0.86	0.27–3.37	<0.0001
PCT (ng/dL)	0.10	0.09–0.17	0.11	0.08–0.22	0.840
Na (mmol/L)	137.3	136.0–138.6	137.0	135.9–138.2	0.296
K (mmol/L)	5.1	4.7–5.5	5.1	4.7–5.5	0.653
pH	7.40	7.37–7.43	7.40	7.39–7.43	0.404
PCO_2 (mmHg)	36	32–40	36	33–41	0.278
ScO_2 (%)	91	88–95	91	88–93	0.071
BR /min	55	48–60	60	50–66	0.008
HR /min	140	131–154	143	136–160	0.142

WBC white blood cell count, *Neu* neutrophils, *Lym* lymphocytes, *CRP* C-reactive protein, *Na* sodium, *K* potassium, *$PcCO_2$* capillary partial pressure of carbon dioxide, *ScO_2* capillary blood oxygen saturation, *BR* breathing rate, *HR* heart rate, *Q1–Q3* medians of the lower and upper halves of data sets

In 10 cases of blood cultures (5.4%), the following bacteria were identified: *Staphylococcus haemolyticus*, 4; *Staphylococcus epidermidis*, 1; *Staphylococcus hominis*, 1; *Corynebacterium*, 1; *Streptococcus parasanguinis*, 1; *Rothia mucilaginosa*, 1; *Micrococcus luteus*, 1; and *Streptococcus hominis*, 1 case. Each positive result was then analyzed in terms of a probable bacterial coinfection or suprainfection.

We failed to substantiate any significant difference in the laboratory or clinical characteristics of the children with falsely positive (contaminated) and negative blood culture (Table 2). Among the patients with positive blood culture, procalcitonin content, which is the most specific marker of bacterial infection, was assessed in eight out of ten children, and it was increased in none of the children. Likewise, increased CRP was noticed in four children, with the highest value of 63 mg/L. However, in the remaining children, CRP was not increased, which counters the presence of any full-fledged bacterial involvement. A WBC level exceeding 20,000 cells/μL was noticed in one patient only (Table 3). Further, repeated blood

Table 2 Clinical and laboratory characteristics of children with falsely positive (contaminated) and negative results of blood cultures

	Blood culture falsely positive (contaminated)		Blood culture negative		p
Parameter	Median	Q1–Q3	Median	Q1–Q3	
Age (months)	116	47–143	88	42–155	0.353
Length of stay (days)	11	8–12	9	8–12	0.604
WBC ($\times 10^3$cells/μL)	10.05	7.42–11.90	10.65	8.90–13.50	0.548
Neu (%)	32.8	22.2–52.9	25.4	14.8–36.0	0.129
Lym (%)	49.6	35.1–55.7	58.1	48.1–68.5	0.052
CRP (mg/L)	10.38	3.02–30.52	2.02	0.41–8.70	0.055
PCT (ng/dL)	0.11	0.10–0.27	0.10	0.08–0.17	0.641
Na (mmol/L)	135.6	135.3–137.0	137.4	136.0–138.7	0.402
K (mmol/L)	5.0	5.0–5.3	5.1	4.7–5.5	0.808
pH	7.40	7.40–7.45	7.39	7.37–7.43	0.246
PCO_2 (mmHg)	35	34–43	36	32–41	0.112
ScO_2 (%)	93	88–101	91	88–94	0.375
BR/min	50	48–62	55	48–60	0.686
HR/min	140	134–150	140	130–154	0.891

WBC white blood cell count, *Neu* neutrophils, *Lym* lymphocytes, *CRP* C-reactive protein, *PCT* procalcitonin, *Na* sodium, *K* potassium, *$PcCO_2$* capillary partial pressure of carbon dioxide, *ScO_2* capillary blood oxygen saturation, *BR* breathing rate, *HR* heart rate, *Q1–Q3* medians of the lower and upper halves of data sets

Table 3 Clinical and laboratory characteristics of children with RSV-related respiratory infection, with falsely positive (contaminated) blood cultures

Patient	WBC ($\times 10^3$ cells/μL)	Neu (%)	Lym (%)	CRP (mg/dL)	PCT (ng/dL)	Hospital stay (days)	Antibiotic
1	11.00	41.0	48.0	62.99	0.10	11	Yes
2	11.90	23.0	61.0	None	0.11	15	Yes
3	21.30	53.0	32.2	14.65	0.42	13	Yes
4	7.00	68.1	23.2	4.00	1.60	7	Yes
5	15.70	52.9	35.1	10.38	None	12	Yes
6	11.70	31.1	50.7	30.52	0.10	10	Yes
7	6.50	34.5	48.5	3.02	None	11	No
8	7.42	22.2	55.7	0.28	0.12	11	Yes
9	9.10	10.5	70.1	<0.10	0.06	8	No
10	8.30	18.3	54.9	0.13	0.10	8	No

WBC white blood cell count, *Neu* neutrophils, *Lym* lymphocytes, *CRP* C-reactive protein, *PCT* procalcitonin

cultures did not confirm the initially positive results. Therefore, all of the results were qualified as sample contamination and not a true bacterial infection. Out of the 10 children with positive blood culture, 7 obtained antibiotic treatment. None of the patients with positive blood cultures had to be transferred to the ICU.

A total cost of blood cultures performed amounted to €1980. Had the blood culture been performed in every patient, the cost would reach €5490. The cost of additional tests related to falsely positive (contaminated) culture results was €260 in ten patients in whom a bacterium was identified. If blood cultures were performed in every patient, and the assumed risk of a contamination leading to a positive result would be 5.4% as outlined above, then additional studies would be performed in 24 children, generating about €624 additional costs (Table 4).

A total reimbursement from the Polish National Health Fund for this cohort of patients (i.e., 512 children diagnosed with RSV infection as above outline) reached €386,957. The chunk of costs, which would have been generated had blood culture been performed in every patient (€5490) would correspond to 1.4% of the total reimbursement. The cost of falsely positive results and additional tests (€625 euro) would use 0.16% more of the reimbursement. Altogether, the additional (unnecessary) costs related to performing blood cultures (and to false positive results) in a single pediatric ward would reach about 1.6% of the sum reimbursed by the National Health Fund (Table 4).

4 Discussion

For many years, it has been believed that RSV patients are "safe" in terms of a serious bacterial infection. Positive blood cultures have been found in very few, not exceeding 1.2% of patients (Levine et al. 2004; Titus and Wright 2003; Hall et al. 1988), with the majority of findings being a fraction of one percentage point (Luginbuhl et al. 2008; Greenes and Harper 1999). Cebey-López et al. (2016), however, performed a prospective multicenter research in which bacterial presence in the blood was assessed in 66 RSV-positive children using PCR for most commonly occurring contagions. Outstandingly, the authors found bacterial suprainfection in seven (10.6%) children, including four cases of *S. pneumoniae* and two cases of *Haemophilus influenzae*. Although this figure is manyfold higher than the average reported in the past aforementioned studies, the authors conclude that this is still not a high incidence of bacteremia, particularly taking into account that the PCR-positive children were not vaccinated against pneumococcal disease. Nonetheless, bacteremia should be considered in case of a severe disease course. The difference between the PCR and past studies may be explicable by the use of conventional blood cultures in the latter. Another reason might be a bias in the patient selection. All of the patients with bacteremia in the PCR study, required respiratory support in the setting of ICU, which may reflect the severity of infection and suppressed immune response. In addition, stay in ICU, in itself, is

Table 4 Expenditure generated by performing blood cultures

	Cost (EUR)	Percentage of hospital costs
1. Blood cultures	–	–
Actually performed in the study group	1980	0.51%
2. Blood cultures –	–	–
(a) Had they been performed in every patient	5490	1.40%
(b) Additional septic analyses had blood cultures been performed in every patient	624	0.16%
3. Total costs of 2 (a) and (b)	6114	1.56%

conducive to bacterial suprainfection. Interestingly, gram-negative bacteria were not identified in any of the patients of the PCR study, the bacteria that usually involve a more serious condition with enhanced inflammatory markers, longer oxygen therapy, and respiratory support (Suárez-Arrabal et al. 2015). In contradistinction, in the present study, we set out to assess the frequency of performing blood cultures in patients hospitalized in a regular pediatric ward, and repeat cultures from patients transferred to the ICU were not taken into consideration. We found no confirmed cases of bacteremia in RSV-related infection. Thus, our findings lend support to those previous literature data that point to a negligible or null rate of bacteremia accompanying RSV-related infections, which obviates the need, in a vast majority of cases, to perform confirmatory blood culturing.

This study has some limitations. Only did 36% of patients have a blood culture performed. Moreover, we failed to compare the results of conventional blood culturing with those of more sophisticated molecular techniques. However, molecular techniques have not yet been implemented into the conventional management of bronchiolitis or any other lower respiratory tract infections, and our goal was to investigate the contemporary clinical routine.

Guidelines for the management of RSV-related bronchiolitis underline no need to implement antibiotic treatment (Ralston et al. 2014). This approach seems well accepted, as studies comparing the use of antibiotics vs. placebo in bronchiolitis show no real benefits of the former (Farley et al. 2014; McCallum et al. 2013; Pinto et al. 2012; Kabir et al. 2009; Mazumder et al. 2009; Kneyber et al. 2008; Field et al. 1966). On the other hand, ampicillin or penicillin G is recommended by the Infectious Diseases Society of America as the first-line treatment choice for inpatient infants and children older than 3 months of age having community-acquired pneumonia (CAP), a disease that may likely be underlined by a different causative factor (Bradley et al. 2011). Current guidelines emphasize that distinguishing between bacterial and viral etiology in CAP is unreliable, irrespective of the diagnostic method used, be it clinical course, chest radiography, or acute phase reactants. Thus, the use of antibiotics is justified in every child with CAP, and it is definitely recommended in children younger than 3 months of age (Harris et al. 2011). The only exception may be made for patients aged 4 months to 5 years who undergo a mild course of disease, particularly in those who are vaccinated against pneumococcal disease. Nonetheless, definition of pneumonia, diagnostic criteria of bronchiolitis, and differences between viral and bacterial infections remain debatable. Likewise, chest radiography is of no real benefit in terms of clinical outcome and management of children with acute lower respiratory infection, including acute bronchiolitis (Schuh et al. 2007; Swingler et al. 1998). Radiography remains recommended mostly for patients who run a severe disease course with complications (Ralston et al. 2014).

In a study by Parikh et al. (2014), blood culturing was performed in 15% of patients with bronchiolitis, which generated over $10,000 expenditure a year. The cost of blood cultures in the present study was much lower due to different lab charges. Yet 1.6% of the total expenditure used for blood culturing appears unreasonably spent money, considering an inappreciable diagnostic effect of the procedure.

In conclusion, we found the incidence of bacteremia is negligible in RSV-related infections, using blood cultures as a conventional diagnostic strategy. The study lends support to earlier literature data showing an exceedingly low incidence of bacteremia in such respiratory infections. We believe these findings do not provide support for routine implementation of blood culturing in children hospitalized due to RSV-related infection.

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Conflict of Interest The authors declare no conflict 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 Center of Postgraduate Medical Education in Warsaw, Poland.

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

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Multi-spectral Pattern of Clinical Presentation and the Resultant Outcome in Central Nervous System Tuberculosis: A Single Center Study on the Ubiquitous Pathogen

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Abstract

Central nervous system (CNS) tuberculosis (TB) is a great medical masquerader having a multi-spectral pattern of clinical presentation, thereby complicating early diagnosis and appropriate management. This review article describes clinical presentation of CNS TB in a group of 47 patients, who were managed in the Nobel Medical College and Teaching Hospital in Biratnagar, Nepal during the last 2 years. We evaluated demographic profile, mode of management, and clinical outcome in these patients. The findings were that intracranial TB was present in 27 (57.5%) patients and the spinal involvement was in 20 (42.5%) patients. The most frequent presentation of the former was TB meningitis with hydrocephalus (55.5%) and that of the latter was Pott's spine with abscess in 50% of cases. TB meningitis with hydrocephalus was the commonest cause of mortality (83.3%) among the patients. CNS TB should be considered in the differential diagnosis in patients presenting with equivocal neurological signs and symptoms, especially in TB endemic regions. It seems prudent to commence early antitubercular therapy for safeguarding such patients from poor neurological outcome as well as mortality it harbingers.

Keywords

Central nervous system · Clinical outcome · Differential diagnosis · Meningitis · Pott's spine · Tuberculosis

1 Introduction

Tuberculosis (TB) is one of the major global health concerns. Despite being globally the most common infective cause accounting for mortality, paradoxically up to two thirds of the people afflicted with TB remain undiagnosed (Bloom et al. 2017; Nelson and Zunt 2011). Moreover, easy accessibility to medical centers employing 'directly observed treatment short course' (DOTS), non-compliance to therapy, associated adverse effects, emergence of multidrug resistant TB, resource starved circumstances of the effected regions, and diagnostic challenges are the major hindrances limiting the efforts to effectively control and treat TB (Rock et al. 2008; WHO 1997).

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In Nepal, TB is the 7th leading cause of death. About 300 new cases, mostly within the economically productive age groups, are diagnosed each day (Adhikari et al. 2019). The Terai regional belt is particularly affected by TB, accounting for up to 50% of infection cases nationwide (National Tuberculosis Program 2017). Central nervous system (CNS) tuberculosis is seen in 10% of all TB patients, resulting in large mortality and long-term dependency for survivors (Rock et al. 2008; Sreeramareddy et al. 2008). In this study we evaluated multispectral patterns of clinical presentation and conducted an audit on outcomes in patients with CNS TB.

2 Methods

This is a retrospective observational study, carried out in patients diagnosed with CNS TB, admitted and managed within the last 2 years in the Neuroscience Department of the Nobel Medical College and Teaching Hospital in Biratnagar, Nepal. Hospital files of 47 patients suffering from CNS TB (M/F -35/12, mean age 32 years, range 21–56 years) were reviewed. The diagnosis was based on the clinical history, signs and symptoms, microscopic examination of a person's sputum for acid-fast bacteria (AFB) and for the content of adenosine deaminase, culture for AFB in sputum or other tissue specimens, chest X-ray, Monteux test, cerebrospinal fluid (CSF) analysis for sugar, protein, and total cell count, and polymerase chain reaction to detect *Mycobacterium tuberculosis* in histologic specimens (Gupta and Kumar 2011, Rock et al. 2008; Garg 1999). We included all cases that met the following case definition of definite, probable, or possible CNS TB (Soria et al. 2019; Thwaites et al. 2009):

- Definite CNS TB – either a positive culture for *Mycobacterium tuberculosis* or the presence of AFB
- Probable CNS TB – clinical symptoms of meningitis highly suggestive for CNS TB, along with the isolation of *Mycobacterium tuberculosis* outside the CNS, as well as exclusion of other differential causes of meningitis;
- Possible CNS TB – clinical symptoms of CNS TB with other relevant clinical biomarkers, such as CSF findings suggestive of TB, along with exclusion of other differential causes, but without any definitive isolation of the pathogen

Intracranial TB included a spectrum of presentations comprising the following lesions, as exemplified in Fig. 1:

- Meningitis with hydrocephalus
- Meningoencephalitis
- Disseminated ring lesions
- Tuberculoma
- Abscess
- Dural based lesions
- Cystic lesion with enhancing mural nodule.

All of the patients were managed with antitubercular therapy as *per* our national TB treatment guidelines (Thwaites et al. 2004). Patients who were lost to follow up or those who left the hospital during the course of treatment against our medical advice were discarded from the evaluation.

Patients with TB meningitis of low Vellore grading (Grade 1 and 2), in association with hydrocephalus, were managed first by external ventricular drainage (EVD), followed by ventriculo-peritoneal (VP) shunting (acute phase of meningitis) or by endoscopic third ventriculostomy (ETV) (chronic phase of meningitis) (Yadav et al. 2016). In those with poor Vellore grading (Grade 3 and 4), VP shunt was the only procedure carried out, providing that the patient showed a good neurological improvement. In the patients with a mass lesion, such as tuberculoma, abscess, or mural nodules, surgical excision and aspiration in case of an abscess was performed.

Patients with spinal TB were clinically categorized according to the American Spinal Injury Association (ASIA) grading scale taking

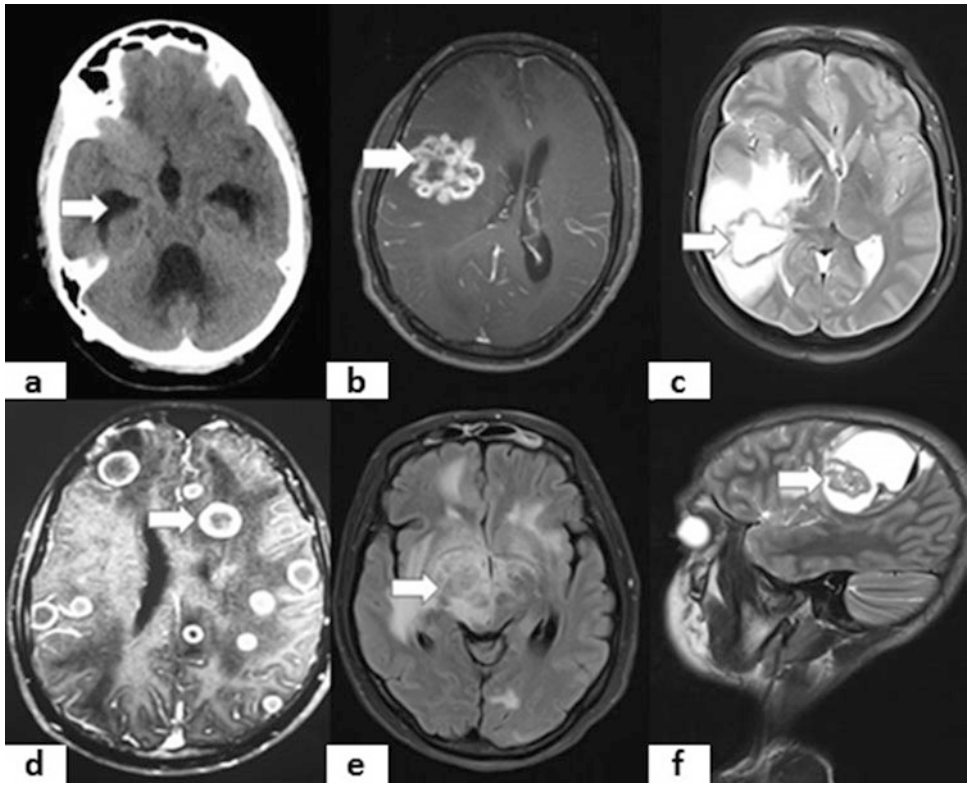

Fig. 1 Multi-spectral presentations of intracranial tuberculosis: (**a**) acute hydrocephalus, (**b**) tuberculoma, (**c**) abscess, (**d**) disseminated ring lesions, (**e**) meningoencephalitis, and (**f**) cystic lesion with mural nodule, all outlined by white arrows

into account their neurological deficits (Jain and Sinha 2005). Spinal TB entailed a spectrum of clinical presentation. We found the following lesions, illustrated in Fig. 2:

- Discitis
- Pott's spine with abscess
- Cord infarction
- Dural based lesions.

A convenience sampling was used to calculate the sample size (n) required for the study. The $n = z^2 \times p \times q/d^2$ formula was applied, where $Z = 1.96$ at 95% confidence interval, $p = 10\%$ prevalence of CNS TB, $q = 1\text{-}p$, and $d = 10\%$ margin of error. The minimum sample size was calculated at 37, whereas the total number of patients included amounted to 47. Data were presented as frequency distribution (counts and percentages) of the occurrence of various complications and grades of CNS TB meningitis and spinal TB of disease cases.

3 Results

The CNS TB patients' demographic characteristics are shown in Table 1. The male to female ratio was 2.9:1. Only did two patients have a history for undergoing treatment for pulmonary TB, whereas three patients had a history of prolonged contact with TB sick individuals. The concurrent HIV infection was unnoticed in this study. Twenty two (63.8%) patients showed clinical improvements in the first three months of interventional treatment, followed by antitubercular therapy.

The most frequent presentation of intracranial TB was meningitis with hydrocephalous accounting for more than half of the cases, in 15 (55.5%)

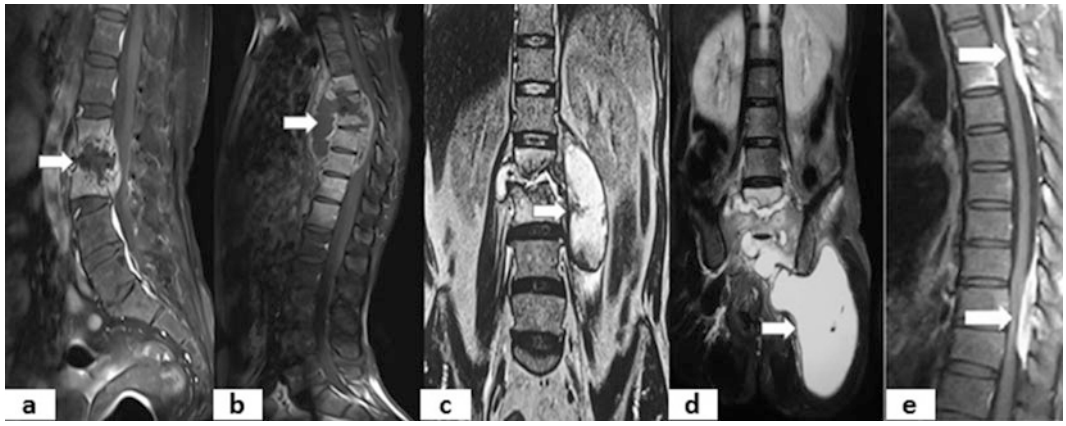

Fig. 2 Multi-spectral presentations of spinal tuberculosis: (**a**) discitis, (**b**) paraspinal abscess, (**c**) psoas abscess, (**d**) sacral abscess, and (**e**) dura based lesions; all outlined by white arrows

Table 1 Demographics of the central nervous system tuberculosis (TB) patients evaluated in the study

Previous history of TB	2 (4.3%)
Contact with TB sick	3 (6.4%)
Concomitant HIV infection	0
Male/female ratio	2.9/1.0
Mean age (range)	32 (21–56) years
Improvement after 3-month treatment	30 (63.8)%
Mortality	6 (12.8%)

out of the 27 patients (Fig. 3). Further, in eight of these 15 patients the condition was severe, graded 3 and 4 on the Vellore scale for TB meningitis. Seven out of the 15 patients were managed with VP shunt, 4 patients underwent ETV, with two of the four having to undergo subsequent VP shunting due to the ETV failure. The least common presentation was that of cystic lesions with nodules observed in only one patient.

Likewise, the most frequent form of spinal TB was Pott's spine with abscess present in 10 patients; followed by discitis that presented in 8 patients. The least frequent presentation was a dura based lesion and spinal cord infarction, noticed in 2 patients each (Fig. 4). Concerning Pott's spine, the thoraco-lumbar region of the spine was involved in 8 cases of spinal TB, followed by cervical and sacral regions involved in 1 case each. The majority of patients (60%) presented with advanced ASIA Grade C and D (Fig. 5).

Four patients with thoracic lesions and progressive neurological deficits underwent lateral extra-cavitory approach (LECA) for decompression and drainage of abscess. Two patients with psoas abscess underwent retroperitoneal drainage of abscess, whereas one patients with pelvic and two patients with paraspinal abscess underwent ultrasound guided percutaneous aspiration of the abscess. Mortality rate was 12.8%, with five cases of high Vellore grade TB meningitis with hydrocephalus and one case of thoracic Pott's spine with ruptured TB abscess complicated with mediastinitis.

4 Discussion

This review shows that CNS TB most often afflicts middle-aged male patients of productive age, with mean age being 35 years (range 21–56 years). We noticed a positive history of being previously treated for pulmonary TB or having a prolonged contact with someone afflicted with TB in only 2 and 3 patients, respectively. The predominance of middle-aged men is in line with data from other studies (Thwaites et al. 2004). Likewise, Soria et al. (2019) found that the majority of the investigated 263 patients with TB meningitis (72.6%) were male and the median age was 35 years (range 18–84 years). In that study, 63 patients (24.0%) had a prior history

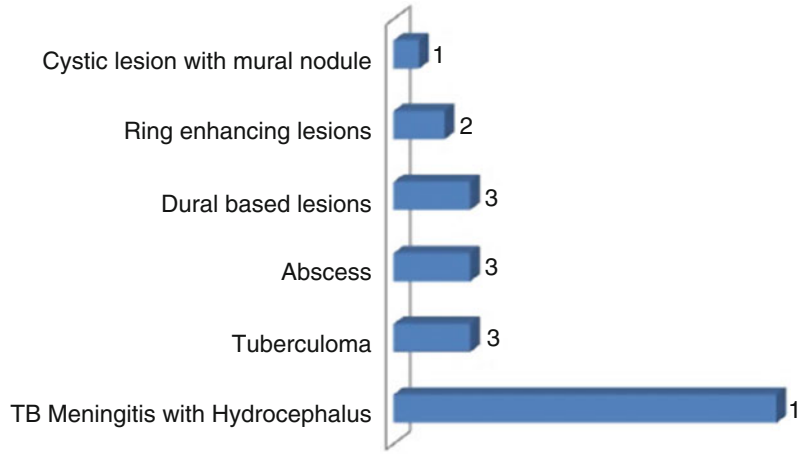

Fig. 3 Distribution of different presentations of intracranial tuberculosis (TB)

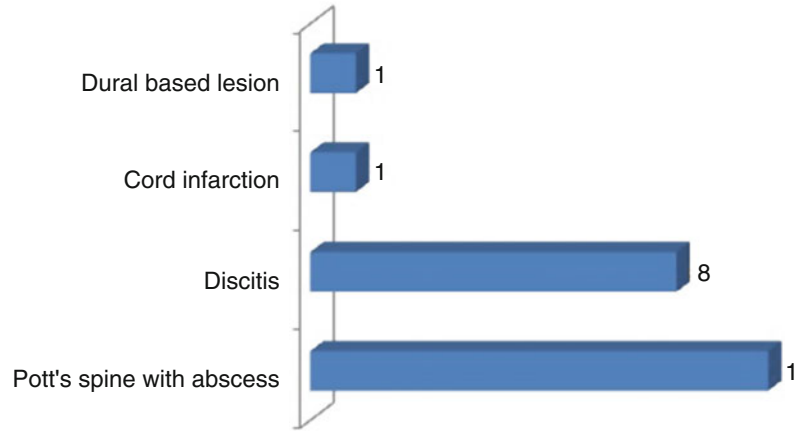

Fig. 4 Distribution of different patterns of presentation in spinal TB

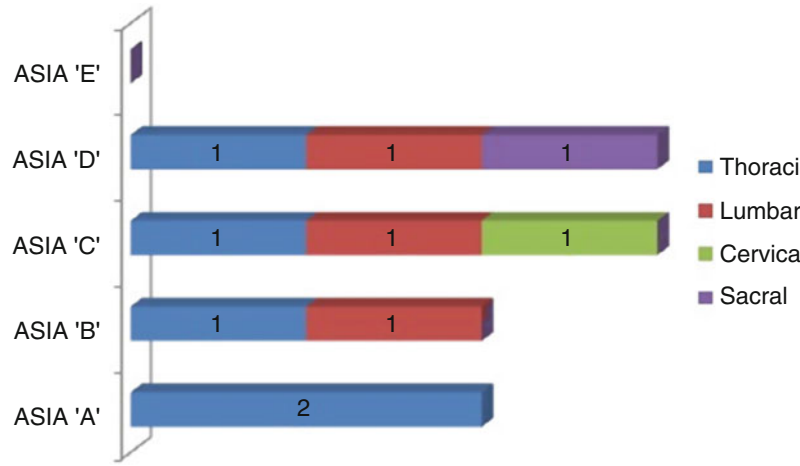

Fig. 5 Regional distribution of the level of spinal cord involvement in Pott's spine condition and the American Spinal Injury Association (ASIA) grading scale of patients with Pott's spine TB

of TB infection, 70 (26.7%) had contact with persons suffering from TB, and 24 (38%) tested positively for HIV co-infection. In another study, all the participants with spinal TB had a history of pulmonary TB and 28% of patients tested positively for HIV infection (Rajshekhar 2009).

In this study, the most common form of CNS TB presentation was TB meningitis with hydrocephalous comprising more than half of the total CNS TB cases. This finding is in line with previous reports that demonstrate the incidence of TB meningitis in 80% of patients diagnosed with CNS TB (Thwaites et al. 2004). However, we noticed no case of co-infection with HIV. In TB meningitis with hydrocephalus, patients with higher Vellore grading (Grades 3 and 4), usually have poor outcome, with mortality reaching 52–100%. Surgery seems to play an unsatisfactory role in such patients as well (Rock et al. 2008). The overall mortality in CNS TB has been reported anywhere between 10% and 57%, being about the middle of this range in the accompanying HIV infection (Soria et al. 2019; Robertson et al. 2018; Dela et al. 2017; El Sahly et al. 2007; Karstaedt et al. 1998). In the present study, there were six patients who died, giving the overall mortality rate of 12.8%. Five out of the six deadly ill patients were in severe condition, with the high Vellore grades and with hydrocephalus. Hydrocephalus *per se* seems the most important determinant of mortality in patients with CNS TB.

The ASIA scale is an effective grading system for neurological deficits in spine TB. A study by Godlwana et al. (2008), performed in 104 spinal TB patients, has shown the thoracic spine involvement in almost 42% of cases. Thirty two percent of them had incomplete paraplegia and 24% had complete paraplegia. In line with those finding, in the present study the thoracic involvement in the form of Pott's spine predominated in patients with spine TB, being present in 50% of patients. A combination of surgical decompression and treatment with antitubecular drugs is needed for the majority of patients with Pott's paraplegia (National Tuberculosis Center (2019)). A period of 12 months of postoperative antitubecular therapy is adequate (Thwaites et al. 2009). Only did one of our patients (2.1%) develop transient hepatitis while on antitubercular therapy. Other studies point to a substantial number of possible adverse effects of the treatment. Sinha et al. (2013) have reported that almost 69% of patients treated with antitubecular drugs come down with adverse reactions, gastrointestinal disorders accounting for about 54% of them. No case of multidrug resistant TB was noticed in the present study. However, the incidence of past multidrug resistant TB has been reported by Soria et al. (2019) in 2.3% patients admitted with TB meningitis, compared with the 3.3% of multidrug resistant new TB cases and 20% of previously treated TB patients in a study of Dela et al. (2017), with almost 50% of them coming from India and China, although the specific organ involvement was not mentioned in the latter study.

In conclusion, CNS tuberculosis is a great medical masquerader having a multispectral pattern of clinical presentation. This disease entity should always be considered in the differential diagnosis while managing patients with CNS disorders, particularly in TB endemic regions. Early implementation of antitubercular therapy can help safeguard these patients from mortality and poor neurological outcome. This study demonstrates that TB meningitis with hydrocephalus is the most common form of presentation of intracranial TB, whereas Pott's spine with abscess is the most common presentation of spinal TB. TB meningitis with hydrocephalus is accountable for the highest mortality in CNS TB patients.

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

Ethical Approval All procedures and studies described in this review were conducted 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 article gained approval from the Institutional Review Committee of the Nobel Medical College and Teaching Hospital (IRC-279/2019).

Informed Consent As there was no current involvement of any human studies in this review article, consent from individual participants was not required.

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Adherence to Therapy in Chronic Obstructive Pulmonary Disease: A Systematic Review

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Abstract

Adherence to therapy plays a key role in treatment optimization and clinical outcome in patients with chronic obstructive pulmonary disease (COPD). The adherence to inhaled medications is poor, ranging from 20% to 60%. In this study we searched Medline and PubMed literature regarding factors that could have an impact on therapy adherence in COPD patients, using the key words "COPD" or "chronic obstructive pulmonary disease" and "adherence". The search was limited to the English language article published between January 2013 and December 2019. Review papers, study protocols, and meta-analyses were excluded. The final material included 25 articles. The evaluation was performed using the Cochrane Review Manager guidelines. The 25 articles represented 29 countries from 5 continents. We assessed adherence to therapy and the impact of selected factors on the adherence in 27,660 COPD patients (60.9% of whom were male, mean age 64 years). The factors affecting adherence were broken down into three categories: sociodemographic, clinical, and psychological. There were two standardized instruments used in the analyzed studies: Test of Adherence to Inhalers (TAI) and self-reported Morisky Medication Adherence Scale (MMAS-8). We found that 46.3% of patients had a moderately good level of adherence to inhaled therapy (TAI range around 50 points), while 41.6% of patients had a high level of adherence to oral therapy. The nature of non-adherence was in most cases inadvertent rather than an erratic or deliberate demeanor (48.5% vs. 38.9% vs. 42.4%, respectively). We conclude that standardized instruments enable the prediction of adherence to therapy and should be used in clinical practice. The assessment of adherence is essential for undertaking interventions to counteract plausible non-adherence. Collaboration between an educator and a psychologist is required to evaluate the patient's motivation and to ensure his comprehension of treatment prescribed.

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Keywords
Adherence to therapy · Clinical practice · COPD · Treatment optimization

1 Introduction

Adherence to treatment plays a key role in the course of chronic obstructive pulmonary disease (COPD). Non-adherence is rather common, as adherence ranges between 20% and 60% of COPD patients. Non-adherence results in symptom exacerbation, frequent hospitalizations, and poor quality of life. COPD was the fourth most common cause of death in 2000. It is estimated that 4.7 million people will die from COPD by 2020, making the disease the third most common cause of death worldwide. Human and economic losses due to non-adherence in COPD have been estimated at $300 billion per year (DiMatteo 2004).

The WHO has identified non-adherence to treatment as one of the most serious health problems. Non-adherence is a fundamental barrier to achieving the expected outcomes of evidence-based treatment. The consequences for non-adherent patients range from health deterioration to death. At the same time, non-adherence has serious health-related and economic ramifications for the entire society. To date, hundreds of factors that affect treatment adherence have been described. Their classification by the WHO comprises five broad categories: (1) socioeconomic factors, (2) healthcare system-related factors, (3) illness-related factors, (4) treatment-related factors, and (5) patient-dependent factors (WHO 2003).

Factors associated with satisfaction and technique of inhaled therapy have been identified as the most significant for COPD treatment adherence, while sociodemographic factors are considered the least significant. The main problem associated with inhaled therapy is a lack of the patients' savvy to use this treatment option. Other substantial predictors of adherence include psychological factors. Beliefs about medication and treatment satisfaction are the most common causes of treatment discontinuation. Chronically treated patients collect experiences and develop their own beliefs regarding the use of specific medications, sometimes also considering the experience of their friends or family members. Another major determinant of adherence is concern about the use of complex inhalers or other devices. In the literature, differences in adherence have been reported between inhaled and oral treatments. Thus, factors that would influence adherence should be considered when selecting specific treatment.

The available reports present contradictory findings regarding the impact of sociodemographic and clinical factors on adherence in COPD patients. In general, there are few studies that include the assessment of the impact of psychosocial factors on adherence to treatment in chronically ill patients. There is also an apparent shortage of the literature describing non-adherence risk factors in COPD patients. Therefore, this study was undertaken to present and evaluate the available literature findings on the influence of sociodemographic, clinical, and psychosocial factors on adherence to therapy in COPD patients.

2 Methods

2.1 Search Strategy

In this study, we searched Medline and PubMed databases for the articles addressing factors that could have an impact on therapy adherence in COPD patients, using the key words "COPD" or "chronic obstructive pulmonary disease" and "adherence" or "MMAS" or "TAI". The search was limited to the English language article published between January 2013 and December 2019. Review papers, study protocols, and meta-analyses were excluded. The final material included 25 articles comprising a total of 27,660 COPD patients. Of this cohort, 16,844 (60.9%) were men of the mean age of 64 years. The evaluation was performed using the Cochrane

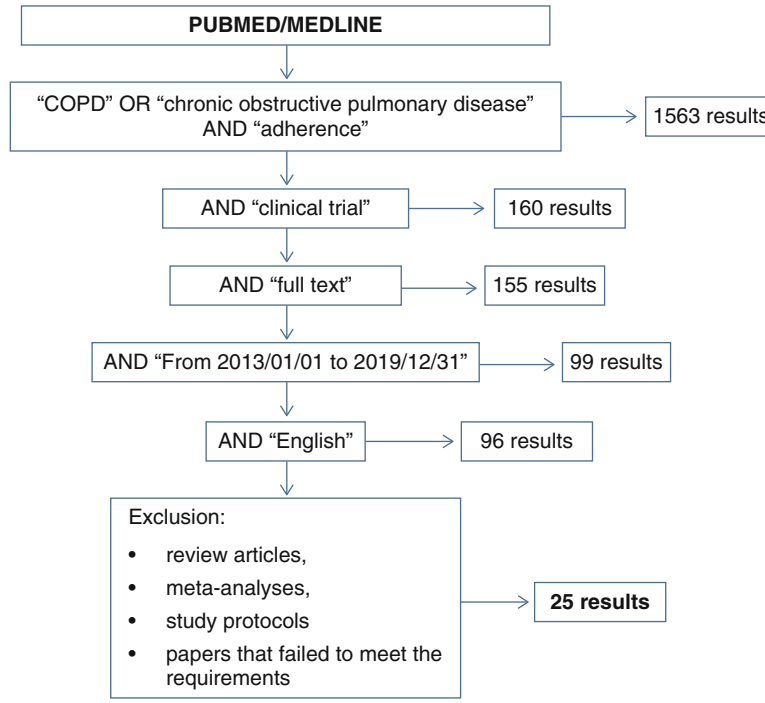

Fig. 1 Study flow diagram

Review Manager guidelines. Subsequent analyses were performed using the Cochrane Review Manager guidelines (Lutje 2019). The search scheme is presented in detail in Fig. 1.

2.2 Statistical Elaboration

Statistical analysis was performed using a fixed effect or random effect model, depending on the heterogeneity of results. For the latter model, the DerSimonian–Laird estimator was used. A p-value <0.05 defined statistically significant associations. The analysis was performed using R software v3.6.1 (R Core Team 2019).

3 Results

There were two main standardized instruments used in the analyzed studies: Test of Adherence to Inhalers (TAI) and self-reported Morisky Medication Adherence Scale (MMAS-8). TAI consists of 10 (short version) or 12 (full version) items. In the 10-item version of the test, each item was scored between 1 (worst) and 5 (best) possible score, yielding a total ranging from 10 to 50 points. The 12-item TAI includes additional two items addressing the healthcare professional, which are scored 1 (bad) and 2 (good), adding the maximum of 4 points. These two items were designed to unravel the inadvertent non-adherent pattern. This pattern was identified when the score for item 11 or 12 was 1. The erratic or deliberate non-adherent behavior pattern was identified when the score for items either 1–5 or 6–10 was ≤ 24, respectively (Plaza et al. 2016a).

The Morisky Medication Adherence Scale (MMAS-8) is a self-reported questionnaire to assess adherence to medication. The scale comprises eight items that assess behaviors and barriers related to the long-term adherence to medication. MMAS-8 score may range between 0 and 8, with the scores <6, 6–7, and 8 corresponding to low, medium, and high adherence, respectively (Morisky et al. 2008).

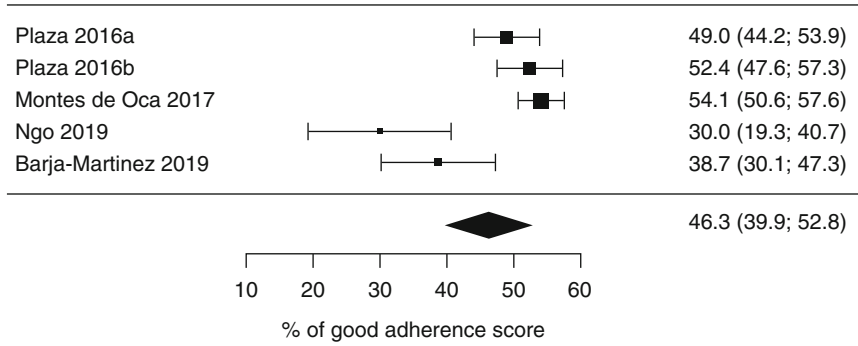

Fig. 2 Percentage of patients with good scores of adherence to therapy in COPD patients

3.1 Test of Adherence to Inhalers (TAI): Five Studies Included in the Analysis

3.1.1 TAI: % of Good Scores of Adherence to Therapy

The analysis shows that 46.3% of patients in the analyzed studies obtained good scores of adherence to therapy (95%CI; 39.9–52.8). The test for heterogeneity demonstrated a considerable heterogeneity of data, with the heterogeneity coefficient of $I^2 = 84.8\%$ ($p < 0.001$). The random effects model was applied in the data elaboration (Fig. 2).

3.1.2 TAI: % of Inadvertent Non-adherence to Therapy

The analysis shows that 48.5% of patients in the analyzed studies showed inadvertent non-adherence to therapy (95%CI: 21.3–75.7). The test for heterogeneity demonstrated considerable heterogeneity of data, with the heterogeneity coefficient of $I^2 = 99.4\%$ ($p < 0.001$). The random effects model was applied in the data elaboration (Fig. 3).

3.1.3 TAI: % of Erratic Non-adherence to Therapy

The analysis shows that 38.9% of patients in the analyzed studies showed erratic non-adherence to therapy (95%CI: 18.3–59.4). The test for heterogeneity demonstrated considerable heterogeneity of data, with the heterogeneity coefficient of $I^2 = 98.9\%$ ($p < 0.001$). The random effects model was applied in the data elaboration (Fig. 4).

3.1.4 TAI: % of Deliberate Non-adherence to Therapy

The analysis shows that 42.5% of patients in the analyzed studies showed erratic non-adherence to therapy (95%CI: 12.0–72.9). The test for heterogeneity demonstrated considerable heterogeneity of data, with the heterogeneity coefficient of $I^2 = 99.6\%$ ($p < 0.001$). The random effects model was applied in the data elaboration (Fig. 5).

3.2 Morisky Medication Adherence Scale (MMAS-8): Six Studies Included in the Analysis

3.2.1 MMAS-8: % of High Scores of Adherence to Therapy

The analysis shows that 41.6% of patients in the analyzed studies obtained high adherence scores (95%CI: 26.7–56.4). The test for heterogeneity demonstrated considerable heterogeneity of data, with the heterogeneity coefficient of $I^2 = 99.3\%$ ($p < 0.001$). The random effects model was applied in the data elaboration (Fig. 6).

3.3 Factors Affecting Adherence to Therapy in COPD Patients

Table 1 shows sociodemographic, clinical, and psychological factors contributing to

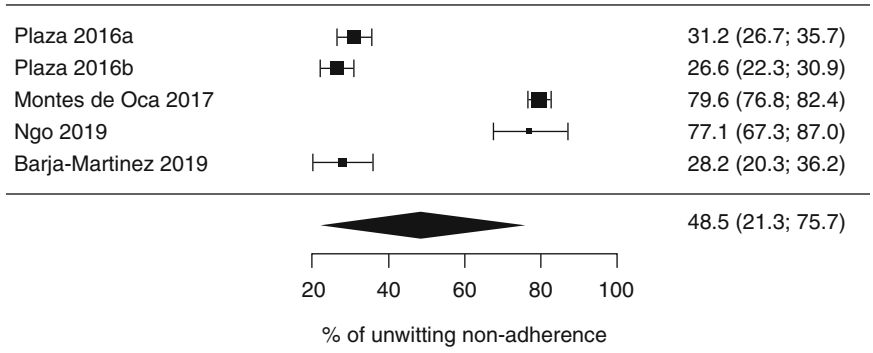

Fig. 3 Percentage of patients with inadvertent non-adherence to therapy in COPD patients

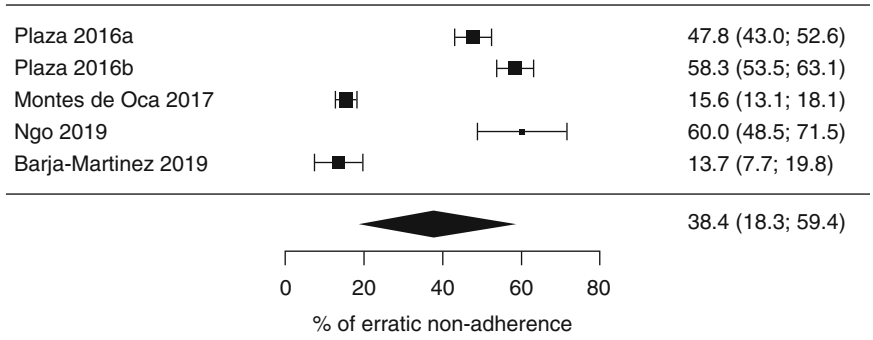

Fig. 4 Percentage of patients with erratic non-adherence to therapy in COPD patients

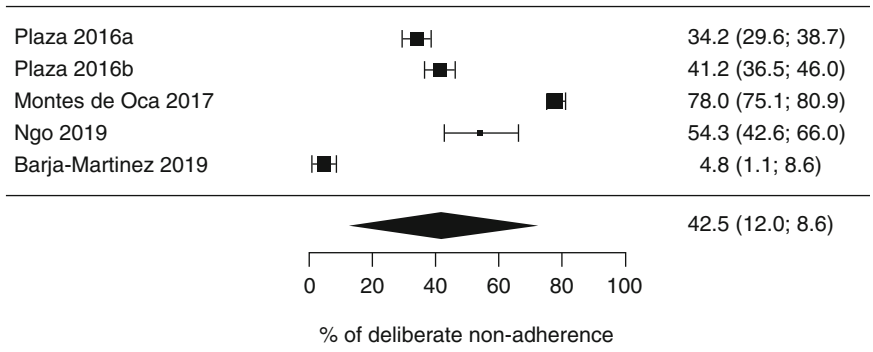

Fig. 5 Percentage of patients with deliberate non-adherence to therapy in COPD patients

non-adherence to therapy in COPD patients. The most commonly reported factors having a negative connotation were those of sociodemographic (low income), clinical (disease duration, severity, and medication), and psychological nature (coping with disease stress, depression, or negative beliefs concerning curability).

Among the factors contributing to better adherence to COPD therapy, age $\geq$ 60 years and male gender are the most commonly reported sociodemographic factors (Table 2). On the clinical side, frequency of hospitalization and the knowledge about the number and type of medications and the use of inhalers also had a

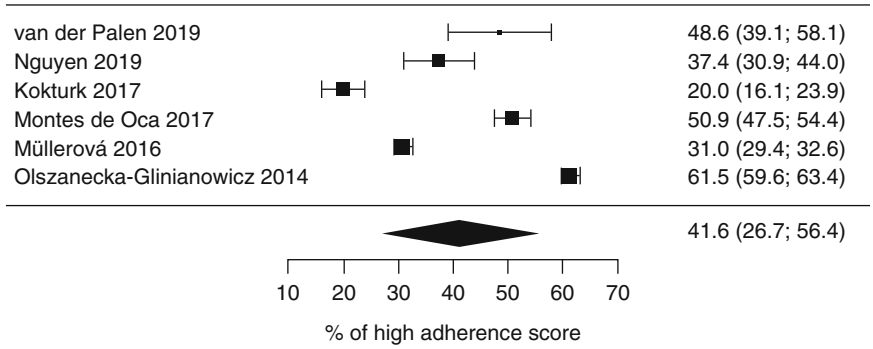

Fig. 6 Percentage of patients with high scores of adherence to therapy in COPD patients

Table 1 Factors decreasing adherence to therapy in COPD patients

Group of factors	Factor	First author and year
Sociodemographic	Income	Liao and Chen (2019)
Clinical	Comorbidities (diabetes, hyperlipidemia)	Liao and Chen (2019)
	Decreasing FEV1	Jouleh (2018)
	Gold stage 2	Jouleh (2018)
		Ivanov (2018)
	Alcohol	Toyama (2019)
	Drugs (bronchodilators, beta-2 adrenergic agonists, xanthines)	Tran (2016)
		Leiva-Fernández (2014)
	Duration of hospitalization	Ngo (2019)
	Duration of COPD	Ngo (2019)
		Olszanecka-Glinianowicz and Almgren-Rachtan (2014)
	Dyspnea (mRC)	Ngo (2019)
	Smoking (number of packs per year)	Ngo (2019)
Psychological	HADS depression 8–10 or > 10	Kokturk (2018)
	Emotional impact of disease	Olszanecka-Glinianowicz and Almgren-Rachtan (2014)
	Negative beliefs about treatment	Toyama (2019)

FEV1 forced expiratory volume in 1 s, *GOLD* Global Initiative for Lung Disease staging, *mRC* Modified Medical Research Council dyspnea scale, *HADS* Hospital Anxiety and Depression Scale

positive influence on adherence to therapy. Satisfaction with inhalation treatment was commonly reported as a strong predictor of better adherence as well.

4 Discussion

This article is a review of recent studies on factors affecting adherence to COPD treatment. Most of the analyzed studies used self-reported measures. The findings demonstrate that COPD patients have lower adherence to therapy than those with other chronic diseases, such as cardiovascular disorders, hypercholesterolemia, osteoporosis, or diabetes. In COPD, adherence rates ranged between 20% and 60%. George et al. (2005), using a patient-reported medication adherence scale, have found a good adherence in 37% of patients. Ágh et al. (2011) have reported that 58.2% of patients had optimum adherence, based on MMAS-8 scores. Plaza et al. (2016b)

Table 2 Factors increasing adherence to therapy in COPD patients

Group of factors	Factor	First author and year
Sociodemographic	Age ≥ 60 years	Barja-Martínez (2019)
		Liao and Chen (2019)
		Kokturk (2018)
		Müllerová (2016)
	Male gender	Liao and Chen (2019)
		Chrystyn (2014)
		Müllerová (2016)
		Kokturk (2018)
	Country	Kokturk (2018)
	Higher educational level (high school or college graduate)	Kokturk (2018)
		Montes de Oca (2017)
	Lower educational level (primary school)	Barja-Martínez (2019)
	Being unemployed	Plaza (2016b)
Clinical	Type of inhaler (soft mist)	Ngo (2019)
	Low % post-BD FEV1	Montes de Oca (2017)
	Low % post-BD FEV1/FVC	Montes de Oca (2017)
	Telemonitoring	Moy (2016)
		Broadbent (2018)
		Pinnock (2013)
	Increasing number of prescribed drugs	Jouleh (2018)
	GOLD-stage 3–4	Jouleh (2018)
		Mısırlıgil et al. (2017)
		Ivanov (2018)
		Olszanecka-Glinianowicz and Almgren-Rachtan (2014)
	Fewer comorbidities	Müllerová (2016)
	Fewer exacerbations	Leiva-Fernández (2014)
		Ngo (2019)
		Montes de Oca (2017)
	Fewer health center visits	Leiva-Fernández (2014)
		Olszanecka-Glinianowicz and Almgren-Rachtan (2014)
		Jouleh (2018)
	Lower CAT	Müllerová (2016)
		Ngo (2019)
		Montes de Oca (2017)
	Fewer devices	Leiva-Fernández (2014)
	Self-reported history of past spirometry	Müllerová (2016)
	No emergency visits due to COPD in past 12 months	Müllerová (2016)
Psychological	Inhaler satisfaction	van der Palen (2019)
		Chrystyn (2014)
	Sense of disease control	Olszanecka-Glinianowicz and Almgren-Rachtan (2014)
	Satisfaction with doctor's management of COPD	Müllerová (2016)
	Fewer maintenance drugs	Chrystyn (2014)
	Positive beliefs about treatment	Olszanecka-Glinianowicz and Almgren-Rachtan (2014)

(continued)

Table 2 (continued)

Group of factors	Factor	First author and year
	Knowledge about treatment and inhalers	Tommelein (2014)
		Poureslami (2016)
		Barja-Martínez (2019)
		Moy (2016)
		Broadbent (2018)
		Dudvarski Ilic (2016)
		Nguyen (2019)

FEV1 forced expiratory volume in 1 s, *FVC* forced vital capacity, *GOLD* Global Initiative for Lung Disease staging, *Post-BD* post bronchodilator test, *CAT* COPD Assessment Test

have found that adherence to inhaled therapy was present in 49% of COPD patients. Referring to the causes of non-adherence, authors report a variety of factors, and the issue is yet debatable in the literature. In the present review, we broke down the factors associated with adherence to COPD therapy into the sociodemographical, clinical, and psychological category, akin to the classification used by WHO (2003).

In screening tests performed in daily clinical practice, the level of adherence in patients treated for asthma and COPD rarely exceeds 50%. In a study by Wiśniewski et al. (2014), only 67% of patients adhered to treatment 30 days after discharge from the hospital. COPD treatment is primarily based on inhalation therapy, although most patients prefer oral medications. In the studies included in this review, the rate of satisfactory adherence ranged between 46.3% for inhalation and 41.6% for oral therapy. This may be exemplified by the findings of Montes de Oca et al. (2017) who reported the scores for 10-item TAI and MMAS-8 questionnaires of 47.4 ± 4.9 vs. 6.8 ± 1.6, i.e., high vs. medium adherence, respectively. In that study, however, the incorrect use of inhalers could influence the adherence level. Patients who adhere better to inhalation than oral therapy usually have a better savvy in using inhalers, and use a variety of inhaler devices and inhaled drugs. Among factors contributing to better adherence, the ones associated with taking medication using an inhaler have been assessed most frequently. In a GAPP study, 23% of patients reported that no time was devoted to discuss with them the use of proper treatment techniques for successful asthma management (Canonica et al. 2007). The literature demonstrates that as many as 90% of patients may inhale medication incorrectly, although the estimate varies depending on the selection of a study sample and on the type of inhaler used. Multiple studies clearly demonstrate the efficacy of education performed by a pharmacist, nurse, or physician in increasing the adherence rate (Broadbent et al. 2018; Dudvarski Ilic et al. 2016; Moy et al. 2016; Poureslami et al. 2016; Tommelein et al. 2014). Broadbent et al. (2018) have shown that patients who receive training from a pharmacist perform a greater number of puffs *per* day compared to the non-trained subjects; mean 48.5% vs. 29.5%, respectively; $p = 0.03$.

Referring to non-adherence, the present review shows that a substantial number of patients (42.5%) purposely chose not to take their medication, which was deliberate non-adherence. On the other side, 48.5% of patients failed to follow the instructions received from medical personnel, which demonstrated inadvertent non-adherence, and 38.9% of patients failed to adhere to treatment due to their lifestyle, which was erratic non-adherence. We also found that psychological factors, such as a belief in the effectiveness of treatment, satisfaction with one's physician, and the ease and training in the inhaler use, count significant for having good adherence. In a study by Olszanecka-Glinianowicz and Almgren-Rachtan (2014), non-adherence is inversely proportional to the sense of control over one's disease and the opinion on the effectiveness of treatment.

In another study, a strong correlation has been reported between adherence to pharmaceutical treatment and patient's perceived health (Wiśniewski et al. 2014). According to Sanduzzi et al. (2014), adverse consequences of non-adherence include a gradual deterioration of quality of life, a sense of the disease being out of control, a greater number of exacerbations, and a higher mortality. Observations from a 3-year-long TORCH study demonstrate a greater than twofold increase in the risk of death and a nearly twofold increase in the risk of rehospitalization in non-adherent patients (Vestibo et al. 2009). These findings were confirmed in the present review, where the number of hospitalizations was a predictor of lower adherence.

The studies reviewed in this article differed in terms of COPD severity. The effect of disease severity on adherence to therapy is a contentious issue. Most studies show a better adherence with increasing severity of disease. Some other studies, however, show the opposite trend, a better adherence when the disease runs a mild course (Liao and Chen 2019; Leiva-Fernández et al. 2014). The discrepancy might be explained by the strength of motivation to continue treatment, associated with persistent symptoms that interfere with the patient's daily functioning. Another consideration concerns the adverse effects of medications, which may affect the patient's willingness to take them. In the GAPP study, patients who categorized their asthma as severe, and those who were treated by specialists, were most likely to discuss the knowledgeable aspects of their condition (Canonica et al. 2007).

The influence of sociodemographical factors on adherence to therapy in COPD patients is yet another debatable issue. In this review, we show that better adherence associates with older age, male gender, and daily functioning. The reason could be that pensioners have more time for regular living, exercising, and following dietary guidelines. On the other side, older patients may have a difficulty to adhere to treatment due to the presence of comorbidities. In contrast, younger patients are more likely to oppose medical advice and tend not to accept their illness, which may lead to non-adherence (Świątoniowska et al. 2018). In the studies reviewed herein male gender predominated among patients. That is in line with a study by Liao and Chen (2019) who have shown that adherence is outstandingly greater in men than women (87.3% vs. 12.7%, respectively). Other studies also show that men are more likely to adhere to therapy than women (Vestibo et al. 2009). The issue is somehow contentious as in a study by Dhamane et al. (2017), 59% of COPD patients are women, but the authors do not show any influence of gender on adherence. Other authors point out that low income and a lack of professional activity undermine adherence to COPD treatment (Liao and Chen 2019; Plaza et al. 2016b), although the underlying reasons for that are not full well clear. In patients experiencing economic difficulties, adherence to disease monitoring may be improved by addressing modifiable barriers such as cost and access (Campbell et al. 2014). There are, however, studies that put into question the association between sociodemographical factors and adherence to therapy (Khadour et al. 2012; Bourbeau and Bartlett (2008).

In conclusion, standardized questionnaire tools enable the prediction of adherence to therapy in COPD patients and should be used in clinical practice. The assessment of adherence is essential for undertaking interventions to counteract plausible non-adherence. Collaboration between an educator and a psychologist is needed to evaluate the patient's motivation and to ensure he comprehends the treatment prescribed.

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

Ethical Approval This is a literature review article that does not contain any current studies or experiments with human participants or animals performed by any of the authors. The writing of this article was accepted by the scientific Review Board of Wroclaw Medical University in Poland.

Informed Consent There are no individual participants included in this review article. Therefore, there was no requirement to obtain individual informed consent.

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How Healthy Is Healthy? Comparison Between Self-Reported Symptoms and Clinical Outcomes in Connection with the Enrollment of Volunteers for Human Exposure Studies on Sensory Irritation Effects

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Abstract

Controlled human exposure studies on sensory irritation effects are usually performed with healthy volunteers. Therefore, in most studies pre-screening by a health questionnaire and a detailed medical examination are combined. The aim of this report is to investigate whether self-reported information about smoking and health status is sufficient or whether additional clinical tests are necessary for a successful and safe enrollment of healthy volunteers. There were 409 volunteers (55% female; 17–57 years; 79% non-smokers) who declared interest in participation in the study. However, 87 subjects failed to meet specific inclusion criteria, and further 138 had to be excluded due to the presence of chronic health problems. In effect, 184 subjects passed the initial questionnaire screening and proceed to further examination. Medical examination included electrocardiogram, blood and urine screening, and an olfactory function test. Atopy status was assessed by skin prick or specific IgE testing. Lung function and a methacholine challenge test were performed to assess respiratory health and bronchial hyperresponsiveness. Overall, only 107 non-smoking subjects (58% female; 19–40 years) who had no respiratory diseases, allergies, or chronic illnesses could be finally selected. Out of the 107 subjects, 8 were excluded due to positive cotinine tests, laboratory test results outside the reference range, or atypical ECGs. In another 12 subjects, obstruction or a bronchial hyperreactivity was diagnosed. Among the remaining 87 healthy subjects, 26 were classified as atopic and further two as hyposmic. In conclusion, although young and non-smoking volunteers considered themselves healthy by questionnaire, 20% showed signs of a heart, liver, or airway disease, and additional 24% were classified as atopics. This suggests that more detailed clinical testing may be necessary to safely exclude those who may adversely react to controlled exposure with sensory irritants.

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Keywords
Clinical examination · Clinical outcome · Exposure study · Health · Self-report · Sensory irritation

1 Introduction

Most human cell and tissue reactions toward inhaled substances cannot be reproduced in animal studies due to physiological and genetic differences. Therefore, controlled exposure studies in humans are of great importance, particularly when investigating sensory irritation of the eyes and the upper respiratory tract. Typically, physiological measurements like eye blink frequency or biochemical analysis of nasal lavage fluid are used to assess reactions caused by trigeminal chemoreception. Even though these responses are based on sensory-mediated defense mechanisms/reflexes and do not represent adverse end points per se, it is thought that if these defense responses are elicited continuously under high and prolonged exposure, they might result in adverse health effects. Nonetheless, along with psychometric ratings of symptoms and the intensity estimates of irritation or odor perception, these physiological measures are used for determining occupational exposure limits (Brüning et al. 2014).

Study exclusion criteria remove participants who are at risk of an adverse outcome due to preexisting medical conditions or whose preexisting physiological properties may alter or mask the expected effects during the study. The risk of an adverse outcome might be associated with the exposure itself, with other experimental procedures used during the study, or with a chance occurrence of pathophysiologic events unrelated to study exposure. For example, a substance with an intensive odor might cause a severe cough attack in a supposedly healthy subject (Claeson et al. 2016), or cycling on an ergometer in order to induce moderate workload during exposure may reveal an existing but unidentified cardiovascular disease. When inflammation markers are to be evaluated, the exclusion of smokers is indispensable since it is known that tobacco smoking decreases the level of exhaled nitric oxide (FeNO) and increases blood eosinophil count (Jacinto et al. 2017). Furthermore, it is known that the most common diseases that can cause a change in the sense of smell include sinonasal disease (Boesveldt et al. 2017), diabetes mellitus (Naka et al. 2010), hypothyroidism (Baskoy et al. 2016), and migraine (Fornazieri et al. 2016).

An additional challenge is the identification of healthy individuals who are more or less susceptible to chemosensory effects. The perception of sensory irritation is highly influenced by personal factors such as anxiety and attitudes toward health risks, and "sensitive" subjects tend to evaluate the symptoms that they perceived at an increased rate, e.g., odor intensity or eye irritation. Therefore, existing research in this area suggests that such non-sensory modulators need to be considered in controlled human exposure studies (Pacharra et al. 2016b; Seeber et al. 2002). To this end, questionnaires that assess chemical sensitivity (Pacharra et al. 2016a), personality traits such as anxiety (Pacharra et al. 2017) or affectivity (Lang et al. 2008), and more objective measures of sensitivity such as the capsaicin-induced cough reflex (Hoffmeyer et al. 2013) or nasal sensitivity to carbon dioxide (Müller et al. 2013) are used.

The aim of the present study was to evaluate the suitability of a questionnaire as the only tool for selecting healthy study participants. To this end, data from six controlled human exposure studies conducted in the exposure laboratory of the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance – Institute of the Ruhr University Bochum (IPA) were re-evaluated with regard to the recruitment of healthy volunteers. The benefit of additional clinical tests such as electrocardiography, spirometry, and bronchial provocation with methacholine, the assessment of atopy status, and an olfactory test were evaluated. In addition, we compared our results with the recruitment strategy of previous sensory irritation studies.

2 Methods

Healthy adults were invited to participate in the study using information sheets published at electronic bulletin boards at the Ruhr University Bochum or the IPA website. In total, there were 409 volunteers (55% female; 17–57 years; 79% non-smokers) who expressed interest in participation in the study. When the subjects contacted the research office, they received a standardized online health questionnaire, assessing chronic diseases, asthma, allergy, smoking habits, and demographic data, which was the first recruitment step (see Table 2 for details). The selection criteria were as follows: age between 18 and 40 years, non-smoker or ex-smoker for more than one year, and a body mass index (BMI) between 18 and 30 kg/m^2. Subjects who reported a physician-diagnosed chronic disease (e.g., sinusitis, migraine, thyroid disease, diabetes, hypertension, gastroesophageal reflux, other chronic diseases, or a neurologic/psychiatric disorder), asthma, allergy (e.g., hay fever), and eczema or who received prescription medications were excluded. Other exclusion criteria included pregnancy or lactation, clinically significant ECG abnormalities, and laboratory results for blood or urine examinations outside of the reference ranges considered clinically significant. The reported smoking status was verified by quantification of cotinine in urine. Eighty seven subjects failed to meet the selection criteria, and further 138 had to be excluded due to the presence of chronic health problems. In effect, 184 subjects passed the initial questionnaire screening and were scheduled for medical examination, which was the final recruitment step (see Table 3 for details).

Subsequently, the subjects completed a medical history and a physical examination by an experienced physician. Blood and urine samples were taken and tested for standard laboratory values, such as blood count, urinary proteins, liver function, and others as well as for specific IgE antibodies. Subjects performed a resting 12-lead ECG with computer-aided protocol interpretation validated by a physician, pulmonary function tests and methacholine inhalation test (MCh test). Additionally, some of the participants performed a skin prick test (SPT), a capsaicin inhalation test, and an olfactory function test with Sniffin' Sticks.

Atopy was assessed by SPT to nine ubiquitous allergens (cat dander, trees, grass, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria alternata, Aspergillus fumigatus*, latex, *Ambrosia elatior*), by a positive (histamine 10 mg/mL) and negative control (saline), or by specific immunoglobulin E (IgE) screening against a mixture of common environmental allergens (*Dermatophagoides pteronyssinus*, cat dander/hair, dog dander, *Cladosporium herbarum*, pollen of timothy, rye, birch, and mugwort (sx1 Phadiatop; ThermoFisher Phadia AB; Uppsala, Sweden)). A positive atopic status was assumed in case of specific IgE concentrations $\geq$0.35 kU/L or by a wheal greater than 3 mm in SPT to one of the allergens listed above.

A pulmonary function test was performed to assess respiratory health. A constant-volume body plethysmograph (MasterScreen®Body, Jaeger GmbH, Wurzburg, Germany) was used for pulmonary function measurements according to the recommendations of the American Thoracic Society (ATS 1995). Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) of the best of three maneuvers were used for further evaluation analyses and for calculating the percent predicted values. According to the Global Lung Function Initiative (Quanjer et al. 2012), lung function measurements were being considered normal in case of FEV_1 and FEV_1/FVC ratio values above the lower limit of normal (LLN).

The MCh bronchial provocation test was carried out to diagnose a possible bronchial hyperresponsiveness. It was performed according to the four-step-one-concentration reservoir test method as described earlier by Baur et al. (1998). MCh was dissolved in phosphate buffered saline (3.3 mg MCh/mL; Provokit, Lindopharm GmbH, Hilden, Germany). The test was continued up to the concentration of MCh needed to produce a 20% fall in FEV_1 from baseline (PD_{20}). Then, PD_{20} was calculated by a linear

interpolation of the dose-response slope defined as the ratio between the percentage FEV_1 decline and the cumulative MCh dose. A $PD_{20} < 0.30$ mg cumulative MCh dose (a total inhaled from the nebulizer) was judged as a positive response. In case of a negative response, changes in FEV_1 after the last test corresponding to a cumulative dose of 0.46 mg MCh were recorded.

The Sniffin' Sticks test is based on pen-like odor-dispensing devices and measures olfactory function with good test-retest reliability and validity (Burghart, Wedel, Germany). It consists of tests for odor threshold (n-butanol, testing by means of a single staircase), odor discrimination (16 pairs of odorants, triple forced choice), and odor identification (16 common odorants, multiple forced choice from 4 verbal items per test odorant) (Hummel et al. 1997). Results are presented as "TDI score", which is the sum of results obtained for threshold, discrimination, and identification measures. Subjects who have functional anosmia score 16.0 TDI points or less, whereas normosmic subjects have a TDI score of 31 or more (Oleszkiewicz et al. 2019; Hummel et al. 2007).

Patients having sensory hyperreactivity (SHR) respond with cough and other airway symptoms at levels of odors and irritants normally regarded as nontoxic. They can be identified either with a questionnaire, the Chemical Sensitivity Scale for Sensory Hyperreactivity (CSS-SHR), or with a standardized capsaicin inhalation cough test. SHR is related to gender (females), rhinitis, and lower airway sensitivity to cold air, but not to age, asthma, or smoking. Thus, MCh and SPT tests are usually negative (Ternesten–Hasséus 2016; Johansson et al. 2002). The CSS-SHR consists of 11 statements, e.g., "In movie theaters, other persons' perfume or aftershave fragrance disturbs me", and quantifies self-reported affective and behavioral reactions to odorous/irritating chemicals (Nordin et al. 2004). A CSS-SHR sum score above 42 was set as a cutoff score for the identification of odor intolerant individuals. The CSS-SHR carries a good test-retest reliability, internal consistency, and validity, and is positively associated with capsaicin sensitivity (Nordin et al. 2013).

For capsaicin challenge test, a single breath dose-response method was applied following closely the methodological recommendations on the assessment of cough (Hoffmeyer et al. 2013). Twelve doses of capsaicin were prepared in the concentration from 0.49 to 1,000 μM. After each dose, the number of coughs was counted. Capsaicin concentration inducing five (C5) or more coughs concluded the test. A value of 2000 μM concentration was assigned if C5-inducing values were > 1000 μM. Five or more coughs at a capsaicin concentration below 15.6 μM was suggested as a cutoff limit for a positive test result (Pullerits et al. 2014).

An additional questionnaire was used to evaluate the self-reported chemical intolerance that focuses on neurovegetative responses, e.g., nausea, to odorous chemicals such as paint or gasoline vapor. The Trigeminal-Mediated Sensitivity (TMS) Scale (Kiesswetter et al. 1999) consists of eight statements rated on a 6-point scale Likert-type scale where "0" is not at all and "5" is very much. The classification into the group of self-reported chemical sensitive subjects (sMCS) was based on a rating with 4 or 5 of at least one out of the eight statements.

The Positive and Negative Affect Schedule (PANAS) (Watson et al. 1988) contains 20 adjectives, 10 of which define rather positive and another 10 rather negative emotions and feelings. These are rated on a 5-point scale Likert-type scale with "1", very slightly/not at all; "2", a little; "3", moderately; "4", quite a bit; and "5" extremely. A sum score above 29 was set as a cutoff limit for the identification of individuals with high negative affectivity (Crawford and Henry 2004).

3 Results

3.1 Overview of Recruitment Strategies of Current Studies on Sensory Irritation

The evaluation of the current literature on controlled human exposure studies with at least 2-h exposure to a sensory irritant indicates that for

most of the studies healthy non-smokers have been recruited on the basis of self-reported information provided by questionnaires together with a screening of blood and urine samples for standard laboratory values. The recruitment strategy in 32 such studies is shown in Table 1.

In most of the studies, between 12 and 40 subjects were examined (men and women, aged between 20 and 40 years). The most common exclusion criteria were smoking, pregnancy, medication, exposure history, and chronic diseases such as diabetes; migraine; chronic disorders of the skin, lungs, and heart; liver diseases; chemosensory deficits, and neurological or psychiatric disorders. The examination by a physician typically comprised medical history and physical examination, including clinical blood chemistry tests. In some cases, a pregnancy test (urine human chorionic gonadotropin) was administered, and specific IgE antibodies to common inhalant allergens (sx1 test) were measured to check atopy/allergy status (Ernstgård et al. 2010a; Sundblad et al. 2004). Further clinical examinations, such electrocardiogram (ECG) (Muttray et al. 2015; Fiedler et al. 2008), skin prick test (SPT) (Ernstgård et al. 2007), pulmonary function measurements (Pacharra et al. 2017), or the measurement of cotinine in urine to confirm the non-smoker status (Lang et al. 2008) were performed less often. A detailed examination of the eyes such as slit-lamp, tear flow rates (Ihrig et al. 2006); of the nose such as nasal endoscopy, active anterior rhinomanometry, acoustic rhinometry, and mucociliary transport time (Muttray et al. 2015); and of olfactory function such as Sniffin' Sticks Test (Kleinbeck et al. 2017) were occasionally carried out.

3.2 Re-Evaluation of Recruitment of Healthy Volunteers for Six IPA Studies

A total of 409 volunteers were interested in participation in the study (median age: 27, range 17–57 years) (Table 2).

Referring to our exclusion criteria, (1) age below 18 or above 40 years, (2) smoker or ex-smoker for less than 1 year, and (3) BMI below 18.0 or above 30.0 kg/m^2, 87 subjects had to be excluded. Furthermore, 138 subjects with a chronic disease, asthma, allergy, skin disease, and receiving prescription medications were not included either. In total, 184 participants passed the initial questionnaire screening (median age 24, range 18–40 years).

Forty-two percent ($n = 77$) (60% female; median age 24, range 18–37 years) volunteers dropped out before taking medical examination. Some of those had scheduling conflicts or changed their mind; some others had to cancel the appointment due to an acute illness. It also happened that at the end of the recruitment period despite eligibility, some of the females were not invited in order to have same size groups of male and female participants. In total, 107 non-smoking healthy subjects (median age 25, range 19–40 years) participated in medical examination (Table 3).

None of the women claimed to be pregnant or nursing. With regard to the blood and urine parameters, four subjects had to be excluded, and another four were excluded due to an atypical electrocardiogram. In 12 subjects, obstruction or bronchial hyperreactivity was diagnosed. Finally, 87 participants passed the medical examination and were classified as healthy participants. Among them, 2 persons were diagnosed hyposmic and 26 were identified as atopic. Because atopic subjects had sensitization but no clinical history, we labeled them "clinically healthy". Two participants had a positive capsaicin test, one of whom was additionally classified as atopic, but none rated positively in the CSS-SHR questionnaire. In addition, seven participants were classified as sMCS, and eight were identified as having high negative affectivity.

4 Discussion and Conclusions

A prerequisite for research exposing human subjects to substances to assist regulators in

Table 1 Controlled human exposure studies on sensory irritants and the recruitment strategies

First author	Year	Medical examination[1]	Smoking	Pregnancy	Chronic disorders	Heart	Lung	Asthma	Allergy	Eyes	Nose	Sensitivity
Dwivedi	2015	yes + blood		hCG		x	x	x	x	x	x	x
Ernstgård	2002	yes + blood		hCG		x	x	x	sx1	x	x	x
Ernstgård	2006	yes + blood		hCG		x	x	x	sx1	x	x	x
Ernstgård	2006	yes + blood		hCG		x	x	x	sx1	x	x	x
Ernstgård	2007	yes + blood		0		x	x		SPT	x	x	x
Ernstgård	2009	yes + blood		hCG		x	x	x	sx1	x	x	x
Ernstgård	2010b	yes + blood & urine	x	hCG		x	x	x	sx1	x	x	x
Ernstgård	2013	yes	x	x		x	x	x	sx1	x	x	x
Fiedler	2008	yes + blood				ECG	Spiro		x	x	x	x
Gminski	2010	yes + blood		x					IgE	x	TDI	FPI-R
Hey	2009	yes				x	x		x	x	x	x
Ihrig	2006	yes	x	0		x	Spiro	MCh	x	Eye	x	PANAS, FPI-R
Juran	2012	yes	x	x		x	x			x	TDI	TMS
Juran	2014	yes		x		x	x	x		x	SOIT	x
Kleinbeck	2008	yes + blood		x		ECG	Spiro	x	x	x	x	x
Kleinbeck	2017	yes + blood		x		ECG	Spiro	x	x	x	TDI	x
Lang	2008	yes	Cotinine				Spiro			x	Rhino	PANAS
Müller	2013	yes		0						EBR>20	x	PANAS, CO_2
Muttray	2009	yes + blood & urine		0		ECG	Spiro	x	x	x	TDI & HNO	x
Muttray	2015	yes +blood & urine		0		ECG	Spiro	x	x	x	TDI & HNO	x
Pacharra	2016	yes +blood & urine				ECG	x	x	x	x	TDI	TMS, NEO-FFI
Pacharra	2017	yes				x	Spiro, FeNO		SAR, IgE	x	TDI	STAI-T, TMS
Sundblad	2004	yes	x	hCG		x			sx1	x	x	x
van Thriel	2003	yes	x	0		x	x			x	x	TMS
van Thriel	2003	yes	x	0		x	x	x	x	x	x	TMS
van Thriel	2005	yes	x	0		x	x			x	x	TMS
van Thriel	2007	yes	x	0		x	x			x	x	TMS
van Thriel	2007	yes		0		Ergo	Spiro	x	x	x	TDI	x
van Thriel	2010	yes + blood		x		Ergo	Spiro			x	x	x
Walinder	2005	yes	x	x		x	x	x	sx1	x	x	x
Walinder	2008	yes	x	x		x	x	x	sx1	x	x	x
Ziegler	2008	yes + blood		x						x	x	PANAS

[1]Even though the medical examination by a physician was never described in detail, it normally comprises medical history and physical examination (e.g., vital sign check, blood laboratory). Gray color indicates "assessed by questionnaire" only; "x" indicates "not specified", i.e., neither queried nor objectively evaluated; "0" indicates a study with "only male participants". *hCG,* pregnancy test, urine human chorionic gonadotropin; blood, clinical blood chemistry tests; *Cotinine,* cotinine in urine; *CO_2,* nasal sensitivity to carbon dioxide (Müller et al. 2013); *ECG,* electrocardiogram; *Ergo,* fitness test with a bicycle ergometry (exercise electrocardiography); *Spiro,* spirometry and sometimes also body plethysmography; *MCh,* bronchial provocation with methacholine; *FeNO,* fractional exhaled nitric oxide; *sx1,* sx1 > 35 kU/L; *IgE,* total IgE (> 100 kU/L); *SPT,* skin prick test; *SAR,* confirmed seasonal allergic rhinitis (SAR): (a) a history of seasonally occurring symptoms which correspond to allergic rhinitis, (b) a report of a medical diagnosis of seasonal allergic rhinitis in the past, and (c) a concentration of allergen-specific IgE in the serum consistent with reported allergens (e.g., grass pollen; tree, or hazel pollen) and indicative of allergic rhinitis; eye, ocular surface area examination with a slit-lamp and tear flow rates measured with paper strips; *EBR*, >20; eye blinking frequency above 20 blinks/min; *HNO,* nasal endoscopy, A scan sonography of the paranasal sinuses, active anterior rhinomanometry, acoustic rhinometry, or measurement of mucociliary transport time; *TDI,* composite threshold discrimination identification for olfactory function with the Sniffin' Sticks Test (Hummel et al. 1997); *SOIT,* Scandinavian odor-identification test for hyposmia/anosmia (Nordin et al. 1998); *TMS,* self-reported general or trigeminal-mediated sensitivity (Kiesswetter et al. 1999); *PANAS,* negative and positive affect schedule (Watson et al. 1988; *STAI-T,* state-trait-anxiety inventory (Laux et al. 1981); *FPI-R,* Freiburger Persönlichkeitsinventar (Fahrenberg et al. 1994); *NEO-FFI,* neuroticism (Borkenau and Ostendorf 2008); and *Rhino,* rhinomanometry

establishing occupational exposure limits is ethical compliance (NASEM 2017). Studies on the exposure of people to sensory irritants usually involve healthy individuals who are unlikely to respond to the controlled exposure conditions with a negative result. It is expected that the observed chemosensory effects are transient and completely reversible.

Questionnaire recruitment of healthy volunteers provides a relatively inexpensive, quick, and efficient way to obtain health screening information from a large sample. Although the questionnaire method is relatively inexpensive and efficient compared to physical exams and tests, the validity of self-reported data may be questionable (Brener et al. 2003).

Table 2 Recruitment Step 1: Questionnaire screening

Participation interest – online screening questionnaire; $n = 409$	
[183 (45%) male, 226 (55%) female, 322 (79%) non-smoker]	
Exclusion criteria	**Number**
Age (< 18 > 40 years)	26
Smoking (smoker or ex-smoker <1 year)	35
BMI (< 18 > 30 kg/m^2)	26
Chronic disease[a]	71
Asthma (diagnosed by a doctor)	6
Allergy (hay fever complaints)	49
Skin disease (contact allergy, eczema)	12
Non-eligible: $n = 225$	
[103 (46%) male, 122 (54%) female; 17–57 years]	
Eligible: $n = 184$	
[80 (43%) male, 140 (57%) female, 18–40 years]	

[a]Sinusitis, migraine, thyroid disease, diabetes, hypertension, gastroesophageal reflux, other chronic diseases, or a neurologic/psychiatric disorder; *BMI* body mass index

Table 3 Recruitment Step 2: Medical examination

Eligible but dropped out: $n = 77$	
[31 (40%) male, 46 (60%) female, 18–37 years]	
Participation in medical examination: $n = 107$	
[49 (46%) male, 58 (54%) female]	
Exclusion criteria	**Number**
Urine (positive cotinine test)	2
Blood (increased liver values)	2
Electrocardiogram (atypical)	4
Obstruction (lung function test)	5
Bronchial hyperreactivity (MCh test)	7
Failed in medical examination: $n = 20$	
[49 (46%) male, 58 (54%) female]	
Healthy participants: $n = 87$	
[37 (43%) male, 50 (57%) female]	
Characterization criteria	**Number**
Sniffin' sticks test (hyposmic)	2
Atopy (positive SPT or sx1 test)	26
SHR (positive capsaicin test)	2
Sensitivity (questionnaire)	15

SPT skin prick test (a wheal greater than 3 mm), *sx1* specific IgE concentrations $\geq$0.35 kU/L, *SHR* (sensory hyperreactivity). *Sensitivity (questionnaire)* self-reported multiple chemical sensitivity (sMCS) and negative affectivity

The main question of the current study was whether additional time-consuming and costly clinical trials are really necessary to safely recruit healthy study participants. The results show that 20% of the study participants showed signs of heart, liver, or respiratory disease, although young, healthy, and non-smoking volunteers were invited to the medical examination based on the results of the initial health questionnaire results. This indicates that additional clinical testing may be needed to safely exclude those who may respond to the controlled exposure with a harmful reaction.

In addition, 24% were classified as atopic after SPT or sIgE screening, but no allergic symptoms such as watery eyes, runny nose, or sneezing were reported. With regard to the identification of non-allergic subjects, the accuracy of the self-reported medical history data depends, among other things, on the subjects' knowledge of their health status or the willingness to report it, although allergies may not have been diagnosed by a physician.

It is currently being discussed whether individuals with allergic rhinitis belong to a susceptible subpopulation that responds more strongly to increased exposure in terms of concentration and duration, starting at lower exposures relative to the general population. In a recent review, Shusterman (2014) argued that preexisting upper respiratory tract inflammation, as occurs in individuals with seasonal allergic rhinitis (SAR), causes increased upper airway

sensitivity to airborne pollutants. A controlled exposure study in humans (Kleinbeck et al. 2018) with 4-h exposure to formic acid has shown that individuals with clinically confirmed SAR exhibit a greater increase in the blink rate than controls, although the study was conducted outside the pollen season. In addition, a controlled study with ethyl acrylate has found that atopic subjects generally have higher baseline blinking rates than non-atopic ones (Sucker et al. 2019). Therefore, in future controlled human exposure studies, the atopy/allergy status should be determined, and, if necessary, subjects with a particularly high blink rate may be excluded, which has previously been performed only in one controlled human exposure study. In a study investigating the chemosensory effects of formaldehyde, subjects with an eye blinking frequency greater than 20 blinks/min were excluded (Müller et al. 2013).

Finally, it was recommended to consider non-sensory modulators in controlled human exposure studies (Pacharra et al. 2016b; Seeber et al. 2002) as either exclusion or stratification criterion. The results of the present study demonstrate that in the group of eligible healthy participants, a further 10% could be classified as "sensitive" by questionnaire, two subjects were diagnosed as hyposmic, and one another was identified by an objective test as having sensory hyperactivity disorder. Hence, consideration of "sensitive" as an additional influence factor makes the recruitment of study participants even more demanding.

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 recruiting and testing procedures were reviewed and approved by the Ethics Committee of the Ruhr University Bochum, Germany.

Informed Consent All individual participants included in the study gave written informed consent. Prior to participation, the participants received information on the study requirements and a written declaration of consent. They were informed verbally and in writing about the study design, possible dangers, and their freedom to withdraw at any time. They received financial compensation for participation.

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Co-expression of Hsp70 Protein and Autophagy Marker Protein LC3 in A549 Cells and THP1 Cells Exposed to Nanoparticles of Air Pollution

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Abstract

The ability of air particulate matter (PM) to cause reactive oxygen species-driven protein damage is associated with both COPD and lung cancer, but the mechanisms are unsettled. In this study, we investigated the co-expression of Hsp70 and the autophagy marker protein LC3 in A549 cells (alveolar epithelial cell line) and THP-1 cells (monocyte/macrophage cells) grown in media supplemented with 100 μg/mL of four types of PM: carbon black (CB), urban dust (UD), nanoparticulate CB (NPCB), and nanoparticulate CB coated with benzo(a)pyrene (NPCB-BaP). Fluorescent monoclonal antibodies and flow cytometry were used to assess the expression and co-expression of HSP70 and LC3 proteins. Hsp70 expression was significantly increased by all PM, while LC3 was decreased by CB in A549 cells, unchanged by CB and UD in THP-1 cells and increased by NPCB and NPCB-BaP in both cell types. All PMs increased the Hsp70/LC3 ratio in binary scatterplots; the relationship was positive and linear, which may reflect chaperone-dependent autophagy. The UD was the only PM type that affected the slopes of the spatial trend lines and altered binary patterns of Hsp70/LC3 distribution in THP1 cells. These findings provide an insight into the molecular mechanisms regulating proteostasis in PM-exposed cells through the chaperone-autophagy system in the cytoplasm.

Keywords

A549 cells · Air pollution · Autophagy · Carbon black · Nanoparticles · Particulate matter · THP1 cells · Urban dust

1 Introduction

The major air particulate matter (PM)-related health problems are currently recognized, but the mechanisms underlying the development and progression of pathology are unknown. Short-term exposures to PM usually exacerbate pre-existing diseases, especially in the respiratory and cardiovascular systems and increase hospital admissions, while long-term exposures accelerate disease progression and significantly reduce life expectancy (Wong et al. 2016). It is established that ambient PM produces reactive oxygen species (ROS) resulting in lipid peroxidation,

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DNA oxidation, and oxidative protein damage (Shang et al. 2017). In eukaryotic cells, there are two major systems that are responsible for the degradation of damaged proteins: ubiquitin-proteasome system (UPS) that degrades most of the damaged proteins and autophagy that is responsible mostly for decomposing the long-lived proteins, aggregated proteins, and cell organelles (Boczek et al. 2019). Autophagy can be considered as a recycling system that allows to maintain ATP production in cells under stressful conditions (Crawley et al. 2019). The major step in this pathway is the formation of a double-membrane autophagosome over a damaged structure and fusion with a lysosome (Yu et al. 2018). A widely used marker of autophagy is a microtubule-associated protein 1A/1B-light chain 3 (LC3), which has become a standard for monitoring autophagy.

Both UPS and autophagy need molecular chaperones to ensure proteostasis (Klaips et al. 2018). The 70 kD heat shock protein (Hsp70) is important in intracellular transduction pathways by regulating the folding and activity of signaling proteins. Hsp70 is necessary for both UPS-mediated protein degradation and chaperone-assisted autophagy (Fernández–Fernández and Valpuesta 2018). Experimental data indicate that activation of autophagy may represent an important cellular defense reaction against oxidative stress and an efficient tool to dispose of damaged proteins (Filomeni et al. 2015). We have previously shown that lung alveolar epithelial cells (A549 cell line) exposed to standardized urban dust (UD) have elevated expression of autophagosome-associated protein light chain 3 (LC3) and Hsp70 proteins (Lukaszewicz et al. 2019). The present study was designed to delineate the role of the heat shock response in the regulation of proteolytic systems in PM-exposed cells. We addressed the issue by investigating changes in the central tendency, variability, and spread of the binary data from Hsp70 and LC3 co-expression assay in A549 cells and in a monocyte/macrophage cell line (THP-1 cells), grown with four different PMs.

2 Methods

2.1 Cell Cultures

A549 cells (ATCC® CCL185™) and THP-1 cells (ATCC® TIB202™) were used in the study. The cells were grown in Dulbecco's Modified Eagle's Medium supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), and 10% FBS, or in ATCC-formulated RPMI 1640 medium supplemented with 2-mercaptoethanol to a final concentration of 0.05 mM. They were maintained in an incubator at 37 °C in a humidified atmosphere containing 5% CO_2 and then plated out onto 6- or 12-well plates and grown in control or PM-conditioned medium for 24 h.

2.2 Cell Treatment

Culture media supplemented with PMs were prepared using commercial, standardized UD, which was purchased from the National Institute of Standards and Technology (Gaithersburg, MD). According to the Certificate of Analysis of Standard Reference Material 1649b, the particle size of UD is within a range of 0.2–11.0 micrometers, with a mean size of about 1.0 micrometer. Coarse carbon black (CB; 260 nm diameter, Huber 990; Haeffner and Co. Ltd., Chepstow, UK) was used as a reference substance. Nanoparticle carbon (NPCB: Printex 90; Degussa, Frankfurt, Germany) and NPCB coated with benzo(a)pyrene(NPCB-BaP) were also used in experiments with both cell types. NPCB-BaP was prepared in the laboratory, and the resulting BaP content of the coated particles was 26 mg BaP/g-Printex 90, as measured by high-performance liquid chromatography using a Grom column and fluorescence detection. For experiments, carbon particles were suspended in a cell culture medium at a concentration of 100 μg/mL and were sonicated in a Sonopuls ultrasonic homogenizer (Bandelin; Berlin, Germany) for 30 s before use. PM-conditioned media were used within 5 min of preparation. Cell-free controls were included in each

experiment to assess the interference of particles with each assay.

2.3 LC3 and Hsp70 Expression

LC3 and Hsp70 proteins were analyzed by flow cytometry using specific rabbit monoclonal antibodies recognizing either human Hsp70 (Abcam, Cambridge, MA) or LC3 proteins (Cell Signalling Inc., Danvers, MA) and positive and negative controls. For flow cytometry analysis, cells were fixed in 0.01% formaldehyde for 10 min, permeabilized with NP-40 (0.1% in PBS), washed with PBS, stained with specific rabbit monoclonal antibodies against human LC3B conjugated to Alexa Fluor 488, and rabbit monoclonal antibodies against human Hsp70 conjugated to Alexa Fluor 647 (Abcam, Cambridge, MA). The cells were then washed, centrifuged, and resuspended in 500 μL of ice-cold PBS containing 10% fetal calf serum (FCS) and 0.01% sodium azide. The unstained A549 or THP-1 cells and single (green or red)-stained cells served as controls to calibrate the flow cytometer detectors and compensation. Samples were analyzed with a FACSCanto II flow cytometer (BD Biosciences Systems, San Jose, CA) with the standard filter setup. The green fluorescence from FITC was detected through a '530/30' nm band-pass filter on the FL1 channel, and red fluorescence of the Alexa Fluor 647 was collected in the FL3 channel (>600 nm long-pass filter). All analyses were performed at the low-rate settings with <1000 events/s. Experimental data were plotted as bivariate cytograms, scatterplots, density plots, and fluorescence histograms and were analyzed for the central tendency, variability, and spread using the FlowJo and Flowing Software.

2.4 Data Analysis

Results were expressed as means ±SD of three to six assays. Statistical differences among the mean results were assessed with one-way or two-way ANOVA, followed by Bonferroni post hoc test for selected pairs of data. A p-value <0.05 defined statistically significant differences. The analysis was performed with a commercial Statistica v6.0 package (Statsoft; Cracow, Poland).

3 Results

In preliminary experiments, A549 and THP1 cells were incubated overnight with four different PMs, and then intracellular LC3 and Hsp70 protein expressions were independently quantified using fluorescence-labeled monoclonal antibodies (green for LC3 and red for Hsp70) and flow cytometry detection. None of the PMs by itself significantly altered red or green fluorescence signal. Changes in Hsp70 and LC3 in both cell types are shown in Table 1. CB increased Hsp70 expression in both cell types and decreased LC3 expression in A549 cells by about 40% ($p < 0.05$), but not in THP1 cells. UD increased Hsp70 in both cell types; the increase was more pronounced in A549 cells (greater than sixfold increase) than in THP1 cells (threefold increase) ($p < 0.01$ for both). Considering NPCB and NPCB-BaP, they exerted a similar effect in both cell types. NPCB increased both LC3 and Hsp70, with the highest increase in Hsp70 in A549 cells (by 142%; $p < 0.01$). The effect of NPCB and NPCB-BaP on LC3 expression in THP1 cells (almost twofold increase vs. control cells) was opposite to that of CB (almost 40% decrease vs. control cells). There was no significant difference between NPCB and NPCB-BaP in both parameters tested.

Bivariate cytograms and density scatterplots of double-stained cells (red color for Hsp70 protein and green for LC3 protein) were evaluated for alterations in the central tendency, variability, and dispersion of results. All the corresponding statistical data such as frequency, standard deviations, relative green/red fluorescence ratios, relative areas, and slopes of the spatial trend lines for each group are shown in Table 1 and Fig. 1. All four PMs increased the Hsp70/LC3 ratio (R; a relative increase of Hsp70 vs. LC3), which confirmed our data from single-staining experiments. The highest increase in R was observed in A549 cells grown with UD. It was greater than sixfold and corresponded to the same-size increase in

Table 1 Effects of carbon black (CB), urban dust (UD), nanoparticle carbon black (NPCB), and nanoparticle carbon black coated with benzo(a)pyrene (NPCB-BaP) on autophagy (LC3 expression) and Hsp70 content in human alveolar epithelial cell line (A549 cells) (**a**) and human monocyte/macrophage cell line (THP-1 cells) (**b**). Cells were treated with a 100 μg/mL of CB, UD, NPCB, or NPCB-BaP for 24 h. LC3 and Hsp70 were quantified in double-stained (green/red) cells by flow cytometry. Results are shown as density scatterplots with the central tendency and scatter area (A), slopes of the spatial trend lines (a and a_1), and the mean region-specific Hsp70/LC3 fluorescence ratio (R total)

	A549		THP1	
(a) Single staining	Hsp70	LC3	Hsp70	LC3
Control	100 ± 41	100 ± 18	100 ± 36	100 ± 20
CB	181 ± 95**	61 ± 17*	151 ± 27	131 ± 32
UD	690 ± 166*##	147 ± 31*##	282 ± 74**##	102 ± 19
NPCB	231 ± 74**##^^	185 ± 33**##^	198 ± 42*^	207 ± 41*##^
NPCB-BaP	242 ± 87**##^^	174 ± 36**##	216 ± 53**	175 ± 40*^

(b) Double staining	Hsp70/LC3 ratio (R)	Area	Slope (a)	Hsp70/LC3 ratio (total R)	Area	Slope (a_1)
Control	0.41	100 ± 12	0.93	0.66	100 ± 11	0.90
CB	0.74	56 ± 9*	0.75	0.77	85 ± 9	0.91
UD	2.68	107 ± 13#	0.70	1.35	132 ± 11*##	0.75
NPCB	0.62	69 ± 8^	0.87	0.64	86 ± 10^^	0.87
NPCB-BaP	0.56	81 ± 13	0.87	0.81	86 ± 14^^	0.90

$*p < 0.05$; $**p < 0.01$ – for comparisons with the corresponding control cells
$\#p < 0.05$; $\#\#p < 0.01$ – for comparisons with the corresponding CB-treated cells
$\hat{}p < 0.05$; $\hat{}\hat{}p < 0.01$ – for comparisons with the corresponding UD-treated cells

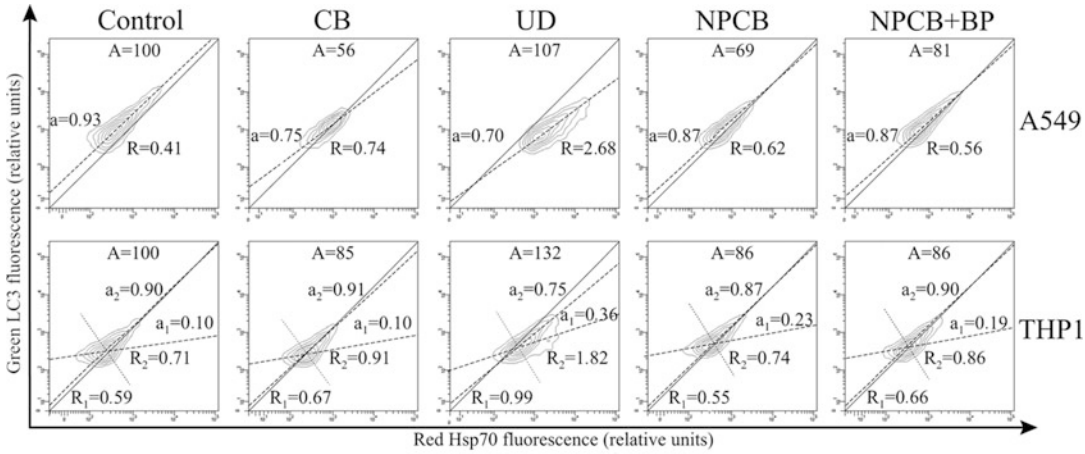

Fig. 1 Effects of carbon black (CB), urban dust (UD) nanoparticle carbon black (NPCB), and nanoparticle carbon coated with benzo(a)pyrene (NPCB-BaP) on the content of LC3 and Hsp70 in human alveolar epithelial cell line (A549 cells) and human monocyte/macrophage cell line (THP-1 cells). Cells were treated with a 100 μg/mL of CB, UD, NPCB, or NPCB-BaP for 24 h. LC3 and Hsp70 were quantified in double-stained (green/red) cells by flow cytometry. Typical results are shown as density scatterplots with the central tendency and scatter area (A), slopes of the spatial trend lines (a, a_1, and a_2), and the mean region-specific Hsp70/LC3 fluorescence ratios (R, R_1, and R_2)

Hsp70 expression noticed in single-staining experiments. In THP1 cells, UD increased the Hsp70/LC3 ratio by about twofold, and NPCB was without a significant effect. In A549 cells, the Hsp70/LC3 association was positive and linear, but in THP1 cells there was a binary pattern of Hsp70/LC3 distribution resulting in the formation of two subpopulations of cells on density

scatterplots. In general, cells with a lower Hsp70/LC3 ratio were less affected by carbon black and nanoparticulate carbon black (R1 and R2 values, respectively, vs corresponding control values in Fig. 1), while UD altered fluorescence scatterplots of all THP1 cells, irrespectively of their Hsp70 and LC3 content. The distribution of bivariate signals was linear in A549 cells but bimodal in THP1 cells suggesting the existence of two cell subpopulations in the latter. When geometric means were used as a cutoff value, the fractions of naïve THP1 cells with lower fluorescence intensity (below the intersection line) had Hsp70/LC3 ratios of about 0.6, and the ratio increased to 0.99 (by about 40%) in UD-treated THP1 cells. This ratio was only slightly affected by other PMs (Fig. 1). In a subpopulation of THP1 cells with a higher fluorescence intensity (above the intersection line), the PM-induced alterations were alike. Moreover, UD increased the Hsp70/LC3 ratio in THP1 cells by about 2.7-fold with a tendency for outlier distributions.

The slopes of the spatial trend lines were unaffected by PM-treatments, except for UD which decreased the slope values by 17% and 25% in THP1 and A549 cells, respectively. Referring to the dispersion of fluorescence scatter, in the cells treated with CB or NPCB or NPCB-BaP, the scatter area decreased maximum by 44% in A549 cells treated with UD. On the other hand, UD failed to affect the scatter area or even increased it by 32% in THP1 cells.

4 Discussion

Exposure to airborne PM increases the risk for cancer and pulmonary and cardiovascular disorders (Pope et al. 2004; Hoek et al. 2002). Clinical and experimental data indicate that the PM-related pathology is linked to oxidative stress (Ovrevik 2019; Shang et al. 2017). It is well evidenced that reactive oxygen species trigger protein, DNA, and lipid peroxidation and generate the production of redox-sensitive, proinflammatory molecules (Pizzino et al. 2017). PM produces an accumulation of misfolded proteins and the activation of many classes of molecular chaperones (Gualtieri et al. 2011). We have previously shown that standardized UD decreases the viability of A549 cells, increases ROS production, and about doubles expression of Hsp70 chaperone protein and LC3 protein. However, in the experimental model used at the time, most changes were not directly related to ROS-mediated glutathione (GSH) depletion (Lukaszewicz et al. 2019). In THP1 cells, both GSH depletion and UD treatment produce stratification of cells into three distinct subpopulations with different patterns of autophagy (Holownia et al. 2019), which is a regulated process of degradation of intracellular components. Autophagy can be viewed either as a constitutive reaction, an effort to survive stress, or a deleterious process. Recent experiments with PM2.5 have confirmed that autophagy is increased in A549 cells (Dai et al. 2019) and in human umbilical vein endothelial cells (Zhou et al. 2018), but the underlying mechanisms are unknown.

The present study aimed to examine the interplay between Hsp70 and LC3 proteins in PM-exposed epithelial and immune cells. This interplay might be essential not only for cell proteostasis but also for the understanding of lysosomal storage diseases, cancer, aging, and neurodegeneration, as Hsp70 may direct, most probably selectively, misfolded proteins to two specific degradation pathways, ubiquitin-proteasome system (UPS) or chaperone-mediated autophagy (CMA), and it is also involved in the regulation of inflammatory signaling (Sulistyowati et al. 2018). UD, a heterogeneous mixture of inorganic and organic chemicals and insoluble molecules, was the most aggressive out of the four tested compounds. It produced a significant increase in Hsp70, especially in A549 epithelial cells, with a less substantial stimulation of autophagy. It seems that metal-dependent oxidative stress may be the major mechanism for an increase in Hsp70 content caused by UD which, in contrast to other PMs, is relatively rich in various metals (Möller et al. 2002). The effects of CB and NPCB or NPCB-BaP were, to some extent, similar to each other but different from UD. Coarse CB should not be considered as a

toxic agent, but published data show that a small-size, nanomolecular carbon can trigger toxicity (Stoeger et al. 2006). Referring to CB and oxidative stress, it has been shown that fine CB is without a significant effect on redox imbalance, which points to a plausible relation between the substance's surface area and oxidative stress it exerts (Zhao and Riediker 2014; Donaldson and Stone 2003).

In the present experimental setting, naïve A549 cells had a relative Hsp70/LC3 ratio of 0.41 with linear distribution and a slope of the spatial trend line of 0.93, which indicate a relative equilibrium and a positive association between both parameters. This equilibrium was affected by different PMs, considering both the central tendency and the "lean" of the spatial trend lines, especially in regard to UD and also CB, but only in A549 cells. In particular, Hsp70 was significantly increased in both cell types, subject to different treatments. However, in cells grown with NPCB and NPCB-BaP, this increase was accompanied by a similar increase in LC3 expression, with only one exception for CB-treated A549 cells, where LC3 content decreased. Plausibly, co-stimulation of Hsp70 and LC3 may reflect the activation of chaperone-dependent autophagy by nanoparticulate carbon exposure.

Toxicity of low-molecular and small-size carbon particles is widely confirmed (Chu et al. 2019). PM2.5 increases oxidative stress and activates MAP kinase pathways in rat cardiac cells (Cao et al. 2016). Caspase-3 and apoptosis increase in L132 cells exposed to PM2.5 (Dagher et al. 2006), and autophagy increases in A549 cells (Deng et al. 2013). In our earlier studies, NPCB caused oxidative stress and DNA damage in A549 cells (Mroz et al. 2007). CB nanoparticles have been reported by other authors to cause cytotoxic injury, to increase the content of proinflammatory chemokines, and to inhibit cell growth (Yamawaki and Iwai 2006). Clinical and animal studies have confirmed the role of CB nanoparticles in aggravating pulmonary disorders such as asthma, lung cancer, pulmonary fibrosis, and systemic cardiovascular disorders (Donaldson et al. 2005). In the present study, CB increased Hsp70 content in both cell types and decreased LC3 in A549 cells but not in THP1 cells. The fluorescence scatter area was significantly reduced after CB in THP1 cells but not in A549 cells. It is, therefore, possible that the heat shock response is a primary cell reaction, while LC3 increase is secondary to increased chaperone levels. Differences between experimental data from carbon-derived probes and UD were evident, but the dose-response experiments would be necessary to shed more light on the molecular mechanisms of these transitions and activations. Nonetheless, UD can be considered a highly reactive mixture, possibly toxic to A549 cells when compared to CB, which produced significantly more compact binary distribution with fewer outlier cells. The effects of NPCB and NPCB-BaP were subtler, in some aspects similar to each other and CB, but a significant fraction of cells treated with both nanoparticulate carbons became highly stimulated as judged from increases in Hsp70 and LC3.

HSP70 has been shown to be required in autophagosome formation. In mouse embryonic fibroblasts, Hsp70 knockout blocked panobinostat-induced formations of autophagic vesicles (Yang et al. 2013). Our present findings suggest that autophagy in A549 cells and THP1 cells exposed to PM may remain under the regulatory control of the heat shock response since the conditions generating the unfolded protein response upregulate both the heat stress response and autophagy (Lukaszewicz et al. 2019). The findings also indicate that the rate of Hsp70 response in THP1 cells was akin to that in A549 cells, or lower as was the case for UD. Also, alterations in LC3 expression were within the same range except for CB treatment of THP1 cells, where LC3 slightly increased in contrast to A549 cells, where it decreased. The most conspicuous difference between both cell types was a binary pattern of Hsp70/LC3 distribution in THP1 cells, with two distinct subpopulations of cells in density scatterplots, i.e., "low" Hsp70 and LC3 cells and "high" Hsp70 and LC3 cells. In general, cells with a lower Hsp70/LC3 ratio were less affected by carbon black and nanoparticulate carbon black, while UD altered fluorescence scatterplots of all THP1 cells irrespective of their Hsp70 and LC3 content.

Both detrimental and beneficial effects of monocyte/macrophage cells have been

documented in the pathophysiology of PM-induced lung diseases (Riches and Martin 2018). Hsp70 decreases inflammation (Khandia et al. 2017), and it is an important inhibitor of apoptosis (Roufayel and Kadry 2019). Hsp70 might thus protect both cell types against PM-induced toxicity. Autophagy is also considered an important transition from monocyte apoptosis to differentiation into macrophages (Zhang et al. 2012), which may be relevant to delayed consequences of PM exposure.

The results presented herein provide an insight into the molecular mechanisms regulating proteostasis through the chaperone-autophagy system in the cytoplasm. An understanding of the interaction between chaperones and autophagy may be critical to recognize the mechanisms involved in PM-induced inflammatory reactions and cancer. Our results suggest that overexpression of Hsp70, most probably due to increased ROS formation, may be an early event caused by cell exposure to PMs. Moreover, co-expression data indicate that chaperone-dependent autophagy plays a role in maintaining cellular homeostasis, especially in NPCB-exposed cells. However, due to complexity of metabolic and signaling interactions of Hsp70 with co-chaperones, membrane transport proteins, enzymes, and lipids, the exact role and relationship between autophagy and the heat shock protein response under toxic stress from PM exposure remain to be determined.

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

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors. The study was accepted by an institutional board for in vitro studies of the Medical University of Bialystok, Poland.

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Whole Blood Assay as a Tool to Describe the Effects of Zinc Oxide Exposure on Innate Immunity

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Abstract

Inhalation of high concentrations of zinc oxide (ZnO) particles may cause metal fume fever. A useful tool to characterize the reactivity of innate immune cells of an individual, e.g., after in vivo exposure, is the whole blood assay (WBA). The measurable outcome of WBA is the release of cytokines, especially pro-inflammatory and pyrogenic cytokines induced by stimulation in vitro. The aim of the study was to evaluate whether inhalation of nano-sized zinc oxide particles modifies the results of WBA from healthy blood donors. Sixteen healthy subjects were exposed to filtered air and ZnO particles (0.5, 1.0, and 2.0 mg/m^3) for 4 h on four different days. Blood was collected before and 24 h after exposure, and ex vivo stimulation of the whole blood was performed using different endotoxin concentrations. The release of interleukin (IL)-1β and IL-8 after 22-h incubation was quantified with specific immunoassays. The dose-response relationship of ex vivo stimulation with different endotoxin concentrations was not affected by previous ZnO exposure. However, based on the previously established calculation models, changes due to ZnO exposure could be described. The range of cytokine release in WBA was calculated for the whole group of blood donors, for the subgroups of low and high responders (each $n = 8$), and on the individual level. Most changes were observed after 0.5 mg/m^3 ZnO exposure. Higher ZnO exposure did not yield higher effects. We conclude that the effects of inhalation of nano-sized ZnO particles in blood of healthy donors using the WBA could be determined. However, it should be noted that cytokine release as outcome of WBA is not a marker of disease.

Keywords

Cytokine release · Endotoxin · Immunity · Interleukins · Nano-sized particles · Whole blood assay · Zinc oxide

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1 Introduction

Whole blood assay (WBA) is an immunological assay based on cytokine release of blood cells. The test can be used to describe pyrogenic

activity of materials. On the other hand, fresh human blood is described as a suitable tool to get information about the immune reaction of a blood donor (He et al. 2018; Liebers et al. 2009, 2018; Wouters et al. 2002). The principle of WBA is the measurement of cytokine release after ex vivo stimulation of fresh human blood with endotoxin, whereupon monocytes are the main target cells. Endotoxin serves as pathogen-associated molecular pattern (PAMP) which is a known trigger of innate immune cells. The hypothesis is that changes in immune reactivity after in vivo exposure to hazards like zinc oxide (ZnO) may be visible in modified responses of blood cells to endotoxin in vitro.

ZnO fumes occur at several workplaces especially during welding. Inhalation may result in zinc fever which includes several flu-like symptoms (Greenberg and Vearrier 2015). Systemic inflammatory effects of inhaled nano-sized ZnO particles have been observed. In a study of Monsé et al. (2018), different exposure circumstances were monitored by assessing several effect parameters, such as symptoms, body temperature, the blood inflammation markers C-reactive protein (CRP) and serum amyloid A (SAA), and lung function of the subjects after exposure. Since a concentration-response relationship between the inhalation of nano-sized ZnO particles and the inflammation markers has been demonstrated at concentrations below the occupational exposure limit for ZnO, the authors conclude to reassess the exposure limit at the workplace.

Thus, ZnO is an interesting substance to follow the question of whether the effects of inhalation exposure are detectable in the in vitro-test system of WBA. However, the immune system is a changeable network of cellular and humoral components, even within daily circadian rhythm (Cermakian et al. 2013). Accordingly, reproducibility of a test with fresh human blood is questionable. Nonetheless, Wouters et al. (2002) have shown that cytokine response of individuals is reproducible in tests repeated two times. The design of a ZnO exposure study of Monsé et al. (2018) allowed for the first time to obtain blood of healthy volunteers at six different time points during a period of 3 months. Based on that design, repeated results of WBA with blood from a group of 16 healthy donors were evaluated in a previous article, without any further exposure (Liebers et al. 2018). Reproducibility was checked using different calculation models, and a normal range of cytokine release for the group was determined. In addition, according to the cytokine response, a ranking within the group was calculated, dividing the participants into high and low responders. It has been shown that the characteristic of high or low cytokine release is stable over time. However, since the interindividual variation was relatively high, a model for the individual evaluation was developed, based on the median absolute deviation (MAD). The model enabled to define a normal range of cytokine release for each subject.

The present study is a continuation of the evaluation to assess whether in vivo ZnO exposure affects ex vivo cytokine release of cells using the WBA. Therefore, blood of healthy volunteers was assayed after ZnO exposure, and the results were compared to those without exposure. The blood cell responses induced with different endotoxin concentrations were determined using the quantification of IL-1β and IL-8 release. To address the issue of variability of ex vivo cytokine release, the effects of ZnO were described in the following three different ways: deviation from the normal range calculated for the whole group, subdivision into low and high responders, and at the individual level. These ways of data elaboration were also used in our previous article on the subject (Liebers et al. 2018).

2 Methods

2.1 Study Group

Exposures to ZnO were performed in the exposure unit of our institute (see details in Monsé et al. 2018). Eight female and eight male volunteers, aged 19–42 years (median 26), were included in the study. Participants were healthy non-smokers. The subjects were exposed to filtered air and ZnO particles (0.5, 1.0, and 2.0 mg/

m^3) for 4 h, with 2-week intervals for each subject. The subjects were generally at rest, except for short periods of moderate physical activity on a cycle ergometer set to a work load of 60 watt. Exposures were randomized and double blinded, with the exception of that to 2.0 mg/m^3, which was not blinded according to instruction given by the Ethics Committee. Medical examinations were performed before, directly after, and approximately 24 h after exposure, and a questionnaire was answered. Heparinized blood was drawn before and 24 h after the beginning of filtered air or ZnO exposure. WBA was initiated within 3 h after venipuncture and was performed between 8 and 10 a.m.

2.2 Cellular Blood Composition

Total cell count and differential cell profile were performed in EDTA blood according to a standard protocol. Differential cell count was within the normal range for all subjects both before and after staying in the exposure unit. Leukocytes ranged between 2800 and 10,900/μl blood before exposure and between 2900 and 9000/μl blood after exposure.

2.3 Whole Blood Assay (WBA)

A normal range of ex vivo cytokine release without ZnO exposure was determined in the same group of volunteers in an earlier study, with six blood samples drawn from the same individual (Liebers et al. 2018). In the current study, WBA was performed before and 24 h after staying in the exposure unit, once *per* individual and *per* exposure. Overall, a total of ten blood samples from each subject were available, four of them after exposure (Fig. 1). Fresh whole blood was incubated with five different concentrations (1, 10, 40, 100, and 1000 pg/ml) of endotoxin (*Escherichia coli*; Haemochrom Diagnostica, Essen, Germany, CSE *E. coli* O113:H10) in the RPMI 1640 medium supplemented with glutamine and HEPES (Gibco, Life Technologies, Darmstadt, Germany). Incubation was performed

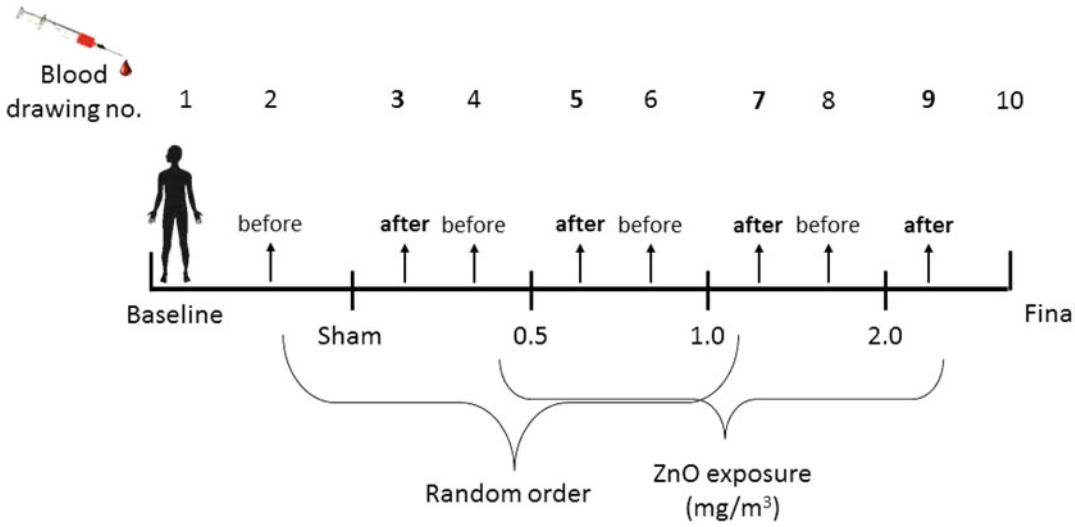

Fig. 1 Scheme of blood drawing for the study. Before and after each exposure scenario, blood collection was performed. In addition, one blood drawing was performed at the beginning and one after the end of the study. With exception of the 2 mg/m^3 ZnO exposure, which was always the final one, the other exposure scenarios were randomly ordered. The time interval between the first and last blood drawing ranged between 3 and 8 months. The time between blood drawings 2, 4, 6, and 8 ranged between 14 days and 3 months. Each of the ten blood samples was stimulated with a panel of six endotoxin concentrations. Subsequently, cytokine release in response to the stimulus was measured in each sample

in a total volume of 1 ml for 22 h at 37 °C (800 µl RPMI +100 µl endotoxin +100 µl blood). Sham exposure was performed leaving out the endotoxin. After centrifugation at 10,000 x *g* for 2 min, cell-free supernatants were aliquoted and frozen at −70 °C until use.

2.4 Cytokine Quantification

Release of cytokines was expressed by and quantified as the content of IL-1β and IL-8 in the cell-free supernatant, using a monoclonal "sandwich" enzyme immunoassay kit (IL-1β, DuoSet™; R&D Systems, Bio-Techne, MN; IL-8: Becton Dickinson, Bio Science, San Diego, CA) with a sensitivity range of 3.9–250 pg/ml for IL-1β and 3–200 pg/ml for IL-8, according to the recommendations of the manufacturer. All samples were measured in two to three different dilutions, and the results were accepted if the coefficient of variation (CV) was below 25%, otherwise the measurements were repeated.

2.5 Data Elaboration

The median, CV, and other percentiles were used to describe changes in the content of IL-1β and IL-8 in response to endotoxin stimulation in the blood. One-sided paired *t*-test was used to estimate the effect of ZnO compared to sham exposure. The problem of multiple comparisons was addressed by using the Bonferroni correction, in which the overall desired statistical significance level α (=0.05) was divided by the number of hypothesis tested. We proposed a specific range for IL-1β and IL-8 consisting of the median ± double MAD (median absolute deviation) for each individual subject and each endotoxin concentration (Liebers et al. 2018). Values above or below the range were interpreted as probable changes of immune reactions after ZnO exposure. Calculations were performed using commercial statistical packages of SAS v9.4 (SAS Institute, Cary, NC) or GraphPad Prism 7 (GraphPad Software, San Diego, CA).

3 Results

3.1 ZnO Effects in Whole Blood Assay (WBA): Evaluation of the Group of 16 Subjects

3.1.1 Normal Range Calculated as 25th and 75th Percentiles of Cytokine Release

If cells were not stimulated with endotoxin, spontaneous release of IL-1β is usually under the detection limit (<3.9 pg/ml in 14 subjects). In contrast, after 0.5 mg/m^3 ZnO exposure, blood from 8 out of the 16 subjects released IL-1β without endotoxin stimulation in a range of 13–80 pg/ml. Compared to sham exposure, this effect was significant (Fig. 2a). This effect on the spontaneous release of cytokine of ZnO exposure was also detectable for IL-8. In contrast to IL-1β, the increase in spontaneous IL-8 was significant after 2 mg/m^3 ZnO exposure compared to sham (Fig. 2b).

Furthermore, on the basis of six repeated WBA of the 16 subjects, without previous ZnO exposure, a normal range of cytokine release was calculated for IL-1β and IL-8 in reference to the 25th and 75th percentiles (Liebers et al. 2018). This range was between 50 and 304 pg/ml for IL-1β after stimulation with 10 pg/ml endotoxin and between 293 and 819 pg/ml after stimulation with 40 pg/ml endotoxin. In reference to these normal ranges, after exposure to 0.5 mg/m^3 ZnO, cytokine release was out of the range in WBA of seven and eight subjects, respectively. Exposure to higher ZnO concentrations resulted in significant changes in IL-1β release only in WBA of three or four subjects, respectively.

For IL-8, the normal range after stimulation with 10 pg/ml endotoxin was 372 to 6136 pg/ml. After stimulation with 40 pg/ml endotoxin, it increased to 1291–10,861 pg/ml. In reference to this calculation, WBAs of five to seven subjects were out of the range after 0.5 mg/m^3 ZnO exposure, while only two to five subjects were out of the range after 1 or 2 mg/m^3 ZnO exposure.

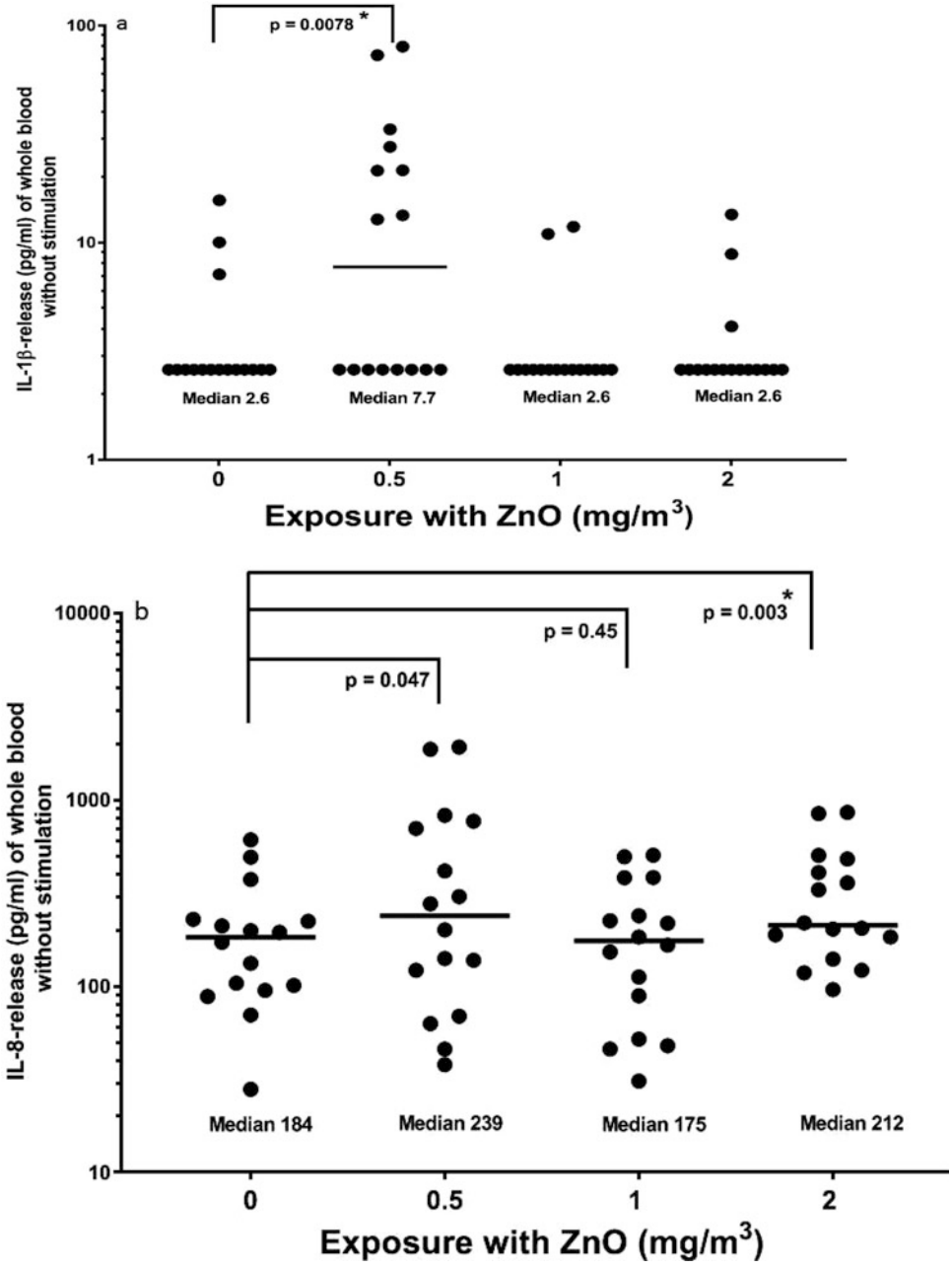

Fig. 2 Spontaneous ex vivo IL-1β (**a**) and IL-8 (**b**) release in whole blood assay (WBA). The blood was taken from 16 subjects after sham or ZnO exposure

3.1.2 Comparison of Cytokine Release Before and After ZnO Exposure

To answer the question of whether ZnO exposure effects are detectable in WBA, data before exposure were set as 100%. The change of cytokine release was described as a percentage in this calculation. When comparing the results of WBA after the four exposure scenarios (0, 0.5, 1, and 2 mg/m^3 ZnO), it turned out that there was a change in cytokine release after 0.5 mg/m^3 ZnO.

There were median increases by 24% and 84% for IL-1β and IL-8, respectively. After sham and 1 or 2 mg/m^3 ZnO exposure, the median cytokine release remained unchanged (Figs. 3a, b).

3.2 ZnO Effects in Whole Blood Assay (WBA): Differences in Cytokine Release in Low and High Responders

The endotoxin-induced dose-response relationship of cytokine release has been previously described (Liebers et al. 2018; Liebers et al. 2009). This dose-dependent response to endotoxin remained generally unaffected by previous exposure of subjects to ZnO. However, ZnO effects were detectable concerning the content of cytokines released in the high responder group (Figs. 4a, b).

3.2.1 IL-1β Release

The subjects were divided into high and low responders according to IL-1β-release in six repeated WBAs, without the exposure setting (Liebers et al. 2018). Using this high/low-responder classification differences were verified after ZnO exposure. While the dose-response relationship was still detectable in both groups, a distinctly higher IL-1β release after 0.5 mg/m^3 ZnO exposure primarily concerned the high responder group (Fig. 4a).

3.2.2 IL-8 Release

IL-8 release was measured in parallel to IL-1β release in the same cell-free supernatants. In contradistinction to IL-1β, spontaneous release of IL-8, even without endotoxin stimulation, was detectable in a range of 28–610 pg/ml after sham exposure (median 184 pg/ml). After exposure of the subjects to ZnO, spontaneous IL-8 release changed to a median of 239 pg/ml (range 38–1923) after 0.5 mg/m^3 ZnO, 176 pg/ml (range 31–505) after 1 mg/m^3 ZnO, and 212 pg/ml (range 96–858) after 2 mg/m^3. Therefore, compared to sham exposure, a significant increase of spontaneous IL-8 release after 2 mg/m^3 ZnO exposure was detectable. Classification into low and high responders due to IL-8 release, without previous ZnO exposure, revealed that the effects of ZnO concern only high responders (Fig. 4b), like it also was the case for IL-1β.

3.3 ZnO Effects in Whole Blood Assay (WBA): Individual Evaluation Based on Median Absolute Deviation (MAD) Model

Individual ranges of cytokine release were calculated based on MAD. In our previous publication, we described median plus/minus double MAD of six repeated WBAs as a normal range for each individual based on the analysis of the 16 subjects (Liebers et al. 2018).

3.3.1 IL-1β Release

Double MAD of ex vivo IL-1β release for each subject, representing the individual range of reactivity is shown in Fig. 5a, b**.** Overall, in the blood from 10 out of the 16 subjects, WBA resulted in IL-1β release beyond the individual range after exposure to 0.5 mg/m^3 ZnO, whereas for higher ZnO concentrations, that was detectable for WBA results of seven (1 mg/m^3 ZnO) and four (2 mg/m^3 ZnO) individuals. In summary, ZnO effects regarding WBA and the parameter IL-1β release were detectable in four to ten of the subjects, without the influence of ZnO exposure dose.

3.3.2 IL-8 Release

Likewise, double MAD of ex vivo IL-8 release was calculated, referring to WBA of the 16 subjects. Furthermore, an individual cytokine release range using the MAD model was documented for each subject separately (Fig. 5c, d). Similarly to IL-1β, the deviation of IL-8 release from the individual range was significant after 0.5 mg/m^3 ZnO. Stimulation with 10 pg/ml and 40 pg/ml endotoxin induced IL-8 release in blood from 7 out of the 16 subjects beyond the individual range, which was most prominent after stimulation with 40 pg/ml endotoxin (Fig. 5d). Exposure to higher ZnO concentrations resulted in four (1 mg/m^3 ZnO) and six (2 mg/m^3 ZnO) subjects demonstrating IL-8 release beyond the

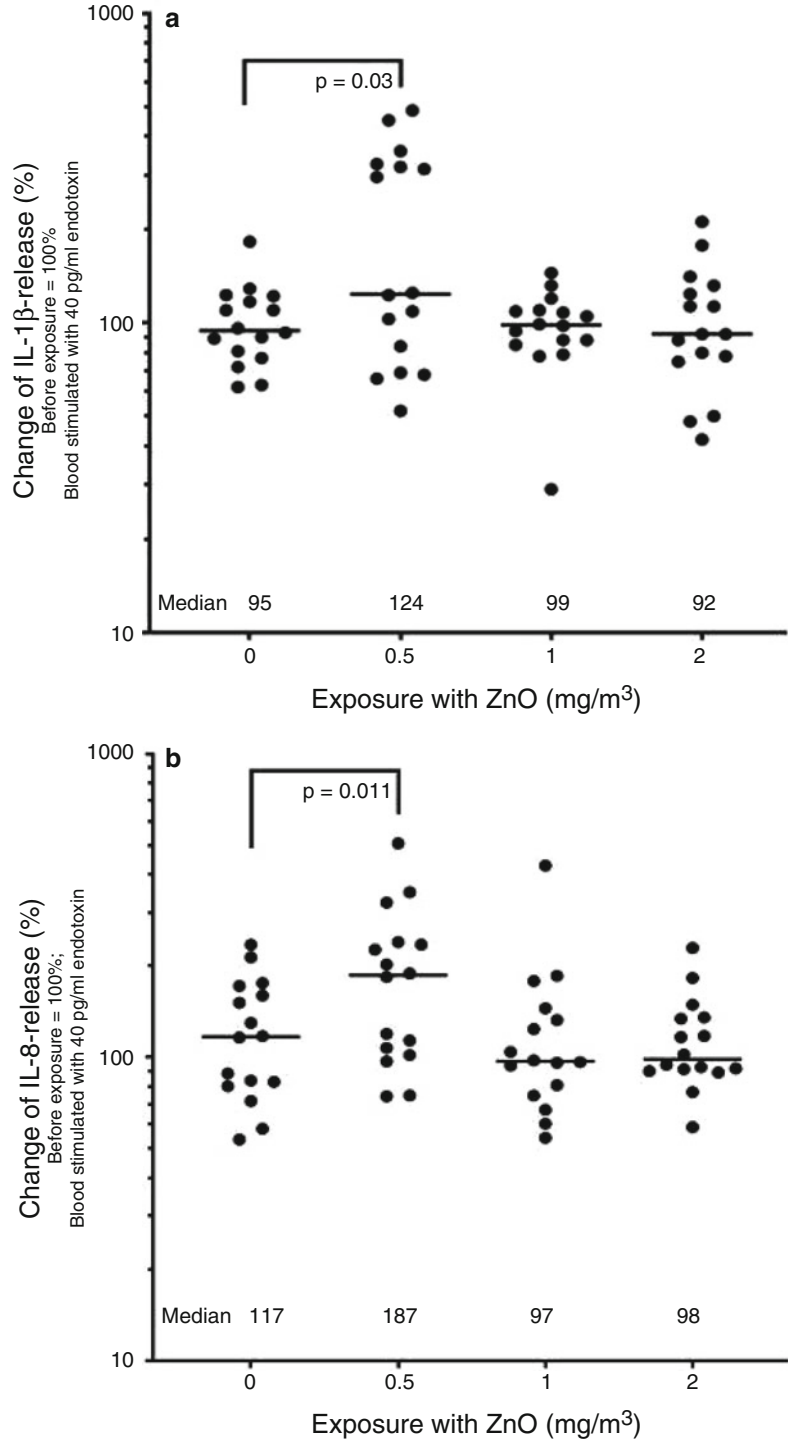

Fig. 3 IL-1β (**a**) and IL-8 (**b**) release after ZnO exposure expressed as percent of the control level before exposure. The increases in both cytokines were significant after 0.5 mg/m^3 ZnO exposure

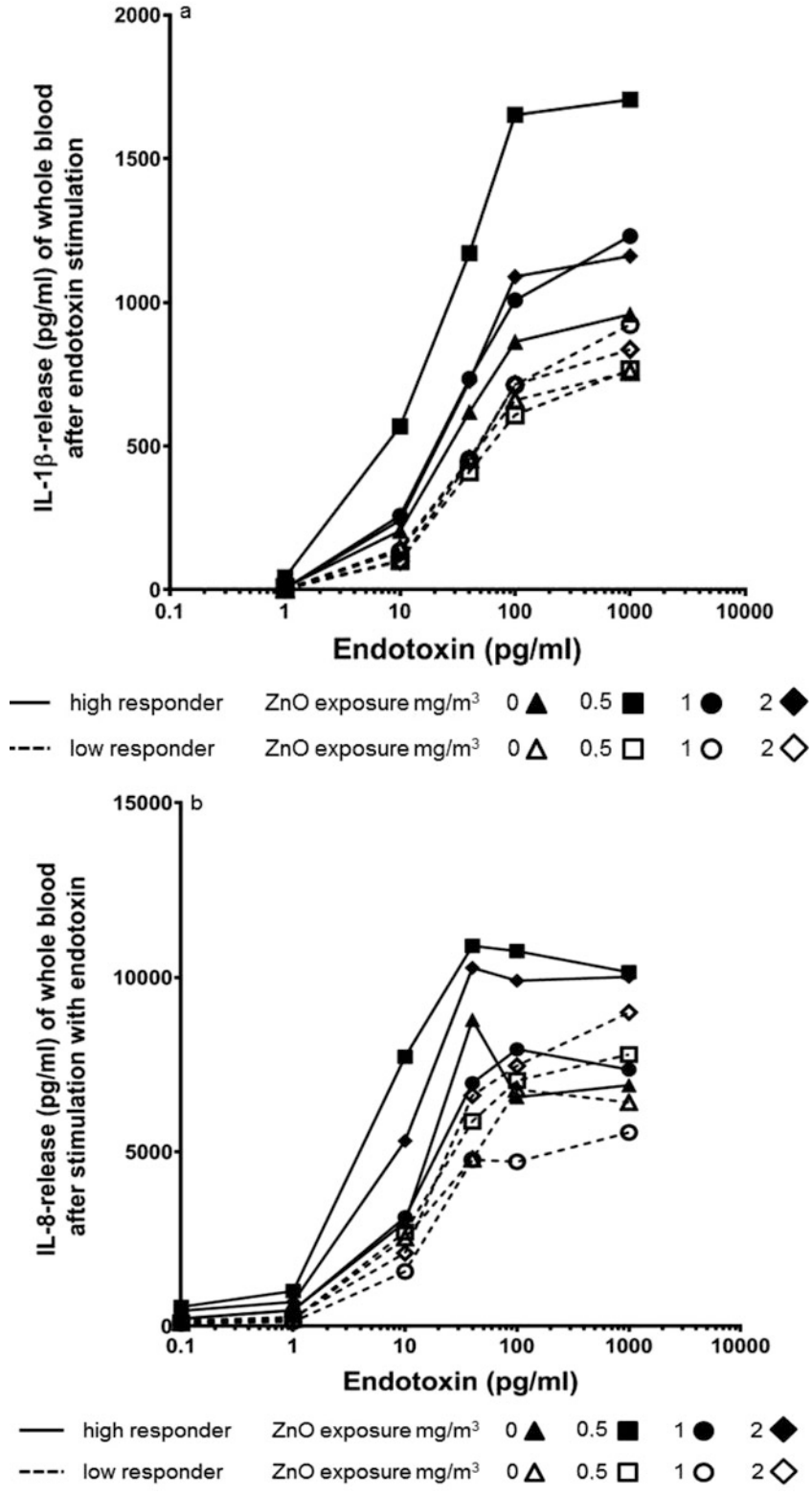

Fig. 4 Median cytokine release (IL-1β) (**a**) and IL-8 (**b**) of 16 subjects measured in whole blood assay (WBA) after stimulation with different endotoxin concentrations, categorized in high ($n = 8$) and low ($n = 8$) responders due to six repeated WBAs, without experimental ZnO exposure

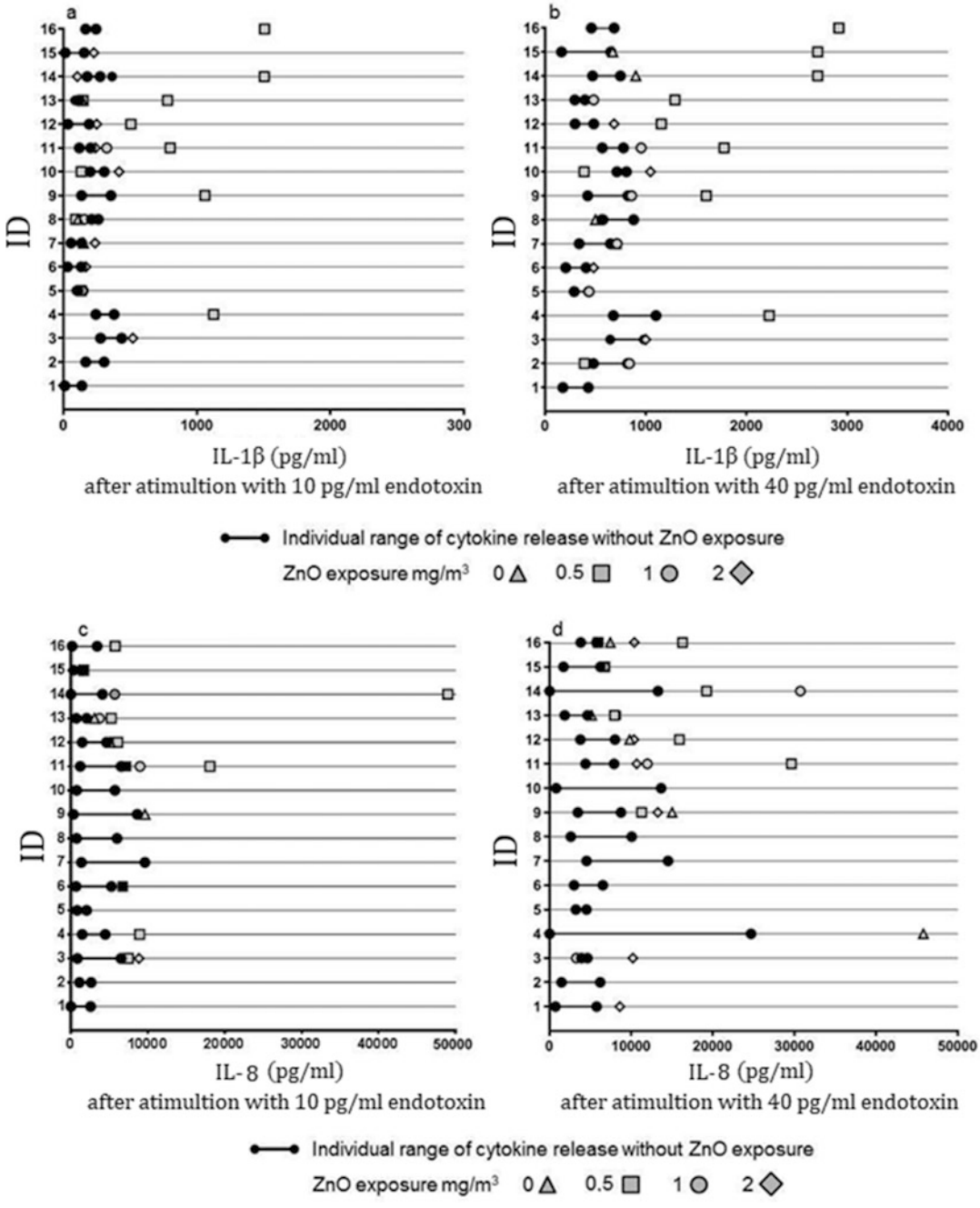

Fig. 5 Individual IL-1β (**a**, **b**) and IL-8 (**c**, **d**) release in WBA after stimulation with 10 pg/ml and 40 pg/ml endotoxin. Double median absolute deviation (MAD) was used for calculation of the individual range (black line between dots). The dots represent the calculated upper and lower limit of cytokine release in WBA for each individual. Cytokine release in WBA after the subject stayed in the exposure unit was indicated when the value was outside the individual range. ID, subject number

individual range. In summary, ZnO effects regarding WBA (outcome IL-8) were detectable in four to seven of the subjects, without dose-response relationship.

There was no association fund between leucocytes or monocytes and cytokine release (data not shown). Changes in cytokine release associated neither with IL-6 nor with SAA or CRP assessed in the subjects' serum.

4 Discussion

This study focused on the effects of exposure to experimental nano-sized ZnO particles in a low concentration range (0.5, 1, and 2 mg/m^3) on ex vivo cytokine release. IL-1β and IL-8 were quantified in cell-free supernatants after in vitro whole blood stimulation from 16 subjects with different endotoxin concentrations. In a previous study, a normal range of cytokine release was calculated for this group as well as for each of the 16 individuals (Liebers et al. 2018). In reference to those data, the effects of ZnO were analyzed with WBA in the present study. No dose-response relationship due to ZnO exposure could be found. However, striking changes in cytokine release after exposure to 0.5 mg/m^3 ZnO were obvious in all calculation models. Finally, since the variation between individuals was high, it seems to be most appropriate to evaluate the exposure effects with WBA regarding the subgroups of high and low responders and also on the individual basis.

To evaluate (sub)toxic and inflammatory effects of ZnO, diverse test models from different working groups have been so far applied. Cytokine release in vitro from human or mouse cell lines has been investigated (Chen et al. 2015; Saptarshi et al. 2015; Sahu et al. 2014). Inflammatory markers have also been investigated in human serum after inhalation exposure to ZnO (Krabbe et al. 2019; Monsé et al. 2018). It has to be considered that in vitro experiments using cell lines show results after direct stimulation with ZnO, whereas human inhalation studies refer to inflammatory markers measured in the serum of the exposed subjects.

The present study refers to the inhalation study of Monsé et al. (2018) and addresses the issue of whether blood cells of human subjects change their ability of endotoxin-induced cytokine release ex vivo after ZnO exposure. Inhalation of ZnO fumes at higher concentrations may have a health impact on the entire body and is known as cause of metal fume fever (Greenberg and Vearrier 2015). Besides fever, typical symptoms include throat irritation, cough, minor respiratory symptoms, metallic taste, and flu-like symptoms, such as a general feeling of illness, myalgia, or headache. Typically, the symptoms occur after a latency period of 4–12 h and resolve within 48 h. Individuals who are affected by ZnO fumes are employees who work with galvanized steel sheets like welders, galvanizers, or car makers. So far, occupational exposure limits of 5–10 mg/m^3 ZnO exist in Germany, the Netherlands, Sweden, Denmark, and the USA (Vogel and Cassee 2018). A reassessment of these exposure limits was suggested by Monsé et al. (2018) who have described a concentration dependent effect on symptoms and on the inflammatory serum markers (CRP and SAA) for ZnO exposure above 0.5 mg/m^3.

A persistent increase of systemic inflammatory markers, such as SAA, CRP, or IL-6, in the serum after zinc and copper exposure has been described from several working groups (Baumann et al. 2016, 2018; Markert et al. 2016). Krabbe et al. (2019) have analyzed serum and nasal secretions concerning the inflammation markers in subjects exposed to zinc- and copper-containing welding fumes under controlled conditions. They documented a sustained increase in CRP, SAA, and IL-6 over the entire course of exposure. In spite of the increase of inflammation markers, subjects were asymptomatic. In contrast to the inflammatory serum markers, cytokine release of whole blood was unaffected in a dose-dependent manner in the present study. However, changes in the cellular response were prominent after exposure to 0.5 mg/m^3 ZnO and were most obvious in the high responder group.

Five (IL-8) and six (IL-1β) out of the eight high responders were male. Thus, an influence of gender for the classification into the high- or

low-responder group cannot be excluded. Aulock et al. (2006) have described a stronger innate immune response of males with regard to cytokine release of whole blood. Nonetheless, the definition of a high responder is not identical with male gender, and it rather suggests individualized effects.

Sahu et al. (2014) investigated the size- and time-dependent effects of ZnO on toxicity and inflammatory potential of human monocytes in vitro. They measured viability, phagocytosis, and cytokine induction in a human monocyte cell line (THP-1), comparing the effect of nano- and micro-sized particles. They found a significantly higher release of IL-1β and IL-8 induced by nano-sized ZnO compared to micro-sized ZnO. These results were time-dependent: IL-1β was measurable not before 24 h, while IL-8 showed a time-dependent increase and a significant decrease after 48 h concerning micro-sized particles. Likewise, an influence of time was documented by Kuschner et al. (1998). They investigated the U937 monocytic cell line, stimulated with ZnO suspensions. A dose-dependent release of IL-8 was verified after 8 and 14 h, but not after 3 h. Further, Saptarshi et al. (2015) studied (sub)-toxic levels of ZnO particles in the A549 epithelial cell line. The highest IL-8 release was found after 5–6 h of incubation with ZnO. Those results raise the question of whether blood sample drawing 24 h after onset of exposure in the current study was the optimum time point concerning the investigation of WBA. Time dependency may explain the missing reaction to higher concentrations of ZnO exposure. Possibly, to document the effects of 1 or 2 mg/m^3 ZnO exposure using WBA, the blood should have been drawn much earlier. This hypothesis will be investigated in further studies.

A description of the effects of ZnO exposure in an in vitro model needs well-considered comparisons. In the present study, the analysis of the results was performed before and after exposure, concerning both ZnO and sham exposures. Sham exposure is an important control since subjects stayed in the exposure unit and ran through short periods of moderate physical activity on a cycle ergometer similarly the condition during exposure. The results of WBA showed significant differences in cytokine release of unstimulated cells, comparing ZnO exposure to sham. This points out that cells were partially triggered by ZnO for spontaneous cytokine release and that the effect was not a consequence of physical activity or the exposure situation itself. However, whether the described changes in cytokine release were really due to ZnO inhalation or to some other substances could not be answered with certainty in the setting employed.

While in the present study blood of ZnO exposed subjects was investigated in WBA, Bleidorn et al. (2019) used blood of non-exposed subjects and stimulated it in vitro with zinc- and copper-containing welding fume. They found a significant release of IL-6, IL-8, and TNF-α for all concentrations (0.1–100 μg) of welding fume. Comparing then the IL-6 results from the in vitro study with an exposure study of 15 volunteers, they found somehow corresponding data. That makes them suggest WBA may be a tool to replace human exposure studies. However, this proposal is to be viewed critically since in vitro data are not a marker of disease. Furthermore, it should be kept in mind that zinc is involved in numerous aspects of human cellular metabolism and plays an important role in the balance of pro- and anti-inflammatory cytokines in response to lipopolysaccharides (Maywald et al. 2017; Pyle et al. 2017; Bonaventura et al. 2014). Diverse studies show that free zinc ions are utilized for immune cell receptors like Toll-like receptor (TLR)-4. Chen et al. (2015) have shown that TLR-4-deficient mice are less reactive to ZnO nanoparticles in regard to pulmonary inflammation than wild-type mice are. Brieger et al. (2013) have shown that zinc ions are involved in various reactions leading to TLR-stimulation in murine macrophage cell lines. Zinc-related signals affect the TLR-4-induced production of the inflammatory cytokines IL-β and IL-6 and also the antiviral immune responses mediated by IFN-β. Therefore, in future studies, it would be interesting to evaluate changes in human blood regarding TLR expression in response to ZnO inhalation.

5 Conclusions

This study demonstrates that the whole blood assay (WBA) is a tool to describe the effects on the human immune response after inhalation studies. Evaluating data of 16 human subjects with defined ZnO exposures, effects in regard to ex vivo cytokine release were investigated. The basis for this study was a previous study (Liebers et al. 2018) in which six repeated WBAs of each individual had been performed, enabling the setting of a range of cytokine release for the group as well as the separate individuals. Those data become an essential basis to describe the exposure effects and to separate effects from normal biological variations.

In the present study, significant changes in cytokine release from blood cells were found especially after exposure to 0.5 mg/m^3 ZnO and partially after 2 mg/m^3 ZnO as well. A clear dose-response relationship between inhalation ZnO exposure and in vitro cytokine release from blood cells could not be verified. Most interestingly, the effects did not concern all of the participants, suggesting that exposure effects should be evaluated on the individual basis. At any rate, it should be noted that WBA is not a direct measure of health effects or inflammation. Increased cytokine levels after in vitro stimulation may mirror the normal reaction of a healthy immune system.

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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 Ruhr-Universität Bochum (No. 4929–14).

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

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Depression and Serum Content of Serotonin in Adult Patients with Atopic Dermatitis

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Abstract

Atopic dermatitis (AD) is a chronic skin disease with the etiology not yet conclusively established. Recent reports demonstrate the role of serotonin (5-hydroxytryptamine; 5-HT) in the pathogenesis of AD. The aim of this study was to investigate the relationship between the serum content of serotonin and depression in adult patients suffering from severe AD. There were 31 patients of the median age of 41 years enrolled into the study, who suffered from AD since childhood, and a control group that consisted of 14 healthy subjects. AD was diagnosed on the basis of Hanifin and Rajka criteria. The severity of skin lesions was assessed with the SCORing Atopic Dermatitis (SCORAD) index and that of depression with the Montgomery-Åsberg Depression Rating Scale (MADRS) questionnaire. We found that all of the patients with severe AD characterized by SCORAD >50 had depression. Depression was classified as mild and moderate according to the MADRS score. Serotonin content was significantly lower in the patients with severe AD (MADRS >12), and there was an adverse relation between the serotonin content and the score of depression, the features not noticed in the control group. We conclude that severe AD, as expressed by the intensification of skin lesions, associates with depression and with the lowering of serum serotonin content. The findings point attention to the cognitive and affective problems in AD patients which could worsen the course of the skin disease.

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Keywords
Affective symptoms · Atopic dermatitis · Depression · Serotonin · Skin lesions

1 Introduction

Atopic dermatitis (AD) is a chronic skin disease of yet unknown pathogenesis (Weidinger and Novak 2016; Leung and Guttman-Yassky 2014). From the epidemiological standpoint, there is a global increase in the prevalence of AD which now affects as much as 20% of the population in the developed countries. The prevalence of AD in Poland is estimated at 4% (Sybilski et al. 2015). Pruritus is the most conspicuous symptom that accompanies eczematous lesions, which are usually located in typical body regions, such as the extensor involvement in infants or children and flexural lichenification in adults (Hanifin and Rajka 1980). The disease results from complex genetic, epigenetic, environmental, and immunological interactions with an overlapping epidermal barrier defect (Nowicki et al. 2015).

Recently, a growing body of research has focused on the coexistence of AD and a number of other nonatopic conditions, such as skin infections, cardiovascular diseases, cancer, and, interestingly, mental disorders that involve depression and suicidal attempts (Brunner et al. 2017). The pathogenesis of depression is at present underlain by the monoaminergic hypothesis, in which dysfunction of serotonergic neurotransmission place a key role. The synthesis and release of monoamines is, to a great extent, influenced by inflammatory cytokines (Gałecki and Talarowska 2018). Recent reports have pointed attention to a key role of serotonin (5-hydroxytryptamine; 5-HT) also in the pathogenesis of AD (Rasul et al. 2016; Kawana et al. 2010; Lonne-Rahm et al. 2008). Therefore, the aim of this study was to examine the relationship between the blood level of serotonin and the severity of depression in adult patients suffering from AD.

2 Methods

This study was performed in a group of 31 adult patients (17 women and 14 men) of the median age of 41 years who had developed AD in childhood. The control group consisted of 14 healthy volunteers, gender- and age-matched. Basic characteristics of the groups are presented in Table 1. The diagnosis of AD was confirmed by a dermatologist and an allergist, according to the Hanifin and Rajka (1980) criteria. The severity of skin lesions was determined based on the SCORing Atopic Dermatitis (SCORAD) index, where score over 50 points indicates severe AD (SCORAD 1993). The lowest SCORAD result we found in this study was 50.4 points and the highest was 80.4 points (median of 61.5 points), pointing to the very severe disease. Exclusion criteria were as follows: lack of consent to participate in the study, age below 18 years, inflammatory comorbidities, mild-to-moderate severity of AD lesions, systemic therapy with immunosuppressive, antihistamine or psychotropic drugs, and phototherapy during 6 months preceding the study. The severity of depression was assessed with a validated Polish version of the Montgomery-Åsberg Depression Rating Scale

Table 1 Characteristics of the study groups

		Patients ($n = 31$)	Controls ($n = 14$)
Gender; *n* (%)	Male	13 (41.9)	8 (57.1)
	Female	18 (58.1)	6 (42.9)
Age (years); median (min-max)		41 (24–75)	42 (22–73)
Education; *n* (%)	Secondary	15 (48.4)	2 (14.3)
	Tertiary	16 (51.6)	12 (85.7)
SCORAD (points); median (min-max)		61.5 (50.4–80.4)	0.0

SCORAD SCORing Atopic Dermatitis index

Table 2 Severity of depression according to the Montgomery-Åsberg Depression Rating Scale (MADRS) in adult atopic dermatitis (AD) patients and control subjects

MADRS score	Depression	AD patients ($n = 31$)	Controls ($n = 14$)
0–11	None; *n* (%)	0	14 (100%)
12–19	Mild; *n* (%)	5 (16.1%)	0
20–29	Moderate; *n* (%)	26 (83.9%)	0

Table 3 Severity of depression according to the Montgomery-Åsberg Depression Rating Scale (MADRS) and severity of skin lesions according to SCORing Atopic Dermatitis (SCORAD) index in adult atopic dermatitis (AD) patients

		SCORAD	
Depression	Patients ($n = 31$)	Median (min-max)	*p*
Mild	5	52.2 (50.4–60.5)	0.002
Moderate	26	63.2 (52.4–80.4)	

Table 4 Severity of depression according to the Montgomery-Åsberg Depression Rating Scale (MADRS) vs. serum serotonin content in adult atopic dermatitis (AD) patients and in controls

	AD patients	Controls	
	Median (min-max)	Median (min-max)	*p*
MADRS (score)	24 (18–28)	4 (1–7)	<0.001
Serotonin level (ng/mL)	85.7 (45.0–110.3)	294.9 (220.4–394.5)	<0.001

(MADRS) (Montgomery and Asberg 1979). Blood for the 5-HT assessment was drawn from the elbow vein in both patients and control subjects between 7.00 and 9.00 a.m. The samples were left for clot for 2 h at room temperature and then were centrifuged at 3500 RPM for 10 min, frozen, and stored at −80 °C until use. The serum content of 5-HT was assayed using a commercial ELISA kit (R&D System, Minneapolis, MN).

Data were presented as medians and minimum-maximum values. The Kruskal-Wallis and Mann-Whitney U tests were used to compare differences between the serum 5-HT content and AD severity in the groups with mild and moderate depression. Relationships among these indicators were evaluated with Spearman's rank correlation coefficient. A p-value of <0.05 defined statistically significant differences. The evaluation was performed using a commercial StatSoft Statistica v13.1 package (Dell Software; Round Rock, TX).

3 Results

All of the AD patients had depression according to the MADRS scale as opposed to none of the controls (Table 2). A positive significant correlation was found between the severity of skin lesions and the severity of depression ($r = 0.64$, $p < 0.001$). Patients with moderate depression had a significantly higher SCORAD score compared to patients with mild depression ($p = 0.002$) as presented in Table 3.

The median blood serum content of 5-HT was 85.7 ng/mL (min-max: 45.0–110.3 ng/mL, $p < 0.001$) in the AD patients who had mild-to-moderate depression, whereas it was outstandingly higher in the control subjects with no depression (median: 294.9 ng/mL; min-max: 220.4–394.5 ng/mL) (Table 4). In this study we noticed no AD patients who would not have a degree of depression according to the MADRS scale (Table 2). There was no appreciable difference in the content of 5-HT depending on the severity of depression ($p = 0.54$) (Fig. 1).

4 Discussion

Contrary to a popular belief that atopic dermatitis is a childhood disease, the incidence of AD among adults is on the rise. In a recently

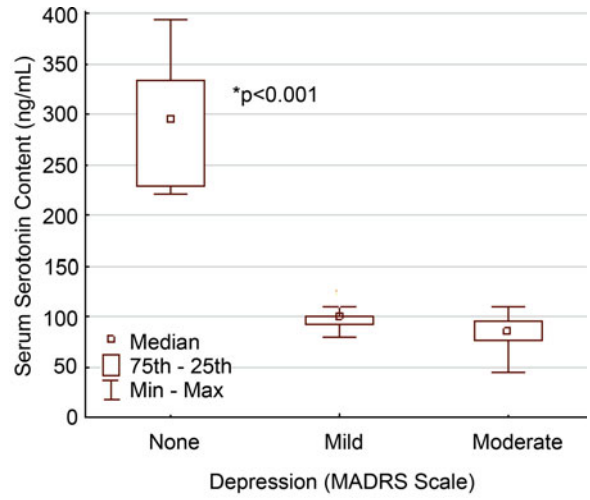

Fig. 1 Serum 5-HT level in atopic dermatitis patients with mild-to-moderate depression, according to the Montgomery-Åsberg Depression Rating Scale (MADRS), and in healthy control subjects without depression symptoms. The asterisk denotes a significantly higher 5-HT content in the control subjects when compared to AD patients with mild and moderate depression

published study of Barbarot et al. (2018) involving adult populations in the USA, Canada, Japan, and Europe, the disease was observed in 4.9% of adults. The literature abounds with research on the coexistence of AD and depression. A study by Cheng et al. (2015) conducted in a group of 8208 Taiwanese adolescents and adults revealed that AD is a risk factor for developing depressive disorders. Similar conclusions were reached by Wei et al. (2016), who have examined patients suffering from atopic diseases. The Northern Finland Birth Cohort study consisted of the observation of 12,058 children born in 1966 and followed up to the age of 31, with focus on the possible development of atopy. The study has revealed a threefold increase in the incidence of depression (hospitalized) in both men and women suffering from atopic diseases, with AD being diagnosed in 691 individuals (Timonen et al. 2001). In a Polish study of Chrostowska-Plak (2013) entailing 89 patients, significant relationships have been noticed between patient-reported pruritus, severity of depression (evaluated by the Beck questionnaire), and the impairment of quality of life (assessed by the Dermatology Life Quality Index). A study of Vinnik et al. (2017), which included 56 AD patients, has found a significant seasonal variation in the rate of depressive symptoms evaluated by the Hamilton Depression Scale. The results of the present study corroborated the previous observations in that the adult patients with AD are significantly more prone to depression. It is worth noting that severe AD and depressive symptoms were found in all of the patients investigated. Thus, the severity of AD dermatitis predisposes to the development of depression, which is consistent with the observations of other authors (Kim 2012). The risk of developing affective disorders by patients with AD apparently remains underrated, and the notion of "psychodermatological care", postulated in the recently published European guidelines for AD treatment, is marginalized (Wollenberg et al. 2018).

Serotonin (5-HT) is a highly hydrophilic biogenic amine derived from the exogenous amino acid tryptophan due to the action of decarboxylases. The main source of 5-HT are gastrointestinal cells, platelets, immune cells (lymphocytes, monocytes and macrophages), mast cells, and central nervous system neurons particularly the dorsal raphe nucleus (Herr et al. 2017; Kim 2012). After release, 5-HT is subjected to a reuptake mechanism underlain chiefly the serotonin reuptake transporter (SERT). High SERT expression is shown by enterocytes, platelets, and neurons of the central

and peripheral neural systems. Twenty-one subtypes of serotonin receptors (presynaptic and postsynaptic) are identified. They are structurally stratified into seven classes: 5-HT_1 (subtypes: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}), 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7. The main function of serotonin is neurotransmission (Kritas et al. 2014). The so-called serotonin concept of depression pathogenesis assumes a dampening of serotonin neurotransmission resulting from dysfunction of its receptors, particularly 5-HT_{1A} and 5-HT_{2A} (Carhart-Harris and Nutt 2017; Rasul et al. 2016).

The role of 5-HT in the pathogenesis of AD has been confirmed by Hosogi et al. (2006) and Rasul et al. (2013) who show this monoamine is responsible for histamine-independent pruritus occurring in AD lesions. In a study of Rasul et al. (2016) consisting of 28 patients (18 women and 10 men), expression of 5-HT, 5-HT_{1A}, and 5-HT_{2A} receptors, along with SERT, has been examined immunohistochemically in both lesional and non-lesional skin. The expression of 5-HT_{1A} and that of SERT were higher in lesional skin, whereas that of 5-HT_{2A} was higher in non-lesional skin. Furthermore, the severity of depression, assessed by MADRS, correlated positively with 5-HT_{1A} expression and adversely with 5-HT_{2A} expression. In non-lesional skin, expression of 5-HT_{2A} correlated positively also with disease severity, assessed by SCORAD.

5-HT plays a key role in communication between the immune and nervous systems due to its pleiotropic effect on various immune cells (Herr et al. 2017; Kim 2012), including modulation of T lymphocytes which largely contribute to the development of AD. Katoh et al. (2006) have shown that platelet-derived 5-HT, along with 5-HTR1 and 5-HTR7 receptors, induces the conversion of monocytes into dendritic cells which also play a role in the AD pathogenesis. Soga et al. (2007) have confirmed that 5-HT plays an essential part in activating monocytes and preventing their apoptosis. That study has also revealed a significantly higher serum 5-HT content in 11 patient suffering from AD (SCORAD = 37 points) when compared to the control subjects. A role of 5-HT in the pathogenesis of AD is also confirmed by reports on the efficacy of serotonin reuptake inhibitors in treatment of this disease (Eskeland et al. 2017; Ständer et al. 2009). However, it is difficult to relate those findings to the present observations due usually to a limited number of patients in the previous studies, lower severity of skin lesions, and a lack of the assessment of depression.

In conclusion, this study demonstrates that the severity of skin lesions and pruritus in adult atopic dermatitis correlated with the intensity of depressive symptoms. Moreover, a significant decrease in 5-HT serum content was noticed in AD patients when compared to healthy control subjects. We conclude that it is advisable to monitor the affective and cognitive brain function in patients suffering from AD. Depression if unnoticed could lead to otherwise treatable exacerbation of the skin condition.

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 Bioethics Committee of the Medical College of the Jagiellonian University in Cracow, Poland.

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

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Evaluation of Nocturnal Respiratory Complaints in Pregnant Women

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Abstract

Snoring during pregnancy increases the risk of low Apgar score and low birth weight of newborns. Snoring women are twice as likely to be diagnosed as having preeclampsia when compared to non-snoring ones. Snoring may also be linked to, albeit it is not a prerequisite for, apneic events at sleep. The aim of this survey-type study was to evaluate the occurrence and severity of nocturnal respiratory complaints in a group of 312 pregnant women. Problems associated with snoring and other nasopharyngeal symptoms were reported by 60% of women. Complaints were more frequent in patients with a higher body mass index. The symptoms were significantly more frequent in the group of snorers. The results of this study suggest a pattern of basic features in pregnancy, such as snoring, overweight, and upper airway symptoms, which all ought to direct attention of care givers to the diagnostics of sleep-related breathing disorders. The early diagnosis would enable to undertake the measures to prevent preterm labor and to avoid postpartum complications in both mother and newborn.

Keywords

Airways · Newborns · Pregnancy · Respiratory complaints · Sleep apnea · Snoring

1 Introduction

Nocturnal breathing disorders constitute a heterogeneous group of ailment leading to disruption of physiological rest during sleep. One of the primary symptoms is snoring. It may be the only sign, but it is often accompanied by shortness of breath. Snoring, which is referred to as an acoustic respiratory sound caused by the vibration of the soft throat's elements, is not synonymous with a disease. Nevertheless, when snoring appears during a short nap or in a vertical body position, it may suggest a pathology. The throat is the only element of the upper respiratory tract that does not have a cartilage bone scaffolding. Excess fat around the neck of obese people may exert a direct pressure on the throat area, reducing its lumen and facilitating airway collapse during

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sleep, often accompanied by hypercapnia, which all is historically described as the obesity hypoventilation or Pickwickian syndrome (Chung et al. 2016; Littleton and Mokhlesi 2009). Fat tissue in the neck is the most important element that, beside anatomical obstacles in the nasal cavity and throat, can also lead to obstructive sleep apnea syndrome (Dzieciolowska-Baran et al. 2009). Intermittent hypoxia occurring during apneic events negatively affects the organ systems, notably, the cardiovascular system (Beninati et al. 1996).

Changes in sleep pattern and architecture occur in normal pregnancy starting from the first trimester. Weight gain during pregnancy may predispose to sleep disordered breathing (Peppard et al. 2000). In addition, diaphragm elevation caused by the enlargement of the uterus during pregnancy leads to a reduction of pulmonary residual capacity and expiratory backup volume, airway narrowing, and inadequate ventilation, causing a decrease in oxygen diffusion at the alveolar capillary membrane (Weinberger et al. 1980; Knuttgen and Emerson 1974; Craig and Toole 1975). In a healthy pregnant woman in the third trimester, mild hypoxia is observed during wakefulness and sleep (Bourne et al. 1995; Awe et al. 1979).

An increase in blood volume causing congestion and swelling of the nasal mucosa may lead to narrowing of the airways within the nasal cavity. High levels of estrogen also have a vasodilatory effect and increase mucous secretion. In the third trimester, vasomotor rhinitis occurs in 20% of pregnant women (Mabry 1986). Up to 42% of women in the 36th week of pregnancy complain of congestion of the nasal mucosa and rhinitis (Bende and Gredmark 1999). The mechanisms above outlined increase negative pressure during inspiration, which may interrupt breathing (Pilkington et al. 1995; Haponik et al. 1983).

Mother sleep is essential for the fetus well-being, since the uterine blood flow and secretion of neurohormones, especially growth hormone, reach their peak during sleep (Blyton et al. 2004). Even slight declines in maternal arterial oxygen content may jeopardize the effective delivery of oxygen to the fetus. Studies on sleep disorders in pregnant women point to increased risk of premature delivery, intrauterine growth restriction, lower Apgar scores in newborns, and even an increase in neonatal mortality (Louis et al. 2014). In this study we set out to evaluate the occurrence of nocturnal upper airway complaints in pregnant women in an attempt to sort out the symptoms that could point to a true breathing disorder during sleep. The study was based on a self-assessment questionnaire.

2 Methods

The study was conducted in the third trimester of pregnancy in 312 women, who were hospitalized at the Department of Obstetrics and Gynecology or were outpatients of the Independent Public Clinical Hospital No. 2 in Szczecin, Poland. Participation in the survey was voluntary, and it was preceded by written informed consent. A conversation was held with each patient to highlight the problem of sleep disorders. For the purpose of the study, we created a questionnaire consisting of 26 open-ended and closed-ended questions. The questions concerned demographic and anthropometric characteristics of the pregnant women, clinical history, upper airway symptoms and complaints, sleep architecture, comorbidities, and social activities. It required 5 min to complete the questionnaire, to lessen to the minimum a burden for pregnant women related to filling it in. Routine obstetric checkups, including ultrasound examinations, were performed to evaluate the state of pregnancy, the fetal weight, and blood flow through selected vessels such as umbilical artery, middle cerebral artery, and uterine arteries.

Continuous data were presented as means ±SD and nominal data as percentages. Data distribution was checked with the Shapiro-Wilk test. The kurtosis and skewness of data distribution were calculated. Differences between the mean values were compared with a *t*-test. Nominal data of the survey were compared with Pearson's chi-squared test. When there were significant differences in the chi-square table, a surplus between the observed and expected values was

analyzed. In case the values were too small, the Yates correction was applied. A significance of differences in the incidence of comorbidities in the snoring and non-snoring groups was assessed with the sample structure indicators. A p-value <0.05 defined statistically significant differences.

3 Results

3.1 Demographic and Anthropometric Characteristics of Pregnant Women

In the entire cohort examined, the mean age of pregnant women was 31.3 ± 5.9 years (range 17–46 years). Women aged 27–36 years (58.7%) were the most numerous, and those aged over 37 years (18.9%) were the least numerous. The median height was 167 cm. Over 50% of women placed in the 163–172 cm range. The shortest patient was 148 cm tall, and the tallest was 192 cm. Concerning body weight, about half of pregnant women placed in the 71–90 kg range (Fig. 1). In 137 pregnant women (43.9%), body weight increased from the beginning of pregnancy to the third trimester by >10 kg. An increase <10 kg was present in 110 women (35.2%). There were 37 (11.9%) current tobacco smokers among all of the pregnant women examined. The mean time of addiction to tobacco was 9.5 ± 5.8 years (median 8 years). The longest period of addiction was 20 years, and the shortest was 1 year.

Nocturnal breathing complaints were reported by 187 (60%) of the pregnant women examined and was underlain by the accompanying snoring and other upper airway symptoms. This group was referred to as "snorers" in further analysis. Women who did not report nocturnal symptoms of a breathing disorder were referred to as "non-snorers".

The mean age of pregnant women in the "snorers" subgroup was 31.3 ± 5.9 years. The youngest woman in this subgroup was 18 years old, and the oldest was 46 years old. The mean age in the "non-snorers" subgroup was 31.5 ± 5.6 years. The youngest woman here was 17 years old, and the oldest was 44 years old. The age differences between the two subgroups of pregnant women were insignificant ($p = 0.60$).

There were no statistical differences in the percentage of smokers between the pregnant "snorers" and "non-snorers" (Chi^2, $p = 0.49$) (Fig. 2). Concerning the level of education, 8% had primary, 36.2% had secondary, and as many as 53.2% had tertiary education. In the "non-snorers" subgroup, there were significantly more people with university education, whereas in the "snorers" subgroup, higher education prevailed; the difference between the two was significant ($p = 0.05$) (Fig. 3).

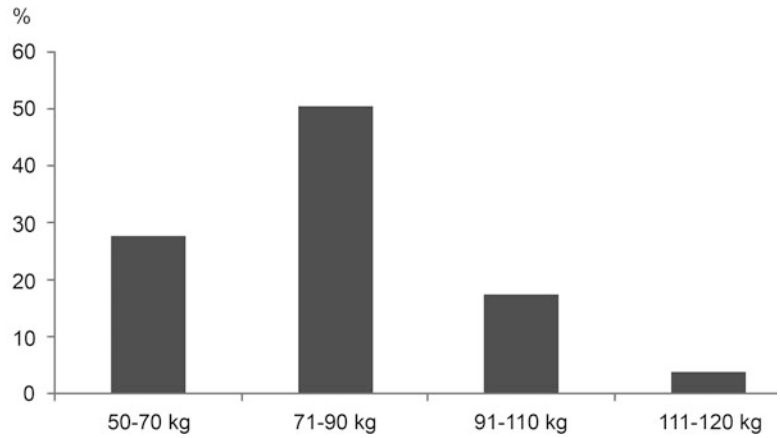

Fig. 1 Percentage distribution of body mass in the third trimester of pregnancy in the entire cohort examined (n = 312)

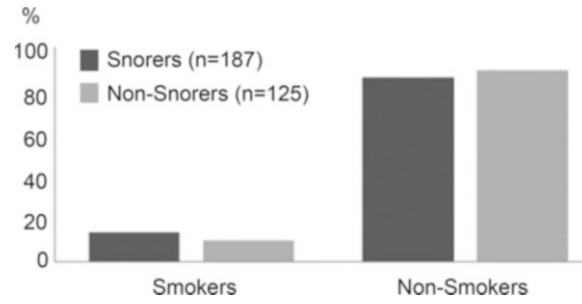

Fig. 2 Percentage distribution of current smokers and nonsmokers in "snorers" and "non-snorers" during pregnancy

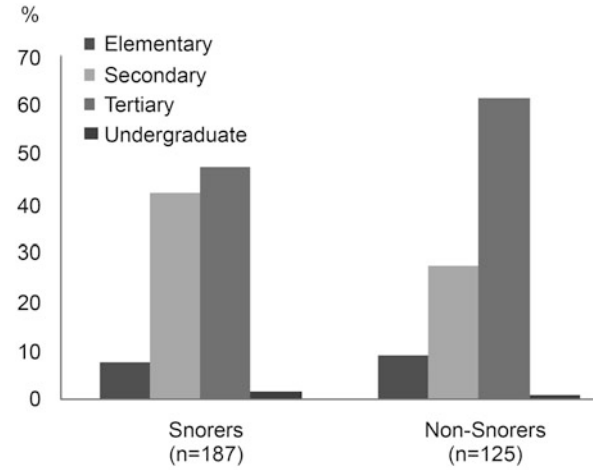

Fig. 3 The level of education in "snorers" and "non-snorers" during pregnancy

Table 1 Body weight in pregnant women with ("snorers") and without ("non-snorers") nocturnal breathing symptoms

Pregnant women	*n*	Median (kg)	Min–Max (kg)	Variance	Skewness	Kurtosis
"Snorers"	187	80	53–127	230.8	0.52	0.10
"Non-snorers"	125	77	51–119	168.5	0.43	0.23

The body weight of the majority of "snorers" in the third trimester was between 71 kg and 90 kg, with the median of 80 kg. For comparison, the median body mass was 77 kg in the "non-snorers" subgroup (Table 1). Differences in weight gain during pregnancy were insignificant between the "snorers" and "non-snorers" (Chi^2 Pearson's: 1.81, df = 4, p = 0.77) (Fig. 4).

Nutritional status was evaluated on the basis of body mass index (BMI) in the first and third trimester of pregnancy. Overall, the mean BMI in the group of "snorers" was significantly greater in the first trimester when compared to "non-snorers": 27.4 ± 5.2 kg/m^2 vs. 26.0 ± 4.8 kg/m^2, $p = 0.02$. This proportional difference between the two groups persisted into the third trimester: 29.5 ± 5.0 kg/m^2 vs. 28.4 ± 4.6 kg/m^2, respectively, $p = 0.04$. In detail, "snorers" in the first trimester of pregnancy were less often overweight but more often obese than "non-snorers." The difference persisted into in the third trimester. Percentage distribution of BMI categories in pregnant "snorers" and "non-snorers" in the third trimester of pregnancy is shown in Fig. 5.

Eighty-three (44.3%) out of the 187 "snorers" and 55 (44.0%) out of the 125 "non-snorers" were

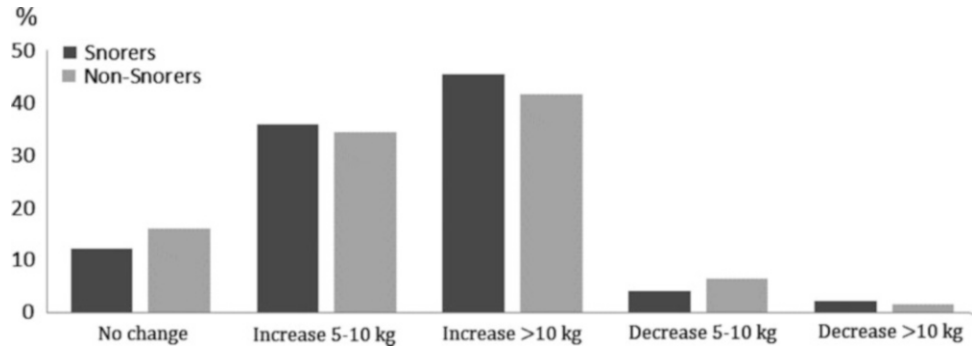

Fig. 4 Body weight changes in "snorers" and "non-snorers" in the third trimester of pregnancy

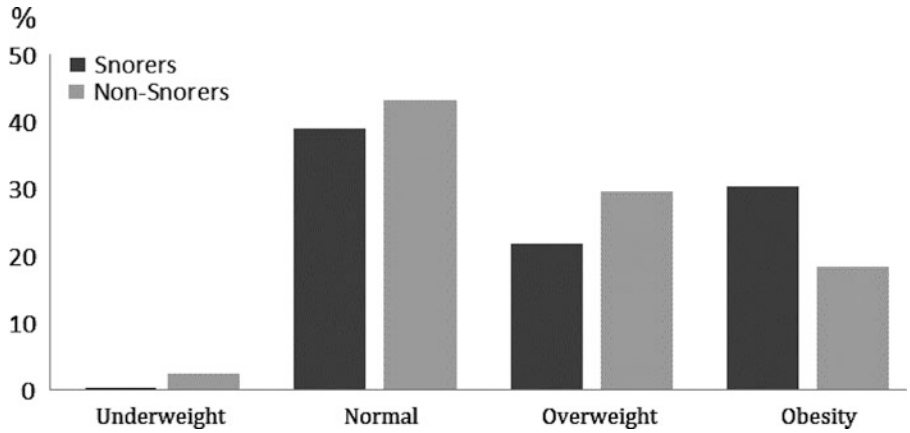

Fig. 5 Percentage distribution of body mass index (BMI) categories in "snorers" and "non-snorers" in the third trimester of pregnancy

primiparous. The remaining women already had one or more children. There were single cases of having six and eight children. Out of the multiparous women, 15.7% had a history of premature birth, which ended before the 37th week of pregnancy. The percentage distribution of premature births was not significantly different between the two subgroups (Fig. 6).

In the entire cohort, 159 (51.0%) out of the 312 pregnant women reported coexisting systemic diseases. There were 78 cases of diabetes, 42 of hypothyroidism, 40 of hypertension, and 54 cases of asthma or allergy, with overlapping diseases in some cases. Frequency distribution of each disease did not differ between the "snorers" and "non-snorers" (Pearson's Chi^2: 23.6, df = 23, $p = 0.43$) (Fig. 7).

3.2 Upper Airway Symptoms in Pregnant Women

Laryngological problems were diagnosed in 158 (50.6%) out of the 312 pregnant women. The most frequent complaint was chronic rhinitis, which was noticed in 90 women. The next frequent was a feeling of a dry throat, noticed 62 women. Nasal septum deviation was diagnosed in 33 women, and another woman had an external deformation of the nose. Thirty-four women reported having two problems in the upper airway simultaneously, including 19 who complained of chronic rhinitis and dry throat together. Nasal septum deviation and chronic rhinitis occurred in six, and tonsillar hypertrophy accompanied by chronic runny nose in three women. Three women reported having three

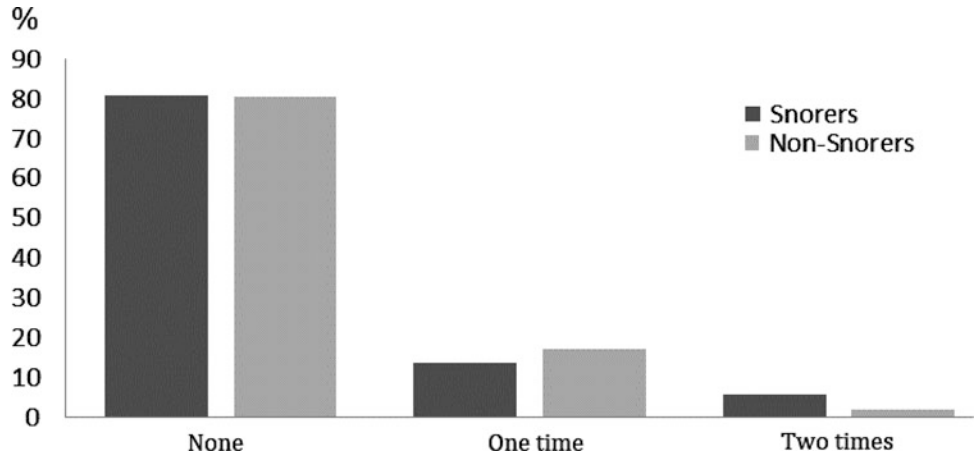

Fig. 6 Percentage distribution of premature birth in pregnant "snorers" and "non-snorers"

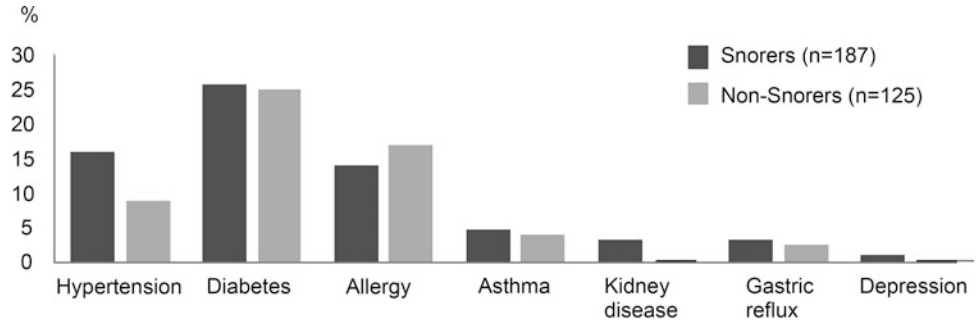

Fig. 7 Percentage distribution of accompanying systemic diseases in pregnant "snorers" and "non-snorers"

symptoms simultaneously such as chronic rhinitis, tonsillitis, and dry throat. There also was nasal edema, chronic stuffy nose, hoarseness, or bruxism in occasional cases.

Among the "snorers", only one pregnant woman was aware of having apneic episodes. The others reported snoring, mainly occasional after a plentiful meal or the use of alcoholic beverages. More than half of the "snorers" (57.7%) were informed about the occurrence of disturbing symptoms by partners and 13.3% by other family members, and 7.7% were used to waking up spontaneously. Snoring was not embarrassing for 48.2% of the women, while 22.4% of them considered snoring an issue, forcing half of them to have a separate bedroom. Three quarters of "snorers" reported that change in body weight during pregnancy was of no effect on the nocturnal breathing complaints, about a quarter reported the enhanced complaints with weight gain, and two women noticed a decrease in symptoms.

One hundred and thirty women (69.5%) out of the 187 "snorers" were not aware of the harmful effects of sleep-related breathing disorders and did not feel any need for treatment. Only did 24 women (12.8%) have a sense of the impact of breathing disorders on health. Sixteen women (8.5%) wished to initiate treatment due to the embarrassing symptoms rather than for medical reasons. The remaining "snorers" failed to express an opinion on the issue. One hundred and forty-three women failed to employ any kind of treatment to reduce the severity of symptoms. In the remaining "snorers", the long-term use of decongestant nasal drops was the

most frequently remedy employed. In single cases, pregnant women used oral medicines, and two women attempted to reduce weight as a remedy for respiratory symptoms.

4 Discussion

In this survey we found that 60% of pregnant women manifested upper airway complaints, with the accompanying snoring. This finding is similar to that of Facco et al. (2010) who have reported, in a cohort of 202 pregnant women, the 28% prevalence of snoring in physiological pregnancy and the 75% prevalence in preeclampsia. Currently, there are no explicit recommendations regarding screening for sleep-related breathing complaints in pregnant women. The Berlin Questionnaires, the STOP-Bang Questionnaires, and the Epworth Sleepiness Scale have been mostly developed to screen for obstructive sleep apnea in middle-aged and older persons (Lapin et al. 2018; Chung et al. 2012; Abrishami et al. 2010; Netzer et al. 1999). There is a lack of studies verifying the usefulness of these questionnaires in pregnant women. Nonetheless, snoring is the prime symptom raising suspicion of sleep-related breathing disorders (Dzieciolowska-Baran et al. 2009). It is estimated that *ca* 24% of pregnant women suffer from some kind of breathing disorders in the second and third trimesters of pregnancy. The percentage of affected women significantly increases in preeclampsia and in pregnancies complicated by intrauterine growth restriction (Izci et al. 2003). Despite a widespread occurrence of sleep-related breathing complaints in pregnancy, the diagnosis is rather rare, which might be due to a lack of public awareness of the meaning of symptoms and their effects on the course of pregnancy and perinatal outcome (Bourjeily et al. 2013). Therefore, we presumed in this study that the risk factors for sleep-related breathing disorders, which are well defined for the general population such as age, cigarette smoking, or overweight (Dzieciolowska-Baran et al. 2010), could also apply to pregnant women. We failed to show any effect of cigarette smoking, to the extent that smoking was continued into the pregnancy just by 10% of women, on the development of nocturnal breathing symptoms. Likewise, we failed to find any age-dependent difference between pregnant "snorers" and "non-snorers". Both groups were rather closely age-matched, which hinders the assessment. However, within the middle-age span of 17–46 years of age, increasing age was not conducive to the development of nocturnal breathing complaints. The influence of age of pregnant women on the appearance of nocturnal breathing complaints remains contentious, as Pien et al. (2014) have reported a relationship between breathing disorders and age and also BMI in pregnancy.

Studies on sleep-related breathing disorders, also during pregnancy, point to overweight and obesity as plausibly the most important risk factor (Lockhart et al. 2015; Olivarez et al. 2010). Dominguez et al. (2018) have convincingly confirmed that risk in a recent study in extremely obese pregnant women. In line with those findings, in the present study we demonstrate that overweight and obese pregnant women, who accounted for about 60% of "snorers" had a significantly higher mean BMI at the beginning of pregnancy when compared to "non-snorers". Among the "snorers", 27.8% presented with the first-degree obesity, 12.2% with the second-degree obesity, and 2.6% with the third-degree obesity. There were significantly more women with BMI in the normal range among the "non-snorers". We also found that the initial maternal BMI was a determinant triggering nocturnal breathing complaints, mostly snoring, in the third trimester, with the weight gain having been about equal in both groups as pregnancy continued. Disturbing symptoms from the nasopharynx, such as dry throat or chronic rhinitis, were noticed more frequently in pregnant "snorers" when compared to "non-snorers", which could have to do with an obstructive tendency caused by the accumulating fat tissue in the facial skeleton in case of a higher BMI. "Snorers" exhibited an increase in symptoms along the pregnancy course and associated them with increased of body weight. Antony et al. (2014) have reported an increase in the incidence of

snoring from 9.5% in the first trimester to 25.8% in the third trimester. Likewise, a prospective observational study by Pien et al. (2005) have revealed apneic episodes and increased frequency of loud snoring pregnancy.

In the present study, as many as 51% of pregnant women reported the occurrence of comorbidities. Diabetes was noticed in 25% of the women. High-caloric diet and a tendency to avoid physical effort in pregnancy seem conducive to the development of gestational diabetes, an increasing serious health concern (Chan et al. 2009). The impact of diabetes on maternal and fetal health is well-known. Hyperglycemia associates with increased risk of fetal macrosomia, preeclampsia, primary cesarean section, or preterm delivery (Lowe et al. 2012; Lindsay 2009). A high diabetic morbidity points to the need of identifying modifiable risk factors for impaired glucose tolerance in pregnancy. Evidence indicates that nocturnal breathing disorders are one such factor associated with poor glucose tolerance and possibly gestational diabetes (Buxton et al. 2010; Donga et al. 2010). Experimental studies have shown that short and interrupted sleep decreases sensitivity to insulin when compared to longer sleep (Louis and Punjabi 2009; Bosy-Westphal et al. 2008). Intermittent nocturnal hypoxia changes glucose metabolism (O'Keeffe and St-Onge 2013). Further, metabolic abnormalities improve as a result of treatment of nocturnal breathing disorders (Dempsey et al. 2010).

Arterial hypertension was the next common comorbidity noticed in this study. Overall, hypertension occurred in 12.8% of women. The prevalence of hypertension was almost twofold greater in "snorers" (Fig. 7), albeit the difference failed to reach statistical significance. Repeated hypoxic episodes are conducive to peripheral vasoconstriction resulting in increased activity of the renin-angiotensin-aldosterone pathway, which may lead to maternal hypertension (Mistry et al. 2019) and, in turn, to the mitigation of blood supply to the placenta and fetus. These mechanisms underlie the association of sleep-related breathing disorders with restricted fetus growth and low birth weight (Conti et al. 1988; Sherer et al. 1991; Lefcourt and Rodis 1996). In a study of Franklin et al. (2000), which involved a cohort of 502 pregnant women, hypertension is present in 14% of snorers compared to 6% of non-snorers. In another study, preeclampsia, associated with hypertension, is present in 10% of "snorers" compared to 4% of "non-snorers" (Edwards et al. 2000). Our present findings are somehow at variance with those previous studies above outlined, as the incidence of diabetes, hypertension, and also other less frequent comorbidities such as allergies, thyroid, or kidney disorders, albeit tending to be greater in "snorers", did not differ significantly from those in "non-snorers". A lack of statistical significance might stem from a rather small number of women in each disease group and from the inherent bias of questionnaire studies, related to subjectivity of responses given by respondents.

In conclusion, this study shows that snoring in pregnancy is much commoner when compared to the population at large. It is usually accompanied by, or a result of, upper airway malfunction, most often expressed in the form nasopharyngeal symptoms. Pregnancy *per se* increases vulnerability to infection (Kourtis et al. 2014) and to respiratory distress associated with dysfunction of pharyngeal muscular activity (Balserak 2015), in effect increasing snoring. These symptoms undergo exacerbation during sleep, leading to nocturnal respiratory complaints. The nighttime exacerbation raises the possibility of pregnancy impact on brain-driven circadian homeostasis. We believe we have shown in this study that snoring in pregnancy, even when accompanied by other complaints from the facial skeleton, is a mild symptom that is not necessarily linked to a significant pathology such as apneic episodes. Yet it is prudent to periodically screen pregnant women for the presence of nocturnal breathing complaints to safeguard from the potential mortality and harm they harbinger to mother and fetus.

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 project was approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval no. KB-0012/19/17).

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

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Cardiovascular Function in Obstructive Sleep Apnea Patients with Controlled Hypertension

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Abstract

This study investigated hemodynamic characteristics of obstructive sleep apnea (OSA) accompanied by hypertensive disease in obese men, in whom blood pressure was pharmacologically controlled within the normal range, not exceeding 140/90 mmHg. There were 21 severe OSA patients (mean age 54.1 ± 9.3 years, apnea-hypopnea index of 47.1 ± 18.8 episodes per hour) included in the study, in whom OSA was diagnosed with polysomnography. The control group consisted of healthy normotensive age-matched subjects. Hemodynamic profile was recorded nonivasively with impedance cardiography. Brachial blood pressure and radial artery tonometry were performed to capture and reconstruct peripheral radial and central aortic pressure waveforms in both groups of subjects. Compared to healthy men, OSA patients had a significantly higher body mass index (BMI); the mean increase in BMI amounted to 6.4 ± 1.2 kg/m^2. The patients also presented significant differences in the hemodynamic profile. The difference consisted of a faster heart rate, higher peripheral pulse pressure, and reduced blood flow acceleration and velocity indices, describing myocardial contractility. Notably, the significance of hemodynamic differences in OSA patients disappeared in the analysis adjusted for the outstanding increase in BMI. In conclusion, the findings strongly suggest that obesity rather than the hypertensive disease *per se* is a source of hemodynamic consequences in OSA patients.

Keywords

Arterial blood pressure · Body mass · Cardiovascular function · Hemodynamics · Hypertension · Obesity · Obstructive sleep apnea

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1 Introduction

Obstructive sleep apnea (OSA) is a potentially severe breathing problem occurring during sleep, with a complex and heterogeneous etiology (Leão et al. 2016). Typically, OSA is accompanied by repeated episodes of obstructive apneas and/or hypopneas caused by perpetual collapses of the upper airway during sleep. OSA is a lifestyle disease with the prevalence among people over 50, estimated at 4% in men and 2% in women (Young et al. 1993). The disease severity is defined by the number of apnea-hypopnea events per hour, the apnea-hypopnea index (AHI). Mild OSA ranges from 5 to 15 events per hour, moderate from 16 to 30, and severe over 30 (Lattimore et al. 2003). OSA patients, particularly with more advanced disease, are often overweight or obese. A broad spectrum of comorbidities and complications secondary to OSA includes cardiovascular and metabolic disorders, including hypercholesterolemia (Can et al. 2009), hypertension, ischemic heart disease, heart failure, cerebrovascular events, diabetes, pro-inflammatory and pro-oxidative propensity, stroke, and mood disorders such as depression (Hui et al. 2019; Mannarino et al. 2012; Kearney et al. 2005; Worsnop et al. 1998; Levy et al. 1996; Rodgers et al. 1996; Neaton and Wentworth 1992).

Several cardiovascular risk factors, signs and symptoms, and complications overlap between OSA and hypertension, having a mutually potentiating effect and adversely affecting clinical outcome. There are also indications of increased arterial stiffness and altered arterial pressure waveform or hemodynamic variables in both hypertensive and OSA patients, although specific characteristics of changes may be different in either disorder (Doonan et al. 2011; Stein et al. 2008; Drager et al. 2007; McEniery et al. 2005). Hypertensive patients have a greater stroke volume, which is often reduced in OSA patients (Shivalkar et al. 2006; Weiss et al. 1996). However, since the majority of OSA patients suffer from hypertension, it is difficult to separate the effects of both disorders.

A contemporary polypharmacological approach to hypertension treatment is usually effective in achieving the target arterial blood pressure (BP) of less than 140/90 mmHg in OSA patients (Whelton et al. 2018), but it is unclear whether reduced BP would normalize changes in the hemodynamic profile. Thus, in this noninvasive study, we investigated the hemodynamic phenotype and the characteristics of peripheral and central blood pressure wave forms in hypertensive men suffering from OSA, in whom BP was pharmacologically controlled below the target level above outlined. The hemodynamic profile was compared to that present in the healthy normotensive age-matched peers.

2 Methods

The study included 21 men (mean age 54.1 ± 9.3, range 37–78 years) with diagnosed severe OSA, with apnea-hypopnea index (AHI) >30. The diagnosis was established on the basis of overnight polysomnography using the Embla S4000 setup (Embla Systems LLC, Natus Medical Incorporated, Pleasanton, CA), according to the guidelines of the American Academy of Sleep Medicine guidelines (Iber et al. 2007). The examination assessed different sleep stages, jaw muscle tone, eyeball movement, oxygen saturation, heart rate, nasal and oral airflow, chest and abdominal movement, and sleep position. The following variables were derived: apnea-hypopnea index, number of breathing disorders per hour of sleep, number of hypopnea events per hour of sleep, and the percentage of oxygen saturation of arterial blood (SaO_2).

An additional criterion for the inclusion into the OSA group was the accompanying hypertensive disease, with BP pharmacologically kept below 140/90 mmHg as evidenced by every morning measurements at home before taking medicines. Exclusion criteria were the following: cardiac pacemaker, persisting atrial fibrillation or paroxysmal atrial fibrillation in the preceding 3 months, decompensated heart failure, acute coronary syndrome, pericarditis, valvular heart disease, pulmonary hypertension, cardiomyopathy,

abnormal thyroid, renal or liver function, and the use of antiarrhythmic drugs.

The control group consisted of 46 healthy normotensive, asymptomatic age-matched men (mean 55.1 ± 9.3, range 37–71 years) not taking any medicines, with a normal picture of 12-lead ECG and a score below 10 points on the Epworth Sleepiness Scale. The possibility of OSA was excluded in these subjects on the basis of a thorough clinical and laboratory investigation. Therefore, polysomnography was not performed in the healthy subjects. All of the study participants underwent a detailed cardiovascular and pulmonary examination, including pulse wave analysis and cardiac impedance measurement. To start off, brachial BP was measured automatically with an M5 Blood Pressure Monitor (Omron Healthcare Co., Kyoto, Japan) on both arms in the sitting position and the arm cuff and a bracelet-like piezoelectric tonometer placed over the radial artery, to measure changes in artery tone, were attached to the Colin Blood Pressure Monitor 7000 (Colin Medical Technology, Komaki, Japan). Readings from the brachial cuff were used as reference values for the continuously recorded pressure waveforms over the radial artery by the piezoelectric transducer. The acquired analog signal was transferred in real-time to the SphygmoCor Mx device (AtCor Medical, West Ryde, Australia) for immediate reconstruction, using a validated transfer function (Townsend et al. 2015; O'Rourke and Pauca 2004; Gallagher et al. 2004) of the central pressure waveform in the ascending aorta. Both radial and reconstructed aortic pressure waveforms were captured and analyzed every minute by Sphygmocor software (Schneider et al. 2018). Averaged values of the following variables were taken into consideration:

- HR – heart rate,
- SBP, DBP, PP – systolic and diastolic blood pressure and pulse pressure, measured separately for the radial (peripheral) and reconstructed aortic (central) pressure waveforms,
- PPA – pulse pressure amplification, a ratio of peripheral to central pulse pressure,
- MBP – mean blood pressure, assumed to be equal in both aorta and peripheral arteries,
- CAI – central augmentation index,
- CAP – central augmentation pressure.

Impedance cardiography was investigated using a tetrapolar-band configuration, with two Ag/AgCl electrodes around the neck and another two around the upper abdomen. In detail, electrodes were bilaterally placed at the extrapolated crossings of mid-axillary lines with the neck base and xiphisternal levels. The electrodes were connected to the cardiac impedance monitor Niccomo (Medis GmbH, Ilmenau, Germany) and the signal recorded continuously for 5 min after a 15-min acclimatization period. The following hemodynamic variables were calculated using a modified Bernstein formula (Mannarino et al. 2012; Wiegand and Zwillich 1994):

- ACI – acceleration index, i.e., the maximum rate of change in blood velocity related to changes in aortic blood acceleration,
- CO – cardiac output,
- SV – stroke volume,
- SVR – systemic vascular resistance,
- VI – velocity index, i.e., the maximum rate of impedance change related to changes in aortic blood velocity.

In addition, anthropometric measures were taken such as body weight and height, waist and hip circumferences, and the derivatives such as body mass index (BMI) and waist-to-hip ratio were calculated.

Data were expressed as means ±SD. Data had normal distribution, checked with the Shapiro–Wilk test of normality. Comparisons between groups were performed with a two-tailed unpaired *t*-test. Since BMI significantly differed between the OSA patients and healthy men, the analysis of covariance (ANCOVA) adjusted for BMI was applied to compare the hemodynamic variables which turned out to be significantly different in the *t*-test comparison. The ANCOVA results are shown as the estimated marginal mean

(EMM) ± SE. A p-value <0.05 defined statistically significant differences. The MedCalc statistical software package for Windows v17.5 (MedCalc, Ostend, Belgium) was employed for the analysis.

3 Results

Among the OSA patients, two (9.5%) had diabetes, six (28.6%) were current smokers, and seven were ex-smokers (33.3%), not smoking for the minimum of 6 preceding months. The mean AHI was 47.1 ± 18.8 per hour, hypopnea index was 16.8 ± 11.6 per hour, and the SaO_2 dropped to 76.4 ± 8.8%. Among healthy age-matched men, 14 (30.4%) were current and 12 (26.1%) ex-smokers. Table 1 shows a summary of continuous data for clinical and hemodynamic characteristics in both groups of subjects. In the main, OSA patients were obese, having increased body mass, and waist-to-hip ratio. The mean augmentation of BMI in OSA patients, compared to healthy subjects, was by 6.4 ± 1.2 kg/m^2. The patients also had a significantly faster heart rate, higher peripheral PP, and lower ACI and VI values. There were no significant differences in the values of SBP, DBP, MBP, and central PP between the two groups.

The significance of differences in the hemodynamic variables between the OSA patients with optimally treated hypertension and the control healthy subjects, noticed in a direct t-test comparison, disappeared in the ANCOVA analysis after adjustment for BMI (Table 2).

4 Discussion

This study shows that male hypertensive patients suffering from severe OSA, with controlled arterial blood pressure not exceeding 140/90 mmHg, had a different hemodynamic phenotype than the healthy age-matched peers did. The difference consisted of a faster heart rate, higher peripheral pulse pressure, and reduced blood flow acceleration and velocity indices describing myocardial contractility. However, we noticed that these differences in the peripheral and central blood pressure waveforms disappeared in the analysis adjusted for the outstanding increase in BMI in OSA patients, whose BMI and body weight were, on average, over 6 kg/m^2 and 20 kg greater, respectively. This intriguing finding strongly suggests that obesity rather than the hypertensive disease per se is a source of hemodynamic consequences in OSA patients. In clinical practice, effective reduction of increased BP is achievable in most of the hypertensive patients, including those with severe OSA (Lavie et al. 2000). However, even when BP is controlled, most of the OSA patients remain obese (Khan et al. 2013; Peppard et al. 2000), and obesity treatment seems the most challenging part of the current medical management (Tuomilehto et al. 2013).

The issue of a relation of OSA to cardiovascular system structure and function is contentious. Whereas some studies show that OSA associates with different features of arterial pressure waveforms or arterial walls, others fail to support such findings. In severely obese OSA patients, desaturation index seems to correlate with the properties of the arterial pressure waveform assessed at the common carotid artery (Doonan et al. 2011; Dubern et al. 2010; Weiss et al. 1996). In contrast, Bakker et al. (2011) have found no significant differences in peripheral or central BP between severe OSA patients and healthy controls. In a case study, Koren et al. (2015) have reported that AHI does not associate with the maximum carotid intima-media thickness, carotid artery diameter, arterial pulse wave velocity, or the augmentation index. In other studies, however, OSA leads to a blunted vascular endothelial response, hypercapnia-mediated increase in sympathetic neural activity, hyperactivity of the renin-angiotensin-aldosterone axis, vascular dysfunction, and premature atherosclerosis (Walter et al. 2013; Stein et al. 2008; Bradley and Floras 2003; Gordon et al. 1967). There is also an apparent relation of OSA to type 2 diabetes mellitus, insulin resistance, metabolic syndrome, and obesity (Koren et al. 2015; Redline et al. 2007).

In the main, overweight and obesity are known determinants of central arterial pressure and other

Table 1 Clinical characteristics and hemodynamic pulse wave variables in OSA patient and control healthy subjects

Variables	OSA ($n = 21$)	Controls ($n = 46$)	p-value
Age (years)	55.1 ± 9.3	54.1 ± 9.3	0.680
Body weight (kg)	104.4 ± 16.8	83.4 ± 14.4	<0.0001
Body height (cm)	177.2 ± 7.0	176.0 ± 5.6	0.460
BMI (kg/m^2)	33.3 ± 5.1	26.9 ± 4.3	<0.0001
Waist-to-hip ratio	1.0 ± 0.1	0.9 ± 0.1	<0.0001
HR (beats/min)	71.0 ± 13.0	63.7 ± 7.7	<0.006
MBP (mmHg)	87.6 ± 11.2	90.8 ± 10.6	0.270
Peripheral SBP (mmHg)	118.9 ± 11.4	118.6 ± 12.0	0.910
Peripheral DBP (mmHg)	72.2 ± 11.1	76.5 ± 9.6	0.120
Peripheral PP (mmHg)	46.7 ± 7.8	42.1 ± 6.7	0.015
Central SBP (mmHg)	105.8 ± 9.7	108.8 ± 12.6	0.350
Central DBP (mmHg)	73.7 ± 12.1	77.8 ± 10.4	0.160
Central PP (mmHg)	32.1 ± 7.0	31.1 ± 6.2	0.530
CAP (mmHg)	5.4 ± 4.3	6.7 ± 4.1	0.260
CAI (%)	120.2 ± 15.7	127.9 ± 16.9	0.083
PPA	1.5 ± 0.2	1.4 ± 0.2	0.067
SV (mL)	93.9 ± 14.1	101.2 ± 15.0	0.095
CO (L/min)	6.8 ± 1.2	6.7 ± 1.0	0.660
SVR (dyn s/cm^5)	1052.4 ± 233.4	1104.2 ± 197.0	0.400
ACI (/100 s^2)	55.4 ± 20.9	75.8 ± 27.6	0.008
VI (/1,000/s)	37.4 ± 10.5	49.6 ± 13.7	<0.002

Results are means ±SD. *BMI* body mass index, *HR* heart rate, *MBP* mean blood pressure, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *CAP* central augmentation pressure, *CAI* central augmentation index, *PPA* pulse pressure amplification, *SV* stroke volume, *CO* cardiac output, *SVR* systemic vascular resistance, *ACI* acceleration index, *VI* velocity index

Table 2 Results of ANCOVA adjusted for BMI showing the comparison of parameters which differed significantly in direct, unadjusted comparison by the unpaired *t*-test

Variables	Controls	OSA	p-value
HR (beats/min)	64.7 ± 1.5	68.9 ± 2.4	0.160
Peripheral PP (mmHg)	42.5 ± 1.1	45.7 ± 1.7	0.160
ACI (/100 s^2)	68.7 ± 3.3	72.3 ± 5.5	0.600
VI (/1,000/s)	45.8 ± 1.6	46.4 ± 2.6	0.860

Results are estimated marginal means (EMM) ± SE. *HR* pulse rate, *PP* pulse blood pressure, *ACI* acceleration index, *VI* velocity index

hemodynamic alterations (Drager et al. 2005; Minoguchi et al. 2005; Jelic et al. 2002). BMI associates with the thickness of carotid intima-media or arterial pulse wave velocity. Bäckdahl et al. (2018) have shown that a substantial reduction in BMI over a 2-year period (from 39.4 ± 3.5 kg/m^2 to 26.6 ± 3.4 kg/m^2) is accompanied by a significant decrease of aortic pulse wave velocity (7.8 ± 1.5 m/s *vs.* 7.2 ± 1.4 m/s, respectively). Moreover, the subcutaneous adipocyte volume and the expression of the *COL4A1* gene in the white adipose tissue before the BMI reduction predicts a decrease in the pulse wave velocity. Nagahama et al. (2004) have shown that the brachial-ankle pulse wave velocity is higher in OSA patients with excess body mass, even without other cardiovascular risk factors. Thus, if adipose tissue is related to arterial stiffness, it is plausible that obesity in OSA patients could contribute to cardiovascular dysfunction more than the blood pressure level *per se*. This plausibility remains to be verified in

the investigation comparing the hemodynamic effects of controlled *vs*. uncontrolled hypertension in OSA patients as well as in obese OSA and obese non-OSA patients, which requires alternative study designs.

In this study, a specific selection of obese OSA patients only, whose arterial blood pressure was controlled within the normal range, remains a limiting factor. Another limitation may be the use of cardiac impedance, rather than the gold standard thermodilution method, for the evaluation of cardiovascular dynamics. However, cardiac impedance is often a preferable choice due to its noninvasiveness (Zhao et al. 2017; Morris et al. 2016; Beck et al. 1997). To this end, we also employed noninvasive radial applanation tonometry with the reconstruction of the aortic pressure waveform, using a validated transfer function in order to describe the central and peripheral arterial pressure waveforms in more detail.

Despite these limitation we believe we have shown that obese hypertensive OSA patients, with controlled arterial blood pressure, have a different hemodynamic phenotype when compared to healthy age-matched subjects. The hemodynamic differences disappeared after adjustment for the increase in body mass index in OSA patients. These findings raise the possibility that the OSA-related hemodynamic differences could be a consequence of obesity rather than the hypertensive disease per se. The corollary is that a reduction in excessive body weight should be a main therapeutic target in OSA patients, aside from the control of hypertension and other cardiovascular or metabolic risk factors.

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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 Poznan University of Medical Sciences (approval no. 278/14).

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

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Diagnosis of Sleep-Disordered Breathing in the Home Environment

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Abstract

Polysomnography, a gold standard for the diagnosis of sleep-disordered breathing, is a complex investigation requiring access to the sleep laboratory. Thus, sleep-disordered breathing could be underdiagnosed. The aim of this paper was to investigate the feasibility and effectiveness of self-performed investigation of obstructive sleep apnea (OSA) in the home setting, using a portable device, and to assess the comfort and simplicity of the procedure from the patient's perspective. The study included 68 middle-aged patients (21 women and 47 men), who were examined at home with the ApneaLink Air device in search for the underlying reason of reported nighttime snoring and occasionally disordered breathing pattern. The apnea-hypopnea index was quantified and matched with body mass index (BMI), age, and other characteristics. OSA was diagnosed in 37 patients (27 men and 10 women): 22 had mild, 4 had moderate, and 11 patients had severe OSA. All cases of severe OSA were present in men. Patients with severe OSA had significantly higher BMI than those from the other groups. All of the patients pointed to the comfort and ease of the diagnostic device. We conclude that home diagnosis of OSA is a relatively easy and cost-effective way to substitute for the hospital-linked polysomnography, particularly in severely ill patients who have a movement difficulty. A wider implementation of home-based diagnosis of OSA may substantially increase the number of patients investigated in a short time span, also leading to the plausibly upward correction of the disease prevalence.

Keywords

Disordered breathing · Portable diagnostic device · Self-diagnosis · Sleep apnea · Sleep-disordered breathing

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1 Introduction

Sleep-disordered breathing is a heterogeneous group of conditions causing the disruption of physiological sleep. The first sign of a disorder is snoring. Sometimes it is a lone symptom but often is accompanied by hypopnea which, in turn, is a harbinger of apneic episodes. Apnea is diagnosed when breathing ceases for longer than

10 s and occurs more frequently than five times per hour. Sleep apnea is classified into three grades depending on the number of apneic episodes per hour: mild, 5–15; moderate, 16–30; and severe, >30 episodes. Arterial blood oxygen saturation decreases during sleep apnea, which makes sleep-disordered breathing a serious, health-threatening, and potentially lethal disease. Repetitive waking arousals make night rest ineffective, dysregulate blood pressure and metabolic controls, lead to daytime sleepiness, and reduce cognitive performance and overall quality of life (Sjösten et al. 2009).

The most common type of sleep apnea, accounting for *ca*. 85% of cases, is obstructive apnea (OSA), in which airways become narrowed or obstructed due to a collapse of the pharyngeal muscles due to either dysfunction of neural activity running down from the brain or local thickening of surrounding fatty or lymphatic tissues. The much rarer central sleep apnea, unconnected to a narrowing of airway lumen, is usually driven by cerebrovascular-related causes (Javaheri 2005; Javaheri et al. 2017). The incidence of OSA in the population of highly developed countries is 2–5% among middle-aged women, 3–7% in men, and over 12% in older men (Jennum and Riha 2009; Duran et al. 2001). That is, however, most likely a gross underestimation as apneic episodes are easily missed and thus the disease is undiagnosed (Simpson et al. 2013). One reason for the situation is insufficient access of patients to professional diagnosis which requires overnight sleep monitoring. This investigation is performed when a suspicion of the disease arises, most often on the basis of nighttime breaks in breathing noticed by the patient's partner (Kunisaki et al. 2016). Polysomnography remains the gold standard for the diagnosis, requiring a hospital setting, being pricey and complex, and rather uncomfortable for the patient. There has been an apparent need for easier and more cost-effective methods of sleep monitoring, usable for large-scale screening, in particular avoiding a complexity of in-hospital stay. Some older studies showed that home self-investigation toward the diagnosis of OSA may be of high value, with sensitivity as high as 90% (Flemons et al. 2003). Recent technological advances in portable and remote medical care have made the home diagnosis of OSA an effectively developing area of investigation.

The goal of this study was to determine the diagnostic feasibility of an automated portable device for the monitoring of breathing pattern at sleep in the home. We analyzed the value of information acquired from the home sleep and breathing investigation for the diagnosis of sleep-related breathing disorders. We also gathered the patients' opinion regarding such a home-based investigation, with particular emphasis on the simplicity of use and the patient's comfort.

2 Methods

There were 68 patients enrolled into the study: F/M – 21 (31%)/47 (69%). The patients were seen by ear, nose, and throat (ENT) specialists due to suspected sleep-disordered breathing, such as frequent, occasional, or regular snoring, shallow breathing, or episodes of breathlessness reported by bed partners. The mean age was 54.0 ± 13.6 years in women (33–82 years) and 47.0 ± 9.9 years in men (range 22–74 years). The difference in age between women and men was significant ($p < 0.05$), and it showed that older women are by no means spared from snoring and suspicion of sleep-disordered breathing. Both snorers and their partners were surveyed using separate questionnaires, designed specifically for this study. The questionnaire for the snorer consisted of 31 items that concerned the demographic and anthropometric features, the type of breathing distress at sleep, a degree of disturbance and embarrassment it caused, recent changes in the patient's body mass, and if there was such a change, how it affected the respiratory distress. The questionnaire for the partner consisted of items concerning the type of breathing disorders noticed and the willingness and motives to help the patient in further medical procedures to resolve the health issue. These preliminary questionnaires were a requirement to qualify for

a portable diagnostic device to be taken home by the patient. The survey answers were not subject to statistical evaluation nor were they matched with the results of diagnostic tests.

Each patient obtained an ApneaLink Air recorder (ResMed, Warsaw, Poland) to use it in the comfort of one's own bedroom, along with verbal and printed instructions and basic training on how to use it for sleep monitoring during a night. The recorder is a lightweight portable sleep testing device that is capable of recording up to five channels of information: respiratory effort, heart pulse, oxygen saturation, nasal flow, and snoring. The device needs to be assembled with a chest belt, pulse oximeter, and a nasal cannula to measure the airflow during breathing. The cannula is connected to a pressure transducer, providing an apnea-hypopnea index (AHI) per hour of the sleep recording time. Aside from AHI, the device is capable of automated analysis of the hypopnea index, airflow limitation, snoring, and oxygen desaturation index, and it distinguishes obstructive from central apneic episodes. The variables recorded have configurable thresholds, meeting the recommendations established for hypopnea scoring by the American Academy of Sleep Medicine and the Centers for Medicare and Medicaid Services. The device also is capable of detection of Cheyne-Stokes breathing pattern and helps identify patients who should be referred to an in-lab sleep study. The device also allows manual scoring if required for a recheck of data. After the completed overnight test, the device was returned to the healthcare provider, and an easy-to-interpret report was generated with a color-keyed AHI or risk indicator, which was subsequently discussed with the patient.

Data were expressed as means ±SD. Distribution of variables that was checked with the Shapiro-Wilk test was calculated. Differences between the arithmetic means of independent variables with normal distribution (age of patients) were assessed with Student's *t*-test. For the AHI data, which showed a skewed distribution, the Mann-Whitney U test was used. Differences in the mean values of AHI depending on age and BMI were assessed with one-way ANOVA and a post hoc least significant difference (LSD) test. A p-value <0.05 defined statistically significant differences throughout the analysis.

3 Results

3.1 Apnea-Hypopnea Index (AHI) Depending on Gender, Age, and Body Mass Index (BMI)

Overall, OSA was detected in 37 (54.4%) patients, in detail in 27 out of the 47 men and in 10 out of the 21 women, out of the 68 study subjects. There were 11 (7.6%) patients with severe OSA (AHI = 43.3 ± 10.9 episodes *per* hour), all of them being men. Thirteen men had mild OSA (AHI = 9.2 ± 2.5 episodes *per* hour) and another three had moderate OSA 18.6 (AHI = 18.6 ± 1.5 episodes *per* hour). Mild OSA was detected in 9 women (AHI = 7.7 ± 2.5 episodes *per* hour), and another 1 woman had moderate OSA (AHI = 7.2 ± 2.5 episodes *per* hour), and another 1 woman had moderate OSA with AHI of 15.8 episodes *per* hour.

Taking both men and women OSA patients together, the mean age of patients was 52.4 ± 11.4 years in mild OSA, 62.8 ± 10.9 years in moderate OSA, and 48.2 ± 8.5 years in severe OSA. The age differed among these three groups of OSA severity was significant, being significantly greater in moderate OSA than that in severe OSA. It was also greater than that the age of healthy subjects ($p < 0.05$; one-way ANOVA with post hoc LDS). Concerning the BMI, it was significantly greater in patients suffering from mild and severe OSA than that in the healthy subjects, with the apparent lack of a significant difference between the patients with moderate OSA and healthy subjects ($p < 0.01$; one-way ANOVA with post hoc LDS) (Table 1).

Table 1 Distribution of study patients by apnea-hypopnea index (AHI), age, and body mass index (BMI)

	AHI	Age (years)	BMI (kg/m^2)
Healthy ($n = 31$)	2.4 ± 1.2 (0.5–4.6)	46.0 ± 14.3 (22–66)	26.7 ± 3.5 (22.1–35.6)
OSA ($n = 37$)			
Mild ($n = 22$)	8.6 ± 2.6 (5.0–13.1)	52.4 ± 11.4 (33–82)	30.4 ± 4.7 (21.6–41.8)
Moderate ($n = 4$)	17.9 ± 1.9 (15.8–19.6)	62.8 ± 10.9 (48–74)	26.0 ± 2.2 (23.1–28.4)
Severe ($n = 11$)	43.3 ± 10.9 (32.2–65.5)	48.2 ± 8.5 (34–60)	32.7 ± 5.9 (43.6–34.5)

Data are means ±SD. *AHI* apnea-hypopnea index (episodes *per* hour of sleep time), *OSA* obstructive sleep apnea, *BMI* body mass index

3.2 Assessment of the Patient's Experience with Performing the Sleep Test

Upon the return of the ApneaLink Air device, patients were asked to describe their experience with using it. Only did one patient give negative comments, complaining about the complicated way of using the switches, but despite the trouble he managed to complete the examination. Forty-six patients (67.6%) assessed the comfort and ease of using the device very well. Twenty-two subjects (32.4%) expressed a positive opinion, mentioning only a minor discomfort associated with the attachment of a nasal cannula and the fear of oximeter sensor slipping off the finger. Four out of these patients also were confused by the flashing diode and were unsure whether the device was on or off. Some patients were also somehow emotionally stressed because of being tested at sleep. In general, the device did not restrict changing the body position at sleep, nor did it disturb sleep in any way. Comparison by patients of the ApneaLink Air device to Holter blood pressure or hear rate testing came out advantageous to the former.

The data acquired indicate that breathing disorders during sleep in women tended to occur at a later age but were milder than those in men, although the incidence of OSA noticed in this study was lower in women than men. The analysis of the age structure of patients with sleep-disordered breathing demonstrates, in general, the association of OSA with age. Interestingly, however, patients with most severe OSA were younger than those with moderate OSA (48.2 ± 8.5 vs. 62.2 ± 10.9 years of age, respectively), even though a reverse relationship was noted concerning BMI (Table 1). In fact, the most senior patients with moderate OSA appeared physically quite lean as their BMI was within the normal range, comparably to the healthy subjects.

All of the tested patients who were diagnosed with OSA or snoring were offered guidance concerning further diagnostic procedures and management. Subjects with abnormal BMI were advised about nutritional measure and physical activity enhancing interventions to lose weight. Some of the overweight and obese patients qualified for corrective surgery of the soft palate or nasal patency, but the decision was delayed until after the achievement of body weight reduction. Polysomnography was recommended in a few patients with moderate and severe OSA in whom the pathogenesis of the disease appeared more complex. This group mostly included patients with coexisting cardiovascular diseases.

4 Discussion

The use of home sleep monitoring devices for the diagnosis of breathing disorders at sleep has been the subject of a lasting debate. The most debatable issue has been of whether such devices may merely help detect a breathing problem or could be used to set the final diagnosis, based on which a specific treatment could be implemented. Another issue is the diagnostic sensitivity of such devices and whether every patient could be self-tested with a portable device and if not what would be the selection of patients for this kind of testing. To answer these questions, the results obtained with portable devices have been compared to those

from polysomnography (Pack 2015). According to the recommendations of the American Sleep Disorders Association (ASDA), polysomnography is the ultimate method for the diagnosis of sleep-disordered breathing. It is much more sensitive than other tests are since it provides a host of recordings of variables during natural sleep, including the electroencephalogram (EEG), electromyogram (EMG), electrooculogram (EOG), electrocardiogram (ECG), airflow, respiratory movements of the thorax and abdomen, arterial oxygen desaturation, snoring, and others. Such a complex analysis cannot be done with portable sleep monitoring devices. The ASDA distinguishes four types of monitoring devices depending on the number of channels recording information. Level I devices are eight-channel monitors (EEG, EOG, ECG, EMG, airflow, respiratory effort, oxygen saturation, body position). Level II devices are seven-channel monitors (EEG, EOG, chin EMG, ECG or heart rate, airflow, respiratory effort, and oxygen saturation). Level III devices are four-channel monitors, but they do not cover EEG, and the simplest level IV devices record one or two variables, which usually are airflow and oxygen saturation.

Polysomnography belongs to Level I category. The advocates of polysomnography emphasize its better sensitivity and specificity, when compared to home sleep testing, which translates into the more effective treatment planning of OSA, which is of particular importance in the case of continuous positive airway pressure (CPAP) therapy (Pack 2015). The opponents, on the other side, point to the high diagnostic costs of using polysomnography and postulate the use of home sleep testing instead, as in the case of severe OSA with a high AHI, the diagnostic yield is comparable with the use of either (Freedman 2015). The opponents, or rather those who are distrustful of HST, draw attention to the fact that superficial, simplified perception solely based on the costs of the diagnostic test is a short-sighted strategy, and the economic benefits associated with the identification and treatment of patients, as well as the costs of misdiagnosis should be considered. The opponents particularly focus on the effective treatment and argue that polysomnography remains "the cornerstone for diagnosis in patients suspected of having comorbid sleep disorders, unstable medical conditions, or complex sleep-disordered breathing" (El Shayeb et al. 2014).

The ApneaLink Air device has been available for several years. The device records up to five channels of information. Nonetheless, it appears fairly effective in detection of sleep-disordered breathing, with sensitivity and specificity of at least 80% for AHI of ten episodes *per* hour of sleep. The greatest sensitivity and specificity of 91% and 95%, respectively, were found for AHI $\geq$15 episodes *per* hour. At AHI >10 episodes *per* hour, specificity decreases leading to a greater number of false-positive results (Erman et al. 2007). Thus, the device seems most useful for OSA screening in high-risk adults, e.g., obese patients with metabolic disorders, which helps implement a prompt treatment. Notably, there also is a promising report on the use of ApneaLink Air in pediatric patients with obesity and suspected OSA (Lesser et al. 2012). That study involved 25 children and adolescents aged 9–18 years with BMI $\geq$95th percentile for age/gender, all of whom were regular snorers. The authors point to the accuracy of the device, comparably to that of polysomnography, in the identification of OSA and to its sensitivity even at low AHI values. That is a finding that underscores the utility of portable screening devices in view of the increasing obesity in children and its relation to the development of OSA. An early diagnosis and treatment of OSA in children is essential for counteracting the impact of the disease on neurocognitive functions and the risk of cardiovascular complications in adulthood.

ApneaLink Air has also been reported to unravel the presence of central breathing disorders, particularly of the Cheyne-Stokes type (Weinreich et al. 2009). Such breathing disorders often occur in patients with cerebrovascular pathologies, damage to the respiratory brain stem network, or opiate and barbiturate overuse. The device is recommended for the diagnosis of mixed central and peripheral episodes of airway obstruction, making it a sensitive pretest that enables the prioritization of such patients for polysomnography.

In conclusion, a self-screening of nighttime respiration in the home setting, using a mobile device, is useful for the diagnosis of sleep-disordered breathing. The major advantages of such screening include a good sensitivity and specificity of testing, a good cost-effectiveness ratio, simplicity and convenience of testing and data analysis, and a good compliance of patients. Such screening also is useful in a prompt identification of patients who would be suitable candidates for the full-fledged in-lab sleep polysomnography study.

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 Bioethics Committee of the Pomeranian Medical University in Szczecin, Poland.

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

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