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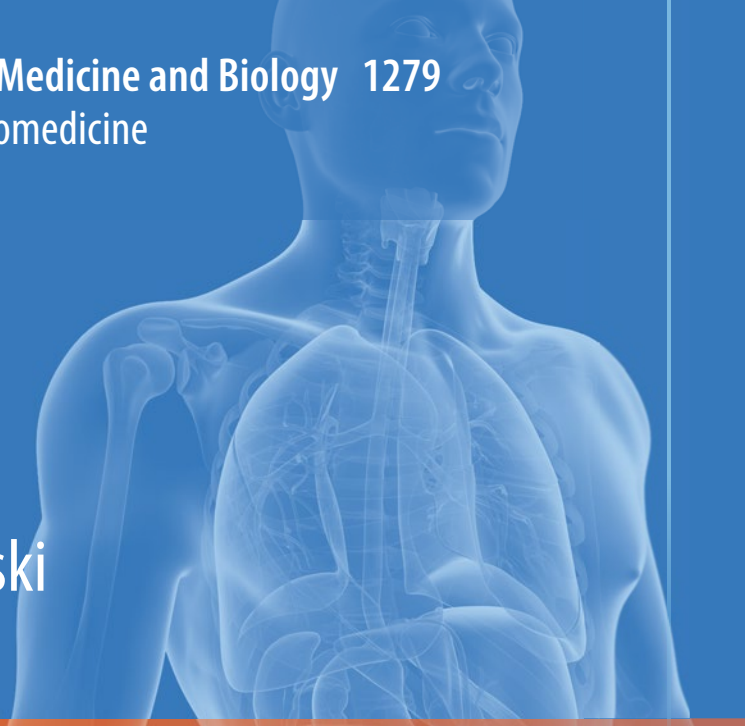

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Chitinase 3-Like 1, Nestin, and Testin Proteins as Novel Biomarkers of Potential Clinical Use in Colorectal Cancer: A Review

Anna Szymańska-Chabowska, Jan Juzwiszyn, Beata Jankowska-Polańska, Wojciech Tański, and Mariusz Chabowski

Abstract

Colorectal cancer is the third most commonly diagnosed cancer in males and the second most common in females. Only 10–20% of patients are diagnosed at the early stage of disease. Recently, the role of novel biomarkers of the neoplastic process in the early detection of colorectal cancer has been widely discussed. In this review, we focused on the three novel biomarkers that are of potential clinical importance in diagnosing and monitoring colorectal cancer. Chitinase 3-like 1 protein, also known as YKL-40, and nestin and testin proteins are produced by colorectal cancer cells. YKL-40 protein is a marker of proliferation, differentiation, and tissue morphogenetic changes. The level of YKL-40 is elevated in about 20% of patients with colorectal cancer. An increased expression of nestin indicates immaturity. It is a marker of angiogenesis in neoplastic processes. Testin protein is a component of cell-cell connections and focal adhesions. The protein is produced in normal human tissues, but not in tumor tissues. Downregulation of testin increases cell motility, spread, and proliferation, and decreases apoptosis. The usefulness and role of these biomarkers, both alone and combined, in the diagnostics of colorectal cancer should be further explored as early cancer detection may substantially improve treatment outcome and patient survival.

Keywords

Biomarkers · Colorectal cancer · Nestin · Testin · YKL-40

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1 Introduction

Colorectal cancer significantly contributes to a reduction in life expectancy. According to data from the Global Burden of Disease Study, the number of deaths due to colorectal cancer has increased from 490,200 in 1990 to 771,100 in 2013, which is over a 57% increase (GBD 2013 Mortality and Causes of Death Collaborators 2015). Colorectal cancer is the third most commonly diagnosed cancer in males and the second in females (Torre et al. 2015). The cancer constitutes a major health care and socioeconomic problem. In 2013, colon and rectum cancers ranked eight as the cause of years of life lost in the developed countries, including Poland. In European countries such as the Netherlands and Sweden, these cancers were classified as the fourth leading cause of years of life lost. A lower survival rate is observed in elderly patients and in those from certain geographical areas such as Eastern Europe (Holleczek et al. 2015), possibly due to poor and delayed diagnosis. At an early stage, colorectal cancer does not manifest specific signs and symptoms, so that it is often diagnosed in advanced stage. Maringe et al. (2013) have reported that only 10–20% of colorectal cancer patients are diagnosed when the cancer is in stage A of Duke's classification (Duke 1932). The difference in survival between high- and low-income countries may be associated with different stages of the disease at the time of diagnosis in respective countries. Thus, the availability of reliable screening tools and biomarkers is of essential importance for the diagnostics and management of the disease. Recently, the role of novel biomarkers in the neoplastic process and early detection of colorectal cancer has been widely discussed (Corbo et al. 2017; Das et al. 2016). In this review we focused on three novel biomarkers that have been under scrutiny as being of potential clinical importance in colorectal cancer diagnosis and monitoring. These biomarkers are chitinase 3-like 1 protein, also known as YKL-40, and nestin and testin proteins; all produced by colorectal cancer cells.

2 Biomarkers and Risk Factors Associated with Colorectal Cancer

An unhealthy diet, including consumption of processed meat products and alcohol, obesity, sedentary lifestyle, and smoking, can all contribute to the development of colorectal cancer (González et al. 2017; Friedenreich et al. 2016; Ferrari et al. 2007). Mitigating the effects of such modifiable risk factors may reduce the level of colorectal cancer morbidity (Johnson 2017; Doleman et al. 2016; Botteri et al. 2008). Patients at high risk are advised to participate in screening programs. Such programs are, however, of rather low sensitivity and specificity for the detection of colorectal cancer and for the avoidance of the cancer high invasiveness. Patients tend to avoid tests such as sigmoidoscopy or colonoscopy, despite the evidence of efficacy of these tests in the diagnosis of colorectal cancer. Therefore, the identification of new blood-derived biomarkers, which patients would be more complaint with, or noninvasive molecular tests would greatly benefit treatment outcome and survival.

The term tumor biomarker was coined in 1988 as a medical subject heading, and it was defined as "molecular products metabolized and secreted by neoplastic tissue and characterized biochemically in cells or body fluids". Biomarkers are indicators of tumor stage and grade and are useful for monitoring the response to treatment and prognosticate recurrence. Biomarkers are represented by a host of molecules such hormones, antigens, amino acids, nucleic acids, enzymes, polyamines, and specific cell membrane proteins and lipids (Goossens et al. 2015).

Currently, there are only two protein biomarkers detectable in blood serum, which are approved for clinical use in colorectal cancer. The carcinoembryonic antigen (CEA) is one of the two. Preoperative elevation of CEA is associated with reduced overall survival. A lack of postoperative normalization of CEA level may indicate an incomplete tumor resection and thus portends a recurrence. CEA monitoring helps identify

patients with metastases, which particularly concerns the liver (Goldstein and Mitchell 2005; Duffy 2001). A disadvantage of CEA is that it lacks sensitivity in the early stages of colorectal cancer. It can also be increased in patients without cancer, being secreted in response to various inflammatory processes, e.g., hepatitis, inflammatory bowel disease, pancreatitis, chronic obstructive pulmonary disease, or oxidative stress in diabetic patients and smokers (Hasan and Mohieldein 2015; Tanaka et al. 2010; Fukuda et al. 1998).

Another biomarker widely used in colorectal cancer detection is fecal hemoglobin (f-Hb), which is usually detected using an immunochemical test. The test does not require dietary or medication restrictions. One or two stool samples are enough to conduct the test. The overall sensitivity and specificity of FIT in colorectal cancer is 79% and 94%, respectively, although these parameters may change due to inter-lab differences in cutoff values used for positive FIT results. The optimum cutoff value is still debatable (Lee et al. 2014; Tanaka et al. 2010). The use of FIT as a screening tool has been shown to decrease mortality associated with colorectal cancer (Chiu et al. 2015; Giorgi et al. 2015). The disadvantage of the test is its lower unsatisfactory sensitivity for detection of early stage cancers. To this end, the search for novel more accurate biomarkers continues (Chiu et al. 2013).

3 Chitinase 3-Like 1 (YKL-40) Protein in the Diagnosis of Colorectal Cancer

Chitinase 3-like 1 (CHI3L1), also known as YKL-40 protein, is a human cartilage glycoprotein-39 (HC-gp-39) that was first described in 1992 as a protein secreted by the MG-63 human osteosarcoma cell line (Johansen et al. 1992). In addition to cancer cells, YKL-40 also is secreted by inflammatory and stem cells. In normal tissues, high YKL-40 expression has been noticed in embryos and fetuses where the processes of proliferation, differentiation, and tissue morphogenetic changes are highly active (Johansen et al. 2007). The blood content of YKL-40 in healthy adults is fairly stable, with the median of 43 μg/L as determined in a study by Johansen et al. (2008) and 40 μg/L in the Danish NORDIC VII study that included 3130 subjects (Tarpgaard et al. 2014), with increasing levels with age. An elevated plasma level of YKL-40 has reported in patients with rheumatoid arthritis, cardiovascular diseases, diabetes, chronic obstructive pulmonary disease, asthma, liver fibrosis, severe infections, as well as in patients with cancers inter alia breast, lung, prostate, colorectal, and gastric cancers (Schultz and Johansen 2010; Roslind and Johansen 2009). Such wide spectrum of different pathologies where YKL-40 increases indicates a lack of specificity. Therefore, comorbidities should be taken into account when assessing the diagnostic utility of YKL-40 investigated for a primary disease.

Kawada et al. (2012) have noticed significant differences in plasma content of YKL-40 between control subjects and patients with stage I/II and stage III/IV colorectal cancer. Those authors have also reported increased YKL-40 mRNA expression in cancer tissue when compared with normal adjacent tissues. Therefore, elevated YKL-40 content may be related to a more aggressive phenotype of cancer, having a high metastatic potential. Cintin et al. (2002) have reported that preoperative serum YKL-40 content rises above the age-corrected 95th percentile of healthy volunteers in 19% of patients with colorectal cancer. In a study by Johansen et al. (2015), YKL-40 is elevated in 20% of patients with colorectal cancer, 15% with rectal cancer, 11% with adenoma, 9% with other nonmalignant diseases of the digestive tract, and 8% with no pathological endoscopic findings. Likewise, Tarpgaard et al. (2014) have found that plasma YKL-40 is higher than the upper normal level in 40% of non-resectable metastatic colorectal cancer.

Postoperatively, YKL-40 may decrease in patients who have it within the normal range before surgery, and that may be a positive

prognostic sign. The elevated level of YKL-40 appears to be an independent prognostic of short survival, based on a study including 603 patients who underwent a primary large bowel resection for colorectal cancer (Cintin et al. 1999). In another study, Cintin et al. (2002) have shown that patients who underwent curative tumor resection and had high serum YKL-40 6 months post-surgery were burdened with increased risk of death. In both studies, the YKL-40 content was independent of the serum CEA content. A comparison between YKL-40 and CEA contents performed by Ye et al. (2014) has revealed that YKL-40 is less accurate for diagnosing colorectal cancer, but better for diagnosing tumor recurrence. YKL-40 also appears of help in diagnosing the early-stage colorectal cancer and in monitoring recurrences when combined with CEA.

A high content of YKL-40 is an independent prognostic of poor response to preoperative chemoradiotherapy in locally advanced rectal adenocarcinoma, although the prognostic value of the protein for survival has not been confirmed. It is also worth mentioning that tumor expression of YKL-40, assessed by immunohistochemistry, takes place in 62% of cases in (Senetta et al. 2015). In a study by Johansen et al. (2015), blood content of YKL-40 was higher in patients with colon and rectal cancer than in those with adenoma and other nonmalignant diseases. In patients with colorectal cancer, content of YKL-40 correlated with the stage of cancer, and it was lower in patients with a rectal tumor. Comorbidities are associated with an increase in YKL-40. Thus, the protein is thought to be a good prognostic of colorectal cancer in patients without comorbidities. Ye et al. (2014) have found higher levels of blood YKL-40 in patients with recurrent and metastatic colorectal cancer than in those with the primary diagnosis of colorectal cancer or shortly after surgery. Liu et al. (2014) have confirmed the presence of a link between shorter survival and a high blood YKL-40, but they failed to notice any relationship between progression-free survival and the type of chemotherapy regimen or the histologic type of tumor. In 510 patients with metastatic colorectal cancer in the NORDIC VII study, high pretreatment plasma YKL-40 content is associated with shorter progression-free survival and with overall survival (Tarpgaard et al. 2014).

4 Mechanisms of Action of YKL-40

YKL-40 is produced by various types of restricted cells including colonic epithelial cells and macrophages. The content of this protein is elevated not only in colorectal cancer but also in other bowel diseases such as inflammatory bowel disease, ulcerative colitis, and Crohn's disease (Kamba et al. 2013; Koutroubakis et al. 2003; Vind et al. 2003). Chen et al. (2011) have reported that the expression of YKL-40 is significantly increased in colonic epithelial cells of non-dysplastic mucosa in patients with ulcerative colitis harboring neoplastic lesions when compared with patients without dysplasia and with healthy subjects. A greater expression of YKL-40 concerns more invasive and metastatic tumors. The YKL-40 also has a growth-stimulating effect akin to that of insulin-like growth factor-1, and it stimulates the migration of colonic epithelial cells. Additionally, YKL-40 stimulates the production of interleukin-8 (IL-8) and tumor necrosis factor-α by activation of nuclear factor kappa B (NF-κB) signaling pathway in a human colon cancer cell line (SW480 cells). Other studies confirmed the presence of NF-κB in inflamed intestinal mucosa and a strong association between increased NF-κB activity and colorectal carcinogenesis in the animal model (Popivanova et al. 2008; Rogler et al. 1998). Elevated YKL-40 in epithelial cells may not only suggest inflammation-associated malignant transformation but may also control intestinal inflammation and promote dysplasia in colonic cells (Chen et al. 2011). The findings of Kawada et al. (2012) have shown that overexpression of YKL-40 leads to increased chemotaxis of macrophages and to angiogenesis associated with increased release of IL-8 and monocyte chemoattractant protein-1 (MCP-1) from SW480 cells. Studies in vitro have shown that angiogenic activity of YKL-40 is independent of vascular

endothelial growth factor (VEGF). The effects of YKL-40 on endothelial cell activation may be blocked by silencing S1 expression in the cells or by an anti-YKL-40 antibody (Shao et al. 2009).

5 Nestin in Colorectal Cancer

Nestin is a class VI intermediate filament protein whose expression is upregulated in many kinds of tumor and some other tissues in both experimental models and human samples (Matsuda et al. 2013; Ishiwata et al. 2011). The protein is expressed mainly in neuroepithelial stem cells and is often present in tissues that undergo a repair process. Increased expression of nestin indicates immaturity (Ehrmann et al. 2005; Mokry et al. 2004). Nestin appears a useful marker of microvessel density (MVD) which is a prognostic factor in neoplastic malignancies, including colorectal cancer (Amoh et al. 2005). A significant correlation between MVD and liver metastases shows that tumors' potential to grow, spread, and metastasize is related to the angiogenesis in and around cancer tissue (Choi et al. 1998; Tomisaki et al. 1996). MVD is an important prognostic factor, but the most essential information is provided by the number of newly formed blood vessels because they have leaky and weak basement membranes, which enables the entry of tumor cells into circulation and further spread. In contrast, it is much more difficult for malignant cells to penetrate the endothelial layer of a mature microvessel.

Many commonly used endothelial markers stain and identify both the newly formed small blood vessels and the already existing larger blood vessels. Teranishi et al. (2007), using an animal model, have shown that the CD34-labeled pattern of colorectal cancer blood vessels differs from that of the nestin-labeled pattern. CD34 is detected in the endothelial cells of larger blood vessels with a median diameter of 9.67 μm, whereas nestin is in smaller microvessels of the median diameter of 9.06 μm. In human colorectal cancer tissue, there are significant differences in the median diameter of blood microvessels, ranging from 8.82 to 6.30 μm. Stronger expression of nestin than CD34 also is found in the infiltrating border of a tumor. Further, MVD determined by nestin labeling appears a better prognostic factor for survival than MVD determined by CD34.

Reports on the connection between increased nestin expression and clinical characteristics of colorectal cancer are scarce. Tajima et al. (2014) have demonstrated a patient with an aggressive, undifferentiated carcinoma of the descending colon where there was an overexpression of several proteins including nestin, which prognosticated a poor prognosis. The patient experienced a recurrence 39 days after surgery and died 2 months later. Using clinical samples of colorectal cancer, Li et al. (2015) have shown that nestin is associated with tumorigenesis as its expression is higher in cancer tissue than in normal tissues. These authors further show nestin labeling in the endothelium of small-sized tumor vessels and in stromal cancer cells. Additional in vitro tests show that a knockdown of nestin arrested the cell cycle at S phase and inhibited the proliferation and migration of colorectal cancer cells. Such findings demonstrate that nestin can be used not only as a prognostic marker, but that it also gives hope for the development of a new therapeutic option for cancer patients.

6 Testin Protein in Colorectal Cancer

The testin protein is encoded by a gene which is located at human chromosome 7q31 within the common fragile chromosomal region FRA7G. This locus is susceptible to cancer-associated chromosomal aberrations which may play a role in the oncogenic process (Tatarelli et al. 2000). The protein is a component of cell-cell connections and focal adhesions. It can interact with other types of focal adhesion proteins and connect the actin cytoskeleton to the extracellular matrix. There structures, along with integrin receptors, play a role in cell motility, spreading, proliferation, and apoptosis (Coutts et al. 2003). The protein is produced in normal human tissues, but not in tumor tissues, such as breast, prostate, liver, ovarian cancer, *in utero* glioblastoma, and others (Hu et al. 2015; Yongbin et al. 2014;

Chene et al. 2004). Downregulation of testin can be due to a loss of heterozygosity of the testin gene or by hypermethylation of its promoter. Clinically, a reduced level of testin protein is found in 89% of patients with glioblastoma. Additionally, downregulation of testin associates with a worse outcome and a shorter survival time. It seems a biological plausibility that enhancing testin expression could attenuate the malignant character of cancer cells (Fu et al. 2015. Bai et al. 2014).

In colorectal cancer, Li et al. (2015) have noticed a significantly lower level of testin mRNA and protein expression when compared with adjacent tumor-free tissue samples. Those authors have also found an adverse association between the histological grade, but not TNM stage, of colorectal cancer and the level of testin. Studies *in vitro* have shown that both testin mRNA and protein expression are remarkably reduced in nine colorectal cancer cell lines, but not in the two kinds of normal human colon cells. Further, in vitro overexpression of testin reduces the colony formation efficiency, inhibits cell growth, and increases mRNA and protein expression of pro-apoptotic proteins, whereas the opposite is present when testin is knocked down. The suppressive effect of testin on colorectal cancer cells has also demonstrated in the murine model.

7 Conclusions

The role of biomarker proteins such as YKL-40, nestin, and TES in carcinogenesis is not yet full elucidated. The proteins are associated with cancer aggressiveness and prognosis. A combined assessment of several biomarkers may improve diagnosis and may better prognosticate recurrence and survival. There is a need for further studies to explore the molecular mechanisms underlying the development of colorectal cancer.

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Ethical Approval This review article does not contain any studies with human participants or animals performed by any of the authors.

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Self-Reported Survey on Allergy Symptoms Among First-Year Students in Veterinary Medicine: A Preamble to the AllergoVet Cohort Study

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Abstract

Practical work in veterinary medicine provides exposure to various allergens which could increase the risk of sensitization. With the ongoing prospective "AllergoVet" study, we are investigating patterns of allergic symptoms and sensitization among veterinary students. Here, we report the results of the introductory self-reported survey on the presence of allergies in the first-year students of veterinary medicine, who had begun their studies in the years 2013–2016. The survey included 553 students who answered a questionnaire (63% response rate, 83% female ratio). The median age was 20 years (IQR: 19–22 years). About half of the responders indicated an interest in participating in the prospective study ("candidates"). Those who were uninterested in participating were termed "noncandidates". A doctor's diagnosis of any kind of atopic disease was reported by every fourth student. Hay fever was reported by 71 (13%) and allergic asthma by 38 (6.9%) students. The prevalence of hay fever in "candidates" (n = 294) and "noncandidates" (n = 259) was 13.5% and 12.2%, respectively. Allergic asthma was reported by 9.9% of "candidates" and 3.5% of "noncandidates", the difference being significant (p = 0.003). We conclude that the prevalence of self-reported allergic symptoms in the first-year students of veterinary medicine is similar to that in the general population. Pre-existing allergic asthma may have increased the motivation for the enrolment into the longitudinal "AllergoVet-study".

Keywords

Allergy · Animal allergens · Asthma · Atopic eczema · Hay fever · Sensitization

1 Introduction

Practical work in veterinary medicine provides exposure to various allergens with an increased risk for developing allergic sensitization to animal allergens and allergic symptoms, such as rhinitis, conjunctivitis, asthma, or dermatitis (Moghtaderi et al. 2014). However, epidemiological data on the prevalence of allergic symptoms in veterinarians are scarce and inconsistent. In this regard, Schelkle et al. (2017) collected two questionnaire-based cross-sectional surveys from 512 and 596 veterinarians in Germany in 2006

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and 2012. The authors observed quite stable prevalence rates of allergic symptoms affecting the airways over the time. Bronchial asthma was reported by 5.1% and 5.6% and allergic rhinitis by 17.0% and 20.2% of the respondents, respectively. These prevalence rates were not elevated when compared to those present in the general population. The authors could not rule out a healthy worker effect, as veterinarians who develop severe allergic symptoms may leave their occupation or may switch from a job in practice to a job in a laboratory or administration. Hence, the necessity of more longitudinal studies on this issue has arisen. Data from the German statutory accident insurance show that 0.1% of veterinarians in practice have to abandon their profession because of allergic asthma upon a 5-year period (Nienhaus et al. 2005).

Veterinarians in practice are supposed to be at risk for sensitization to animal allergens, but there might also be a substantial exposure during the training at the university. In this regard, Samadi et al. (2012) conducted a cross-sectional study on students of veterinary medicine at Utrecht University in the Netherlands in 2006. This three-stage longitudinal study demonstrates an increasing prevalence rate of rhinitis, conjunctivitis, asthma, and dermatitis. About 25.8% of the responders reported a history of allergy before the enrolment to the veterinary faculty. The first-time occurrence of allergic symptoms during the study was reported by another 8.7% of the students. The authors demonstrate an increase in the self-reported allergic symptoms in the farm-animal students over the three successive study stages (3.4%, 8.1%, and 20%). Paradoxically, prevalence rates of sensitization assessed via blood tests seem to decrease over time.

To enhance the knowledge on the development of sensitization and the occurrence of symptoms in veterinary students, the prospective "AllergoVet" study was conducted in cooperation with the veterinary faculty of the Justus Liebig University in Giessen, Germany. The veterinary faculty in Giessen is one of the five faculties in Germany altogether offering 1100 seats for veterinary medicine to more than 5000 applicants each year. The study, approved by the Ethic Committee of the Ruhr University Bochum, started with the enrolment of 71 freshmen and baseline examination in 2013. The enrolment ended in 2016 with a total of 313 students recruited. The average response rate for that longitudinal study was 36%. The present article is a different ramification and an extension of that study. In order to learn more about the prevalence of allergic symptoms in the basic population of veterinary students in Giessen and about a possible selection bias, we distributed a short questionnaire regarding allergic symptoms to all freshmen from 2013 to 2016. The data of the evaluation of this short questionnaire are presented herein.

2 Methods

2.1 Population

Five hundred fifty-three out of the 880 freshmen from the Department of Veterinary Medicine at the Justus Liebig University in Giessen, Germany, volunteered to answer a questionnaire on the doctor's diagnosis of their past allergic diseases. The survey was conducted in 2015 and 2016, the response rate was 63%, and the female/male ratio was 5/1. The median age was 20 years (IQR: 19–22 years), and min-max was 17–42 years.

We used the data from two reputed previous population surveys performed by the Robert Koch Institute as a reference for comparison of the prevalence rate noted in the present study with the lifetime prevalence rate in the general population (Lange et al. 2014; Kamtsiuris et al. 2013). For matching the age structure of the study population, we considered the data from the German Health Interview and Examination Survey for Adults (DEGS1) and the German Health Interview and Examination Survey for Children and Adolescents (KiGGS). KiGGS started collecting health data from a population-based sample in the year 2003 and provided the first nationally representative information on allergic diseases and sensitization (Kurth 2007; Schlaud et al. 2007). The first wave of KiGGS data was collected from 12,368 children and adolescents at 163 sample

points via a phone survey in Germany during 2009–2013. For the matching comparison with the present survey, we used the data restricted to the age group 14–17 years of that survey (Schmitz et al. 2014). On the other hand, the first wave of DEGS data concerned the period 2008–2011, and it was part of the health monitoring system at the Robert Koch Institute. The survey consisted of interviews, examinations, and tests on the targeted population of residents aged 18–79 years at 180 sample points in Germany (Langen et al. 2013). Here, for the matching comparison with the present survey, we used the data restricted to the age group 18–29 years (Haftenberger et al. 2013; Kamtsiuris et al. 2013). The absolute frequencies were calculated from the prevalence rates and total numbers, if not reported in literature.

2.2 Data Elaboration

The prevalence of allergic diseases diagnosed by a doctor was presented as crude rates derived from the number of cases by 100 respondents. Two-sided Fisher's exact test was used for the calculation of 95% confidence interval. Each difference between two rates was tested for significance using Fisher's exact test. Calculations were performed using the interoperable software tool Epi Info™ provided by the US Centers for Disease Control and Prevention.

3 Results

The results from 460 female freshmen responding to our request on allergic symptoms are given in Table 1. The prevalence rate and frequency were as follows: 12.4% (n = 57) for hay fever, 7.0% (n = 32) for allergic asthma, 8.5% (n = 45) for atopic eczema, 9.1% (n = 42) for allergic contact dermatitis, and 4.8% (n = 22) for animal allergy. About one quarter of the surveyed female students (22.8%, 95% CI 18.7; 27.6) reported that they had ever been diagnosed with at least some kind of atopic disease – hay fever, allergic asthma, or atopic eczema. Overall, the prevalence rate of allergic symptoms in the present study was comparable to that in the previous KiGGS population survey where it amounted to 27.9 per 100 persons; the difference between the two surveys was not statistically significant. However, atopic eczema (14.6%) and allergic contact dermatitis (21.2%) were most prevalent in female adolescents of the KiGGS survey (Schlaud et al. 2007). Bronchial asthma was most frequently reported by female participants of the DEGS1 survey (12.8%). In both cases, these results were significantly different from the present findings ($p < 0.001$ and $p < 0.005$, respectively). On the other hand, hay fever was less prevalent among the present student populations than it was in both surveys referenced.

The pattern of allergic symptoms was quite similar in 93 male freshmen, with hay fever in 15.1% (n = 14), allergic asthma in 6.5% (n = 6),

Table 1 Prevalence rate (%) of self-reported allergic diseases (doctor's diagnosis) in females with 95% confidence intervals (95% CI) and counts (n)

	AllergoVet (n = 460)			KiGGS 1; age 14–17 years (n = 1590)			DEGS1; age 18–29 years (n = 547)		
Female	(%)	95% CI	n	(%)	95% CI	n	(%)	95% CI	n
Hay fever	12.4	(9.4; 15.9)	57	16.3	(14.5; 18.2)	259	19.0	(15.9; 22.6)	104
Bronchial asthma	7.0	(4.8; 9.8)	32	6.7	(5.5; 8.2)	107	12.8	(9.9; 16.4)	70
Eczema	9.8	(7.1; 13.1)	45	14.6	(12.8; 16.7)	232	6.6	(4.8; 9.1)	36
Urticaria	1.7	(0.8; 3.3)	8				1.6	(0.7; 3.7)	9
Atopic disease (any)	22.8	(18.7; 27.6)	105	27.9	(25.7; 30.3)	444			
Allergic contact dermatitis	9.1	(6.6; 12.3)	42	21.2	(19.1; 23.5)	337	8.9	(6.3; 12.5)	49
Food allergy	4.8	(3.0; 7.2)	22				7.6	(5.4; 10.6)	42

Table 2 Prevalence rate (%) of self-reported allergic diseases (doctor's diagnosis) in males with 95% confidence intervals (95% CI) and counts (n)

	AllergoVet (n = 93)			KiGGS 1; age 14–17 years (n = 1470)			DEGS1; age 18–29 years (n = 526)		
Male	(%)	95% CI	n	(%)	95% CI	n	(%)	95% CI	n
Hay fever	15.1	(8.5; 24.7)	14	20.3	(18.3; 22.5)	298	16.1	(12.5; 20.4)	85
Bronchial asthma	6.5	(2.4; 14.0)	6	7.2	(6.0; 8.6)	106	11.3	(8.3; 15.1)	59
Eczema	2.2	(0.3; 7.8)	2	11.3	(9.7; 13.1)	166	6.3	(4.1; 9.7)	33
Urticaria	0		0				1.3	(0.6; 2.6)	7
Atopic disease (any)	19.4	(11.8; 31.0)	18	29.7	(27.4; 32.2)	437			
Allergic contact dermatitis	2.2	(0.3; 7.8)	2	7.1	(5.9; 8.6)	104	2.7	(1.6; 4.3)	14
Food allergy	1.1	(0.0; 6.0)	1				4.3	(2.5; 7.4)	23

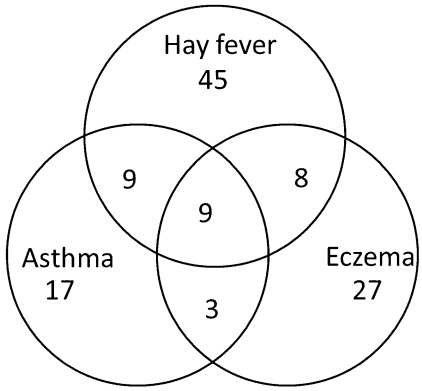

Fig. 1 Distribution of self-reported allergic symptoms among freshmen of both genders

atopic eczema in 2.2% (n = 2), allergic contact dermatitis in 2.2% (n = 2), and animal allergy (excl. insect venom) in 1.1% (n = 1) of participants (Table 2). Only 18 male students (19.4%) did report any diagnosis of atopic disease in the past time, which was significantly fewer than the 29.7% reported in the KiGGS1 survey ($p < 0.05$). Hay fever and bronchial asthma also were rarer than those noticed in both KiGGS1 and DEGS1 surveys, which failed to reach a significant difference. In contradistinction, the rate of eczema was significantly higher in the present study, 11.3% vs. 2.2% in KiGGS1 ($p < 0.005$).

The distribution of multiple symptoms from the present survey is displayed in a Venn diagram (Fig. 1). Allergic asthma was associated with hay fever in 18 students. A set of three atopic symptoms, diagnosed by a doctor, was reported by nine students. Overall, 435 students (77%) had never reported any allergic symptom, confirmed by a doctor, and 409 (74%) not even reported ever making any suspecting self-observations.

About half of the responders, further referred to as "candidates", indicated an interest in participating in the main study. The prevalence rates in "candidates" (n = 294) and "noncandidates" (n = 259) were as follows: hay fever 13.5% vs. 12.2%, allergic asthma 9.9% vs. 3.5%, eczema 9.5% vs. 7.3%, allergic contact dermatitis 8.8% vs. 6.9%, and animal allergy 4.1% vs. 3.9%, respectively (Table 3). Atopic diseases, in the main, tended to be more frequent in "candidates" than in "noncandidates" (23.5% vs. 20.8%, respectively). The exception was a significant higher prevalence rate in allergic asthma in "candidates" ($p = 0.006$). Additionally, Table 3 provides more details on the distribution of causes of allergies among the respondents. Grass and pollen were reported the most frequent triggers of allergic symptoms (13.9%), followed by house dust mites (10.1%). Animal allergens attested by a doctor triggered allergies in 4% of the respondents, with negligible differences between "candidates" and "noncandidates".

4 Discussion

In a cross-sectional survey conducted among freshmen at the faculty for veterinary medicine of the Justus Liebig University of Giessen in Germany, lifetime prevalence rates for allergic asthma and hay fever tended to be lower than the rates reported by both participants of the KiGGS1 survey aged 14 to 17 years (Schlaud

Table 3 Prevalence rate (%) of potential participants "candidates" (AllergoVet+) and "noncandidates" (AllergoVet-) with 95% confidence intervals (95% CI) and counts (n)

	AllergoVet + (n = 294)			AllergoVet – (n = 259)			Total (n = 553)	
	(%)	95% CI	n	(%)	95% CI	n	(%)	n
Females	78.9		232	83.4		216	80.7	446
Hay fever	12.2	(8.6; 17.0)	36	13.5	(9.4; 18.8)	35	12.8	71
Bronchial asthma	9.9	(6.6; 14.2)	29	3.5	(1.6; 6.6)	9	6.9	38
Atopic eczema	9.5	(6.3; 13.8)	28	7.3	(4.4; 11.5)	19	8.5	47
Urticaria	1.0	(0.2; 3.0)	3	1.9	(0.6; 4.5)	5	1.4	8
Atopic disease (any)	23.5	(18.3; 29.7)	69	20.9	(15.7; 26.4)	54	22.2	123
Allergic contact dermatitis	8.8	(5.8; 13.0)	26	7.0	(4.1; 11.0)	18	8.0	44
Grass and pollen allergy	14.3	(10.3; 19.3)	42	13.5	(9.4; 18.8)	35	13.9	77
Food allergy	4.4	(2.4; 7.6)	13	3.9	(1.9; 7.1)	10	4.2	23
House dust allergy	10.9	(7.5; 15.4)	32	9.3	(5.9; 13.8)	24	10.1	56
Animal allergy	4.1	(2.1; 7.1)	12	3.9	(1.9; 7.1)	10	4.0	22

et al. 2007) and the DEGS1 survey (Langen et al. 2013). Likewise, prevalence rates for atopic eczema were lower than those observed in the KiGGS1 survey. About every fourth female freshman and every fifth male freshman reported at least one doctor's diagnosis of an atopic disease in the past. Although the questionnaire-based surveys are appropriate investigation tools, the consistency between reported symptoms and the actual lifetime prevalence of allergic diseases cannot be evidenced. In this regard, atopic eczema in childhood could be reported more frequently if parents had been interviewed as was the case in the KiGGS study. The lack of such interviews in the present study could result in a lower prevalence of atopic eczema when compared to the KiGGS study.

A limitation of the comparison of prevalence rates could arise from different time periods of data collection for the three surveys. KiGGS1 was performed in 2005, DEGS1 was conducted from 2008 to 2011, and the present survey was from 2013 to 2016. Interestingly, KiGGS1 revealed an outstanding prevalence for allergic contact dermatitis in 21.2% of female adolescents (Schlaud et al. 2007). In this regard, the authors discussed a causal effect from the use of cheap jewelry and cosmetics by girls. Hence, lower rates of contact dermatitis in the present study could have something to do with a minor use of jewelry and cosmetics in our study population. But the difference could also be due to a general downward trend in contact dermatitis over time (Langen et al. 2013). However, a prevalence rate of 22.8% for any kind of atopic disease in the first-year students in the present study is in accord with the observation of 25.8% of allergic veterinary freshmen at Utrecht University reported by Samadi et al. (2012).

In the year 2016, our cohort was closed at a total of 313 students. We suppose that most of the 294 freshmen who signalled their willingness to participate in the prospective AllergoVet study ("candidates") during this survey actually became participants. Most of the observed differences between this group and the students who stated disinterest ("noncandidates"; n = 259) were statistically insignificant, except for the prevalence of bronchial asthma in "candidates". Asthma patients among the students seemed particularly interested in participation in the AllergoVet study due to their personal situation. A pre-existing allergy might have increased the motivation to participate in this prospective study.

5 Conclusions

The majority of freshmen in veterinary medicine in Germany are well-educated female students between 17 and 22 years of age. From this upstream survey, we learn that about every fourth freshmen in veterinary medicine had a diagnosis of an atopic disease in the past, 13% were

suffering from hay fever, and 7% were suffering from bronchial asthma. The prevalence rates are not higher than those in the general population. The prevalence rate for atopic diseases was slightly higher for potential study participants than for nonparticipants. Bronchial asthma was significantly more frequent among potential participants. A minor selection bias should be considered in the interpretation of results of the subsequent longitudinal AllergoVet study.

Conflicts of Interest The work is part of the IPA project 109-AllergoVet funded by the intramural budgets provided by the German Social Accident Insurance (DGUV) to the Institute for Prevention and Occupational Medicine (IPA). Practical realization of the study was supported by the Unfallkasse Hessen (especially I. Thullner) and the University for Veterinary Medicine, Giessen (especially Prof. Dr. St. Arnhold and Prof. Dr. S. Tacke). The authors also want to express their gratitude to all students who participated in the study. The authors are independent from the DGUV in the study design and the interpretation of the results and have no other conflicts of interest to declare.

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

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

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The Pattern of Sensitization Influences Exhaled and Nasal Nitric Oxide Levels in Young Adults

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Abstract

Nitric oxide (NO) from upper (nasal NO, nNO) or lower airways (fractional exhaled NO, FeNO) is considered a surrogate marker for Th2-type inflammation, which is influenced by atopy. The aim of this study was to analyze nNO and FeNO in regard to qualitative and quantitative aspects of sensitization. We evaluated 244 non-smoking young adults. All of them were first-year students recruited for a longitudinal study. An inhalation allergy screening tool was used for atopy definition (specific immunoglobulin E (sIgE) to sx1 $\geq$ 0.35 kU/L), and also sIgE response to three inhalant perennial allergens, house dust mite (HDM, d1), cat (e1), and dog (e5), was determined in the non-pollen season. With respect to sx1, 100 subjects could be classified as atopic. Sensitization to one, two, or three perennial allergens could be demonstrated in 46, 10, and 16 students, respectively. The subjects with positive IgE response to sx1, but not sensitized to HDM, cat, and/or dog, had FeNO levels comparable to those of non-atopic subjects (13.5 vs. 13.0 ppb, respectively; $p = 0.485$). These levels were significantly lower compared to atopic subjects being sensitized to any perennial allergen (19.0 ppb; $p = 0.0003$). After grouping the atopic subjects for perennial sensitization patterns, significantly higher FeNO could be detected in subjects with poly-sensitization ($n = 26$; 26.0 ppb) compared to the mono-sensitized ones ($n = 46$; 18.0 ppb; $p = 0.023$). Regarding nNO, no differences could be observed. Applying a two-way ANOVA, we could reveal a significant correlation of specific HDM-IgE CAP-class with FeNO ($p < 0.0001$) and nNO levels ($p = 0.007$). Finally, a significant relationship was found between nNO and FeNO for the whole cohort ($p < 0.0001$). In summary, our findings support the argument that atopy and perennial sensitization should be considered for the interpretation of NO.

Keywords

Airway inflammation · Biomarker · Exhaled nitric oxide · Nasal nitric oxide · Sensitization

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1 Introduction

Measurement of nitric oxide (NO) either from upper (nasal NO; nNO) or lower airways (fractional exhaled NO, FeNO) may be used as a surrogate marker for Th2-type inflammation, e.g., airway eosinophilia (Jatakanon et al. 1998). FeNO is recognized as an important noninvasive tool in clinical practice to clarify the etiology of respiratory symptoms (Häussermann et al. 2013). Moreover, it has been demonstrated of value for monitoring levels of airway eosinophilic inflammation and to guide therapy (Dweik et al. 2011). There is a high basal activity of nitric oxide synthase (NOS) in the nasal epithelium and paranasal sinuses resulting in a 100-fold higher nNO level compared to NO produced in the lower respiratory tract (Lundberg et al. 1995).

So far, with the exception of ciliary disorders characterized by a dramatic decrease of nNO, measurement of nNO is not a routine diagnostic method (Wodehouse et al. 2003). There are few data demonstrating the usefulness of nNO in terms of its being a biomarker of inflammation (Antosova et al. 2017). Most studies have been performed in clinical cohorts, and the general population-based studies are lacking. nNO increases in allergic rhinitis (Arnal et al. 1997). Lower nNO levels are shown in patients with allergic rhinitis after treatment with topical steroids compared to non-treated ones (Kharitonov et al. 1997). Consistently, increasing nNO levels have been provoked by specific allergen challenge in subjects with allergic rhinitis due to molds (Boot et al. 2007). However, nNO profiles are almost identical after *A. fumigatus* and placebo inhalation (Stark et al. 2005). Somewhat conflicting, nNO levels have also been shown to fall after specific challenge (Serrano et al. 2012). The interpretation of nNO level is complex as inflammation causes nasal congestion and thereby could reduce nasal passage of NO-rich air through the paranasal ostia. nNO is reduced in patients with nonallergic rhinitis, chronic sinusitis, and nasal polyps (Colantonio et al. 2002). Moreover, different methodological approaches used for nNO measurements have to be taken into account when making comparisons between studies (Hoffmeyer et al. 2019; Antosova et al. 2017).

Beside technical and analytical issues, different subject characteristics cause NO variations (ATS/ERS 2005). Concerning FeNO in adults, age, cigarette smoking, atopy, and gender have to be considered (Taylor et al. 2007; Olin et al. 2006; Kharitonov et al. 1995). In a systematic review, higher FeNO in subjects with allergic rhinitis has been reported compared to either healthy or atopic individuals, although healthy and atopic individuals have similar values (Linhares et al. 2011). Elevated FeNO is detected in non-asthmatic subjects with allergic rhinitis in the non-pollen season especially in perennially sensitized subjects (Henriksen et al. 1999). The level and type of allergic sensitization affect FeNO in subjects with atopic asthma, with perennial allergens having the greatest effect (Olin et al. 2004). In addition, there is a positive association between FeNO and the increasing number and maximum diameter of each positive skin prick test (Ho et al. 2000).

Methodological issues that cause variations in nNO levels have been widely addressed (Hoffmeyer et al. 2019; de Winter–de Groot and van der Ent 2009). Further data are needed on the subject characteristics and environmental modifiers. Moreover, data simultaneously assessing upper and lower airway NO levels are limited (Gupta et al. 2014). In subjects suffering from allergic rhinitis, nNO is not significantly different from healthy controls in the non-pollen season. In contrast, FeNO is significantly elevated in perennially sensitized subjects (Henriksen et al. 1999). Also, an increasing nNO level with the number of perennial sensitizing allergens is described (Krantz et al. 2014). In the present study, we investigated the level of nNO and FeNO, as indirect markers of inflammation in the upper and lower airways, in relation to atopic status and pattern of perennial sensitization reflected by specific serum IgE in non-smoking young adults.

2 Methods

2.1 Study Population and Allergic Sensitization

Within the framework of the AllergoVet longitudinal study, 244 first-year students in a university were evaluated. A questionnaire concerning exposure, respiratory symptoms, and smoking habits was completed. All of the young adults were healthy never-smokers and free of respiratory infection (Table 1). Measurements of specific IgE (sIgE) were done by ImmunoCAP 250 (Thermo Fisher Scientific, Uppsala, Sweden), and sIgE values ≥0.35 kU/L were considered positive. Three inhalant perennial allergens, house dust mite *Dermatophagoides pteronyssinus* (HDM, d1), cat (e1), and dog (e5) dander, and a mixture of common inhalant allergens (sx1, Phadiatop, Thermo Fisher Phadia AB, Uppsala, Sweden) were used for sIgE testing. The sx1 mixture, apart from the three inhalant perennial allergens, also contains the seasonal allergens of timothy grass pollen, rye grass pollen, *Cladosporium herbarum*, birch pollen, and mugwort pollen. A positive atopic status was assumed in case of any positive sIgE value. Perennial sensitization with elevated sIgE level to HDM antigen (0.40 and 0.59 kU/L) but sx1 below the cutoff level (0.26 and 0.30 kU/L, respectively) was found in only two students. The students were grouped by the number of positive results to perennial allergens into mono-sensitized ($n = 1$) and poly-sensitized ($n > 1$). Positive results for perennial sensitization were further classified according to the sIgE level into six categories (CAP-class): CAP-1; 0.35 – <0.7 kU/L, CAP-2; 0.7 – <3.5 kU/L, CAP-3; 3.5 – <17.5 kU/L, CAP-4; 17.5 – <50 kU/L, CAP-5; 50 – <100 kU/L, and CAP-6; ≥100 kU/L. The sum of CAP-class test results from the three perennial allergens (HDM, cat, and dog) was calculated and referred to as a total perennial allergy score (Henriksen et al. 2001). All of the subjects were tested in the month of November without the influence of seasonal pollen allergens. The subjects were informed not to perform any strenuous physical activity within 60 min before the NO measurements.

2.2 NO Measurements

FeNO and nNO measurements were performed with the handheld NIOX MINO® system (Circassia, Bad Homburg, Germany) according to the published recommendations (ATS/ERS 2005). The NIOX MINO® employs an electrochemical sensor with a measurement range of 5–1700 ppb and a good short-term repeatability demonstrating a coefficient of variation of 10% (Marthin and Nielsen 2013).

The subjects were studied in a convenient seated position. FeNO was measured during active oral exhalation at a flow rate of 50 mL/s. Concerning nNO, different techniques have been applied, and the highest values were found using the method of a closed velum (de Winter–de Groot and van der Ent 2009). Recently, we could demonstrate higher values and a better short- and long-term repeatability using the breath-holding than the tidal-breathing method for nNO measurement (Hoffmeyer et al. 2019). We validated those results in the current study and confirmed the presence of differences between the two methods in favor of the breath-holding in the Bland-Altman analysis (data not shown). The Bland-Altman analysis of distance for the two methods revealed a mean nNO difference of 50% (243 ppb) and SD of the difference of 51%, confirming our previous results of 51% (243 ppb) and 35%, respectively. Therefore, nNO measurement was done during breath-holding with the mouth shut. Nasal olive was applied into a nostril and hold in place by the participant while the contralateral nostril was left open. The aspiration was done at a flow rate of 5 mL/s and automatically stopped after 45 s.

2.3 Statistical Analysis

Data were expressed as medians with interquartile ranges (IQR, 25th; 75th percentile). The

Table 1 Subject characteristics of 244 non-smoking first-year students

		No atopy	Perennial sensitization	Seasonal sensitization only[a]
Gender	*n* (F/M)	144 (133/11)	72 (59/13)	28 (22/6)
Age	Years	19 (19; 21)	19 (19; 21)	19 (19; 22)
BMI	kg/m^2	21.4 (20.0; 23.3)	22.4 (20.3; 24.1)	22.6 (21.0; 25.2)
sx1	kU/L	0.09 (0.06; 0.13)	11.1 (3.47; 32.38)	6.13 (0.49; 17.33)
Asymptomatic	*n* (F/M)	125 (116/9)	36 (28/8)	11 (9/2)
Rhinitis only	*n* (F/M)	12 (11/1)	25 (21/4)	9 (8/1)
Asthma only	*n* (F/M)	4 (3/1)	1 (0/1)	2 (1/1)
Rhinitis + asthma	*n* (F/M)	3 (3/0)	10 (10/0)	6 (4/2)

F female; *M* male; *BMI* body mass index; *sx1* IgE antibodies to a mixture of ubiquitous allergens (atopy screen); data are continuous variables with medians and interquartile ranges (IQR)
[a]sx1 positive without IgE response to HDM, cat, and dog

D'Agostino and Pearson omnibus normality test, cross-checked by the Kolmogorov-Smirnov test, was used to assess data distribution. A two-sided significance level of 0.05 was chosen for all tests. The Bland-Altman analysis was performed to compare two methodological approaches. Atopics and non-atopics were compared using a *t*-test or Mann-Whitney U test as appropriate. Differences between multiple groups were assessed with one-way ANOVA. Correlations between measured parameters were assessed with the Pearson test or Spearman rank test, where appropriate. A two-way ANOVA was used to examine the influence of qualitative aspects, e.g., mono- vs. poly-sensitization, and quantitative aspects, e.g., intensity of sensitization on nitric oxide levels. In this analysis, the independent continuous variable for intensity of sensitization ("sIgE concentration") was transferred into a categorical variable ("CAP-class"). Data were analyzed and visualized by GraphPad Prism v7.04 software for Windows (GraphPad, San Diego, CA).

3 Results

3.1 Characteristics of Subjects

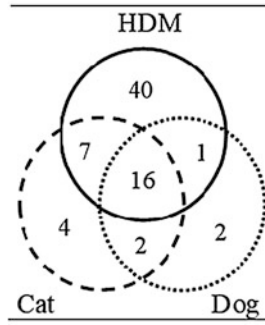
Females accounted for the majority of the 244 students ($n = 214$; 88%), with 81 of them being atopic (38%). In comparison, significantly more of the 30 male participants could be classified as atopic ($n = 19$; 63%, $p = 0.01$). Overall, 100 atopics could be identified in the non-smoking first-year students. The proportion of atopics sensitized to perennial allergens ($n = 72$) was similar in females ($n = 59$; 72%) and males ($n = 13$; 68%). More than 50% of the atopics in comparison to only 13% of non-atopics reported respiratory symptoms (Table 1) which occurred mainly in the pollen season. At the time of the study, all of the students were asymptomatic.

No significant gender differences were evident regarding sensitization to any particular perennial allergen (Table 2). Overall, 64 (26%), 29 (12%), and 21 (9%) subjects were sensitized to HDM, cat, and dog allergen, respectively. They were divided according to mono- or poly-sensitization to perennial allergens. In detail, 40, 4, and 2 subjects were mono-sensitized to HDM, cat, or dog, respectively. Poly-sensitization to 2 allergens was found in 10 subjects (HDM/cat, $n = 7$; HDM/dog, $n = 1$; and cat/dog, $n = 2$), and 16 subjects were poly-sensitized against all of these 3 allergens tested. Positive results for perennial sensitization were further classified according to the sIgE levels into six CAP-classes (Fig. 1). Concerning HDM, CAP spectrum was quite similar, except CAP-class 1 which was only seen in HDM mono-sensitized subjects. A stronger sensitization against cat or dog (CAP >2) was only observed in case of poly-sensitization.

Table 2 Sensitization and specific IgE antibodies

		All ($n = 244$)	Females ($n = 214$)	Males ($n = 30$)	p-value*
sx1	n (%)	100 (41%)	81 (38%)	19 (63%)	0.010
	kU/L	8.8 (2.1; 31.6)	8.8 (2.4; 32.4)	11.1 (1.8; 24.7)	0.722
HDM (d1)	n (%)	64 (26%)	52 (24%)	12 (40%)	0.177
	kU/L	9.3 (1.7; 20.3)	8.9 (1.8; 25.1)	13.1 (0.7; 15.8)	0.626
Cat (e1)	n (%)	29 (12%)	25 (12%)	4 (13%)	0.765
	kU/L	2.3 (0.6; 7.3)	3.4 (0.7; 7.5)	0.7 (0.6; 5.8)	0.444
Dog (e5)	n (%)	21 (10%)	18 (8%)	3 (10%)	0.730
	kU/L	0.9 (0.5; 3.2)	0.8 (0.4; 3.2)	2.8 (0.4; 3.3)	0.740

Data are continuous variables with medians and interquartile ranges (IQR); *females vs. males; sIgE values ≥0.35 kU/L were considered positive



CAP-class	1	2	3	4	5	6
HDM						
Mono (n=40)	10	5	14	8	1	2
Poly (n=24)		6	8	5	4	1
Cat						
Mono (n=4)	1	3				
Poly (n=25)	7	6	10	1		1
Dog						
Mono (n=2)	2					
Poly (n=19)	6	8	5			

Fig. 1 Perennial mono- and poly-sensitization and respective strength of sensitization (CAP-class). *CAP-class* sensitization categories (see Methods), *HDM* house dust mite

3.2 Sensitization and NO Measurements

The subjects sensitized to seasonal allergens only had the out-of-season FeNO levels similar to those of non-atopic subjects (13.5 (11.0; 17.8) ppb vs. 13.0 (10.0; 17.0) ppb, respectively, $p = 0.485$). These FeNO levels were significantly lower compared to atopic subjects being sensitized to any of the perennial allergens (19.0 (15.0; 31.5) ppb; $p = 0.0003$) (Fig. 2a). Those who were sensitized to perennial allergens were further stratified according to mono- or poly-sensitization. The results for FeNO in relation to a specific perennial sensitization are depicted in Fig. 2b. After grouping for sensitization patterns, significantly higher FeNO was detected in the subjects with poly-sensitization ($n = 26$; 26.0 (16.0; 44.5) ppb) compared to mono-sensitization ($n = 46$; 18.0 (13.8; 23.3) ppb; $p = 0.023$). There were no differences in FeNO regarding the anamnestic seasonal rhinitis and/or asthma compared to subjects without seasonal symptoms, irrespective of underlying atopy (non-atopic $p = 0.574$; atopic $p = 0.452$) (data not shown).

Regarding nNO, there were no differences between poly-sensitized (664 (552; 764) ppb) and mono-sensitized subjects (665 (487; 841) ppb; $p = 0.875$). nNO was similar in non-atopic and atopic subjects outside the pollen season, independent from the pattern of sensitization, e.g., seasonal only or perennial. The nNO levels were 626 (462; 756) ppb, 612 (421; 758) ppb, and 664 (512; 791) ppb for non-atopic, seasonal only and perennial sensitized. nNO was not significantly influenced by intermittent clinical symptoms of rhinitis and/or asthma (non-atopic $p = 0.952$; atopic $p = 0.110$; data not shown). There were higher, but not statistically different, nNO levels in atopic subjects with intermittent rhinitis (720 (570; 838) ppb) compared to asymptomatic ones (607 (419; 732) ppb; $p = 0.070$).

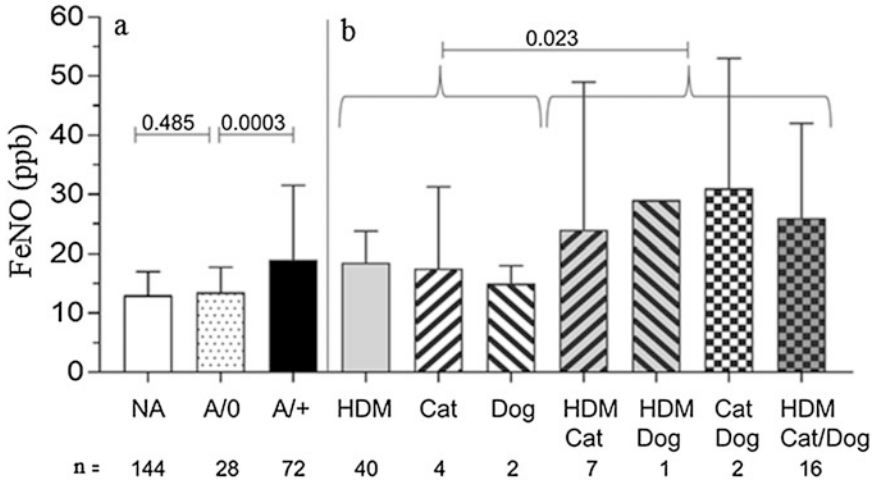


Fig. 2 FeNO in relation to atopic status (**a**) and to specific perennial sensitization (**b**). A group of subjects with poly-sensitization was compared to that with mono-sensitization (medians with interquartile ranges). *NA* non-atopic; *A/+* sensitization against the following allergens (without perennial allergens (A/0)); *HDM* house dust mite

Referring to the pattern analysis, apart from comparing mono- and poly-sensitized subjects, the strength of sIgE content has to be considered. Due to a small number of subjects mono-sensitized against animal antigens as shown in Fig. 1, these subgroups were not suitable for further analysis. However, the results for HDM sensitization were suitable for further qualitative and quantitative analysis. The influence of qualitative aspects, e.g., mono- vs. poly-sensitization, and quantitative aspects, e.g., intensity of HDM sensitization in terms of sIgE content or CAP-class on FeNO and nNO, is shown in Fig. 3a, b, respectively. In two-way ANOVA, correlation of HDM CAP-class with either FeNO or nNO level could be revealed. In contrast, there was no modulation by qualitative aspects. Thus, intensity of sensitization against HDM, irrespective of additional perennial sensitization, has a major influence on FeNO levels. Additionally, no significant interaction was found between CAP-level and qualitative aspects of sensitization (mono- or poly-sensitization) (FeNO $p = 0.819$; nNO $p = 0.451$). Similar results were noticed when NO was analyzed regarding the total perennial allergy score (data not shown).

3.3 Correlation of FeNO and nNO

A significant relationship was found between NO of the upper and lower airways for the whole cohort ($r = 0.438$; $p < 0.0001$). Linear regressions for nNO and FeNO of subjects without ($n = 144$; $r = 0.384$, $p < 0.0001$) and with atopy ($n = 100$; $r = 0.490$, $p < 0.0001$), considering either perennial ($n = 72$; $r = 0.509$, $p < 0.001$) or seasonal only sensitization ($n = 28$; $r = 0.440$, $p = 0.022$), were all significant (Fig. 4a, b). In addition, stratification by the intensity of sensitization revealed significant relationships for mono-sensitized ($n = 46$; $r = 0.534$, $p = 0.0002$) and poly-sensitized subjects ($n = 26$; $r = 0.443$, $p = 0.023$).

4 Discussion

In this study, we demonstrate an influence on FeNO and nNO of sensitization pattern in young adults without respiratory symptoms. Since NO measurement could be confounded by smoking, the investigation was restricted to non-smoking subjects (Alexanderson et al. 2012; Olin et al. 1998; Kharitonov et al. 1995). The

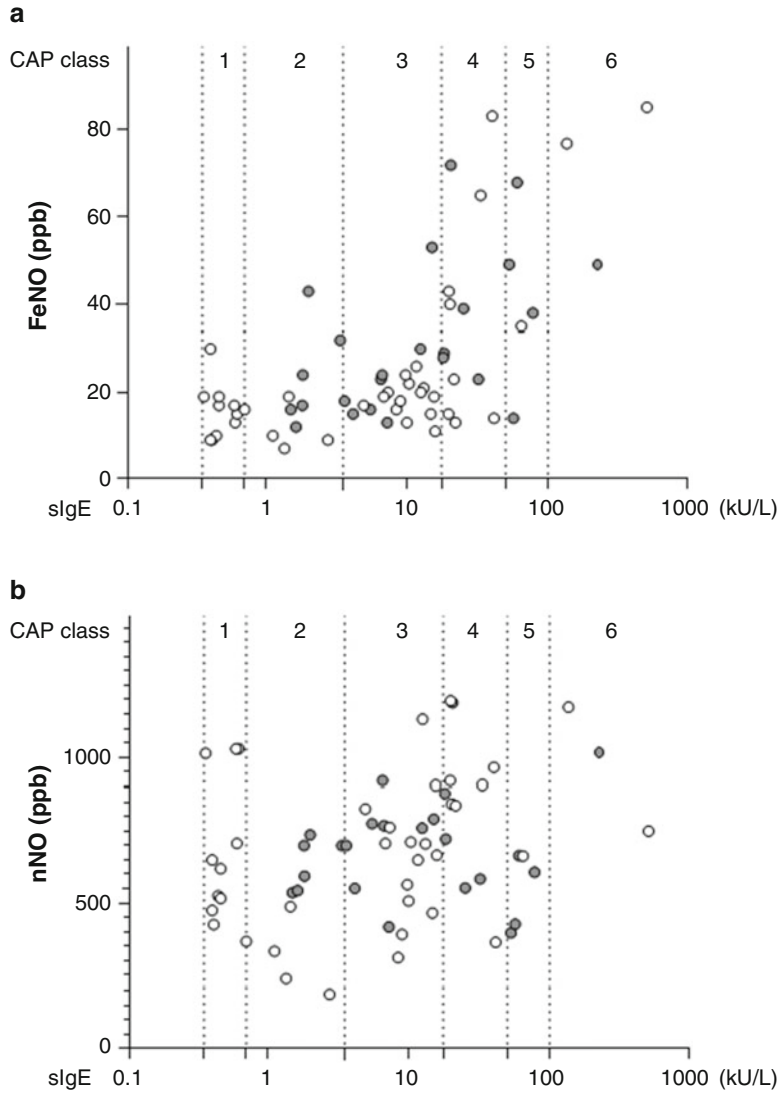


Fig. 3 Correlation between CAP-class of HDM sensitization and the level of exhaled (**a**) and nasal (b) nitric oxide (NO). Results are stratified by mono-sensitized (m, open symbols) and poly-sensitized subjects (p, solid symbols). Two-way ANOVA:
(**a**) CAP df 5, F = 8.56; $p < 0.0001$, m/p df 1, F = 0.200; $p = 0.656$
(**b**) CAP df 5, F = 4.64; $p = 0.007$, m/p df 1, F = 0.286; $p = 0.595$

main finding was that NO levels in the non-pollen season were influenced by the pattern of perennial allergen sensitization. The pattern of sensitization was evaluated according to qualitative aspects such as mono- vs. poly-sensitization and quantitative aspects such as the strength of sensitization in terms of CAP-class. Both FeNO and nNO were measured with routinely recommended methods (ATS/ERS 2005). Concerning nNO, we used the breath-holding method whose results were in line with those published previously (Hoffmeyer et al. 2019).

Aside from the skin prick test, sIgE antibodies have been used to identify sensitization in atopic subjects without asthma or rhinitis (Rouhos et al. 2008). Referring to sIgE levels, we identified in this study 100 (40%) atopic students, the proportion in accord with the most recent DEGS survey from Germany, in which 45% adults aged 18–29 were atopic as assessed from positive sIgE to sx1

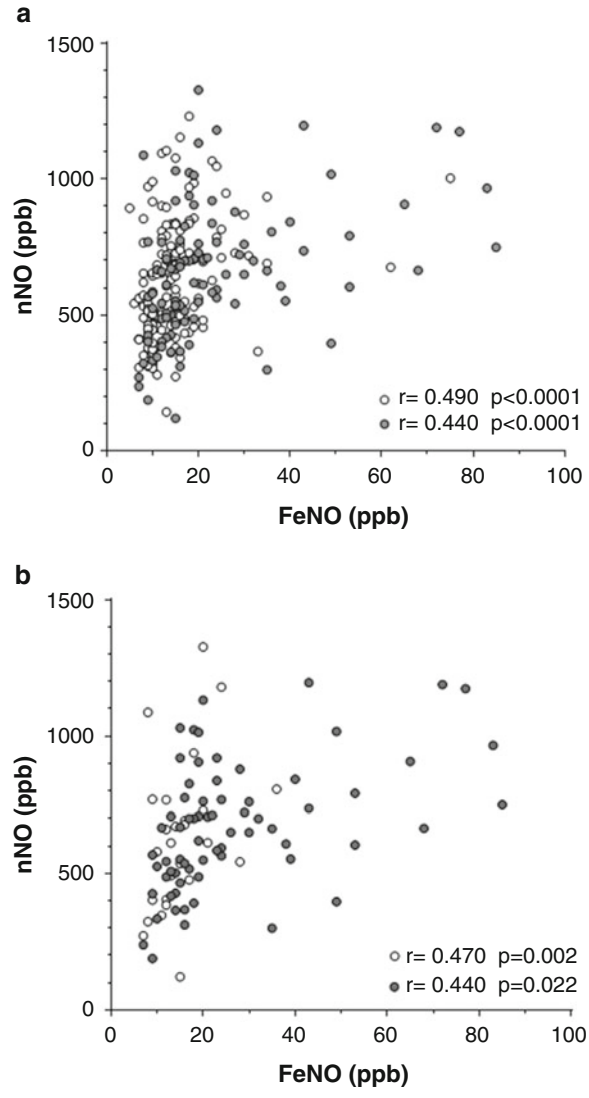


Fig. 4 Correlation between FeNO and nNO levels. Results are stratified by non-atopic (open circles) (**a**) and atopic (solid circles) subjects (**b**) and by the pattern of sensitization: seasonal only (open circles) and perennial (solid circles)

(Haftenberger et al. 2013). With respect to specific allergen sensitization to HDM, our results are in line with the DEGS survey reporting the prevalence of 26% in young adults aged 18–29 years. We found that 70% of the non-smoking atopic students were sensitized to perennial allergens and 30% to seasonal allergens only. These proportions correspond to those defining atopy from a positive response to skin prick test, amounting to 69% and 31%, respectively (Rouhos et al. 2008).

We noticed higher FeNO levels outside the pollen season in case of perennial sensitization than those in atopic subjects without perennial sensitization and non-atopic healthy subjects. This is in line with the literature sources reporting atopy and allergic rhinitis as two important FeNO modifiers. In asymptomatic atopic adults, elevated FeNO was seen compared to non-atopic subjects (Horváth and Barnes 1999). In addition, increased FeNO was found in asymptomatic subjects sensitized to HDM (Moody et al. 2000).

Other studies performed in non-smoking asymptomatic adults have shown similar FeNO content in atopic compared to healthy subjects (Rouhos et al. 2008; Berlyne et al. 2000). However, they failed to consider the pattern of sensitization. FeNO has been similar in non-atopic and in low-sensitized atopic asthmatics (Ekroos et al. 2009). Referring to the kind of sensitization, the highest levels of FeNO are shown in asthmatic patients sensitized to perennial allergens compared to those in non-atopic asthmatics or in the presence of seasonal allergens (Olin et al. 2004). Continuous exposure to perennial allergens might have a stronger effect on lower airways compared to pollen occurring seasonally (Warm et al. 2015). Concerning the impact of allergic diseases, the majority of studies have revealed a significantly higher FeNO in allergic rhinitis compared to healthy subjects (Linhares et al. 2011). In non-smoking male military conscripts recently diagnosed as having atopic asthma, the median FeNO levels are significantly higher than those in subjects with non-atopic asthma, both being significantly higher than those in healthy controls (Ekroos et al. 2009). A modulation of FeNO content by the measurement method, either in or out of season, has been reported (Prieto et al. 2002). Simpson et al. (1999) have found higher FeNO levels in subjects with sensitization who are exposed to relevant allergens compared to those without current exposure.

Aside the kind of sensitization, e.g., perennial versus seasonal only, our results support the importance of qualitative aspects. In children, a relationship between elevated FeNO and the exacerbation of rhinitis or the number of positive wheals in skin prick test has been noticed (Cardinale et al. 2005). In extensive analysis, Rouhos et al. (2008) have defined the degree of atopy, apart from the number of positive reactions, as a sum of wheal diameter. A total prick wheal sum serving as a proxy for the strength of sensitization has also been applied in other studies (Ekroos et al. 2009; Ho et al. 2000). Likewise, a sum of immunologic test results, e.g., sIgE from perennial indoor allergens, is referred to as a total perennial allergy score (Henriksen et al. 2001). The identification of atopic subjects in the present study relied on serologic IgE levels. Accordingly, apart from the sIgE content, we also used the IgE CAP-class as a proxy for the strength of sensitization. The number of subjects sensitized against HDM enabled the in-depth evaluation. Applying two-way ANOVA, we could reveal a significant correlation of specific HDM-IgE CAP-class with both FeNO and nNO levels. In poly-sensitized subjects, no CAP-class "1" HDM-IgE level could be observed. These results demonstrate that the intensity of sensitization against HDM is a major modulator of NO levels. Whether this is also the case for the other perennial allergens could not be shown due to a limited number of subjects respectively sensitized.

Different methods have been applied for the measurement of nNO (Hoffmeyer et al. 2019; de Winter–de Groot and van der Ent 2009). All these methods are based on the generation of airflow through nasal cavities aspirating or insufflating air via one nostril while the velum is closed. Moreover, a steady NO plateau is not produced before a washout phase. We found similar nNO values in non-atopic and atopic subjects independent from the pattern of sensitization, e.g., seasonal only or perennial. However, atopic subjects with intermittent rhinitis had higher levels of nNO than the asymptomatic ones. This is in line with a higher nNO content in patients with perennial and/or seasonal rhinitis with and without active symptoms compared with healthy controls (Arnal et al. 1997). nNO content seems elevated in allergic rhinitis in some but not all studies when compared to healthy controls (Struben et al. 2006). In this respect, similar nNO levels in subjects with allergic or perennial rhinitis, compared to control subjects, have been reported in a Norwegian general population study (Henriksen et al. 1999). It is to mention that allergen exposure geographically differs both quantitatively and qualitatively. For instance, in another study from Norway, exposure to HDM has been

substantially lower than that to cat allergen (Alexanderson et al. 2012). Interestingly, a significant correlation between the nNO level and strength of sensitization in terms of CAP-class was noticed in the present study for HDM mono-sensitized subjects. This finding is in line with the association between nNO content and skin prick reactivity (wheal diameter) to house dust mite found in a study of Moody et al. (2000). An association of high levels of cat sIgE with increased nNO has been also reported (Alexanderson et al. 2012). A small number of only four subjects mono-sensitized to cat allergen in the present study were insufficient to further elaborate on this association.

There is evidence that allergic rhinitis and asthma share common immunologic features addressed as "unified allergic airway". Both conditions are characterized by the presence of activated Th2-lymphocytes and increased Th2/Th1 cytokine ratio (Jatakanon et al. 1998). There is a continuum from atopic-specific sensitization in the otherwise healthy condition to clinically manifested allergic diseases, such as allergic rhinitis or allergic asthma. On the cellular level, a continuum exists from basal activity of immunologically competent cells, stimulation, and finally inflammatory response. There is a continuous and high basal activity of nitric oxide synthase (NOS) in the nasal epithelium and paranasal sinuses (Lundberg et al. 1995). NOS in the lower airways increases by induction of iNOS under the influence of Th2 cytokines (Dweik et al. 2011). FeNO levels may determine clinical expression of atopy because atopy can be considered an immune disorder associated with increased airway inflammation (Jang et al. 2013). Recently, a significant positive correlation between FeNO and nNO in non-smokers has been reported (Alexanderson et al. 2012). After exclusion of possible confounders, such as age, gender, and the use of inhaled corticosteroids, a correlation between surrogates of inflammation in the upper and lower airways in different airway disorders has been reported. A connection between nNO and FeNO is most evident in healthy subjects and in patients with allergic rhinitis. The present findings support a statement by Williamson et al. (2010) that even in the absence of airway disease an individual variation in NO expression exists. Thus, increased FeNO outside the season for typical aerogen allergens in the asymptomatic young adults with perennial sensitization of the present study may indicate a state of subclinical inflammation.

This study has some potential shortcomings. Firstly, the findings are based on a cross-sectional evaluation. Secondly, young adults were all students of high socioeconomic status, and they were predominantly females of a narrow age range. Thirdly, stratification of the sensitization status was based on sIgE to sx1 and on three indoor aeroallergens only. We did not include specific pollen or food allergens in this study. Fourthly, HDM was a predominating allergen in mono-sensitized students.

In summary, our findings support the argument that atopy and perennial sensitization should be considered for the interpretation of NO measurement results. Out of the pollen season, both FeNO and nNO levels were associated with the presence and strength of perennial sensitization. Finally, we could demonstrate a significant relationship between NO originating from the upper and lower airways supporting the unified (allergic) airway concept.

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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 University Bochum in Germany. The participants received financial compensation for taking part in the study.

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

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Discrimination Between Atopic, Allergic, and Asthmatic Volunteers for Human Exposure Studies on Sensory Irritation

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Abstract

Atopic, allergic, and especially asthmatic subjects might be particularly susceptible to sensory irritation induced by airborne chemicals compared to healthy individuals. Therefore, a good characterization of subjects is essential in inhalation exposure studies on sensory irritants. A total of 105 volunteers, 87% of whom reported to be non-allergic, participated in a medical examination that included skin prick test (SPT), measurements of total IgE, specific IgE (sIgE) to an ubiquitous allergen mix (sx1), and fractionated exhaled nitric oxide (FeNO), as well as pulmonary function and methacholine test. The median value of sIgE to sx1 was 0.20 kU/L (0.07–91.3 kU/L) and correlated significantly with total IgE (28.8 kU/L (2–756 kU/L)) and FeNO (14 ppb (5–100 ppb)). Forty-three subjects (41%) had sIgE to sx1 $\geq$ 0.35 kU/L and were classified as atopic. Thirty-five subjects, all also sx1-positive, were positive in SPT. Obstruction, small airway disease, and/or bronchial hyperreactivity were diagnosed in 18 subjects. Receiver operating characteristics (ROC) were performed to check whether signs of sensitization are useful to discriminate subjects with and without airway diseases. However, sx1, total IgE, FeNO, and SPT reached only low areas under the curve (AUC: 0.57–0.66). Although predominantly young and, according to their own statements, mostly non-allergic subjects participated in the study, almost half of them were atopic, and 10% had airway disease or bronchial hyperreactivity. This indicates that the validity of self-reported data might be inaccurate. In summary, diversified investigations of the allergy-related health status appear necessary for a thorough characterization of subjects for exposure studies on sensory irritants.

Keywords

Allergy · Asthma · Atopy · IgE · Exhale nitric oxide · Inhalation exposure · Sensory irritation · Volunteers

1 Introduction

In human risk assessment, animal data are the major source of evidence when evaluating the effects on the upper respiratory tract of sensory irritation. Nevertheless, due to major differences

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in anatomy, physiology, and airflow dynamics between rodents and humans, controlled human inhalation exposure studies are considered the gold standard for assessing acute and reversible sensory irritant effects (Brüning et al. 2014). Human studies are often criticized for the inherent variability of subjective and objective sensory irritation effects. Therefore, the characterization of a study group and the recruitment of either healthy volunteers or carefully selected subjects with predispositions to measurable reversible physiologic or biomarker responses are of particular importance. During the recruitment process, participants who are at risk of adverse outcome are excluded (Rosenkranz et al. 2020).

Since allergic inflammation results from a specific pattern of cellular and humoral responses leading to the activation of the innate and adaptive immune system, allergic subjects and those who are predisposed to developing an allergy (atopics) are likely to respond differently or more sensitively than non-allergic ones to sensory irritants. In a study of nasal sensory function, 31 subjects with seasonal allergic rhinitis (SAR) have shown a significantly lower perceptual threshold to propanol than 29 subjects without SAR ($p < 0.05$) (Shusterman et al. 2003). In another study, inhalation of 15 ppm acetic acid for 15 min has resulted in a significantly higher nasal airflow obstruction in 8 subjects with SAR compared to 8 subjects without SAR (Shusterman et al. 2005). Furthermore, in a controlled human exposure study with formic acid (Kleinbeck et al. 2018) or with ammonia (Pacharra et al. 2017), subjects with SAR have a higher blink rate than controls. Likewise, atopic subjects have a higher blink rate compared to non-atopic ones in a controlled exposure study with ethyl acrylate performed by Sucker et al. (2019). A systematic review of experimental studies on health effects of short-term exposure to airborne chemicals has confirmed that asthmatics are more sensitive to sulfuric acid and sulfur dioxide, but not to ozone and nitrogen dioxide (Johansson et al. 2016). In addition, studies on inhalation exposure show that deposition of ultrafine carbon particles in distal airways and alveoli is greater in patients with asthma than in healthy subjects (Chalupa et al. 2004; Daigle et al. 2003), which may be caused by enhanced breathing in the former due to increased dead space ventilation. Therefore, a good characterization of subjects regarding atopy, allergy, or asthma is essential in inhalation exposure studies on sensory irritants, which requires time-consuming and expensive multistage recruiting procedures. The aim of the present study was to retrospectively verify whether all tests carried out were necessary for a successful characterization of a study group. In addition, the study addressed the issue of a match between the subjective (health questionnaire) and objective (measured) data collected on atopy, allergy, and asthma.

2 Methods

2.1 Study Design

A total of 105 volunteers, 87% of whom reported to be non-allergic, participated in the study. All of them were investigated at the Institute for Prevention and Occupational Medicine of the German Social Accident Insurance, Institute of the Ruhr University (IPA) in Bochum, Germany, during the recruitment process for controlled human exposure studies on the effects of sensory irritation. Healthy adults were invited to participate in the study using information sheets published via electronic job advertisements or on the homepage of the institute. The volunteers interested in participation filled a standardized online health questionnaire assessing demographic data, smoking habits, and chronic diseases, including asthma and allergies. Inclusion criteria were age 18–40 years and never smoked tobacco or quit smoking at least 1 year before the study. Subjects who reported to be pregnant, having asthma, another chronic illness, or on prescription drugs were excluded. Since it was planned to include atopic subjects in upcoming studies, a number of subjects also were involved who reported a previous positive allergy test. Some of them reported allergic symptoms such as rhinitis and conjunctivitis, but never asthma. Subjects who met the inclusion criteria in the health questionnaire were invited to a medical examination, consisting of an interview by a physician and clinical tests.

Smoking habit was assessed by the questionnaire and verified by urine cotinine content. Arterial blood pressure was measured and a resting 12-lead ECG was performed. Blood samples were taken and investigated for standard laboratory values. In the case of clinically relevant findings or a positive cotinine test, the examination was terminated, and the subject was excluded from further analysis.

2.2 Atopy

Atopy was assessed by skin prick testing (SPT) and measurement of specific IgE antibodies (sIgE). A panel of nine common inhalant allergens (cat dander, trees, grass, latex, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, *Aspergillus fumigatus*, and *Ambrosia elatior*) and histamine 10 mg/mL (positive) and saline (negative control) solutions were used for SPT. Total IgE as well as sIgE to a mixture of common environmental allergens (*Dermatophagoides pteronyssinus*, cat and dog danders, *Cladosporium herbarum*, and pollen of timothy, rye, birch, and mugwort) (sx1 Phadiatop; ThermoFisher Phadia AB; Uppsala, Sweden) were measured with the ImmunoCAP 250 system. A positive atopic status was assumed in case of a wheal diameter greater than 3 mm in SPT to one of the allergens listed above or sIgE concentrations to sx1 $\geq$ 0.35 kU/L.

2.3 Lung Function, Methacholine Test, and Exhaled Nitric Oxide

Pulmonary function was assessed with spirometry and body plethysmography (CareFusion, Würzburg, Germany) as described earlier (Miller et al. 2005; Wanger et al. 2005). Reference values were chosen according to the Global Lung Function Initiative (Quanjer et al. 2012). By convention, the lower limit of normal (LLN) for forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), FEV_1/FVC, mean expiratory flow at 25% of the FVC (MEF25), and maximal mid-expiratory flow (MMEF) is designed to be the fifth percentile. Cases with FEV_1/FVC < LLN were classified as obstructive, those with normal FEV_1/FVC but MEF25 or MMEF < LLN as small airway disease. Methacholine test was performed as previously described with a reservoir method (Merget et al. 2005). Bronchial hyperresponsiveness was assumed when FEV_1 decreased $\geq$20% from baseline or specific airway resistance (sRt) doubled and increased to $\geq$2 kPa $\times$ s with a cumulative dose of $\leq$300 μg methacholine. Fractional exhaled nitric oxide (FeNO) measurement was performed using a Niox Mino device (Aerocrine, Solna, Sweden) according to the recommendations of the American Thoracic Society and the European Respiratory Society (ATS/ERS 2005).

2.4 Statistical Analysis

Results were reported as medians with ranges or counts and percentages and were compared using the Mann-Whitney U test for continuous variables and Fisher's exact test for dichotomous variables. Spearman's correlation coefficient (r) was used to assess the strength of relationship between pairs of variables. A p-value <0.05 defined statistically significant differences.

Receiver operating characteristic (ROC) plots are one possible graphical presentation for describing and comparing diagnostic tests. The area under the curve (AUC) is a global measure for the diagnostic performance of a test. The AUC ranges from 0 to 1. ROC plots were performed using the clinical diagnoses of obstruction, small airways diseases, and bronchial hyperreactivity based on spirometry and body plethysmography. AUCs were calculated to compare the ability of sIgE to sx1, total IgE, FeNO, and SPT to discriminate between subjects with and without airway disease/bronchial hyperreactivity. The analysis was performed using a commercial GraphPad Prism v7.04 statistical package (GraphPad Software; La Jolla, CA).

3 Results

Among the 105 subjects who completed medical examination and clinical testing, there were 14 (13%) who reported a previous positive allergy test, with 10 of them having allergic symptoms such as rhinitis and/or conjunctivitis. No subject showed symptoms at the time of the examination. With the exception of methacholine test in four cases, there were no other missing values. Personal and clinical data of the whole study group are summarized in Table 1 (left column). The group consisted mainly of young subjects and slightly more women than men (median age: 24 (19–37) years; 56% female). Forty-three subjects (41%) had sIgE to sx1 $\geq$ 0.35 kU/L, and 35 of them (81%) also showed at least 1 positive SPT. There was no positive SPT without elevated sIgE to sx1. About half of the atopics reacted in SPT to grass pollen (58%) and/or the house dust mite *Dermatophagoides pteronyssinus* (49%). Besides the atopy markers (sIgE and SPT results), the main differences in clinical data between the 43 atopic (Table 1, middle column) and 62 non-atopic subjects (right column) were higher total IgE ($p < 0.0001$), higher FeNO ($p = 0.01$), and lower FEV_1/FVC ($p = 0.01$) in atopics. However, there was no significant difference in the proportion of subjects with obstruction ($p = 0.57$), small airways disease ($p = 0.99$), and bronchial hyperreactivity ($p = 0.12$) between the two groups. When comparing atopics who reported allergic symptoms ($n = 10$) with atopics without symptoms ($n = 33$), there was no significant difference in any of the parameters listed in Table 1 (data not shown).

In addition to the finding that all SPT-positive subjects were also sIgE-positive to sx1, the number of positive SPT results correlated with the

Table 1 Demographic and clinical data of the whole study group and the two subgroups (atopics and non-atopics)

	All (n = 105)	Atopics (n = 43) sx1 $\geq$ 0.35 kU/L	Non-atopics (n = 62) sx1 < 0.35 kU/L
Age, years, median (range)	24 (19-37)	24 (19–32)	24 (19–37)
Gender, n (%)	59 (56.1%) female	21 (48.8%) female	38 (61.3%) female
BMI, kg/m^2, median (range),	22 (16–35)	23 (18–35)	22 (16–34)
Total IgE positive ($\geq$100 kU/L), n (%)	22 (21.0%)	18 (41.9%)	4 (6.5%)
Total IgE positive, kU/L, median (range)	28.8 (<2–756)	83.4 (2.9–756)	15.7 (<2–138)
sIgE sx1 positive ($\geq$ 0.35 kU/L), n (%)	43 (41.0%)	43 (100.0%)	0 (0.0%)
sIgE sx1, kU/L, median (range)	0.20 (0.07–91.30)	5.04 (0.36–91.30)	0.10 (0.07–0.33)
At least one positive SPT, n (%)	35 (33.3%)	35 (81.4%)	0 (0.0%)
D. farinae, n (%)	14 (13.3%)	14 (32.6%)	0 (0.0%)
D. pteronyssinus, n (%)	21 (20.0%)	21 (48.8%)	0 (0.0%)
Cat hair, n (%)	12 (11.4%)	12 (27.9%)	0 (0.0%)
Alternaria alternata, n (%)	4 (3.8%)	4 (9.3%)	0 (0.0%)
Aspergillus fumigatus, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Tree pollen (mix 1), n (%)	16 (15.2%)	16 (37.2%)	0 (0.0%)
Gras pollen, n (%)	25 (23.8%)	25 (58.1%)	0 (0.0%)
Latex, n (%)	1 (1.0%)	1 (2.3%)	0 (0.0%)
Ambrosia, n (%)	2 (1.9%)	2 (4.7%)	0 (0.0%)
More than three SPTs positive, n (%)	11 (10.5%)	11 (25.6%)	0 (0.0%)
FeNO, ppb, median (range)	14 (5–100)	16 (5–100)	13 (5–67)
FEV_1/%FVC, median (range)	82.4 (65.5–95.8)	80.5 (65.5–93.8)	84.1 (66.0–95.8)
Obstructive, n (%)	3 (2.9%)	2 (4.7%)	1 (1.6%)
Small airways disease, n (%)	8 (7.6%)	3 (7.0%)	5 (8.1%)
Bronchial hyperreactivity[a], n (%)	11 (10.5%)	7 (16.3%)	4 (6.5%)

[a]4 missings; *BMI* body mass index, *sIgE* specific IgE antibodies, *FEV_1* forced expiratory volume in one second, *FVC* forced volume vital capacity, *FeNO* fractional exhaled nitric oxide, *SPT* skin prick test

sx1-sIgE values ($r = 0.81$; $p < 0.0001$) (Fig. 1a). Although less pronounced, the correlation between the sx1-sIgE values and the levels of FeNO ($r = 0.24$; $p = 0.014$) was significant (Fig. 1b).

Thirteen (93%) out of the 14 subjects who had reported a previous positive allergy test were positive in both sIgE and SPT. Of the 91 subjects who reported being non-allergic, 30 (33%) were atopic, 22 (24%) with positive results in both sIgE and SPT (Fig. 2).

In 18 (17%) subjects, airway obstruction or small airway disease and/or bronchial hyperreactivity were diagnosed. Out of the 14 subjects, who reported a previous positive allergy test, 1 showed features of obstruction and bronchial

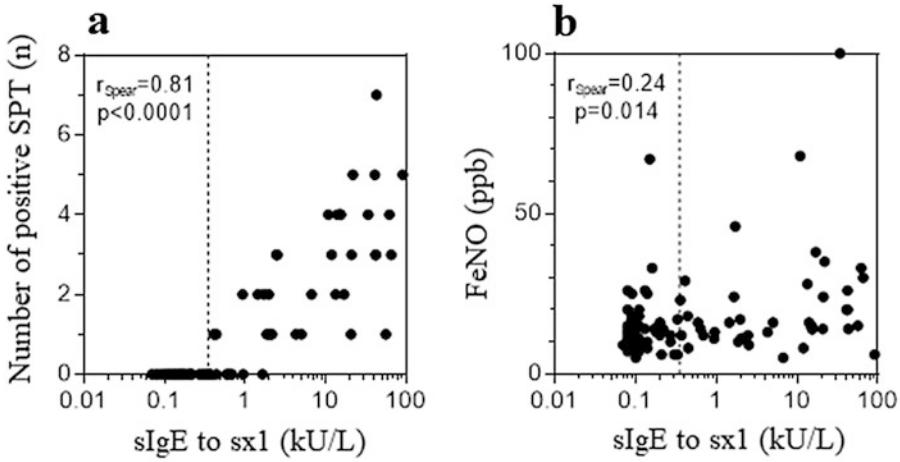

Fig. 1 Correlation between sx1-sIgE and skin prick test (SPT) results (as a number of positive SPT reactions) (**a**) and between sx1-sIgE and fractionated exhaled nitric oxide (FeNO) results (**b**). The dotted line indicates the cutoff for a positive sx1-sIgE result (≥0.35 kU/L)

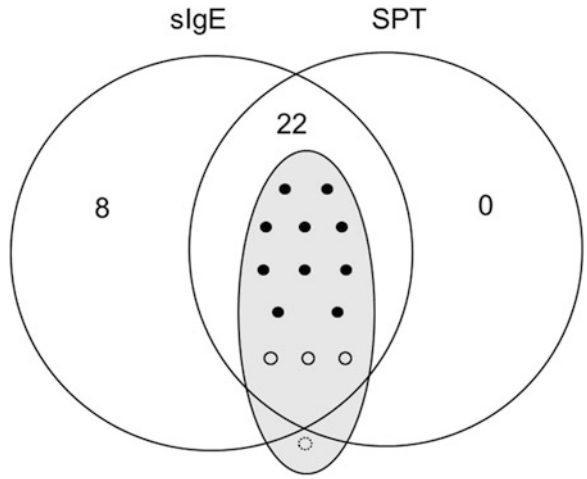

Fig. 2 The Venn diagram showing the concordance of positive sIgE results (against sx1) and SPT results (at least one positive SPT). In addition to the number of subjects indicated by numerics, the subjects who reported a previous positive allergy test (n = 14) are depicted as dots in the grey oval (filled dots: subjects reporting allergic symptoms). The dot outside the bottom intersection (circular dotted outline) represents a subject who had reported a previous positive allergy test but who did react neither in sIgE test nor in SPT

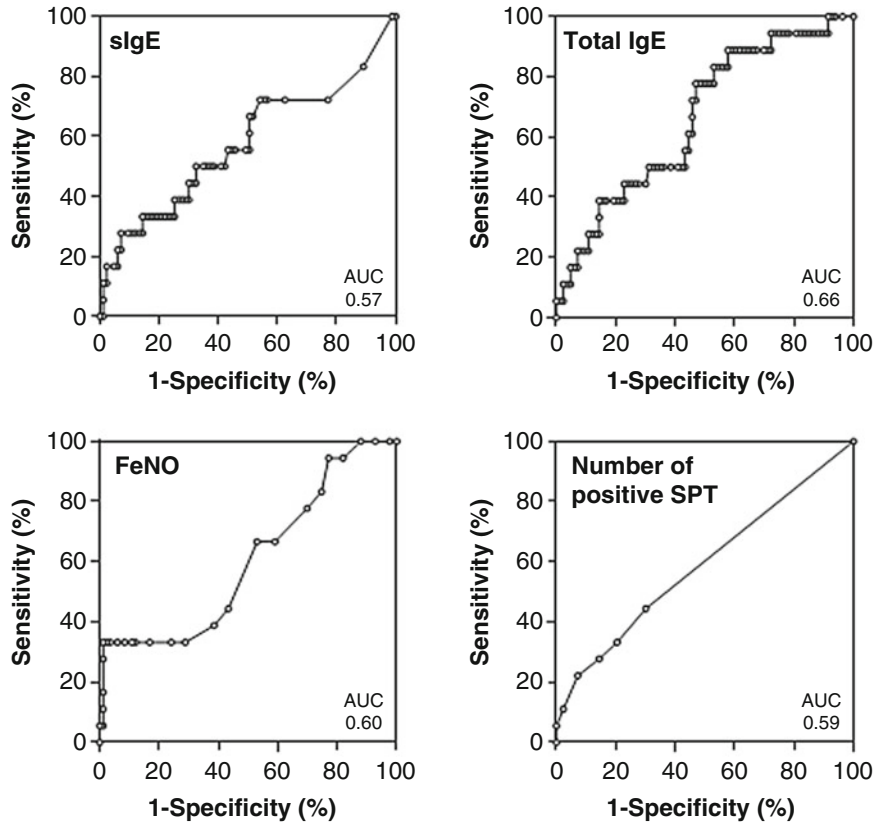

Fig. 3 Receiver operating characteristic (ROC) plots. The content of sIgE to sx1, total IgE, FeNO, and the number of positive SPT reactions were evaluated in 105 subjects under the angle of discriminating power for the diagnosis of obstruction, small airway disease, and/or bronchial hyperreactivity. *AUC* area under the curve, *sIgE* specific IgE antibodies, *FeNO* fractional exhaled nitric oxide, *SPT* skin prick test

hyperreactivity. To check whether signs of sensitization are useful to discriminate subjects with and without obstruction, small airway disease, and bronchial hyperreactivity, ROC curves were performed (Fig. 3). However, AUC reached rather low values for sIgE to sx1 (AUC = 0.57), total IgE (AUC = 0.66), FeNO (AUC = 0.60), and SPT (as a number of positive reactions; AUC = 0.59), which indicates that no variable standing alone would be useful to correctly discriminate the subjects.

4 Discussion

Since individuals with atopy, allergy, and/or asthma are suspected to be particularly susceptible to sensory irritants, extensive examinations were carried out to thoroughly characterize the study group. For this, a time-consuming and expensive multistage recruiting procedure was used. Out of the 105 young subjects who participated in the medical examination, 41% were atopic. Although this percentage is in line with the most recent "German Health Interview and Examination Survey for Adults (DEGS)" in which 45% of 18 to 29-year-olds were atopic (positive sIgE to sx1) (Haftenberger et al. 2013), it must be taken into account that 87% of our study group stated in the health questionnaire that they were non-allergic. That the outcome of a questionnaire could be inaccurate is in agreement with a literature review showing that self-reports of different types of health-risk behaviors are affected by both cognitive and situational factors (Brener et al. 2003). In fact, studies

investigating the congruence between measured and reported values for height and weight of subjects found an underreporting for weight and over-reporting for height (Neermark et al. 2019; Gorber et al. 2007). In a recent study on young and according to the initial health questionnaire for healthy volunteers, 20% showed signs of a heart, liver, or airway disease in medical examinations (Rosenkranz et al. 2020). In addition, the accuracy of self-reported information depends on the knowledge of health status. For example, in our study the subjects may have not known that they had an atopy/allergy or they may have not been willing to report it. Since 13 (93%) out of the 14 subjects, who reported a previous positive allergy test, and 30 (33%) out of the 91 subjects, who reported being non-allergic, were diagnosed as atopic, positive information in the questionnaire appears more reliable than negative information. Therefore, although the questionnaire method is relatively fast and cheap in comparison to physical examinations and clinical measurements, the validity of self-reported data, particularly on atopy and allergy, might be inaccurate.

Another question was whether all medical examinations of our study were relevant or whether the identification of subjects with atopy/allergy identified all subjects with respiratory problems. In this context it is known that for some allergens there is a close correlation between the results of sIgE and SPT. Similar to our findings, a recent study has shown a concordance between sIgE and SPT for ten tested allergens of 74–88% (Knight et al. 2018). In view of these results, the SPT might be redundant in our recruitment scheme. However, since SPT results are immediately available and in contrast to sIgE to the mixture sx1 also provide information on sensitization to perennial or seasonal allergens, it seems appropriate to test both SPT and sIgE to sx1. Taking into account the FeNO, a study on 28 asthmatic subjects has found a positive correlation between FeNO level and the number as well as diameter of positive SPT results (Ho et al. 2000). Also in our study, significantly higher FeNO levels in atopic than in non-atopic subjects were found.

In some studies, signs for eosinophilic inflammation, such as a high level of sIgE or FeNO, have appeared useful predictors for allergic asthma. The ROC analysis of the data of 96 preschool children has shown that FeNO is a good discriminator (AUC = 0.91) between children with probable asthma and healthy controls (Malmberg et al. 2003). In a group of 107 symptomatic bakers, high content of rye flour-sIgE (AUC = 0.83) and clear SPT results (AUC = 0.81) have been good predictors for an asthmatic reaction during a challenge test (van Kampen et al. 2008). However, in the present study, neither sIgE to sx1 nor total IgE, FeNO, or SPT were identified as useful tools to discriminate subjects with and without obstruction, small airway disease, or bronchial hyperreactivity (AUCs: 0.57–0.66).

Although young and according to their own statements mostly non-allergic subjects participated in the medical examination, almost half of them showed signs of atopy, and 10% had airway disease/bronchial hyperreactivity. The accuracy of self-reported data on a lack of atopy and allergy was questionable. In contradistinction, positive statements on the presence of allergy seemed mostly reliable.

In conclusion, we have shown that multiple medical investigations, even though involving enhanced effort and cost, should be included into the recruitment scheme for a successful discrimination between atopic, allergic, and asthmatic study participants.

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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 recruitment procedure and the exposure studies were approved by the Ethic Committee of the Ruhr-University Bochum in Germany.

Informed Consent All participants provided written informed consent and received financial compensation for participation.

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Impaired Coordination and Recruitment of Muscle Agonists, But Not Abnormal Synergies or Co-contraction, Have a Significant Effect on Motor Impairments After Stroke

Sharon Israely, Gerry Leisman, and Eli Carmeli

Abstract

Movement synergies, muscle co-contraction, and decreased motor drive to muscle agonists were suggested to be major factors in motor impairments after stroke. The purpose of this study was to investigate the major muscle mechanisms contributing to motor impairment after stroke. Twelve healthy and 13 post-stroke patients participated in this observational study. Both groups participated in a single experimental session, performing hand pointing movements in multiple directions, during which EMG was assessed. Additionally, the patients underwent the Fugl-Meyer assessment. A set of features from the electromyography (EMG) signal and co-contraction ratios were used to compare the capacity to modulate the muscle activity between the two groups of participants. A correlation analysis was applied between the Euclidian distances of each target and the Fugl-Meyer scoring assessment in the post-stroke patients. We found that impaired modulation of muscle activity in post-stroke patients was characterized by significantly increased Euclidian distances between the EMG features of different target directions and by a higher variability between muscle activation compared to healthy subjects. Impaired capacity to modulate muscle activity significantly correlated with the impairment status. In conclusion, impaired motor performance post-stroke systematic disturbance in the control signal to limb muscles, which manifests as decreased capacity to modulate muscle activity, rather than co-contraction of muscle antagonists or stereotyped movement patterns.

Keywords

Electromyography · Motor drive · Motor performance · Muscle activity · Muscle contraction · Stroke

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1 Introduction

Brunnstrom (1970) has described six stages of recovery of voluntary movements post-stroke, reflecting the patient's capacity to isolate a movement of a particular limb segment. According to the magnitude and location of the lesion, the restoration of arm motor function begins with flaccid paralysis, followed by return of reflexes, voluntary movement within a synergy, movements out of synergy, and finally restoration of normal movement patterns. The movement synergy is here understood as the stereotypical movement patterns emerging during voluntary movement execution, reflecting the incapacity to dissociate limb segment movements from one another (Levin et al. 2009; Krakauer 2005).

The Fugl-Meyer scale enables the classification of post-stroke patients' impairment based on the recovery stages (Fugl Meyer et al. 1975). The scale is widely used in clinical settings (Nelson et al. 2018; Harris–Love et al. 2015; Mirela Cristina et al. 2015; Ohn et al. 2013). It has been validated to represent the motor impairment status post-stroke, mainly through construct validity studies that correlate the score with those of other clinical scales (Wei et al. 2011) or with activities of daily living status (Gladstone et al. 2002). Movement synergies of different severity are commonly seen in post-stroke patients and are suggested to reflect the degree of motor impairment. However, most studies focus on the chronic stage of post-stroke recovery, when the majority of recovery had already took place (Wagner et al. 2007). In the first post-stroke month, when motor recovery is substantial, movement synergies may not be pronounced as spasticity is not yet developed. Moreover, muscle activation pattern does not necessarily reflect the capacity to move out of synergies. Beer et al. (2000) have reported that impaired modulation of muscle activity post-stroke should neither be attributed to movement synergies nor to co-contraction ratios, but rather to systematic disturbance in the control signal to limb muscles. Wagner et al. (2007), on the other hand, have suggested the changes in muscle activity in the subacute post-stroke phase reflect improvements in hand reaching. Hand reaching movements require simultaneous activation of multiple muscles upon two joints, as opposed to stereotypical movement patterns, which makes the execution of such movements difficult for post-stroke patients. When reaching to different locations, healthy subjects change the activation amplitude of muscles in the time domain. For instance, reaching across the body may require a greater involvement of the pectoralis and anterior deltoid muscles than when reaching to the body side typically executed by the middle deltoid muscle and scapular stabilizers (Israely et al. 2017a, b). Likewise, reaching to the body top involves a greater involvement of the deltoid muscles than when reaching to a lower body target.

In the present study, we set out to investigate the muscle mechanisms contributing to the motor post-stroke impairment whose degree was evaluated on the Fugl-Meyer scale. In detail, we investigated the notion that clinical manifestations of motor impairment could have to do with muscle activation pattern during a hand reaching task. We reasoned that different movement strategies and impaired coordination in post-stroke patients might be captured by the time domain properties of the electromyography (EMG) signal. Therefore, the ability to change the activation properties of muscles during different motor tasks could be inferred from the EMG signal modulation. Figure 1 illustrates the proposed concept of muscle activity modulation during movements targeted to different locations, in both post-stroke and healthy subjects.

2 Methods

2.1 Participants

The study was conducted at the Bait-Balev Rehabilitation Center in Nesher, Israel. There were two groups of participants: 13 post-stroke patients and 12 control healthy subjects. Both groups participated in a single experimental session with the EMG assessment. In addition, the patients underwent the Fugl-Meyer assessment to enable the evaluation of a correlation between

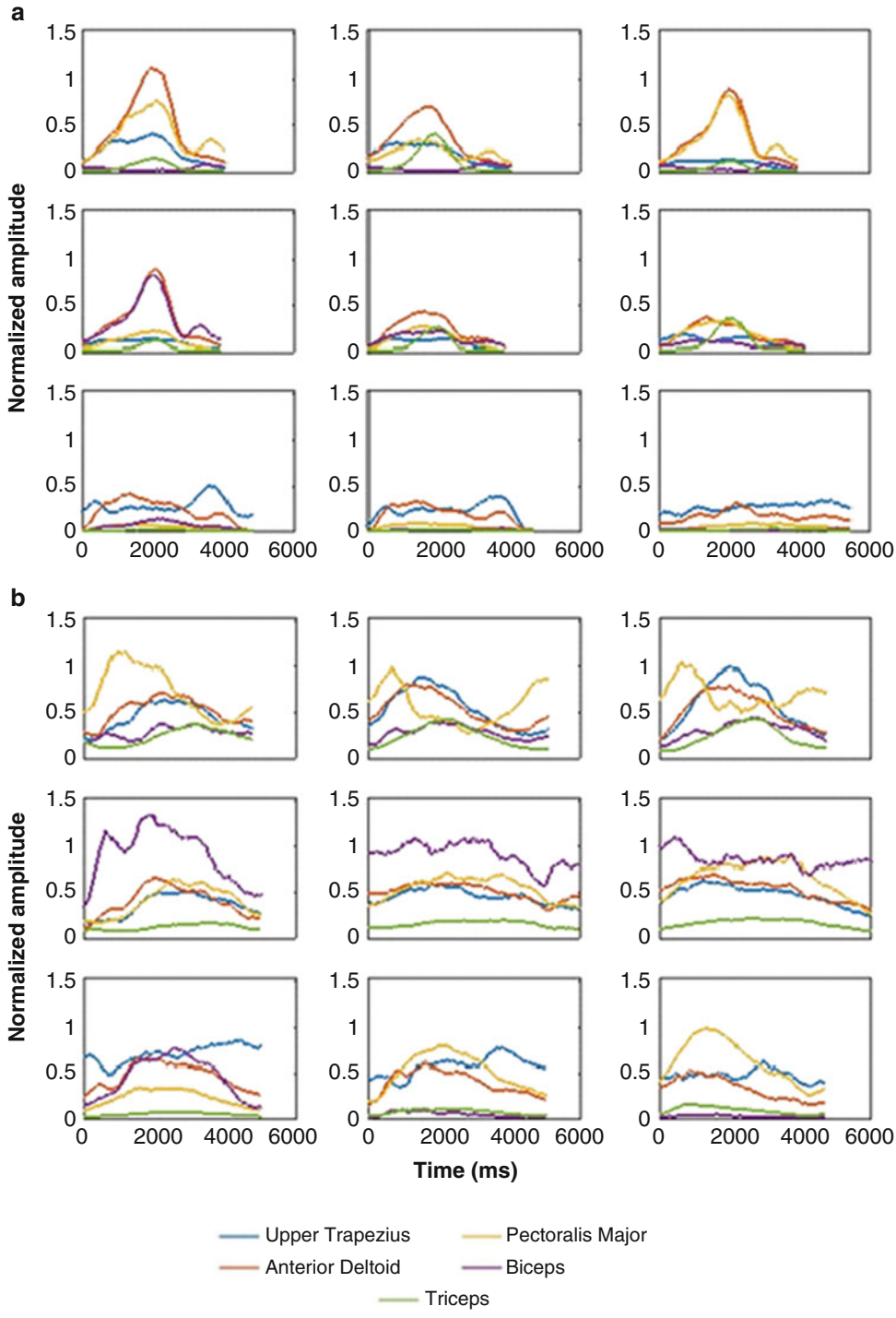

Fig. 1 Changes in muscle activation in the time domain for different movement directions, referred herein as modulation of muscle activity. Participants executed hand pointing movements to nine targets located in front of them while monitored by EMG. (**a**) EMG tracings of five muscles in a healthy subject while executing the movements to nine targets; (**b**) same traces in a post-stroke individual. Note that muscle activation in the healthy subject is more phasic, especially in the upper six movement targets, the overall exertion is smaller, and the time to

clinical score and EMG data. Cerebral stroke and hemiparesis of the upper extremity. Exclusion criteria, other central nervous system disease, and significant visual or hearing deficits. Participants' characteristics are summarized in Table 1. Post-stroke patients had mild (Fugl-Meyer score > 38; $n = 4$)-to-moderate (Fugl-Meyer score > 50; $n = 9$) motor impairment. Control subjects were contacted over the phone. The groups were about matched by the number of participants and sex, but differed by age ($p = 0.027$).

2.2 Study Protocol

Post-stroke patients were tested using the affected arm, whereas healthy subjects were tested using their self-reported dominant arm. Maximum voluntary contractions (MVC) were measured by standard muscle testing (Hislop and Montgomery 2002). The subject sat in front of a table with his forearm resting on it comfortably. The hand reaching spatial device was located as indicated in Fig. 2a. Subjects were requested to point to each target five times with a voice cue activated by EMG software every 10 s, for 45 pointing movements. The target pointing order was constant for all participants. Figure 2b illustrates the target order for a subject with a right-hand dominance. The order for the left-hand dominant subjects was horizontally mirrored but the same in the vertical dimension.

The hand reaching spatial device was composed of two vertical rods to which there were attached three semicircular shelves. Each shelf contained three movable pointing pins adjustable left- and rightward. The three shelves were located 10, 35, and 60 cm above the table. The device was positioned at the maximum hand reaching distance in front of the tested shoulder, with the middle pin (target 5) aligned with the shoulder in the sagittal plane. The side pins were positioned at a 45° angle to the shoulder joint at either side.

2.3 Electromyography

Surface EMG was recorded from eight muscles of the shoulder girdle and arm: trapezius; deltoid anterior, medial, and posterior fibers; pectoralis major; infraspinatus; biceps; and triceps (Trigno 8 setup, Delsys; Boston, MA). Electrodes were placed in accordance with the guidelines of the Surface Electromyography for the Non-Invasive Assessment of Muscles (SENIAM)–European Community Project (Hermens et al. 1999). MVC were performed prior to data collection. A 1-min rest period followed each MVC assessment to limit the possibility of fatigue. EMG signals were band-pass filtered at 20–450 Hz and were sampled at 2000 Hz.

EMG recordings were organized as an 8 by T matrix X^{8XT} for each target, where 8 was the number of muscles and T was the number of time samples. Net noise was filtered out at 50 Hz, followed by a mean subtraction. This was followed by root mean square calculations using overlapping windows of 50 samples. The mean baseline EMGs for each trial were subtracted from the averaged data. EMG data were normalized in accordance with the 70% MVC for each muscle.

Each muscle from each EMG dataset and from each of the 9 movement directions was expressed by 12 constant features. Extracted features were chosen according to the authors' considerations to construct a good low dimensional representation of the original EMG data matrix. Figure 3 details

Fig. 1 (continued) task completion is shorter when compared with those in the post-stroke individual. The post-stroke activation pattern is more tonic, especially for the six lower targets of movement. The pectoralis muscle (yellow trace) is highly activated on all of the nine movement directions. In contrast, in the healthy subject, the pectoralis muscle is strongly activated in phasic pattern for the upper three targets of movement and for the mid-left target, which corresponded to the across-body movement, and is barely active in the three lower targets

Table 1 Participants' demographics and baseline measures

Post-stroke patients	Age	Sex	Dominant hand	Side affected	Stroke type	Location	Time since stroke (days)	FM (score)	Shoulder pain (score)
1	78	F	R	L	Ischemic	R-MCA	22	53	44
2	67	F	R	R	Ischemic	L-MCA	32	51	40
3	78	M	R	L	Ischemic	R-IC	20	54	60
4	81	F	R	L	Ischemic	R-IC and Th	13	58	35
5	68	F	R	R	Ischemic	L-Tl	13	53	7
6	76	M	R	L	Ischemic	R-IC and BG	25	51	27
7	68	F	R	R	Ischemic	L-IC	9	54	0
8	89	M	R	R	Ischemic	L-pons	25	42	0
9	79	M	R	L	Hemorrhagic	R-Th	14	38	2
10	71	M	R	R	Ischemic	L-MCA	20	60	0
11	63	M	R	L	Ischemic	R-MCA	15	46	0
12	82	F	R	L	Ischemic	R-BG	26	41	0
13	81	M	R	L	Ischemic	R-MCA	20	59	0
Control subjects									
1	78	M	R	R					
2	71	F	R	R					
3	74	M	R	R					
4	54	M	R	R					
5	58	M	L	L					
6	65	M	R	R					
7	67	F	R	R					
8	70	F	L	L					
9	78	F	R	R					
10	79	M	L	L					
11	70	F	R	R					
12	67	M	R	R					

R right *L* left, *MCA* middle cerebral artery, *IC* internal capsule, *Th* thalamus, *Tl* temporal lobe, *BG* basal ganglia, *FM* Fugl-Meyer score. The Fugl-Meyer scale evaluates the degree of motor impairment with 0 (maximum) and 66 (minimum impairment). Shoulder pain was assessed on a visual analogue scale (VAS) with 100 (maximum) and 0 (no pain)

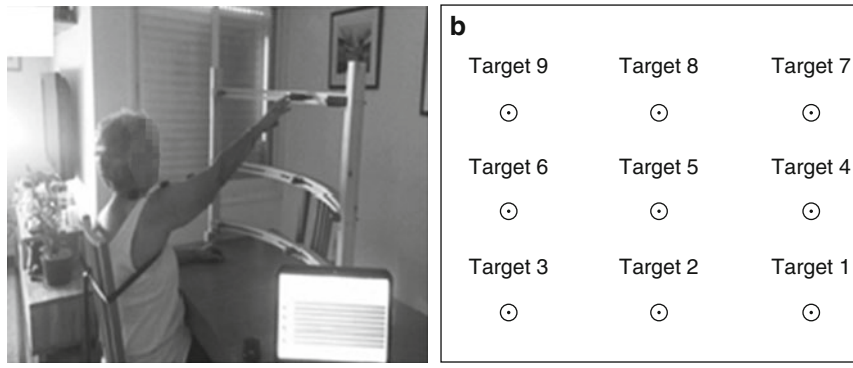

Fig. 2 The hand reaching spatial device. (**a**) Subjects were asked to reach with their dominant hand (control group) or the impaired hand (patient group) to nine different targets that were located in each participant's maximum hand reaching range of motion. (**b**) Representation of the order and direction of the targets for a subject with a dominant right hand

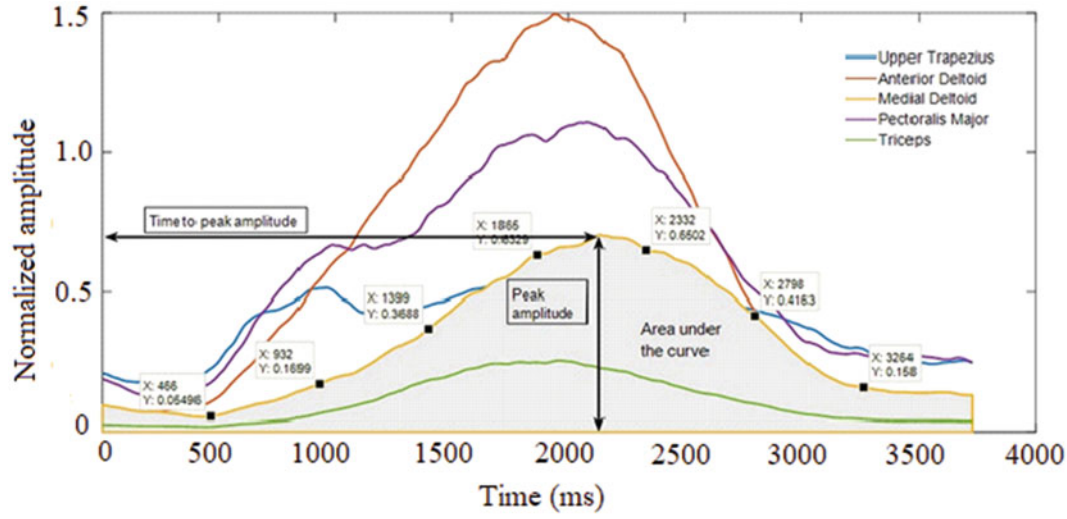

Fig. 3 A set of 12 features was extracted from the EMG signal of each muscle to compare inter-group differences in its modulation in response to different movement directions. The features comprised the time and amplitude of the first peak, the time and amplitude of the second peak, and the total area under the curve. In cases of more than two peaks or less than two peaks the relevant features were replaced as detailed in the method section. Additional seven features comprised the amplitude of seven data points equally distributed on the time domain plot. In the figure, only five muscles are shown and only one is marked by the set of features. In the study, features were extracted from eight muscles and for each movement direction. Accordingly, each subject was represented by a feature matrix for further analysis

the chosen features selected by the algorithm. The analysis was performed using Matlab (MathWorks Inc., Natick, MA).

2.4 Data

For each movement direction $i \in [1, 9]$, the EMG data $X^{8 \times T}$ was expressed as $X^{8 \times 12}$. For the first inter-group comparison, each $X^{8 \times 12}$ matrix was converted to $X^{1 \times 96}$, $f \in [1, 96]$ vector. Accordingly, each participant was expressed by $X^{9 \times 96}$ matrix ($X^{i \times f}$) for all nine movement directions. This was followed by calculating the Euclidian distance between all combinations of rows within the $X^{9 \times 96}$ matrix.

The first method applied for the comparison of differences in muscle function between the post-stroke patients and healthy subjects used the Euclidian distances between EMG features, the central fifth point of the reaching space, shown in Fig. 2b, all the other target directions (Table 2). A multivariate analysis of variance (MANOVA) was applied using the eight dependent variables in each subject for inter-group comparison. The Euclidian distance of a muscle feature for reaching each target was correlated with the Fugl-Meyer score. A second method for comparison between the two groups used the same features, however separately for each muscle (Table 3). Accordingly, each muscle tested of each subject was expressed as an eight-dimensional vector. The MANOVA was applied for each muscle separately. The third method of analysis compared the total exertion of individual muscles for each movement direction. An eight-dimensional vector represented each subject for each movement direction (Table 4). The MANOVA was again applied for comparison between the two groups.

In addition, five pairs of muscles were evaluated for co-contraction and compared between the two groups, according to previously proposed method of analysis (Israely and Carmeli, 2016; Kellis et al. 2003). Briefly, the minimum function and the maximum function of common muscle activities were calculated for each muscle pair. For each function, the total area

Table 2 Comparison of the mean Euclidian distances between EMG features for the central point of the reaching space and for all other target directions between post-stroke patients and control healthy subjects

Condition	Target 1	Target 2	Target 3	Target 4	Target 6	Target 7	Target 8	Target 9
Healthy	4.876	4.045	4.919	4.185	5.256	4.700	4.191	5.997
	(0.954)	(1.314)	(1.772)	(1.253)	(1.848)	(1.213)	(1.340)	(1.900)
Stroke	8.867	7.330	9.103	7.997	6.553	7.368	6.738	7.418
	(5.185)	(4.162)	(3.836)	(5.142)	(2.474)	(2.541)	(2.387)	(2.713)
p value	0.017	0.017	0.020	0.022	0.150	0.003	0.004	0.142

Data are means (SD); non-significant values are highlighted in gray. Note: values in the first two rows represent the Euclidian distances between two vectors as detailed in the methods section and accordingly have no units

Table 3 Differences in muscle activity, assessed by the mean Euclidian distances between EMG features of the central point of the reaching space and all other target directions, between groups

Muscle	*p* value	Target 1	Target 2	Target 3	Target 4	Target 6	Target 7	Target 8	Target 9
Upper trapezius	0.027	0.003	0.008	0.002	0.032	0.000	0.029	0.049	0.034
Anterior deltoid	0.119	0.105	0.033	0.006	0.066	0.938	0.246	0.361	0.154
Medial deltoid	0.004	0.010	0.003	0.001	0.013	0.001	0.005	0.011	0.013
Posterior deltoid	0.480	0.522	0.353	0.069	0.132	0.098	0.121	0.197	0.271
Pectoralis major	0.360	0.278	0.038	0.064	0.221	0.615	0.239	0.077	0.955
Infraspinatus	0.000	0.028	0.037	0.001	0.002	0.000	0.000	0.006	0.000
Biceps	0.189	0.438	0.218	0.239	0.100	0.191	0.063	0.012	0.005
Triceps	0.212	0.061	0.083	0.053	0.046	0.353	0.023	0.007	0.054

Multivariate analysis of variance MANOVA. The numbering of targets represents the movement directions depicted in Fig. 2

under curve was calculated. Then, the area of minimum function was divided by that of maximum function. The presence of a correlation between co-contraction indices and the Fugl-Meyer score was investigated.

The Fugl- Meyer scale, which assesses upper extremity motor function, was the main outcome measure. It consists of 33 items scored on a 3-point scale, where 0 represents the inability to complete the test, 1 represents a partial ability, and 2 represents full completion. The items assess reflexes, capacity to move in and out of synergy, and the ability to limit a movement to the shoulder, elbow, or wrist and to grasp various objects (Fugl Meyer et al. 1975). In addition, shoulder pain was assessed on a visual analogue scale (VAS) with 100 (maximum) and 0 (no pain).

There were some missing data in the statistical analysis. Three out of the 13 post-stroke patients managed to reach for the 6 lower targets, but not for the upper 3. The mean amputation technique was applied for the three missing targets in these three participants (Schafer and Graham 2002). When the assumption of homogeneity of variance was violated, ANOVA Welch test was applied instead of the standard MANOVA. Spearman's rank correlation was applied between the Fugl-Meyer score and the mean Euclidian distance of each target direction. A p value <0.05 defined statistically significant differences. The analysis was performed using a commercial SPSS v23 package (IBM Corp, Armonk, NY).

3 Results

3.1 Changes in Muscle Activity Between the Middle of the Hand Reaching Space to Other Movement Directions

Table 2 summarizes the mean Euclidian distances from the middle fifth target to each other target direction in the two groups. Distances between the fifth target and other six targets were significantly different between the two groups

Table 4 Comparison of the mean muscle exertion index, measured as the normalized EMG amplitude for each muscle and for each target direction, between groups. Accordingly, the data are unitless

Muscle		Target 1	Target 2	Target 3	Target 4	Target 5	Target 6	Target 7	Target 8	Target 9
Upper trapezius	Healthy	2,360	1,844	1,918	2,814	2,616	2,543	3,503	3,589	3,652
		(1221)	(1522)	(1791)	(1445)	(1597)	(1763)	(1758)	(1612)	(1699)
	Stroke	7,307	8,643	8,160	11,370	10,422	10,126	9,420	9,399	9,677
		(4285)	(5523)	(5961)	(7431)	(6768)	(6785)	(4271)	(4203)	(5155)
	p value	0.001	0.001	0.003	0.001	0.001	0.002	0.002	0.002	0.005
Anterior deltoid	Healthy	1,569	2,138	2,751	2,036	2,575	3,650	2,371	3,101	4,380
		(734)	(1024)	(1067)	(663)	(1027)	(859)	(941)	(1040)	(977)
	Stroke	4,955	6,242	6,424	6,871	6,955	7,204	6,198	6,999	7,678
		(2137)	(3026)	(2890)	(3298)	(3059)	(3325)	(2563)	(3015)	(3641)
	p value	<0.001	<0.001	0.001	<0.001	<0.001	0.002	0.001	0.003	0.019
Medial deltoid	Healthy	1,504	1,254	1,019	2,049	1,800	1,516	2,744	2,621	2,186
		(334)	(425)	(497)	(658)	(599)	(568)	(906)	(748)	(645)
	Stroke	5,621	6,115	4,713	8,501	6,834	5,894	7,953	7,707	7,197
		(2781)	(4849)	(3626)	(6020)	(4492)	(4195)	(4594)	(5281)	(6281)
	p value	<0.001	0.004	0.003	0.002	0.002	0.003	0.006	0.014	0.033
Posterior deltoid	Healthy	535	345	462	765	436	355	847	534	404
		(293)	(226)	(713)	(638)	(457)	(322)	(364)	(276)	(230)
	Stroke	2,438	3,109	2,341	4,465	3,207	2,703	4,125	3,930	3,887
		(3089)	(5187)	(3104)	(6574)	(4815)	(3911)	(6050)	(6056)	(7183)
	p value	0.047	0.079	0.053	0.066	0.061	0.052	0.121	0.110	0.160
Pectoralis major	Healthy	1,091	1,525	3,441	1,271	1859	4,037	1,073	1,632	3,675
		(1132)	(1024)	(2974)	(1134)	(1729)	(3223)	(940)	(1086)	(2873)
	Stroke	3,742	5,676	8,087	4,441	4,897	6,825	3,138	4,238	5,570
		(4010)	(6234)	(6064)	(5586)	(4233)	(4795)	(3181)	(3604)	(3097)
	p value	0.038	0.035	0.024	0.063	0.030	0.100	0.075	0.051	0.156
Infraspinatus	Healthy	1,306	1,313	1,258	1,493	1,378	1,166	1,569	1,640	1,430
		(198)	(290)	(430)	(459)	(406)	(308)	(498)	(366)	(401)
	Stroke	4,972	5,996	6,307	7,196	6,716	6,755	6,090	6,312	6,474
		(2841)	(4375)	(4667)	(5174)	(4542)	(4944)	(3815)	(4207)	(5010)
	p value	0.001	0.002	0.002	0.002	0.001	0.002	0.005	0.007	0.011
Biceps	Healthy	440	483	462	500	478	454	495	488	441
		(198)	(265)	(231)	(232)	(266)	(236)	(208)	(262)	(205)
	Stroke	2,248	2,765	2,717	3,191	3,004	2,929	1,786	1,849	1,818
		(1670)	(2330)	(2062)	(3168)	(3134)	(2602)	(902)	(940)	(843)
	p value	0.002	0.004	0.002	0.010	0.013	0.005	0.001	0.001	<0.001
Triceps	Healthy	1,451	1,357	925	1,670	1,227	900	1,826	1,582	1,085
		(886)	(958)	(718)	(1228)	(853)	(838)	(1400)	(1187)	(997)
	Stroke	3,569	4,112	3,012	5,666	4,494	3,975	5,290	5,055	4,870
		(2764)	(4660)	(3277)	(5956)	(4465)	(3909)	(5357)	(5149)	(5480)
	p value	0.020	0.057	0.043	0.063	0.023	0.016	0.075	0.064	0.058

Data are means (SD); Welch's ANOVA was applied for comparisons. The numbering of targets represents the movement directions depicted in Fig. 2

($p < 0.05$) in that they increased post-stroke, reducing the possibility for movement synergies. Targets 6 and 9 were placed in such a way that the patients had to execute the hand pointing movements across the body and to the upper portion of the reaching space. These movements required a greater muscle exertion of deltoid and pectoralis muscles also in healthy subjects,

assumingly leading to non-significant inter-group differences. Movements to other locations in space required a smaller effort in healthy subjects but were still demanding for post-stroke patients, which led to significant inter-group differences.

Table 3 illustrates the modulation of each muscle activity in the time domain between different targets for both groups. Using single muscle activity for inter-group comparisons produced less consistent differences that were present only in the upper trapezius, medial deltoid, and infraspinatus but not in the other muscles. That might indicate that differences in muscle activity between the two groups depended on interactions between muscles and not only on the activity of single muscles. With reference to the previous assumption regarding the non-significant inter-group differences for targets 6 and 9, Table 3 does illustrate non-significant inter-group differences for the anterior and posterior deltoids and pectoralis muscles but significant differences for the medial deltoid. The first three muscles are highly active in movements across the body and in the top of the reaching space, i.e., for targets 6 and 9. The non-significant inter-group differences concerning these muscleswith our assumption. However, the significant difference between the medial deltoids was with the assumption.

3.2 Muscle Exertion for Different Movement Directions in the Healthy and Post-stroke Groups

The posterior deltoid, pectoralis major, and triceps demonstrated non-significant inter-group differences in some of the movement directions (Table 4). Non-significant differences in pectoralis for higher target directions were consistent with our assumption of non-significant inter-group differences in the first analysis method for targets 6 and 9. The posterior deltoid was less activated during hand reaching in both groups as indicated by lower exertion values compared to the other muscles, suggesting that this muscle did not play a key role in task completion. A non-significant difference in the triceps muscle was rather surprising, given its crucial role in extending the elbow, a movement reportedly shown to be significantly impaired post-stroke (Tomita et al. 2018; Beebe and Lang 2009). Dysfunction of elbow extension post-stroke may be aggravated due to impaired recruitment of muscle agonist (triceps) or hyperactivity of muscle antagonist (biceps). Both factors are related to the extent of the integrity of the corticospinal tracts, i.e., stroke severity (Lindenberg et al. 2010) and the time lapse from stroke (Harris–Love et al. 2015). In the present study, patients were in the subacute phase post-stroke with mild-to-moderate motor impairment, and they apparently sustained less biceps co-contraction, resisting the elbow extension by the triceps.

3.3 Correlation Between Muscle Activity and Fugl-Meyer Score

Correlation was investigated between the Fugl-Meyer score and Euclidian distances between targets. The mean of each individual modulation matrix, i.e., the Euclidian distance from each of the nine targets to all other eight targets, was calculated, so that each individual was represented by modulation vector. Table 5 illustrates the results in which significant negative correlations indicate that an increased Euclidian distance between target features corresponded to low Fugl-Meyer scores.

3.4 Muscle Co-contractions in the Healthy and Post-stroke Individuals

Muscle co-contraction ratios between pairs of muscles were calculated and compared between the groups of healthy and post-stroke individuals. Table 6 illustrates the p values of MANOVA evaluation, using the co-contraction ratios between five muscle pairs in both groups. The results were not significant in the three movement directions 4, 5, and 7. These three targets were

Table 5 Correlation between the mean Euclidian distance and the Fugl-Meyer score ($n = 13$)

	Target 1	Target 2	Target 3	Target 4	Target 5	Target 6	Target 7	Target 8	Target 9
Spearman's coefficient	−0.549	−0.527	−0.519	−0.554	−0.577	−0.596	−0.557	−0.546	−0.607
p value	0.052	0.064	0.069	0.049	0.039	0.032	0.048	0.053	0.028

Table 6 Comparison of co-contraction ratios for five muscle pairs between healthy subjects and post-stroke patients

	Pairwise comparisons; *p* values				
	Trapezius-anterior deltoid	Anterior deltoid-posterior deltoid	Pectoralis major-posterior deltoid	Anterior deltoid-biceps	Biceps-triceps
Target 1	0.007	0.879	0.781	0.058	0.138
Target 2	0.001	0.057	0.105	0.028	0.304
Target 3	0.021	0.192	0.751	0.025	0.076
Target 4	0.256	0.476	0.251	0.023	0.056
Target 5	0.074	0.757	0.741	0.052	0.028
Target 6	0.007	0.012	0.010	0.001	0.239
Target 7	0.019	0.685	0.043	0.075	0.063
Target 8	0.002	0.008	0.306	0.014	0.092
Target 9	0.001	0.004	0.006	0.002	0.330

located at the body side and required a shoulder horizontal abduction, in contrast to targets 6 and 9 that required reaching across the body with a horizontal adduction. Reaching across the body to targets 3, 6, and 9 showed significant differences in inter-group co-contraction ratios, due assumingly to greater exertions required to complete the task. There were non-significant inter-group differences in the co-contraction ratio in three pairs of muscles. This might reasonably justify the notion that the emergence of stereotyped movement patterns evolves later through recovery and does so in patients with a greater degree of motor impairment. The right-hand column in Table 5, for instance, illustrates that the ratio between the recruitment of the biceps and triceps muscles was preserved in the subacute phase. Accordingly, impaired elbow extension was probably not due to overactivity of the biceps antagonizing the triceps, but rather due to impaired recruitment of the triceps (Molina Rueda et al. 2012; Wagner et al. 2007). These findings suggest that the co-contraction ratios did not play a major role in motor impairments in the subacute phase of the post-stroke patients.

Figure 4 illustrates the means of co-contraction ratios for each pair of muscles separately. It emphasizes that apart from the co-contraction between the trapezius and the anterior deltoid, the other four pairs of muscles were recruited in a similar manner in both groups, despite a greater exertion of muscles in the post-stroke patients (Table 4). Significant inter-group differences between the trapezius and the anterior deltoid could assumingly result from overactivity of the trapezius to compensate for a weaker deltoid (Israely et al. 2017b; Levin et al. 2009; Wagner et al. 2007). Correlations between the Fugl-Meyer score and co-contraction for all of the muscle pairs were non-significant, which is compatible with the non-significant differences in co-contraction ratios between the two groups, reinforcing the assumption that mild-to-moderate motor impairments in the subacute phase of

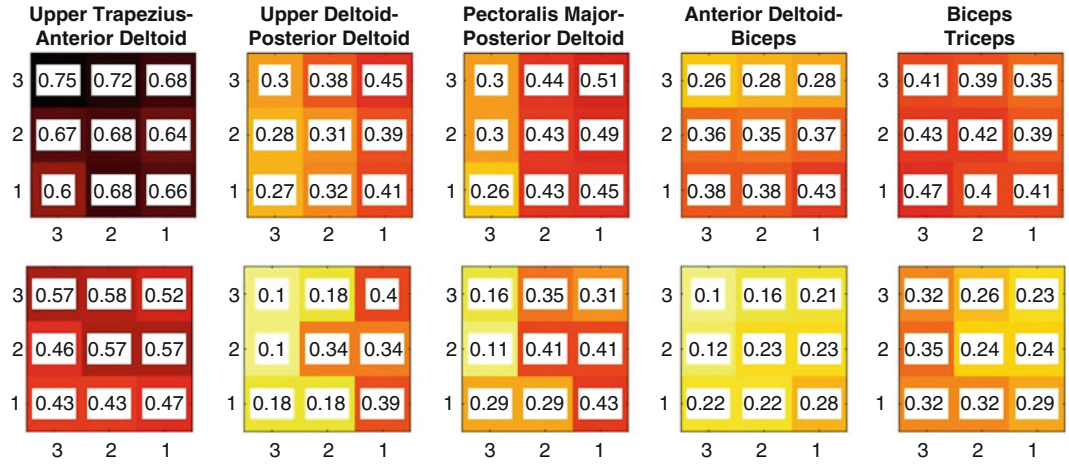

Fig. 4 Mean co-contraction ratios of post-stroke patients (upper row) and healthy subjects (bottom row) of five muscle pairs for nine movement directions. Each column refers to a different pair of muscles. Co-contraction between a pair of muscles was defined as the ratio between the minimum function of the two muscles and the maximum function of the two muscles and was accordingly graded on 0 to 1 scale. Dark colors indicate higher co-contraction ratios, which means that timing and amplitude of recruitment of the two relevant muscles were more similar. Lower values, marked by brighter colors, indicate different recruitment properties of the two muscles. The digits 1, 2, and 3 around the boxes indicate the order of targets executed during hand reaching by the right-handed participants. They applied hand reaching to the first row of targets, followed by the second row and then the third row. In each row, the first applied hand reaching was to column number 1, followed by column number 2 and then column 3. For example, the fifth target is the target in the second row in the second column

stroke patients are not due to increased co-contraction ratios.

4 Discussion

We investigated muscle mechanisms underlying motor impairments, in post-stroke patients, who were mostly categorized as suffering from mild motor impairments, in the subacute post-stroke phase. In this cohort, we evaluated their capacity for executing demanding task, which apparently could not be executed in patients with higher degrees of motor impairment. Three patients could execute hand reaching to only six targets instead of nine targets. During data collection, it was noticed that post-stroke patients struggled to execute this task. To minimize the use of compensation strategies, a trunk belt was used to eliminate trunk displacement, forcing the patients to complete the task without recruiting additional abnormal degrees of freedom. The aim was to study muscle mechanisms that contribute to motor impairments under these circumstances, knowing that the well-defined cohort may limit the generalization of results. Movement synergies were measured by the extent to which patients modulated their muscle activity in the time domain, for different directions. Since movement synergy is characterized by stereotypical movement pattern, we hypothesized that it would be reflected by decreased Euclidian distances between different directions in the post-stroke condition. The second mechanism investigated was co-contraction between five pairs of antagonistic muscles.

Post-stroke patients modulate muscle activity in a different manner than healthy subjects do. Contrary to our assumption, Euclidian distances between targets were significantly increased in post-stroke patients, when compared to healthy subjects. Increased Euclidian distances between targets showed large changes in the pattern of muscle activity between targets, suggesting that motor impairments were probably not due to stereotyped movement patterns. These findings are compatible with previous reports showing that pathologic movement synergies

mostly appear in later stages of recovery (McPherson and Dewald 2019; Pandian and Arya 2012) and in patients with more pronounced motor impairments (Cirstea and Levin 2000). Capacity to move out of synergies and to execute isolated joint movements in the subacute phase of recovery may indicate a better prognosis for recovery of physiologic movement patterns (Krakauer and Marshall 2015; Winters et al. 2015).

In this study we revealed significant negative interactions between the Fugl-Meyer scores and muscle activity. The larger the Euclidian distance between targets, the lower the score. The validity of these interactions in mildly impaired patients should be cautiously considered due to the ceiling effect of proximity to the maximum Fugl-Meyer score. Significant differences in muscle activity modulation between the stroke patients and healthy subjects indicate that motor impairments are manifested by interactions between activities of different muscles rather than by activation properties of single muscles. Although muscle exertion was significantly higher in post-stroke patients (Table 4), co-contraction ratio was not a major factor in motor impairments.

Concerning the movement components above outlined, the Fugl-Meyer score enables the quantification of motor impairment in a clinical setting. Since the Fugl-Meyer scale assesses sub-movements, and as such enables the isolation of segmental movements, a decreased score can be interpreted as movement synergies or increased co-contraction. The present findings were with this notion despite significant correlations between muscle activity modulation and the Fugl-Meyer score. The findings illustrate that larger movement impairments correlated with increased Euclidian distance between different target directions. Movement synergies were not the main source of motor impairments observed. This notion is reinforced by the Euclidian distances of the patients being significantly greater when compared to those of the control subjects. The present findings are in line with those of Beer et al. (2000) who have revealed abnormal spatial tuning of muscle torque at the elbow, used to initiate movements of the paretic limb in hemiparetic patients. Those authors suggest that spatial abnormalities result from systematic disturbances in the control signal to limb muscles rather than muscle weakness, spasticity, or muscle synergies. In the present study, co-contraction ratios between five pairs of muscles were not significantly different between the stroke patients and healthy subjects, suggesting that motor impairments were probably not related to co-contraction between antagonists. These findings are with those of other studies that investigated patients in the chronic stage of recovery from more severe motor impairments (Ohn et al. 2013; Leonard et al. 2006).

Significant correlations between our model of the assessment of muscle activity impairment post-stroke and the Fugl-Meyer scale are not settled with the underlying mechanisms causing motor impairments, i.e., movement synergies and increased co-contractions. In this study patients were examined approximately 3 weeks post-stroke and suffered from mild-to-moderate impairments. Spasticity was not strongly pronounced in these patients during recovery; therefore muscle activations were less prone to stereotypical movement patterns. It seems that during recovery from flaccid paresis, patients scan for a proper group of agonistic muscles to efficiently execute the movement manifest by increased Euclidian distances between targets. A larger task demand probably requires recruitment of additional synergistic muscles for task completion (Israely et al. 2017a).

Our present findings underscore the necessity for differentiation between a degree of motor impairment and the time elapsed since stroke, when formulating a suitable treatment program, as has also been suggested by others (Carmichael and Krakauer 2013). Twitchell (1951) and Brunnstrom (1970) have suggested that control over basic synergies should be achieved in order to execute more complex movements. Accordingly, during early stages of recovery, patients should be aided or encouraged to use these synergies (Cirstea and Levin 2000; Bobath, 1990; Carr and Shepherd 1989).

In the last decades, task-oriented training (TOT) has been indicated to promote functional

independence. TOT was previously defined as treatment approach that focuses on meaningful complex movements, with real-life object manipulation in a real-life environment (Timmermans et al. 2010). Numerous studies have evaluated the efficiency of TOT, but controversies remain as yet unsettled regarding its efficiency for improving motor function (Thant et al. 2019; Almhdawi et al. 2016; Jeon et al. 2015; Pollock et al. 2014; French et al. 2007). The mechanisms for motor improvement, motor learning, and motor compensation remain unsettled as well (Krakauer and Cortés 2018; Kitago et al. 2013; Roby-Brami et al. 2003).

Animal experiments and human studies have demonstrated the necessity to focus on reducing motor impairment by enhancing motor learning in the first 6 months post-stroke (Krakauer et al. 2012; Moon et al. 2009). In that context, therapists should direct toward the execution of motor task with the right movement pattern. In severe cases of stroke, therapists may decrease the degrees of freedom to be used by the patient to ease the motor task or may use assisted approach to bridge the agonist recruitment deficits. Moreover, therapists should apply the commonly known rehabilitation principles for motor learning, which include distributed practice (frequent rest periods within a session), contextual interference (using different objects to manipulate), task specific variability (different textures, weights, arm range of motion, direction of movement), feedback by knowledge of results, and increased task demands (Israely et al. 2017b; Schweighofer et al. 2011; Krakauer 2006). In moderately to severely (Fugl-Meyer <45 points) impaired patients, with increased muscle tone 2 weeks post-stroke (Ashworth scale >2), a greater attention should be taken for preserving joint motion ranges and to decrease a chance for contractures in later stages of recovery (Triccas et al. 2019; Urban et al. 2010; Wissel et al. 2010). Platz et al. (2001) have introduced the arm ability training that implemented the above principles, tailored to post-stroke patients with mild impairment. TOT is considered the intervention of choice in patients with mild motor impairment, as long as the emphasis is placed on correct movement pattern and motor learning principles.

This study has several limitations. A rather small number of participants might affect between-group differences. It also could have an impact on the application of classification algorithms validating our findings. Patients had just mild-to-moderate motor impairments, which makes it difficult to generalize the findings for more severe post-stroke conditions. Nonetheless, we believe we have shown that impaired capacity for modulating muscle activity for different movement directions is a good indicator of the overall motor impairment of the upper extremity in milder post-stroke stages of motor impairment. Our findings suggest that in the subacute phase of recovery, motor impairment cannot be attributed to movement synergies or increased co-contraction between muscle antagonists, but rather to impaired coordination and recruitment of muscles agonists, resulting in increased demand on synergistic muscles. It might be assumed that corticospinal tract integrity may facilitate isolated joint movement out of movement synergies.

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 Review Board of the Bait-Balev Rehabilitation Center in Nesher, Israel, and it was registered at ClinicalTrials.gov#NCT03063151.

Informed Consent Informed consent was obtained from all individual participants included in the study. In addition, the subject presented in the photo of Fig. 2 gave written informed consent permitting the reproduction of his body in both electronic and printed versions of the article.

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Prognostics of Hospitalization Length and Mortality in Patients with Traumatic Frontal Brain Contusions

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Abstract

Traumatic brain injury has ripple effect on the physical, cognitive, behavioral, and emotional domains of quality of life and portends a long-term neurological disability in survivors. In this study we evaluated the prognostic role of demographic and clinico-radiological variables on the hospitalization length and mortality in 71 of patients with frontal brain contusions. The receiver operating characteristic (ROC) plots were performed, with area under the curve (AUC) values, for graphical comparison of variables that would predict mortality and hospitalization length. We found that the best prognostics of mortality were the Glasgow Coma Scale score, the motor function score, and the Rotterdam CT score, with AUC values of 0.873, 0.836, and 0.711, respectively. Concerning the prediction of hospitalization length, the AUC showed inappreciable differences, with the highest values for the Glasgow Coma Scale score, Rotterdam CT score, and the serum cortisol level in a 0.550–0.600 range. Curve estimation, based on multivariate analysis, showed that the scores of motor function, Glasgow Coma Scale, and Rotterdam CT correlated best with the prediction of both mortality and hospitalization length, along with the upward dynamic changes of serum cortisol for the latter. We conclude that basically simple and non-invasive assessment in survivors of acute traumatic brain contusion is helpful in predicting mortality and the length of hospital stay, which would be of essential value in better allocation of healthcare resources for inpatient treatment and rehabilitation and for post-hospital patient's functioning.

Keywords

Clinical outcome · Hospitalization length · Mortality · Traumatic brain contusion

1 Introduction

Acute brain injury is the most common sequelae of all traumatic incidents. Frontal contusions comprise 43.4% of the intracranial pathologies resulting from trauma (Papo et al. 1982). Moreover, such contusions have a ripple effect on the physical, cognitive, behavioral, and emotional domains of quality of life, and they also harbinger variable long-term neurological disabilities

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among the survivors (Kurland et al. 2012). Although most of the patients are initially neurologically preserved, there is a high tendency for rapid progression of brain lesions, leading to sudden brain herniation and eventual death (Chen et al. 2018; Rehman et al. 2008). Brain injury also embarks a significant negative effect on daily lives of patients and caretakers, and it connotes a substantial enduring socioeconomic burden for families and society (Chen et al. 2006).

There is a paucity of neurosurgical studies pertaining to the prognostic value of various clinical factors in predicting the length of hospitalization and mortality among patients with traumatic brain injury. Thus, this study seeks to predict outcome in patients with traumatic frontal contusions based on demographic and clinico-radiological variables referring to the total length of hospitalization and mortality.

2 Methods

2.1 Patients and Study Design

There were 71 patients enrolled in the study with the diagnosis of traumatic frontal contusions who were hospitalized in the Department of Neurosurgery at the Nobel Medical College and Teaching Hospital in Biratnagar, Nepal, during January 2017 and June 2019. Exclusion criteria consisted of significant associated polytrauma with hemodynamic instability, patient's refusal to participate in the study, or leaving the hospital in the course of treatment against medical advice.

We evaluated demographic and clinico-radiological variables that could have a bearing in predicting the length of hospitalization and mortality (outcomes) among patients with traumatic frontal contusions, such as patient age, gender, initial Glasgow Coma Scale, Rotterdam computed tomography (CT) score, assessment of motor function, bilateralism on opposing body sides, changes in the serum cortisol and thyroid hormones from the first to the third day of admission, accompanying polytrauma, management mode, and the presence of other concurrent brain lesions. Study outcomes were the length of hospitalization and the mortality rate in patients with traumatic frontal contusions.

2.2 Sample Size and Data Elaboration

The minimum sample size, required to achieve the study goal, was calculated as 32 patients using the following formula:

$$n = z^2 \text{ x } p \text{ x } q/d^2$$

where z is 1.96 at 95% confidence interval; p, 20% prevalence of traumatic frontal contusions; q, 1-p; and d, 10% margin of error.

The receiver operating characteristic (ROC) plots were performed, with area under curve (AUC) values, for graphical presentation and comparing variables that would be useful for predicting the length of hospitalization and morbidity among patients with traumatic frontal contusions. The analysis of variance (ANOVA) and multivariate logistic regression, along with the logistic coefficient curve, were used to evaluate the effects of different variables, which could affect the two outcome variables: mortality rate and hospitalization length. The analysis was performed using a commercial statistical package of SPSS v16 (IBM Corp., Armonk, NY).

3 Results

In the ROC analysis, the best predictors of mortality in patients with traumatic frontal contusion were the score of the Glasgow Coma Scale, motor function score, and Rotterdam CT score, with the AUC values of 0.873, 0.836, and 0.711, respectively (Fig. 1). One-way ANOVA for predicting mortality yielded a significant p-value only for the three variables aforementioned; 0.001, 0.003, and 0.005, respectively. Concerning the prediction of hospitalization length, the AUC showed inappreciable differences, with the highest values also for the Glasgow Coma Scale score (0.592), Rotterdam CT score (0.589), and the serum cortisol level (0.564) (Fig. 2).

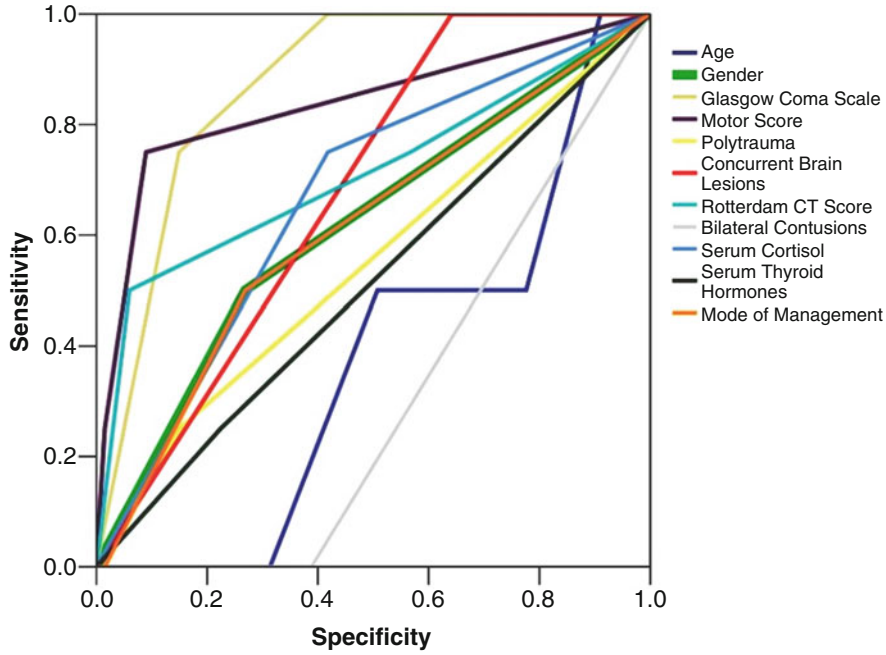

Fig. 1 Receiver operating characteristic (ROC) plots of clinico-radiological variables for predicting mortality among patients with traumatic frontal contusions

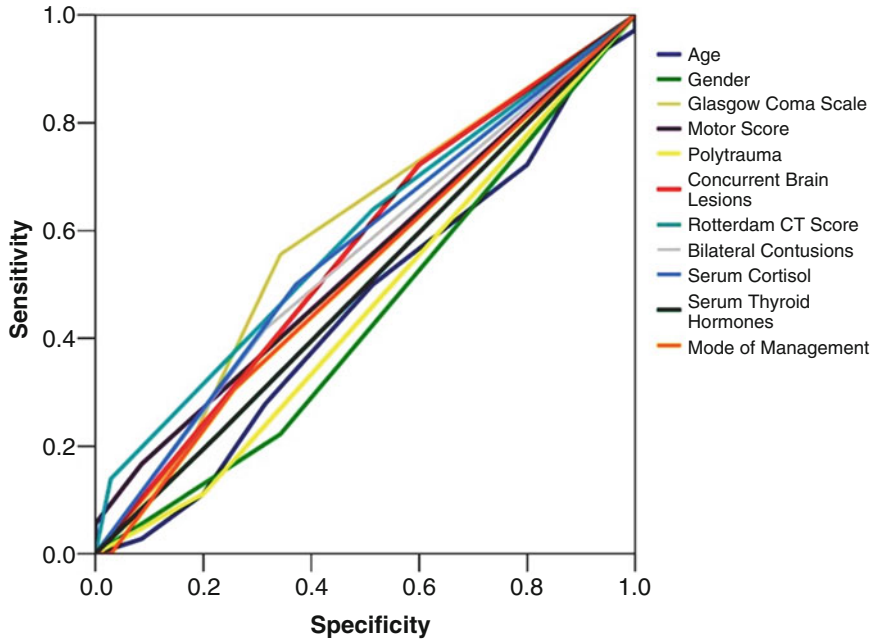

Fig. 2 Receiver operating characteristic (ROC) plots of clinico-radiological variables for predicting hospitalization length among patients with traumatic frontal contusions

In multivariate analysis, curve estimation of the size and direction of the relationship between a predictor, i.e., clinical and radiological variables investigated, and the response outcomes, i.e., mortality and hospitalization length, are depicted in Figs. 3 and 4, respectively, For mortality, the best prediction yielded the motor score ($p = 0.001$), Glasgow Coma scale score ($p = 0.001$), and Rotterdam CT score ($p = 0.047$). For the hospitalization length, prediction was significantly associated with the Glasgow Coma Scale score ($p = 0.001$), Rotterdam CT score ($p = 0.001$), mode of management ($p = 0.001$), serum cortisol content ($p = 0.006$), and the presence of concurrent brain lesions ($p = 0.005$).

4 Discussion

In this study we assessed the efficacy of clinico-radiological and biochemical variables in predicting mortality and hospitalization length of patients with traumatic frontal brain contusions. There is a paucity of studies addressing the issue that is of substantial practical interest in the face of frequency of such injuries and uncertain outcome and complicating management. In the assessment, we used a spectrum of different factors and variables, such as the Glasgow Coma Scale score, the Rotterdam CT score, and the motor function score at presentation, the presence of concurrent brain pathologies, the associated polytrauma, and the dynamics of changes in the content of serum cortisol and thyroid hormones. We also took into account patients' demographics such as age and gender and the management algorithm performed in these patients. The findings, in general, were that the factors above outlined positively correlated with both mortality and hospitalization length in patients with acute frontal brain injury as depicted in curve estimation, based on multivariate regression analysis (Figs. 3 and 4). There were, however, differences in the prognostic power among these factors.

The Glasgow Coma Scale was the most significant item in predicting both mortality and

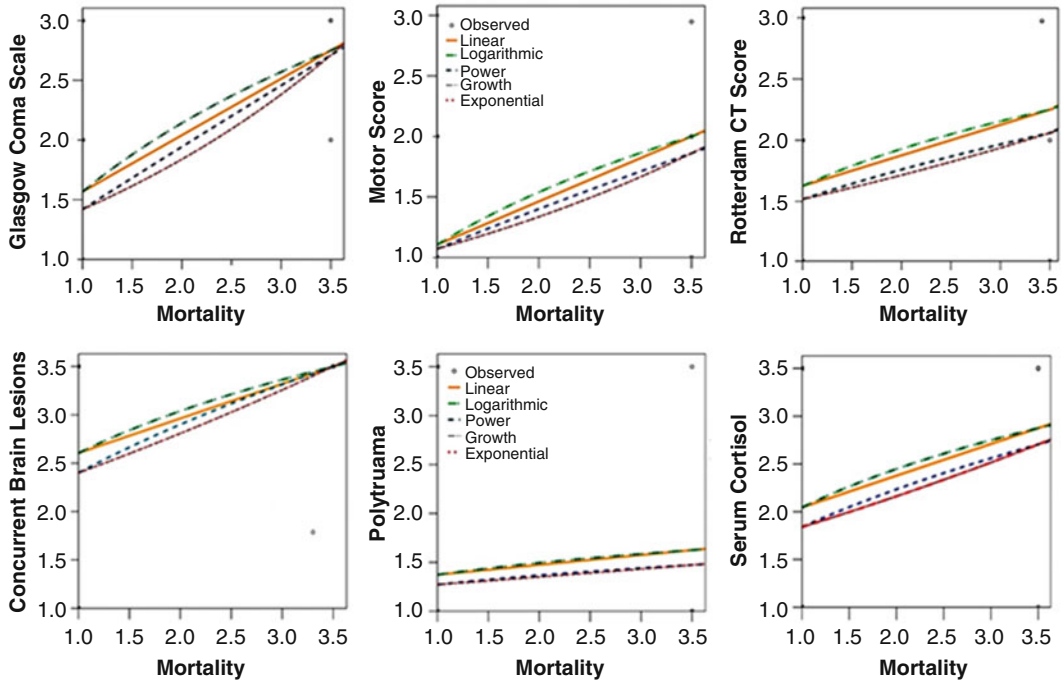

Fig. 3 Curve estimations of clinico-radiological variables being associated with the prediction of mortality among patients with traumatic frontal contusions

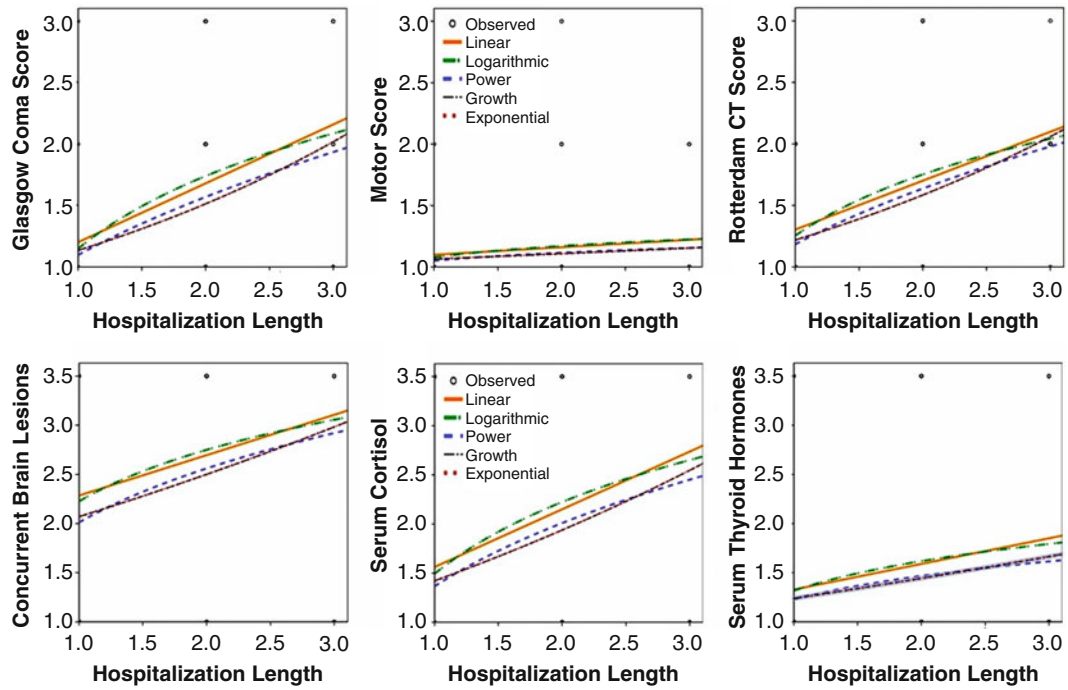

Fig. 4 Curve estimations of clinico-radiological variables being associated with the prediction of hospitalization length among patients with traumatic frontal contusions

hospitalization length in patients with traumatic frontal contusions. For mortality, the AUC value corresponding to this scale in the ROC plot amounted to 0.873. The finding pointing to the essential importance of the scale in the patient assessment is in line with other previous studies on the subject (Arango-Lasprilla et al. 2010; Hung et al. 2004). However, the score of motor function at presentation and the Rotterdam CT score ran closely behind the Glasgow Coma scale score in predicting mortality, in terms of the AUC values. Moreover, the assessment of motor function seems superior and more reliable in some patients, as the eye and verbal components of the Glasgow Coma Scale might be adversely influenced by drugs, alcohol intoxication, or patient intubation. In addition, motor function assessment is the only one clearly and objectively achievable when a full Glasgow Coma Scale cannot be performed. The Rotterdam CT score, which helps evaluate the course of acute traumatic brain injuries (Munakomi 2016), also appeared useful in predicting both mortality and hospitalization length in our patients. The score was a particularly strong determinant regarding mortality, with the AUC of 0.711.

In addition, we found in this study that an upward change in the serum cortisol during the first few days of hospitalization due to frontal brain injury was a significant prognostic of mortality. The role of the assessment of serum cortisol in such patients is a contentious issue. In contradistinction to the present finding, Hannon et al. (2011) have found that patients with a low level of cortisol would rather have increased mortality. That finding has not been supported by a study of Olivecrona et al. (2013) who have failed to substantiate the presence of a relationship between dynamic changes in cortisol and mortality after brain injury. The discrepancy among the studies concerning the relation of serum cortisol to mortality is not readily explicable and requires further exploration using alternative study designs.

Referring to the hospitalization length, in general, the variables investigated showed narrow differences in the ROC plot, within a

0.550–0.600 range of AUC values. The Glasgow Coma Scale score and the Rotterdam CT score appeared the most significant determinants with the AUC of 0.592 and 0.589, respectively. However, the serum cortisol level and polytrauma, including concurrent brain lesions, also ranked high in the ability to determine the hospitalization course. In this study, fractures of long bones accounted for the majority of associated polytrauma. Fractures of the pelvis and lower limbs immobilize patients and delay physical rehabilitation, which increases the risk of pulmonary embolism, prolongs the hospital stay, and increases the risk of mortality. These results are in line with those reported by Tardif et al. (2017) and Stewart et al. (2013), except that the latter study has reported chest injuries, rather than bone fractures, as being prevalent among patients with acute traumatic brain injury. Likewise, patient age was another factor that was found of significance in predicting the length of hospital stay in the present study, with increasing age prolonging the stay. Older age carries the inherent risk of comorbidities, such as pre-existing hypertension, diabetes, or cognitive impairments, which all hinders the management of a treatment course in acute brain trauma, in turn leading to a prolongation of hospital stay (Lazaridis et al. 2015; Vitaz et al. 2003).

A limitation in interpreting the findings of the study is that it was performed in a rather small sample of 71 patients with traumatic frontal brain injury hospitalized in a single tertiary medical center. The results obtained should be validated in larger multi-center prospective randomized trials. Nonetheless, we believe we have shown that basically simple and non-invasive assessment in survivors of acute traumatic brain contusion may be helpful in gaining predictive knowledge on the length of hospital stay and plausible mortality. This knowledge is of essential value for better allocation of healthcare resources concerning the management of inpatient treatment and rehabilitation, which would eventually lead to better outcome and post-hospital quality of life.

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 Nobel Medical College and Teaching Hospital in Biratnagar, Nepal (approval no. 134/2018).

Informed Consent Written informed consent was obtained from the patients, their relatives, or next of kin, depending on the patient's clinical status, of all individual participants included in the study.

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Circulating P-Selectin and Its Glycoprotein Ligand in Nondiabetic Obstructive Sleep Apnea Patients

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Abstract

Selectins and their ligands play an important role in atherosclerosis. The role of these adhesion molecules in the pathogenesis of obstructive sleep apnea (OSA) may be of clinical relevance. Therefore, the aim of this study was to assess the serum content of platelet P-selectin (P-SEL) and P-selectin glycoprotein ligand 1 (PSGL-1) in different OSA stages. The study was performed in nondiabetic patients, aged 32–71, in whom OSA was verified by polysomnography. The apnea/hypopnea index (AHI) was used to stratify OSA stages: AHI <5, no sleep pathology (OSA-0); AHI 5–15, (OSA-1); AHI 16–30, (OSA-2); and AHI >30, (OSA-3). There were 16 patients in each group. P-SEL and PSGL-1 were assessed by ELISA kits. There were no appreciable differences in the patients' glucose or high-specificity C-reactive protein content. We found that P-SEL and PSGL-1 significantly increased from OSA-0 to OSA-3. There were the following positive associations in all OSA patients: P-SEL vs. AHI, PSGL-1 vs. AHI, and P-SEL vs. PSGL-1. In addition, the adhesion molecules are associated with the anthropometric parameters, oxygen saturation, and sleep architecture in the OSA-1 group. We conclude that the adhesion molecules consistently increase in the blood of nondiabetic OSA patients, along with progression of disorder severity.

Keywords

Adhesion molecules · Apnea-hypopnea index · Atherosclerosis · Nondiabetic patients · Obstructive sleep apnea · Selectins · Sleep pathology

1 Introduction

The obstructive sleep apnea (OSA) is a common sleep disorder, with 9–38% the prevalence in the adult population (Senaratna et al. 2017). The essential pathophysiologic feature is a recurrent cessation of breathing during sleep caused by upper airways collapse. The severity of OSA is usually stratified on the basis of the apnea-hypopnea index (AHI) as follows: mild (AHI 5–15), moderate (AHI 15–30), and severe (AHI >30) (Kapur et al. 2017). The most common risk factors for OSA include obesity, male gender, age, and postmenopausal state (Laratta et al. 2017), all of which are akin to the typical

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cardiovascular risk factors. Recurrent episodes of apnea or hypopnea cause many a pathological process, foremost hypoxia–reoxygenation cycles and rapid alterations in the intrathoracic pressure, leading to endothelial dysfunction, oxidative stress, and general atherosclerosis.

Atherosclerosis is a multifactorial inflammatory disease which is expected to be the main cause of mortality in the Western countries (Hansson 2005) due to the development of a spate of cardiovascular and cerebrovascular pathologies. The earliest stage of atherosclerosis is endothelial dysfunction, with dysregulation of thrombosis, redox imbalance, and inflammatory reactions (Gimbrone Jr and Garcia-Cardena 2016), which is followed by migration and adhesion of leukocytes to the endothelium. These processes are mediated by adhesion molecules, notably by P-selectin (P-SEL) which is a transmembrane glycoprotein, stored in endothelial cells and platelets. P-SEL translocates to the cell surface after cell activation (Ley 2003), where the molecule is conducive to leukocyte–endothelium and platelet–endothelium interactions (Budhiraja et al. 2007). Expression of P-SEL is remarkably enhanced in the endothelium overlying active atherosclerotic plaques (Johnson-Tidey et al. 1994). Adhesion of leukocytes to endothelial cells is mediated by the P-selectin glycoprotein ligand-1 (PSGL-1), which is a leukocyte protein responsible for about 90% of P-SEL binding. PSGL-1 also binds to L-selectin and enables the interaction between leukocytes on inflamed endothelial cells (McEver 2001). PSGL-1 deficiency prevents endothelial activation and reduces the risk of atherosclerosis in mice (Luo et al. 2012). Both P-SEL and PSGL-1 play a key role in the early stages of atherosclerosis. Therefore, this study seeks to define the serum content of platelet P-SEL and PSGL-1 in OSA patients.

2 Methods

There were 64 consecutive patients, aged 32–71, with a suspicion of OSA, enrolled into the study. Each patient underwent a physical examination and was subjected to the Epworth Sleepiness Scale (ESS) to determine the subjective diurnal sleepiness. The patients were then referred to in-lab sleep polysomnographic (PSG) examination, performed with an EMBLA S4000-Remlogic setup equipped with Somnologica Studio v5.0 software (Embla Systems, Thornton, CO). The full-night recordings were analyzed manually to verify the data provided automatically. The apnea was defined as a cessation of airflow lasting for more than 10 s. The hypopnea was defined as at least 30% reduction of airflow with at least 4% desaturation, lasting for at least 10 s. On the basis of AHI (the number of apnea and hypopnea episodes per hour), we divided patients into four groups: OSA-0 (no disorder; AHI <5), OSA-1 (mild disorder; AHI 5–15), OSA-2 (moderate disorder; AHI 16–30), and OSA-3 (severe disorder; AHI >30); each group consisted of 16 subjects. Arterial blood pressure, complete blood count, high-sensitivity C-reactive protein (hsCRP), lipid profile, fasting (Glu-0), and 120-min (Glu-120) glycemia during oral glucose tolerance test (OGTT) were determined, using Dimension Xpand Plus Systems (Siemens Healthcare Diagnostics, Deerfield, IL). The content of P-SEL and PSGL-1 was measured by the ELISA method using a DRG Reagent Kit (DRG International Inc., Springfield, NJ) on microplate reader (Sunrise Tecan; Männedorf, Switzerland). The study participants with hsCRP <8.00 mg/L and no diabetes according to the OGTT results were enrolled. The exclusion criteria were as follows: chronic or acute heart diseases, stroke, respiratory failure, chronic kidney or liver disease, current smoking, neoplasm, diabetes mellitus, and current infection.

Data were expressed as medians and interquartile ranges (IQR: 25th–75th). The Shapiro-Wilk test was used to check data distribution. Since the distribution was skewed, nonparametric tests were used in further analysis. Differences among the studied groups were assessed with the Kruskal-Wallis test, followed by a post hoc Dunn's test. The Spearman rank correlation coefficient (rho) was used to investigate correlations among parameters, with the following estimators of a rho strength: weak (0.10–0.29), average (0.30–0.49), strong

(0.50–0.69), very strong (0.70–0.89), and almost full (0.90–1.00). Multiple regression analysis was conducted for relevant parameters. In all of the tests, $p < 0.05$ was taken as an indicator of statistical significance. The analysis was performed using a commercial statistical packet of Statistica v12.0 (StatSoft, Tulsa, OK).

3 Results

Clinical and laboratory characteristics of the study groups are shown in Table 1. The groups differed in the AHI and ESS results. There also were significant differences in the body mass index (BMI) and neck circumference, with the highest values in OSA-3 group. However, there were no intergroup differences in blood pressure, glucose levels during OGTT, total cholesterol, LDL-cholesterol, or triglycerides, nor were there any in the duration of sleep phases expressed as a percentage of the total sleep time neither.

There were significant differences in P-SEL and PSGL-1 content among the OSA groups. P-SEL increased from OSA-0 to OSA-3 (Fig. 1), with the following statistical power of differences of the intergroup comparisons with Dunn's test: OSA-0 vs. OSA-1 ($p > 0.05$), OSA-0 vs. OSA-2 ($p < 0.002$), OSA-0 vs. OSA-3 ($p < 0.001$), and OSA-1 vs. OSA-3 ($p < 0.001$). PSGL-1 also increased from OSA-0 to OSA-3 (Fig. 2), with the following intergroup significance: OSA-0 vs. OSA-1 ($p = 1.0$), OSA-0 vs. OSA-2 ($p = 0.100$), OSA-0 vs. OSA-3 ($p < 0.001$), and OSA-1 vs. OSA-3 ($p < 0.001$).

Associations between P-SEL and PSGL-1 content and other parameters in the OSA groups are shown in Table 2. P-SEL is strongly or very strongly associated with the level of AHI, minimum oxygen saturation, white blood cell count, and total cholesterol. Likewise, PSGL-1 is associated with the magnitude of AHI. On the other hand, duration of non-REM-3 sleep is adversely associated with the content of both P-SEL and PSGL-1.

Multiple regression analysis was used to assess which investigated parameters could independently influence the content of either P-SEL or PSGL-1 (Table 3). Increasing AHI might explain about 95% of linear increase in PSGL-1 in OSA-3 and 83% of linear increase in PSGL-1 in OSA-2. Moreover, elevated PSGL-1 might explain 84% of linear increase in P-SEL in OSA-3.

4 Discussion

The major finding of the present study was that the blood content of the adhesion molecules P-SEL and PSLG-1 in the blood of OSA patients progressively increased from mild to severe OSA. Moreover, there was a significant inverse association between average arterial oxygen saturation and P-SEL content. On the other side, P-selectin is associated rather weakly with the anthropometrical parameters such as BMI or waist and neck circumference. These findings confirm some of the other studies on the issue, although differ in details with yet some other studies. A previous study in 80 male non-smoking Caucasians has also shown a progressive increase of P-SEL along the increase of OSA severity (Cofta et al. 2013). Another one has shown that P-SEL, and also the incidence of silent brain infarction, is significantly higher in moderate-to-severe OSA when compared to the control group of obese patients but not suffering from OSA. Moreover, CPAP treatment reverses the increased P-SEL content (Minoguchi et al. 2007). Dyugovskaya et al. (2008) have shown in a group of 68 patients that P-SEL content is increased in patients with severe OSA when compared to mild OSA. Rather unexpectedly, those authors find the difference in P-SEL loses significance after adjustment to BMI, which may point to a key role of obesity in the enhancement of the molecule content in OSA. A study by Jurado-Gamez et al. (2012) has used the oxygen desaturation index per hour (ODI), as opposed to AHI in the studies outlined above, as a measure of OSA severity. The authors have shown an increase in P-SEL in severe "desaturators" (ODI $\geq$30%) when compared to mild-to-moderate "desaturators" (ODI 5–30%). In another study,

Table 1 Clinical and laboratory characteristics of patients by the severity of obstructive sleep apnea (OSA)

Parameter	OSA-0 (n = 16)	OSA-1 (n = 16)	OSA-2 (n = 16)	OSA-3 (n = 16)	p
AHI (events/h)	1.6 (0.7–3.3)	10.2 (6.5–12.3)	20.2 (17.1–23.2)	54.5 (50.0–59.5)	–
Age (years)	49.5 (42.5–57.5)	56.0 (6.5–12.3)	56.5 (47.0–61.0)	55.0 (50.0–59.5)	ns
EPW (points)	6.0 (4.5–9.5)	7.5 (4.5–11.0)	8.0 (6.0–10.0)	11.0 (5.0–14.0)	<0.0001
BMI (kg/m^2)	26.1 (22.–27.2)	28.4 (26.4–32.1)	28.8 (26.9–30.2)	33.2 (30.1–39.4)	<0.0001
Waist (cm)	90.0 (81.0–96.0)	96.5 (92.5–103.5)	102.5 (90.0–107.0)	105.0 (103.0–121.0)	0.0002
Neck (cm)	38.5 (34.5–41.5)	40.5 (39.5–42.5)	42.0 (38.5–43.0)	45.0 (42.0–46.0)	0.0009
NREM1%	8.0 (4.8–15.2)	7.5 (6.0–10.9)	13.9 (8.2–21.3)	15.5 (6.0–21.9)	ns
NREM2%	31.1 (24.6–49.1)	29.3 (7.9–41.6)	47.1 (27.3–53.6)	50.7 (26.1–67.0)	ns
NREM3%	20.1 (7.9–35.8)	31.5 (22.4–46.1)	15.9 (12.3–38.15)	20.1 (11.5–31.3)	ns
REM%	15.7 (9.8–21.8)	21.0 (10.6–25.8)	12.9 (3.9–23.7)	17.3 (0.0–31.3)	ns
$O_{2\ Sat\ aver}$ (%)	94.9 (94.0–95.8)	93.8 (93.1–94.4)	94.8 (92.05–95.0)	92.0 (90.8–93.3)	ns
$O_{2\ Sat\ min}$ (%)	90.0 (87.5–92.0)	84.5 (80.0–88.5)	82.5 (80.0–86.0)	75.0 (64.5–78.0)	ns
SBP (mmHg)	127 (120–141)	132 (119–144)	134 (128–151)	135 (117–153)	ns
DBP (mmHg)	85 (77–90)	84 (78–90)	87 (80–97)	90 (80–98)	ns
G-0 (mmol/L)	5.07 (4.78–5.31)	5.12 (4.87–5.53)	5.17 (4.81–5.56)	5.41 (5.10–5.84)	ns
G-120 (mmol/L)	5.20 (4.64–5.95)	6.67 (4.88–8.13)	6.08 (4.25–7.47)	6.45 (5.07–8.29)	ns
T-C (mmol/L)	5.3 (4.9–6.2)	5.7 (5.1–6.4)	5.3 (5.0–6.1)	5.3 (4.9–6.0)	ns
TG (mmol/L)	1.3 (0.9–1.7)	1.5 (1.2–2.0)	1.4 (1.0–1.7)	1.7 (1.2–2.3)	ns
HDL-C (mmol/L)	1.7 (1.3–2.0)	1.6 (1.3–1.8)	1.4 (1.2–1.7)	1.4 (1.1–1.5)	ns
LDL-C (mmol/L)	3.4 (3.0–4.3)	3.8 (3.0–4.2)	3.5 (2.8–3.9)	3.6 (3.1–4.0)	ns
WBC (10e9/L)	5.63 (5.19–6.54)	6.56 (5.79–7.30)	7.20 (5.71–8.00)	6.47 (5.52–8.29)	ns
hsCRP (mg/L)	0.80 (0.60–1.40)	1.40 (0.65–2.05)	1.40 (0.45–2.45)	2.00 (1.45–3.00)	0.058
P-SEL (ng/mL)	35.56 (32.65–39.94)	39.49 (37.27–48.14)	49.64 (45.11–52.83)	59.67 (53.96–77.70)	<0.0001
PSGL-1 (ng/mL)	103.3 (94.25–111.9)	115.3 (99.79–139.3)	139.3 (102.6–429.4)	480.0 (110.4–800.0)	0.003

Data are presented as medians and (IQR: 25th–75th); *AHI* apnea/hypopnea index, *EPW* Epworth Sleepiness Scale, *BMI* body mass index, *Waist* waist circumference, *Neck* neck circumference, *NREM* non-rapid eye movement sleep stage, *REM* rapid eye movement sleep stage, $O_{2\ Sat\ aver}$ average arterial oxygen saturation, $O_{2\ Sat\ min}$ minimum arterial oxygen saturation, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *GLU-0* fasting blood glucose level, *GLU-120* blood glucose level at 120 min of oral glucose tolerance test, *T-C* total cholesterol, *TG* triglycerides, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *WBC* white blood cell count, *hsCRP* high-sensitivity C-reactive protein, *P-SEL* P-selectin, *PSGL-1* P-selectin glycoprotein ligand-1, *ns* nonsignificant; p-values correspond to the Kruskal-Wallis test

no difference in P-SEL has been noticed between "non-desaturators" (ODI <10%) when compared with "desaturators" (ODI ≥10%) (Priou et al. 2010). In the experimental rat model, blood P-SEL content increased in the apnea group (Nacher et al. 2007). There are, however, studies failing to show the dependence of blood P-SEL content on OSA severity, although it correlated with BMI (Robinson et al. 2004).

In this study, we also found an increase in the blood content of PSGL-1, a P-SEL glycoprotein ligand, depending on the OSA severity. The association of PSGL-1 and AHI was particularly strong in OSA-2 and OSA-3 groups. PSGL-1 is strongly associated with P-SEL in OSA-3 group. In contrast, PSGL-1 is weakly associated with BMI. It is rather hard to compare our results with the literature data as there is a paucity of studies on the content of PSGL-1 in the course of OSA. In a study by Horváth et al. (2018), the authors fail to report an appreciable difference in

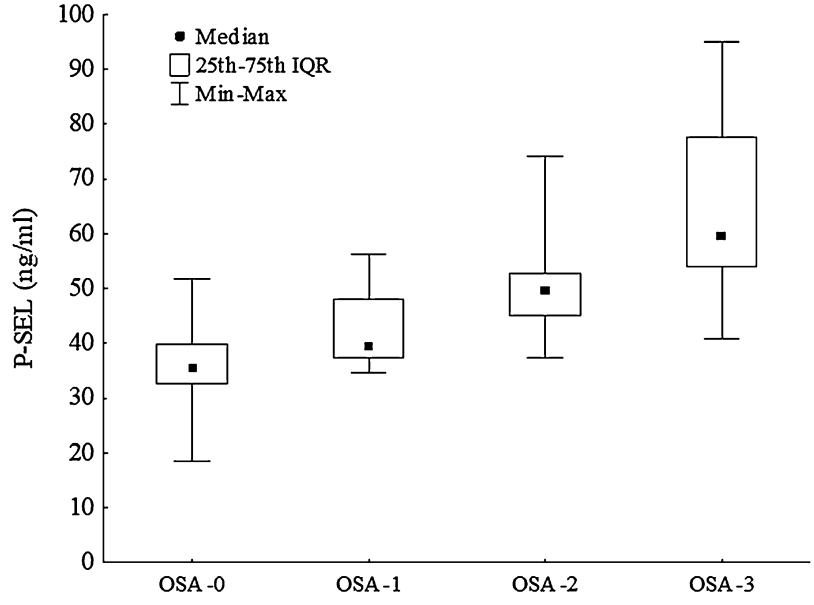

Fig. 1 Blood content of P-selectin (P-SEL) across the groups of increasing obstructive sleep apnea (OSA) severity: OSA-0, no disease; OSA-1, mild; OSA-2, moderate; and OSA-3, severe OSA ($p < 0.001$; Kruskal-Wallis test)

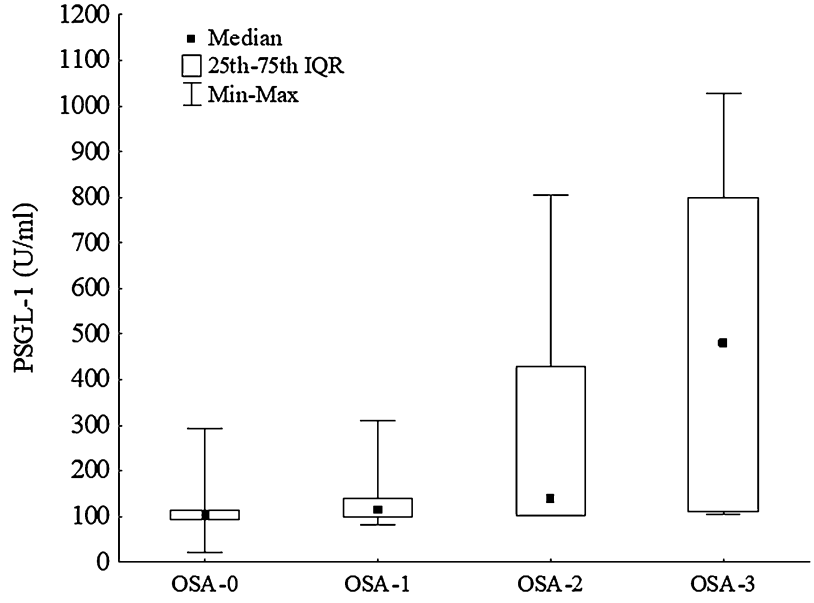

Fig. 2 P-selectin glycoprotein ligand-1 (PSGL-1) across the groups of increasing obstructive sleep apnea (OSA) severity: OSA-0, no disease; OSA-1, mild; OSA-2, moderate; and OSA-3, severe OSA ($p = 0.003$; Kruskal-Wallis test)

PSGL-1 content between OSA and non-OSA patients.

We did not find any appreciable differences in the duration of NREM-3 sleep stage, expressed as a percentage of total sleep time, across all of the OSA groups investigated. Interestingly, strong adverse P-SEL–NREM-3 and PSGL-1–NREM-3 associations were unravelled. Currently, there are no data linking disorders of sleep stages with cardiovascular risk. Further investigations are required to explore whether a reduction in

Table 2 Associations of P-selectin (P-SEL) and P-selectin glycoprotein ligand-1 (PSGL-1) with the investigated parameters, when significant in at least one group of obstructive sleep apnea (OSA) severity (Spearman's rho correlation coefficients)

Molecule	OSA-0 (n = 16)	OSA-1 (n = 16)	OSA-2 (n = 16)	OSA-3 (n = 16)	OSA-1, 2, 3 (n = 52)	All patients (n = 64)
P-SEL						
AHI			0.517	0.503	0.689	0.735
BMI						0.390
Waist					0.328	0.372
Neck		0.520			0.498	0.498
NREM1						
NREM2				0.524	0.391	0.324
NREM3				−0.612	−0.381	−0.268
$O_{2\ Sat\ aver}$					−0.294	−0.384
$O_{2\ Sat\ min}$					−0.402	−0.606
WBC				0.629		0.319
hsCRP						0.265
Glu-0						
Glu-120						
T-C						
PSGL-1			0.559	0.840	0.454	0.466
PSGL-1						
AHI			0.786	0.802	0.554	0.556
BMI						0.284
Neck					0.298	0.337
NREM1						
NREM2						
NREM3				−0.556	−0.306	
$O_{2\ Sat\ aver}$		0.499				
$O_{2\ Sat\ min}$		0.621				−0.274
WBC				0.720		
hsCRP	0.535					0.342
Glu-0			−0.512			
Glu-120				0.503		
T-C			0.690			
LDL-C					0.290	

AHI apnea/hypopnea index, *BMI* body mass index, *Waist* waist circumference, *Neck* neck circumference, *NREM* non-rapid eye movement sleep stage, $O_{2\ Sat\ aver}$ average arterial oxygen saturation, $O_{2\ Sat\ min}$ minimum arterial oxygen saturation, *WBC* white blood cell count, *hsCRP* high-sensitive C-reactive protein, *Glu-0* fasting blood glucose level, *Glu-120* blood glucose level at 120 min of oral glucose tolerance test, *T-C* total cholesterol, *T-C* total cholesterol, *LDL-C* low-density lipoprotein cholesterol

NREM-3 sleep could be an early presage of the development of arteriosclerosis.

Atherosclerosis is a chronic inflammatory disease, resulting from the interplay of dysfunctional endothelium with systemic hemostatic and inflammatory mechanisms (Lui and Sau-Man 2012). It is characterized by the accumulation of lipids and fibrous elements especially in large arteries (Lusis 2000). This process is the main cause of cardiovascular disorders (CVD), including stroke, myocardial infract, and heart failure, the irreversible effects of advanced atherosclerosis. The early stage of atherosclerosis is a challenge for clinical practice, especially since patients could benefit from therapeutic modifications of CVD risk factors. One of the earliest stages of atherosclerosis is leukocyte rolling on activated endothelium, which is

Table 3 Multiple regression analysis showing factors significantly affecting the outcome variables P-selectin (P-SEL) and P-selectin glycoprotein ligand-1 (PSGL-1) in the groups of patients with increasing severity of obstructive sleep apnea (OSA)

Outcome variable	OSA-0	OSA-1	OSA-2	OSA-3
P-SEL	n/a	n/a	n/a	$p = 0.0008$ for
				P-SEL and AHI
				P-SEL and NREM2
				P-SEL and NREM3
				P-SEL and WBC
				P-SEL and PSGL-1
				($\beta = 1.12$; $R^2 = 0.87$)
PSGL-1	n/a	n/a	$p = 0.0004$ for	$p < 0.0001$ for
			PSGL-1 and AHI	PSGL-1 and AHI
			($\beta = 0.67$, $R^2 = 0.83$)	($\beta = 0.42$, $R^2 = 0.95$)
			PSGL-1and Glu-0	PSGL-1 and Glu-120
			PSGL-1 and T-C	PSGL-1 and NREM3
				PSGL-1 and WBC

AHI apnea-hypopnea index, *NREM* non-REM sleep stage, *WCB* white blood cell count, *Glu-0* fasting blood glucose level, *Glu-120* blood glucose level at 120 min of oral glucose tolerance test, *T-C* total cholesterol, β standardized coefficient indicating the strength of the independent influence of a predictor variable on the outcome variable, R^2 coefficient describing the percentage of linear changes of an outcome variable by linear changes of a predictor variable, *n/a* non-applicable, i.e., no variable was related to either P-SEL or PSGL-1

promoted by the adhesion molecules P-SEL and its glycoprotein ligand PSGL-1. Enhanced plasma content of P-SEL reflects both endothelial dysfunction and platelet activation (Blann et al. 2003). Burger and Wagner (2003) have shown that P-SEL contributes to atherosclerosis through mediating the monocyte rolling, recruitment of leukocytes to atherosclerotic lesions, and facilitation of thrombosis.

P-SEL is elevated in the blood of patients with acute myocardial infraction (Chiu et al. 2005; Ikeda et al. 1994). Moreover, an inhibitor of P-SEL suppresses plaque formation, rupture, and the intimal bleeding (Guo et al. 2015). Chung et al. (2009) and O'Connor et al. (1999) have reported the elevation of P-SEL in congestive heart failure, although it failed to associate with the ejection fraction. Interestingly, the authors find no difference in P-SEL depending on the ischemic and nonischemic background of heart failure, nor does the content of P-SEL depend on the use or not of acetylsalicylic acid by patients. P-SEL also is elevated in line with the progressing vascular dysfunction of hypertension (Sanada et al. 2005; Verhaar et al. 1998) and in the early stage of progressing ischemic stroke (Wang et al. 2013).

PSGL-1 is constitutively expressed on the cell surface (Kappelmayer and Nagy Jr 2017; Frenette et al. 2000). It recruits leukocytes and platelets into the atherosclerotic lesions (Huo and Xia 2009; Frenette et al. 2000). It also adversely affects lipid metabolism as reduced content of total cholesterol, LDL-C, and triglycerides and elevated HDL-C are noticed in PSGL-1-deficient mice (Li et al. 2018). Ozaki et al. (2014) have shown that PSGL-1 expression is higher in acute than in stable coronary insufficiency, possibly due to plaque instability (Kitamura et al. 2018). These findings are supported by the experimental studies in the mouse showing that PSGL-1 deficiency is associated with reduced atherosclerosis burden and less endothelial damage (Luo et al. 2012).

Elevated risk of atherosclerosis in OSA patients has been widely discussed. Untreated OSA leads to increased morbidity and mortality. Cardiovascular disorders and their sequelae are the most frequent complications (Lavie et al. 1995). The Sleep Health Heart Study has shown the odds ratio for patients with AHI >11 of 1.27

for coronary artery disease and 1.58 for stoke (Lui and Sau-Man 2012). McNicholas and Bonsigore (2007) consider OSA as an independent risk factor of cardiovascular events and show that CPAP therapy reduces this risk. There also are a lot of data concerning the impact of OSA on stroke. Redline et al. (2010) in a study in about 5,500 patients have shown an association between OSA and ischemic stroke, with the adjusted hazard ratio of up to 2.86 in men. Those authors have also observed a risk of stroke in women with AHI >25, the risk increasing by 6% with each unit of AHI increase from 5 to 25. Similar findings have been reported in other studies (Sharma and Culebras 2016; Yaggi et al. 2005).

We conclude that in nondiabetic obstructive sleep apnea patients, moderate and severe stage of the disease enhances the blood content of the adhesion molecules P-selectin and P-selectin glycoprotein ligand-1 in the disease severity-dependent manner as assessed from the magnitude of the apnea-hypopnea index. The increase in the content of adhesion molecules in OSA is liable to be conducive to enhance propensity for arteriosclerosis and consequently cardiovascular morbidity and mortality of the disease.

Conflict of Interest The authors declare no conflicts of interests in relation to this article.

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

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

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Nutritional Status and Dietary Patterns in Adults with Severe Obstructive Sleep Apnea

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Abstract

Obstructive sleep apnea (OSA) is associated with daytime sleepiness, obesity, and lifestyle and dietary changes. The potential role of diet in OSA has been largely unexplored. The aim of the study was to assess nutritional status and dietary patterns in OSA patients. The study was conducted in 137 adult patients (48 women and 89 men) aged 31–79 suffering from OSA. The following diagnostic procedures were undertaken: polysomnography, anthropometric measurements, and a dietary pattern questionnaire. We found that 128 (93.4%) patients were overweight or obese with the mean body mass index (BMI) of 33.2 ± 6.1 kg/m^2 and weight of 98.0 ± 20.2 kg. The mean percentage of total body fat was $45.0 \pm 5.5\%$ in women and $32.5 \pm 5.5\%$ in men. Obesity was associated with the severity of OSA, expressed by apnea/hypopnea index. We further found that the waist-to-hip ratio in women, but the neck circumference or percentage of body fat in men, characterizes best the OSA patients. Referring to dietary habits, half of the patients consumed white bread on a daily basis, 35.8% of them had whole grain bread in the diet, and only 16.8% consumed fish at least two portions a week. A third of patients used butter as a spread for bread or a source of fat for cooking, 2.9% of them used soft margarine, and 20.4% used olive or canola oil. Fruits and vegetables were consumed by 60% and 38% of patients, respectively. Refined sugar and sweets were used by 31.4% of patients every day. We conclude that excessive body weight, which may portend the development of OSA, is characterized by different anthropometric variables in men and women. Further, improper dietary habits seem conducive to the gain in body weight and thus may be at play in the pathogenesis of OSA.

Keywords

Body fat · Dietary patterns · Nutritional status · Obesity · Obstructive sleep apnea

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1 Introduction

Obstructive sleep apnea (OSA) is one of the very common diseases in the general population. Among the other factors, the prevalence of OSA depends on age and sex and is estimated at 13% in men and 6% in women aged 30–70 years; it has shown a relative percentage increase by 14–55% over the last 20 years (Peppard et al. 2013). OSA is characterized by recurrent collapse of the upper airway during sleep, resulting in partial or complete obstructive of airflow limitation, leading to a decrease in blood oxygenation, subsequent arousals, fragmented sleep, and excessive daytime sleepiness. Untreated OSA is associated with significant comorbidity and can lead to many cardiovascular complications (Kumor et al. 2013). The main risk of OSA is obesity. According to WHO data, the number of obese patients is growing and has significant impact on the healthcare system. In 2016, 39% of women and 39% of men aged 18 and over were overweight worldwide. Fifty to 70% of overweight and obese subjects have OSA (Young et al. 2002). Including epidemiological data, OSA still remains underdiagnosed in obese subjects. Moreover, OSA and obesity are considered independent risk factors for cardiovascular diseases and atherosclerosis progression. The main measure of obesity is body mass index (BMI), strongly correlated with the severity of OSA. Other anthropometric parameters are also closely related to OSA. Waist-to-hip ratio is a reliable correlate of OSA irrespective of gender. Neck circumference is an independent risk factor for male, but not for female OSA patients. These different aspects of obesity may contribute to the pathogenesis of OSA (Lim et al. 2014). BMI, neck circumference, and a high Epworth Sleepiness Scale (ESS) are independently predictive of moderate-to-severe OSA in males, whereas age, neck circumference, and morning headaches are independently predictive in females (Earl et al. 2019). The waist-to-hip ratio and neck measures also associate with OSA severity assessed from arterial oxygen desaturations or apnea/hypopnea index (AHI) (Tom et al. 2018). De Melo et al. (2019) have reported that a higher amount of food intake during the evening period may diminish sleep quality in moderate and severe OSA patients. Those authors also report the presence of a moderate association between the resting energy expenditure or sleep quality and the incidence of OSA, although this association seems insensitive to disease severity.

The present study seeks to define the nutritional status of OSA patients by dietary intake and anthropometric measures and to evaluate the relation of OSA to the obesity metrics, such as BMI, body fat content, muscle mass, neck circumference, and their association with OSA severity, assessed by AHI.

2 Methods

2.1 Patients

There were 137 untreated patients enrolled into the study (M/F, 89/48), who presented symptoms suggestive of OSA and were referred to the in-sleep polysomnography (PSG) investigation (Nox Medical, Reykjavík, Iceland) between June 2017 and June 2018. The assessment of a sleep structure was performed according to the recommendations of Hori et al. (2001). The patients completed a questionnaire on snoring, observed apneas, comorbidities, and medicines used. Sleepiness was assessed with EPS, with scores $\geq$10 classified as excessive daytime sleepiness. All of the patients with previously diagnosed or treated OSA and those after bariatric surgery were not eligible for the study.

The diagnosis OSA was established according to the recommendations of the American Academy of Sleep Medicine, which require an AHI $\geq$5/h of sleep in the presence of typical OSA symptoms or AHI $\geq$15/h, regardless of the clinical manifestation (Kapur et al. 2017). Only were patients with the severe OSA (AHI >30/h) recruited to the study.

2.2 Nutritional Assessment

A physical examination was performed, and the following parameters were recorded: neck circumference, waist-to-hip ratio (WHR), body mass index (BMI, kg/m^2), and the central (visceral) obesity as a WHR >1 and peripheral obesity as a WHR <0.8. Body weight and height were measured using a Seca 799 station and column scales, with the accuracy of ±0.1 kg/cm (Seca, Chino, CA). The interpretation of BMI data followed the classification set by the WHO, i.e., underweight <18.5 kg/m^2, normal weight 18.5–25.0 kg/m^2, overweight 25–30 kg/m^2, and obese ≥30 kg/m^2. Whole-body impedance (BIA, wrist to ankle) was measured using the Maltron BioScan 920-II multifrequency bioelectrical impedance analyzer (Maltron BioScan, Rayleigh, UK) according to the manufacturer's instructions. Before taking the BIA measurement, the patients were instructed with the following guidelines: no heavy exercise 12 h before the test, no large meals or caffeinated products 4 h before the test, and consumption of liquids limited to 1% of body weight or two 8 oz. glasses of water 2 h before the test.

A 62-item non-quantitative food frequency questionnaire (FFQ-6) was used for the assessment of food intake. The questionnaire was used in the form of a table that included selected food products (see Table 4). Patients determined the frequency of food consumption using a 6-point scale, using the following score: daily, 6 points; 4–5 times a week, 5 points; 2–3 times a week, 4 points; once a week, 3 points; 2–3 times a month, 2 points; and rarely, 1 point. The tool collected information on the frequency of consumption of 62 assorted product groups, representing 8 main food groups consumed in the last 12 months.

2.3 Statistical Elaboration

Data were expressed as means ± SD. Differences between groups were evaluated with a *t*-test and one-way analysis of variance ANOVA, followed by post hoc Fisher's test as appropriate. The association between the frequency of consumption of food products and gender was assessed with Pearson's correlation coefficient. A p-value <0.05 denoted statistically significant differences. A commercial statistical package of Statistica v13.3 was used (StatSoft, Tulsa, OK).

3 Results

3.1 Obstructive Sleep Apnea (OSA) Patients' Characteristics

Thirty nine subjects (28.5%) were overweight (25 ≤ BMI ≤30 kg/m^2), and 88 subjects (64.2%) were obese (BMI >30 kg/m^2). According to the International Diabetes Federation criteria for waist circumference, 89.3% of men and 100% of women had abdominal obesity. Further, 95.2% men and 87.5% women have elevated WHR (>0.90 for men and > 0.85 for women). Most of the anthropometric parameters were greater in men than those in women, particularly the neck circumference, waist circumference, WHR, and muscle mass. Only were the hip circumference and body fat statistically greater in women (Table 1).

All of the OSA patients had increased sleepiness measured by the ESS scale. Sleepiness score was higher in women than that in men (11.2 ± 4.9 vs. 10.1 ± 4.8, respectively, $p = 0.239$). In contrast, the mean AHI was higher in men – 39.7 ± 23.9 *per* hour of sleep – and it was somehow lower in women, 31.6 ± 25.3 *per* hour ($p > 0.05$). There was no difference in SpO_2 between genders (Table 2).

In male OSA patients, AHI, and thus disease severity, significantly associated with the neck circumference, hip circumference, waist circumference, body fat, and BMI. In female patients, AHI associated with WHR only (Table 3).

3.2 Frequency of Food Consumption (FFQ Questionnaire Results) in Obstructive Sleep Apnea Patients

About one half of the OSA patients (53.5%) consumed white bread every day. Only did 35.8% of

Table 1 Patients' anthropometric characteristics

Parameter	Men ($n = 89$)	Women ($n = 48$)	p
Age (years)	54.2 ± 12.3	58.4 ± 11.8	0.054
Body mass index (kg/m^2)	32.6 ± 6.1	34.1 ± 6.1	0.176
Neck circumference (cm)	44.5 ± 5.1	39.0 ± 2.1	<0.001
Waist circumference (cm)	110.5 ± 15.7	104.1 ± 11.1	0.020
Hip circumference (cm)	109.3 ± 11.8	114.4 ± 9.7	0.017
Waist-to-hip ratio	1.0 ± 0.1	0.9 ± 0.1	<0.001
Body fat (%)	32.5 ± 5.8	45.0 ± 5.5	<0.001
Muscle mass (kg)	33.8 ± 4.7	21.1 ± 2.8	<0.001

Data are means ± SD; *t*-test

Table 2 Polysomnography parameters

Parameters	Men ($n = 89$)	Women ($n = 48$)	p
AHI (*per* hour of sleep)	39.7 ± 23.9	31.6 ± 25.3	0.090
ESS score (points)	10.1 ± 4.8	11.2 ± 4.9	0.239
Mean SpO_2 during sleep (%)	90.3 ± 12.0	91.8 ± 2.9	0.460
Minimum SpO_2 during sleep (%)	78.0 ± 12.8	80.4 ± 8.1	0.288

Data are means ±SD; *AHI* apnea/hypopnea index – event per hour of sleep, *ESS* Epworth Sleepiness Scale, SpO_2 peripheral blood oxygen saturation (finger pulse oximetry); *t*-test

Table 3 Associations between obstructive sleep apnea (OSA) severity assessed by apnea/hypopnea index (AHI) and anthropometric parameters

	AHI – men		AHI – women	
	r	p	r	p
Age	−0.035	0.767	0.034	0.849
Neck circumference	0.427	<0.001	−0.029	0.871
Hip circumference	0.435	<0.001	0.036	0.842
Waist circumference	0.421	<0.001	0.332	0.055
Waist-to-hip ratio	0.155	0.191	0.388	0.024
Body fat	0.496	<0.001	−0.022	0.901
Body mass index	0.488	<0.001	0.010	0.519

r Pearson's correlation coefficient

patients use grain bread, and 16.8% consume at least two portions of fish a week. Butter was a daily source of fat for 34.3%, soft margarine was for 2.9%, and olive oil or canola oil was for 20.4% of patients. Fruits were daily consumed by 60% and vegetables by 38% of patients. Sugar and sweets were everyday products for 31.4% of patients (Table 4).

The mean frequency of bread consumption was similar in male and female OSA patients, amounting to 2–3 times per week. Milk consumption also was similar in both genders, consisting mostly of 2% fat milk; it varied from once a week to 2–3 times a month. All of the patients showed increased intake of poultry and pork rather than beef (once a week to 2–3 times a week vs. 2–3 times a month, respectively). Consumption of fruits and vegetables, whether fresh or refined, was similar in OSA women and men (Table 5).

4 Discussion

OSA is a common disease worldwide, affecting over 13% of men and 6% of women (Peppard et al. 2013). In a Polish epidemiological study, the diagnosis of OSA has been established in 7.5% of middle-aged population, and the prevalence of

Table 4 Percentage of patients consuming each of the products listed corresponding to the frequency indicated by points from 6 to 1 as indicated in the table legend

	Men (%)						Women (%)					
Food products	6	5	4	3	2	1	6	5	4	3	2	1
White bread	53.9	12.4	12.4	3.4	1.1	15.7	52.1	4.2	10.4	8.3	0.0	20.8
Whole grain bread	37.1	13.5	16.9	9.0	2.2	21.3	33.3	10.4	16.7	8.3	2.1	27.1
Milk 2% fat	25.8	5.6	5.6	2.2	3.4	57.3	12.5	2.1	8.3	8.3	4.2	60.4
Milk 3.2% fat	5.6	6.7	10.1	6.7	3.4	67.4	2.1	8.3	6.3	6.3	0.0	75.0
Pork	9.0	14.6	34.8	24.7	6.7	9.0	4.2	4.2	31.3	29.2	6.3	22.9
Poultry	2.2	21.3	42.7	19.1	4.5	10.1	10.4	8.3	50.0	22.9	0.0	6.3
Fish	1.1	4.5	10.1	31.5	19.1	23.6	2.1	0.0	16.7	31.3	14.6	22.9
Olive oil, canola oil	20.2	7.9	23.6	16.9	5.6	16.9	20.8	12.5	31.3	14.6	6.3	2.1
Butter	31.5	11.2	11.2	6.7	5.6	24.7	39.6	2.1	4.2	2.1	8.3	33.3
Fresh vegetables	25.8	28.1	16.9	7.9	4.5	7.9	35.4	20.8	18.8	2.1	0.0	12.5
Fresh fruits	40.4	13.5	20.2	5.6	5.6	5.6	50.0	20.8	10.4	0.0	2.1	6.3
Sugar and sweets	34.8	6.7	4.5	9.0	9.0	27.0	25.0	4.2	4.2	6.3	2.1	43.8

Food frequency points: daily, 6 points; 4–5 times a week, 5 points; 2–3 times a week, 4 points; once a week, 3 points; 2–3 times a month, 2 points; rarely, 1 point

Table 5 Mean frequency of consumption of food products by gender

	Men	Women	Statistics
White bread	4.7 ± 1.8	4.4 ± 2.0	$\chi2 = 0.809$ df = 5 $p = 0.976$
Whole grain bread	4.1 ± 1.9	3.8 ± 2.0	$\chi2 = 5.01$ df = 5 $p = 0.414$
Milk 0.5% fat	1.3 ± 1.0	1.5 ± 1.2	$\chi2 = 3.98$ df = 5 $p = 0.553$
Milk 2% fat	2.8 ± 2.2	2.2 ± 1.8	$\chi2 = 8.08$ df = 5 $p = 0.152$
Milk 3.2% fat	2.0 ± 1.6	1.8 ± 1.5	$\chi2 = 3.42$ df = 5 $p = 0.635$
Beef	2.1 ± 1.3	2.0 ± 1.1	$\chi2 = 7.10$ df = 5 $p = 0.213$
Pork	3.7 ± 1.3	3.0 ± 1.4	$\chi2 = 8.97$ df = 5 $p = 0.110$
Poultry	3.7 ± 1.2	3.9 ± 1.2	$\chi2 = 10.55$ df = 5 $p = 0.061$
Fish	2.5 ± 1.2	2.6 ± 1.2	$\chi2 = 3.82$ df = 5 $p = 0.576$
Olive oil, canola oil	3.7 ± 1.7	4.2 ± 1.3	$\chi2 = 7.28$ df = 5 $p = 0.200$

(continued)

Table 5 (continued)

	Men	Women	Statistics
Butter	3.8 ± 2.1	3.6 ± 2.3	$\chi2 = 8.17$ df = 5 $p = 0.147$
Soft margarine	1.4 ± 1.2	1.1 ± 0.5	$\chi2 = 3.90$ df = 4 $p = 0.419$
Fresh vegetables	4.4 ± 1.5	4.6 ± 1.7	$\chi2 = 6.36$ df = 5 p = 0.273
Stewed vegetables	2.1 ± 1.9	2.3 ± 1.7	$\chi2 = 5.46$ df = 6 $p = 0.486$
Steamed vegetables	2.7 ± 1.8	3.2 ± 1.8	$\chi2 = 2.78$ df = 5 $p = 0.733$
Fresh fruits	4.7 ± 1.5	5.1 ± 1.4	$\chi2 = 7.12$ df = 5 $p = 0.212$
Nuts and seeds	1.8 ± 1.4	1.7 ± 1.4	$\chi2 = 3.49$ df = 5 $p = 0.625$
Legumes	1.9 ± 1.2	1.8 ± 1.3	$\chi2 = 9.98$ df = 5 $p = 0.076$
Sugar and sweets	3.7 ± 2.2	3.0 ± 2.2	$\chi2 = 6.35$ df = 5 $p = 0.274$

Data were expressed as means ± SD. Food frequency points: daily, 6 points; 4–5 times a week, 5 points; 2–3 times a week, 4 points; once a week, 3 points; 2–3 times a month, 2 points; rarely, 1 point; Pearson's Chi-squared test

OSA was more than threefold greater in men (11.2%) than that in women (3.4%) (Plywaczewski et al. 2008). In the present study, we evaluated the nutritional habits of OSA patients. The most important drawback concerned the high intake of white bread, simple carbohydrates, and the presence of sweets and confectionery in the diet. In addition, a higher than recommended by WHO consumption of red meat and butter leads to high intake of saturated fatty acids. These findings demonstrate a less than healthy or optimum dietary pattern that falls rather far from the standard recommendations in the prevention of cardiovascular or metabolic, such as diabetes, disorders in OSA patients.

Data on food consumption in OSA patients are scarce, although such patients should require nutritional interventions due to the accompanying obesity. Reid et al. (2019) have shown that OSA associates with lower intake of whole grains, higher intakes of red meat, and a lower overall diet quality. Araghi et al. (2013) highlight the role of body weight in managing OSA by showing that weight gain and loss are consistently associated with increasing and decreasing AHI. The estimate is that a 10% weight loss may lead to as much as a 26% reduction in AHI (Peppard et al. 2013). A recent meta-analysis has confirmed that a significant reduction in AHI and other OSA signs and symptoms is caused by weight reduction and lifestyle interventions, the effect of which depended, to an extent, on OSA severity and gender (Carneiro-Barrera et al. 2019).

We further evaluated the patients' body composition and anthropometric parameters. The majority of patients showed overweight or obesity, with BMI and BF exceeding the recommended norms of the American Council

on Exercise (BF% >25% for men and >32% for women). BMI values were greater in OSA men than those in women. We further found that the neck, hip, and waist circumference as well as the percentage of body fat were associated with increasing severity of OSA in men, but not clearly so in women, which underscores the presence of gender differences in the expression of disease severity. Nonetheless, an increased waist-to-hip ratio appears a much more sensitive indicator of OSA severity in women than in men. These findings are in line with the previous studies on sleep-related breathing disorders. Young et al. (2002) have reported an association between the risk of OSA and obesity, WHR, or neck circumference. Ip et al. (2004) and Ip et al. (2001) have shown that higher BMI is accompanied by a higher risk of apnea in Chinese patients, even though the BMI remains in the normal range. Similar conclusions have been reached in the Korean population, where BMI, male gender, and hypertension are closely associated with the risk for OSA (Kang et al. 2014; Kim et al. 2004). Likewise, Udwadia et al. (2004) have reported an association between BMI, neck circumference, and diabetes and the risk for OSA in Indian men. Polesel et al. (2019) have reached the same conclusion in the Brazilian population. These authors argue that waist circumference and waist-to-hip ratio best prognosticate sleep-related breathing disorders in women, whereas neck circumference and waist-to-hip circumference are the best harbingers of mild OSA in men, with BMI remains a factor the most closely associated with severe OSA. However, BMI may not be a perfect parameter for the assessment of OSA severity. Sutherland et al. (2019) have found that South American OSA patients have a larger AHI when compared to African Americans, having a similar BMI. Cho et al. (2016) have also reported that OSA patients have a larger neck circumference than that in healthy control subjects. Further, the authors did not show any significant differences in BMI, waist circumference, or waist-to-hip ratio in OSA patients when compared with a control group, nor have they noticed any differences in neck circumference between Asian and Caucasians patients. In contradistinction, Davidson and Patel (2008) have shown a much stronger association between waist circumference and the severity of sleep-related breathing disorders than that for neck circumference or BMI. In that study, however, only were half of the patients obese, with waist circumference of more than 102 cm. The factors that have the greatest potential for prognosticating the development and identification of sleep-related breathing disorders seemingly are highly multifarious and vary from study to study. Aside from disease severity, these factors depend not only on body built and anthropometric parameters but also on genetic and ethnic differences. From the clinical standpoint, there is a consistent impression that BMI and neck circumference rank first as prognostics of OSA development (Cizza et al. 2014; Simpson et al. 2010; Onat et al. 2009).

In conclusion, most of OSA patients are overweight and obese. There are gender differences in prognosticating the severity of OSA on the basis of anthropometric parameters. In women, waist-to-hip ratio seems an optimum predictor of OSA severity, whereas the neck circumference and waist-to-hip circumference rank first in men. Most of OSA patients run an unhealthy and proarteriosclerotic diet, high in calories, fat, and simple carbohydrates and low in fish, fresh fruits, and vegetables. A nutritional intervention should become a routine part of counseling and management OSA patients.

Conflict 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 Medical University of Warsaw in Poland.

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

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Medication Compliance in COPD Patients

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Abstract

Chronic obstructive pulmonary disease (COPD) is one of the most severe public health problems and a leading cause of death worldwide. One of the main reasons for poor control of the disease is low patient compliance with treatment plan. The aim of the study was to investigate sociodemographic and health status factors that may have an influence on adherence to treatment. There were 106 inpatients (F/M, 42/64; mean age 70 ± 6 years) with COPD enrolled into this retrospective study. Patients completed the Adherence to Refills and Medications Scale (ARMS) to assess adherence to therapy. We found that the mean ARMS score was 23.1 ± 6.8. About 86% of patients had low adherence, and 14% had good adherence (mean score 3.2 ± 2.4). The low-adherence patients were more likely to be older ($p = 0.020$), female ($p = 0.011$), single ($p = 0.019$), not professionally active ($p = 0.049$), hospitalized more often ($p = 0.005$) and for a longer time ($p = 0.046$), feel worse ($p = 0.023$), experience a greater impact of the disease on sleep quality ($p = 0.008$) and daily activities ($p = 0.001$), and had a higher GOLD stage of COPD when compared to patients with good adherence patients ($p = 0.012$). Multiple factor analysis demonstrates that independent adverse predictors of the ARMS score included the following: being single (OR = 3.18), having had more than eight hospitalizations (OR = 1.18), and experiencing dysfunction in daily activities (OR = 1.79). Male gender (OR = 0.77) and longer than 21-day hospitalizations (OR = 0.93) were independent positive predictors of adherence. In conclusion, COPD patients demonstrate a low level of adherence to pharmacotherapy. Adherence is adversely affected by sociodemographic (older age, female gender, being single, and professionally inactive) and clinical factors (more frequent hospitalizations, perception of poor well-being, disordered sleep and daily functioning, and a higher GOLD stage).

Keywords

Adherence to treatment · COPD · Health status · Pharmacotherapy · Sociodemographic factors

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1 Introduction

Chronic obstructive pulmonary disease (COPD) is a leading cause of chronic morbidity and mortality worldwide. It involves a permanent restriction of air flow through the airways, which is typically progressive and associated with a significant chronic inflammatory response of the respiratory tract to inhaled particles and gases. The Global Burden of Disease (GBD) organization estimates that the number of COPD patients in the world exceeds 328 million, including 168 million men and 160 million women (Vos et al. 2012). In 1990, COPD was the sixth most common cause of death, but the mortality is growing. In the year 2000, COPD already ranked fourth, and by 2020, an estimated 4.7 million people will die from COPD, making it the third most common cause of death worldwide (López-Campos et al. 2016). These changes are due to a rise in tobacco smoking, increasing environmental pollution in developing countries, and a longer life expectancy. Epidemiological studies covering the entire Polish population are lacking, but available estimates put the number of COPD patients in Poland at two million (Niżankowska–Mogilnicka et al. 2007).

In most patients, the available pharmaceutical treatment enables full control of the disease, significant alleviation of symptoms, and a reduction of exacerbation risk. One of the main reasons for poor control of the disease is low adherence to treatment plan, including pharmacotherapy, lifestyle and diet modifications, and a lack of cooperation with the medical caretakers. In 2003, WHO listed nonadherence to treatment among the world's most serious health problems, as it constitutes a major obstacle to benefiting from evidence-based therapy (Burkhart and Sabaté 2003). According to a WHO report, less than 50% of chronically ill patients adhere to their treatment, which adversely affects treatment effectiveness, patients' quality of life, and the economic side of treatment. In Poland, the percentage of COPD patients who continue treatment with inhaled medication at the end of 1-year follow-up does not exceed 21% (Kardas et al. 2012).

In literature, there is no clarity yet regarding the impact of certain factors on adherence in COPD. Therefore, the purpose of the present study was to determine the influence of the selected variables on adherence to pharmaceutical treatment in COPD patients.

2 Methods

2.1 Patients and Survey Instrument

This retrospective study included 106 inpatients (F/M, 42/64; mean age 70 ± 6 years) diagnosed with COPD and treated at the Lower Silesian Lung Center in Wroclaw, Poland. The Adherence to Refills and Medications Scale (ARMS) was a survey tool to assess adherence to therapy. Sociodemographic and clinical data were obtained from medical files. The ARMS has been developed by Kripalani et al. (2009) and used in patients with coronary artery disease and other chronic conditions, including hypertension, dyslipidemia, and diabetes mellitus. The survey consists of 12 items gathered in 2 subscales: adherence to taking medications (8 items) and adherence to refilling prescriptions (4 items). Responses are scored on a 4-point Likert-type scale as "none", "some of the time", "most of the time", and "all of the time", assuming the values from 1 to 4, respectively. The score range is from 12 to 48; the lower the score, the better the adherence (Lomper et al. 2018).

Sociodemographic data were obtained from the patient files and concerned gender, age, marital status, residence, education, and professional activity. Clinical data referred to body mass index (BMI), cigarette smoking habit, number and duration of hospitalizations, perceived well-being, impact of the disease on sleep quality and daily activities, and medications. Patients were stratified according to the current GOLD classification of COPD severity, using a four-stage GOLD classification: GOLD I mild, GOLD II moderate, GOLD III severe, and GOLD IV very severe (Le et al. 2019).

2.2 Statistical Elaboration

Continuous variables were expressed as means ±SD and ordinal variables as counts and percentages. Quantitative variables were assessed for significance of differences using the Mann-Whitney U test. Comparison of qualitative variables was performed using the Chi-squared test with Yates's correction for 2 × 2 tables or with Fisher's exact test in case of low counts. The influence of quantitative variables on the dichotomous variable (high vs. low adherence) was analyzed using logistic regression, and the results were presented as odds ratio (OR) with a 95% confidence interval (CI). Multivariate analysis of the independent influence of the variables studied on the quantitative variable was performed using linear regression, and the results were shown as regression model variable values with a 95% confidence interval (95% CI). A p-value <0.05 defined statistical significance of differences and associations. The analysis was performed using the R software v3.6.1 (R Core Team 2019).

3 Results

3.1 Sociodemographic and Clinical Characteristics of Patients in Relation to Adherence to Treatment

A total ARMS score of 12–15 points was interpreted as high adherence and scores of ≥16 as low adherence. Accordingly, low adherence was found in 85.9% of patients and high adherence in the remaining 14.1%; a difference between the groups was significant ($p < 0.05$). In the low-adherence group, the patients predominated who were female, older, widowed, single, and retired (Table 1).

Low-adherence patients were hospitalized more often, and their current hospitalization was longer. Overall, patients in the low-adherence group had more severe disease and poorer perception of well-being and experienced a more significant impact of COPD on daily activities and sleep (Table 2).

Table 1 Sociodemographic characteristics of chronic obstructive pulmonary disease (COPD) patients in relation to the level of adherence to therapy

Variable		High adherence ($n = 15$)	Low adherence ($n = 91$)	All ($n = 106$)	*p*-value
Age (year)	Mean ± SD	66.8 ± 4.81	70.7 ± 6.0	70.2 ± 5.9	0.020
BMI (kg/m^2)	Mean ± SD	25.0 ± 3.3	25.3 ± 6.0	25.2 ± 5.7	0.892
Pack-years	Mean ± SD	28.7 ± 18.1	37.6 ± 24.9	36.3 ± 24.2	0.132
Gender	Female	1 (6.7%)	41 (45.1%)	42 (39.6%)	0.011
	Male	14 (93.3%)	50 (55.0%)	64 (60.4%)	
Marital status	In relationship	13 (86.7%)	44 (48.4%)	57 (53.8%)	0.019
	Single	0 (0.0%)	20 (22.0%)	20 (18.9%)	
	Widowed	2 (13.3%)	27 (29.7%)	29 (27.4%)	
Residence	Urban	5 (33.3%)	27 (29.7%)	32 (30.2%)	0.768
	Rural	10 (66.7%)	64 (70.3%)	74 (69.8%)	
Education	Primary	3 (20.0%)	35 (38.5%)	38 (35.9%)	0.295
	High school	8 (53.3%)	42 (46.2%)	50 (47.2%)	
	College/ university	4 (26.7%)	14 (15.4%)	18 (17.0%)	
Professional activity	Active	8 (53.3%)	21 (23.1%)	29 (27.4%)	0.049
	Unemployed	0 (0.0%)	4 (4.0%)	4 (3.8%)	
	Retired	7 (46.7%)	66 (72.3%)	73 (68.9%)	
Cigarette smoking	Smokers	12 (80.0%)	72 (79.1%)	84 (79.3%)	1.000
	Non-smokers	3 (20.0%)	19 (20.9%)	22 (20.8%)	

BMI body mass index. The p-value corresponds to high vs. low-adherence difference for all items in a given category of variables

Table 2 Clinical characteristics of chronic obstructive pulmonary disease (COPD) patients in relation to the level of adherence to therapy

Variable		High adherence ($n = 15$)	Low adherence ($n = 91$)	All ($n = 106$)	p-value
Number of hospitalizations	1–4	13 (86.7%)	38 (41.8%)	51 (48.1%)	0.005
	5–8	2 (13.3%)	38 (41.8%)	40 (37.7%)	
	>8	0 (0.0%)	15 (16.5%)	15 (14.2%)	
Current hospitalization	< 7 days	12 (80.0%)	37 (40.7%)	49 (46.2%)	0.046
	8–14 days	3 (20.0%)	33 (36.3%)	36 (34.0%)	
	15–21 days	0 (0.0%)	15 (16.5%)	15 (14.2%)	
	> 21 days	0 (0.0%)	6 (6.6%)	6 (5.7%)	
Perceived well-being	Very good	0 (0.0%)	1 (1.1%)	1 (0.9%)	0.023
	Good	11 (73.3%)	35 (38.5%)	46 (43.4%)	
	Moderate	4 (26.7%)	30 (33.0%)	34 (32.1%)	
	Poor	0 (0.0%)	25 (27.5%)	25 (23.6%)	
Impact of COPD on daily activities	None	2 (13.3%)	5 (5.5%)	7 (6.6%)	0.001
	Slight	10 (66.7%)	17 (18.7%)	27 (25.5%)	
	Moderate	2 (13.3%)	28 (30.8%)	30 (28.3%)	
	Significant	1 (6.7%)	18 (19.8%)	19 (17.9%)	
	Very significant	0 (0.0%)	23 (25.3%)	23 (21.7%)	
Impact of COPD on sleep	None	9 (60.0%)	15 (16.5%)	24 (22.6%)	0.008
	Slight	3 (20.0%)	18 (19.8%)	21 (19.8%)	
	Moderate	1 (6.7%)	15 (16.5%)	16 (15.1%)	
	Significant	1 (6.7%)	18 (19. 8%)	19 (17.9%)	
	Very significant	1 (6.7%)	25 (27.5%)	26 (24.5%)	
Feeling rested after a night's sleep	Yes	10 (66.7%)	30 (33.0%)	40 (37.7%)	0.027
	No	5 (33.3%)	61 (67.0%)	66 (62.3%)	
Medications[a]	SAMA	5 (33.3%)	23 (25.3%)	28 (26.4%)	0.535
	SABA	2 (13.3%)	11 (12.1%)	13 (12.3%)	1.000
	LABA	8 (53.3%)	55 (60.4%)	63 (59.4%)	0.814
	LAMA	5 (33.3%)	57 (62.6%)	62 (58.5%)	0.064
	Theophylline	1 (6.7%)	14 (15.4%)	15 (14.2%)	0.690
	Glucocorticoids	1 (6.7%)	30 (33.0%)	31 (29.3%)	0.062
GOLD stage	I	9 (60.0%)	18 (19.8%)	27 (25.5%)	0.012
	II	5 (33.3%)	39 (42.9%)	44 (41.5%)	
	III	1 (6.7%)	22 (24.2%)	23 (21.7%)	
	IV	0 (0.0%)	12 (13.2%)	12 (11.3%)	

[a]Total exceeds 100%, as the item allowed for multiple choices. The p-value corresponds to high- vs. low-adherence difference for all items in a given category of variables, unless otherwise indicated

3.2 Level of Adherence to Treatment

The mean ARMS score was 23.1 ± 6.8 points or 1.9 points *per* question, meaning that the average frequency of adherent behaviors was slightly lower than "some of the time". The mean score concerning "medication taking" subscale was 15.0 ± 4.4 points or 1.9 points *per* question, meaning that the nonadherence to taking medications also was slightly lower than "some of the time". Likewise, the mean score in "prescription refilling" subscale was 8.1 ± 2.7 points or 2.0 points *per* question, meaning that the adherence to getting prescription refills also corresponded to "some of the time" category (Table 3).

Table 3 Adherence to therapy in chronic obstructive pulmonary disease (COPD) patients of the study

ARMS	Range (points)	*n*	Mean ± SD	Mean *per* question
Overall ARMS score	12–48	106	23.1 ± 6.8	1.9
Taking medications	8–32	106	15.0 ± 4.4	1.9
Refills	4–16	106	8.1 ± 2.7	2.0

ARMS the adherence to refills and medications scale

3.3 Multiple Factor Analysis of the Impact of Variables on Adherence to Treatment

Logistic regression demonstrates a single significant independent predictor of low adherence to treatment, which turned out to be male gender, with OR = 0.77, meaning a 23.6% decrease in adherence compared to females ($p < 0.05$). Linear regression demonstrates that the independent significant predictors of a total ARMS score were the following (Table 4):

- Marital status: compared to being married, being widowed increased the score by a mean of 3.2 points.
- Number of hospitalizations: compared to one to four hospitalizations, more than eight hospitalizations increased the score by a mean of 7.5 points.
- Duration of current hospitalization: compared to hospitalization shorter than 7 days, hospitalization longer than 21 days decreased the score by a mean of 8.2 points.
- Impact of COPD on daily activities: compared to no impact, a strong impact increased the score by a mean of 15.6.

3.4 Multiple Factor Analysis for Taking Medications and Refilling Prescriptions

Linear regression demonstrates that the independent significant ($p < 0.05$) predictors of ARMS score in the subscale of taking medications were the following (Table 5):

- Number of hospitalizations: compared to one to four hospitalizations, more than eight hospitalizations increased the score by a mean of 4.8 points.
- Duration of current hospitalization: compared to hospitalization shorter than 7 days, hospitalization longer than 21 days decreased the score by a mean of 5.2 points.
- Impact of COPD on daily activities: compared to no impact, a significant impact increased the score by a mean of 10.1 points.

Significant predictors of ARMS score in the subscale of refilling prescriptions were the following:

- Number of hospitalizations: compared to one to four hospitalizations, more than eight hospitalizations increased the score by a mean of 2.7 points.
- Impact of COPD on daily activities: compared to no impact, a significant impact increased the score by a mean of 5.5 points.

4 Discussion

The literature data indicate that COPD patients have a lower level of adherence than those with cardiovascular disease, hypercholesterolemia, osteoporosis, depression, or diabetes. COPD treatment is primarily based on inhalation therapy, though most patients prefer oral medications. Adherence in patients with inhalation therapy is low. In a study by Wiśniewski et al. (2014), 67% of patients adhered to treatment just for up to 30 days after discharge from the hospital. In

Table 4 Multiple factor analysis of the impact of variables on the level of adherence to therapy in chronic obstructive pulmonary disease (COPD) patients

Variable		Adherence			Total score		
		OR	95% CI	*p*	OR	95% CI	*p*
Age (year)		1.01	0.99; 1.02	0.528	0.11	−0.20; 0.41	0.490
BMI (kg/m^2)		0.10	0.98; 1.01	0.744	−0.15	−0.39; 0.09	0.222
Pack-years		1.00	0.99; 1.01	0.761	−0.05	−0.17; 0.06	0.355
Gender	Female	1	ref.		ref.		
	Male	0.77	0.67; 0.91	0.001	−0.67	−3.21; 1.87	0.601
Marital status	In relationship	1.00	ref.		ref.		
	Single	1.11	0.90; 1.37	0.324	1.13	−2.40; 4.66	0.525
	Widowed	1.02	0.85; 1.24	0.825	3.18	0.06; 6.30	
Residence	Urban	1.00	ref.		ref.		
	Rural	1.12	0.93; 1.36	0.231	−0.16	−3.38; 3.06	0.923
Education	Primary	1.00	ref.		ref.		
	High school	0.90	0.76; 1.08	0.260	−0.99	−4.01; 2.03	0.517
	College/ university	0.89	0.71; 1.13	0.338	−0.73	−4.74; 3.29	0.718
Professional activity	Active	1.00	ref.		ref.		
	Unemployed	1.09	0.75; 1.63	0.658	3.68	−3.12; 10.47	0.284
	Retired	1.06	0.87; 1.31	0.553	−2.89	−6.38; 0.61	0.104
Number of hospitalizations	1–4	1.00	ref.		ref.		
	5–8	1.15	0.88; 1.50	0.323	4.27	−0.31; 8.86	0.067
	> 8	1.18	0.81; 1.72	0.406	7.51	1.03; 13.98	0.024
Current hospitalization	< 7 days	1.00	ref.		ref.		
	8–14 days	0.99	0.79; 1.23	0.914	−2.33	−6.07; 1.41	0.218
	15–21 days	0.95	0.67; 1.36	0.787	−4.49	−10.58; 1.60	0.146
	> 21 days	0.93	0.59; 1.47	0.748	−8.22	−16.04; −0.40	0.040
Impact of COPD on daily activities	None	1.00	ref.		ref.		
	Slight	1.03	0.75; 1.41	0.868	−0.54	−5.91; 4.82	0.841
	Moderate	1.36	0.89; 2.09	0.161	2.64	−4.66; 9.95	0.472
	Significant	1.64	0.99; 2.71	0.060	4.82	−3.81; 13.44	0.269
	Very significant	1.787	0.89; 3.60	0.109	15.60	3.60; 27.59	0.012

(continued)

Table 4 (continued)

Variable		Adherence			Total score		
		OR	95% CI	*p*	OR	95% CI	*p*
SAMA	No	1.00	ref.		ref.		
	Yes	1.01	0.80; 1.30	0.966	−0.32	−4.19; 3.56	0.871
SABA	No	1.00	ref.		ref.		
	Yes	1.28	0.94; 1.74	0.126	2.71	−2.60; 8.03	0.312
LABA	No	1.00	ref.		ref.		
	Yes	1.01	0.82; 1.25	0.893	−0.44	−4.00; 3.12	0.806
LAMA	No	1.00	ref.		ref.		
	Yes	1.07	0.86; 1.34	0.543	0.08	−3.73; 3.88	0.969
Theophylline	No	1.00	ref.		ref.		
	Yes	0.93	0.68; 1.28	0.674	−1.38	−6.81; 4.06	0.615
Glucocorticoids	No	1.00	ref.		ref.		
	Yes	1.20	0.86; 1.68	0.289	−0.94	−6.68; 4.79	0.744
GOLD stage	I	1.00	ref.		ref.		
	II	1.11	0.82; 1.50	0.519	2.54	−2.67; 7.75	0.334
	III	0.92	0.54; 1.57	0.749	7.27	−1.94; 16.47	0.120
	IV	1.01	0.49; 2.06	0.981	9.85	−2.40; 22.09	0.113

Data are odds ratio (OR) 95% confidence intervals (95% CI); *BMI* body mass index; *SAMA* short-acting muscarinic antagonist; *SABA* short-acting inhaled beta-agonist; *LABA* long-acting β2-agonists; *LAMA* long-acting muscarinic antagonists.

screening tests performed in daily clinical practice, adherence to treatment in asthma and COPD patients rarely exceeds 50%. In the present study, good adherence was found in just 14.2% of patients. This is a considerably lower percentage than that found in other studies.

There is an ongoing discussion on the influence sociodemographic variables have on adherence. COPD patients who live alone often nonadhere to treatment. In studies by Bourbeau and Bartlett (2008) and Khdour et al. (2012), sociodemographic factors failed to associate with adherence to treatment. On the other hand, Tashkin (1995) has demonstrated that a stable family situation and available social support are linked to better adherence. Social support is among the most commonly reported predictors in chronically ill patients. Research has repeatedly shown a beneficial impact of social support on quality of life in patients with high blood pressure, acute coronary syndromes, atrial fibrillation, and heart failure (Ji et al. 2019). Social support plays an essential role in reducing stress, anxiety, and depression and in limiting the adverse influence of frailty in elderly patients.

In the present study, one factor that significantly affected adherence to treatment was the patient's daily functioning. COPD patients often quit work due to dyspnea and other symptoms, which decreases the perception of well-being and raises economic difficulties. All has an impact on both motivation to adhere to treatment and prescription refilling. The COPD patients in this study were in the seventh decade and often times with comorbidities, which made them use polypharmacy. The use of multiple drugs

Table 5 Multiple factor analysis of the impact of variables on taking medications and refilling prescriptions in chronic obstructive pulmonary disease (COPD) patients

Variable		Taking medications			Refilling prescriptions		
		r	95% CI	*p*	*r*	95% CI	*p*
Age (year)		0.05	−0.15; 0.25	0.612	0.06	−0.07; 0.18	0.382
BMI (kg/m^2)		−0.09	−0.25; 0.07	0.265	−0.06	−0.16; 0.04	0.230
Pack-years		−0.03	−0.11; 0.05	0.422	−0.02	−0.07; 0.02	0.331
Gender	Female	ref.			ref.		
	Male	−1.12	−2.78; 0.55	0.185	0.45	−0.60; 1.49	0.398
Marital status	In relationship	ref.			ref.		
	Single	0.46	−1.84; 2.77	0.690	0.67	−0.79; 2.12	0.362
	Widowed	2.04	−0.00; 4.07	0.050	1.15	−0.14; 2.43	0.079
Residence	Urban	ref.			ref.		
	Rural	−0.31	−2.41; 1.80	0.770	0.15	−1.17; 1.48	0.819
Education	Primary	ref.			ref.		
	High school	−0.50	−2.47; 1.47	0.614	−0.49	−1.73; 0.76	0.438
	College/ university	−0.22	−2.84; 2.41	0.869	−0.51	−2.16; 1.14	0.538
Professional activity	Active	ref.			ref.		
	Unemployed	2.52	−1.92; 6.96	0.261	1.15	−1.64; 3.95	0.414
	Retired	−2.11	−4.39; 0.17	0.070	−0.78	−2.21; 0.66	0.286
Number of hospitalizations	1–4	ref.			ref.		
	5–8	2.78	−0.22; 5.77	0.069	1.50	−0.39; 3.38	0.118
	> 8	4.79	0.55; 9.02	0.027	2.72	0.06; 5.39	0.045
Current hospitalization	< 7 days	ref.			ref.		
	8–14 days	−0.82	−3.27; 1.62	0.504	−1.51	−3.05; 0.03	0.055
	15–21 days	−2.02	−6.00; 1.96	0.314	−2.46	−4.97; 0.04	0.054
	> 21 days	−5.24	−10.35; −0.13	0.045	−2.98	−6.19; 0.24	0.069
Impact of COPD on daily activities	None	ref.					
	Slight	0.11	−3.40; 3.62	0.951	−2.86	1.56; 0.56	0.559
	Moderate	2.03	−2.74; 6.81	0.398	−2.39	3.62; 0.69	0.686
	Significant	3.41	−2.23; 9.05	0.231	−2.14	4.95; 0.43	0.432
	Very significant	10.11	2.27; 17.95	0.012	0.55	10.42; 0.03	0.030

(continued)

Table 5 (continued)

Variable		Taking medications			Refilling prescriptions		
		r	95% CI	*p*	*r*	95% CI	*p*
SAMA	No	ref.					
	Yes	0.095	−2.44; 2.63	0.941	−2.01	1.18; 0.61	0.608
SABA	No	ref.					
	Yes	2.053	−1.42; 5.53	0.242	−1.53	2.84; 0.55	0.550
LABA	No	ref.					
	Yes	−0.042	−2.37; 2.29	0.972	−1.86	1.07; 0.59	0.589
LAMA	No	ref.					
	Yes	0.985	−1.50; 3.47	0.432	−2.48	0.66; 0.25	0.250
Theophylline	No	ref.					
	Yes	−0.86	−4.42; 2.69	0.63	−2.75	1.72; 0.65	0.647
Glucocorticoids	No	ref.					
	Yes	−1.49	−5.24; 2.26	0.429	−1.81	2.91; 0.64	0.642
GOLD stage	I	ref.					
	II	1.34	−2.07; 4.74	0.436	−0.94	3.35; 0.27	0.267
	III	4.81	−1.21; 10.82	0.115	−1.33	6.24; 0.20	0.199
	IV	5.45	−2.55; 13.45	0.178	−0.64	9.43; 0.09	0.086

Data are regression coefficient *r* 95% confidence intervals (95% CI); *BMI* body mass index, *SAMA* short-acting muscarinic antagonist, *SABA* short-acting inhaled beta-agonist, *LABA* long-acting β2-agonists, *LAMA* long-acting muscarinic antagonists

increases the risk of adverse effects, leading to a belief that the main treatment is rather ineffective or even harmful. As a result, patients often randomly discontinue medications, which worsens the course of a disease.

An association between perception of one's health and adherence has been documented (Wiśniewski et al. 2014). According to Sanduzzi et al. (2014), adverse consequences of nonadherence include a gradual deterioration of quality of life, a sense of lack of control over a disease, and a higher mortality. Likewise, poor treatment outcome associates with poor adherence (Mäkelä et al. 2013; van der Molen et al. 2002). In the present study, both frequent and longer hospitalizations were significant predictors of lower adherence. This finding is in line with those of the 3-year-long TORCH study that has demonstrated a greater than twofold increase in the risk of death and a nearly twofold increase in the risk of rehospitalization in nonadherent patients (Vestibo et al. 2009). On the other hand, frequent COPD exacerbations may increase the perception of treatment necessity and thus portend better adherence.

In the present study, we found that female gender was associated with significantly lower adherence to treatment. That finding is at variance with most other studies, although some studies do report an association between gender and adherence (Kokturk et al. 2018; Müllerová et al. 2016). The influence of gender on adherence may be related to the increasing percentage of women who smoke cigarettes, a habit that may adversely affect daily functioning. Another factor, often reported as a predictor of adherence to treatment,

is age. In this study we found that older age significantly associates with lower adherence. The finding is in line with the reports that show older age is a risk factor for nonadherence due to forgetfulness, cognitive impairment, and polypharmacy (Shrestha et al. 2015). However, the issue remains contentious as some studies show that old patients are more likely to adhere to treatment (Rand et al. 1995; Turner et al. 1995). Yet other studies fail to support any association between age and adherence (Khdour et al. 2012).

We also report in here that nonadherent patients belonged to higher GOLD classes and thus suffered from more severe COPD. Adherence has to do with the severity of respiratory function deterioration. Dyspnea, the most bothersome symptom, is related to a decrease in FEV1% (Duarte–de–Araújo et al. 2018). Nonetheless, a link between COPD severity and adherence is not full clear. Some studies show that more severe course of COPD increases the patients' motivation to counteract distressing symptoms (Ivanov et al. 2018; Jouleh et al. 2018). In contradistinction, Liao and Chen (2019) have reported that as many as 81% of patients with a low severity of COPD, assessed from a proportion of days covered with at least one COPD maintenance medication of more than 80%, adhere to treatment. In the same vein, Leiva–Fernández et al. (2014) have reported that patients with mild-to-moderate COPD use up more prescriptions for inhaled corticosteroids.

In conclusions, we believe we have shown in this study that COPD patients, in the main, demonstrate a low level of adherence to therapy. Older age, female gender, being single and professionally inactive, frequent and longer hospitalizations, poor perception of well-being, and more sever course of COPD are all factors that significantly impact adherence to treatment. Medical practice should include an evaluation of adherence to therapy in COPD patients to achieve the optimum outcome.

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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 Wroclaw Medical University (approval no. 371/2019).

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

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Seasonality of Respiratory Syncytial Virus Hospitalization

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Abstract

Seasonality of respiratory syncytial virus (RSV) infection is an area of limited knowledge. In this study, we set out to get insight into the epidemic characteristics of RSV. We retrospectively evaluated medical files of 512 hospitalizations in children due to RSV infection from January 2010 to July 2017. In this cohort of patients, there were 96.3% of children below 1 year of age; the median age was 2.8 months. The influence of weather condition during the week of hospitalization (WH) and also the preceding week (WP) on the rate of hospitalizations was also assessed. An overview of morbidity data demonstrates that the epidemic RSV season started from Week 50 of a year and lasts until Week 15 of the following year, with a peak between Week 4 and Week 10. The average monthly percentage rate of morbidity *per* year was as follows: December, 12.3%; January, 24.5%; February, 29%; and March, 21.7%. Hospitalizations were positively associated with the minimum and maximum outside air temperature during the WH (62.5% and 59.7%, respectively) and the WP (64.3% and 63.4%, respectively) and with relative humidity (WH 23% and WP 29.8%). A weak association with the wind speed was also noticed (WH 22% and WP 21%), while there was no influence of the level of atmospheric pressure on RSV morbidity. We conclude that seasonality of RSV is present between December and April each year, and morbidity is mostly influenced by minimum-maximum outside air temperature changes. Further epidemiological exploration is required to get a better knowledge on both active and passive immunization against RSV.

Keywords

Airways · Bronchiolitis · Infection · Respiratory syncytial virus · Seasonality

1 Introduction

Lower respiratory tract infections (LRTI) are one of the major health concerns. Respiratory syncytial virus (RSV) is one of the most frequent and virulent pathogens causing LRTI. The exact global burden of RSV infections remains unknown. The estimate is that up to 20% of LRTI might be caused by RSV (Nair et al. 2010). The proportion increases in hospitalized LRTI, reaching 26%, and in children below 2 months of age, reaching 44% of cases (Hall et al. 2013). Clinical course of RSV infection varies from transient events to severe bronchiolitis and pneumonia. A vast majority of bronchiolitis caused by RSV is of particular concern (Hall

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et al. 2009) due particularly to children's young age and poor treatment options (Friedman et al. 2014; Ralston et al. 2014). Necrotizing bronchiolitis or pneumonia may require intensive care unit treatment (Simões et al. 2015).

There are data to suggest that the RSV season directly precedes the influenza outbreak (van Asten et al. 2016). Considering the ease of the RSV transmission and its impact on both morbidity and mortality in all age groups (Richter et al. 2016), intensified measures of preventing the disease deem warranted. To this end, predicting the RSV season is a paramount public health issue. RSV morbidity shows a seasonal pattern in temperate and subtropical latitudes (Tang and Loh 2014), while in the tropical climate, semiannual activity is observed (Hogan et al. 2016; Bloom-Feshbach et al. 2013). There is no effective vaccination against RSV as of yet. One of the hurdles is that the vaccine timing to prevent RSV infections, particularly in the youngest children, is a pivotal but unsettled issue (Caballero et al. 2017; Gálvez et al. 2017). Passive RSV prophylaxis can be currently achieved by administration of a monoclonal antibody, palivizumab, to infants at a high risk of severe RSV infection. The issue of prophylaxis is difficult to resolve due to high costs, differences in seasonality, and varying lengths of the RSV season in the different climate zones (Lee et al. 2017; Sanchez-Luna et al. 2017). Thus, exploration of a more precise RSV infection timeline may help adjust local prevention strategies (Panozzo et al. 2010).

There is a paucity of studies on the RSV incidence. The available data show a reverse association with outside temperature (Oliveira-Santos et al. 2016; Sirimi et al. 2016; du Prel et al. 2009) and a positive one with relative humidity (Nenna et al. 2017). The aim of the present study is to evaluate the RSV epidemiology in connection to meteorological conditions on the basis of RSV-related hospitalization in a single pediatric hospital ward in Warsaw, Poland. The issue was addressed by assessing the annual, monthly, and weekly fluctuations in the disease incidence depending on the weather condition.

2 Methods

In this study, we retrospectively evaluated medical files of 512 children hospitalized due to RSV infection from January 2010 to July 2017. A vast majority of patients, 493 (96.3%) out of the 512, were below 12 months of age. The median age was 2.8 months, ranging from 8 days to 121 months. The infection was diagnosed on the basis of signs and symptoms indicating the involvement of the lower respiratory tract. It was confirmed using a rapid RSV diagnostic test and/or polymerase chain reaction (PCR), performed in a nasopharyngeal swabs. The RSV-related diagnoses, coded according to the ICD-10 manual, were in the order of frequency as follows: bronchiolitis (J21.0; 390 cases), pneumonia (J12.1; 65 cases), and bronchitis (J20.5; 57 cases). The following terms were further referred to in this epidemiological overview:

Epidemic Week a week when at least 2% (*ca* 1/52) of the total number of RSV cases was diagnosed each year.

Epidemic Month a month when at least 8.5% (*ca* 1/12) of the total number of RSV cases was diagnosed each year. Frequency of infection data was calculated as a percentage of all positive cases *per* month each year, and then the percentages were added up and divided by the number of years (7 years for July–December or 8 years for January–June). The mean percentage obtained was used in further considerations.

Epidemic Year a year when at least 13.3% of the total number of cases was diagnosed. The overall number of months evaluated was 90 (or 7.5 years), making a single year 1/90. The following formula was used: $1/90 \times 12$ (or alternatively $1/7.5$) $= 13.3\%$.

Meteorological Data The RSV hospitalizations were referred to the daily outside temperature (maximum, minimum, and mean), mean daily relative humidity, mean daily wind speed, and mean daily atmospheric pressure. The data were

obtained from a measurement station of the Institute of Meteorology and Water Management in Warsaw, Poland, at the Warsaw Chopin Airport, with the coordinates of 52°9′N, 20°58′E, located approximately 14 km away from the hospital. Taking into account the incubation period of RSV infection, leading to delayed hospitalization from infection onset, a 7-day backward adjustment was made in matching the meteorological data.

Data was expressed as arithmetic means ±SD or medians with interquartile range (IQR). Data distribution was checked for normality using the Shapiro-Wilk test. An unpaired *t*-test or Mann-Whitney U test was used as required. The Spearman rank correlation coefficient *rho* was used to evaluate associations between sets of data. 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

A total number of RSV-related cases of infection *per* year varied between 30 in 2010 and 98 in 2013. There were annual fluctuations noticed. For instance, there were 98 (19.1% of total), 71 (13.9% of total), and 93 (18.2% of total) cases, respectively. However, no fixed pattern of annual fluctuations could be observed (Table 1).

Table 2 shows monthly and weekly evaluations in more detail. The months with the greatest morbidity were December (12.3% of cases *per* year), January (24.5% of cases *per* year), February (29% of cases *per* year), and March (21.7% of cases *per* year). The epidemic season extended toward April in 2011 and 2014 (8.8% and 11.3% of cases *per* year, respectively). Most often, epidemic season starts from Week 50 of a year and lasts until Week 15 of the following year, reaching peak between Week 4 and Week 10 (Table 3).

Referring to the meteorological conditions, RSV hospitalizations were associated with outside temperature, relative humidity, and wind speed. No significant association was found with barometric pressure. Temperature is adversely associated with hospitalizations (maximum temperature rho = −0.597, minimum temperature rho = −0.625, and mean temperature rho = −0.613). After adjustment for a 7-day RSV incubation delay, i.e., taking the meteorological data 1 week before hospitalization, correlations for temperature and relative humidity became slightly stronger. The *rho* coefficient for the association of RSV hospitalizations with wind speed was weak, amounting to 0.200 before and 0.188 after the adjustment (Table 4).

Table 1 The monthly and yearly number and percentage of respiratory syncytial virus (RSV) infections during 2010–2017

	2010	2011	2012	2013	2014	2015	2016	2017	SUM	%
Jan	3	16	4	29	13	27	15	27	134	26.2
Feb	11	18	15	27	14	12	12	40	149	29.1
Mar	7	11	14	28	16	7	9	21	113	22.1
Apr	1	5	4	4	8	3	2	3	30	5.9
May	2	1	0	1	4	2	4	2	16	3.1
Jun	1	1	0	0	2	2	0	0	6	1.2
Jul	0	0	0	0	0	0	0	na	0	0.0
Aug	0	0	0	0	0	1	0	na	1	0.2
Sept	0	0	0	0	0	1	0	na	1	0.2
Oct	0	0	0	0	1	1	0	na	2	0.4
Nov	2	0	3	1	0	3	1	na	10	2.0
Dec	3	5	9	8	13	3	9	na	50	9.8
Total number	30	57	49	98	71	62	52	93	512	100
Total percentage	5.8	11.1	9.6	19.1	13.9	12.1	10.2	18.2	–	–

Table 2 Percentage of cases each month and the mean percentage during 7.5 years of the study period

	2010	2011	2012	2013	2014	2015	2016	2017	Mean %
Jan	10.0	28.1	8.2	29.6	18.3	43.6	28.9	29.0	24.5
Feb	36.7	31.6	30.6	27.6	19.7	19.4	23.1	43.0	29.0
Mar	23.3	19.3	28.6	28.6	22.5	11.3	17.3	22.6	21.7
Apr	3.3	8.8	8.2	4.1	11.3	4.8	3.9	3.2	5.9
May	6.7	1.8	0.0	1.0	5.6	3.2	7.7	2.2	3.5
Jun	3.3	1.8	0.0	0.0	2.8	3.2	0.0	0.0	1.4
Jul	0.0	0.0	0.0	0.0	0.0	0.0	0.0	na	0.0
Aug	0.0	0.0	0.0	0.0	0.0	1.6	0.0	na	0.2
Sept	0.0	0.0	0.0	0.0	0.0	1.6	0.0	na	0.2
Oct	0.0	0.0	0.0	0.0	1.4	1.6	0.0	na	0.4
Nov	6.7	0.0	6.1	1.0	0.0	4.8	1.9	na	2.9
Dec	10.0	8.8	18.4	8.2	18.3	4.8	17.3	na	12.3

na nonavailable

Table 3 Percentage distribution of RSV-related hospitalizations by weeks in each epidemic season and the mean percentage in each week on yearly basis during 7.5 years of the study period

Week	2010	2011	2012	2013	2014	2015	2016	2017	Mean %
1	6.7	1.8	0.0	5.1	2.8	9.7	3.9	3.2	4.1
2	0.0	3.5	2.0	0.0	2.8	8.1	5.8	4.3	3.3
3	0.0	5.3	4.1	8.2	4.2	6.5	7.7	5.4	5.2
4	3.3	15.8	0.0	12.2	2.8	11.3	9.6	9.7	8.1
5	3.3	8.8	4.1	8.2	8.5	16.1	5.8	16.1	8.9
6	10.0	8.8	0.0	6.1	1.4	6.5	9.6	7.5	6.2
7	0.0	5.3	8.2	10.2	7.0	1.6	9.6	11.8	6.7
8	16.7	8.8	14.3	5.1	7.0	3.2	0.0	10.8	8.2
9	10.0	5.3	10.2	5.1	1.4	4.8	1.9	7.5	5.8
10	20.0	7.0	8.2	6.1	4.2	3.2	9.6	7.5	8.2
11	0.0	1.8	10.2	7.1	9.9	1.6	0.0	4.3	4.4
12	0.0	3.5	2.0	6.1	7.0	1.6	3.9	3.2	3.4
13	0.0	3.5	4.1	6.1	1.4	1.6	1.9	3.2	2.7
14	0.0	3.5	2.0	1.0	5.6	0.0	0.0	1.1	1.7
15	3.3	1.8	6.1	2.0	4.2	0.0	1.9	1.1	2.6
16	0.0	3.5	0.0	1.0	1.4	1.6	1.9	1.1	1.3
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48	0.0	0.0	4.1	0.0	0.0	1.6	1.9	na	1.1
49	0.0	0.0	6.1	3.1	0.0	0.0	3.9	na	1.9
50	0.0	5.3	2.0	1.0	4.2	1.6	0.0	na	2.0
51	6. 7	3.5	6.1	2.0	5.6	0.0	7.7	na	4.5
52	3.3	0.0	4.1	2.0	8.5	3.2	5.8	na	3.8

na nonavailable

4 Discussion

The findings of this study are generally in line with those present in the literature. The RSV seasonality varies, depending on both geographical location and climatic conditions. Annual epidemic seasons are observed in subtropical and temperate latitudes, while the infection pattern is less clear in tropical climate (Tang and Loh 2014; Bloom-Feshbach et al. 2013). In Europe, most of the research on the matter has been performed in the Mediterranean subtropical climate. The RSV seasons last from December until

Table 4 Correlation coefficient *rho* for the relationship between RSV-related hospitalizations and meteorological conditions

Parameter	Before adjustment	After 7-day adjustment
Maximum temperature	−0.597	−0.634
Minimum temperature	−0.625	−0.643
Mean temperature	−0.613	−0.640
Relative humidity	0.230	0.298
Wind speed	0.200	0.188
Atmospheric pressure	ns	ns

ns nonsignificant

March in Cyprus (Panayiotou et al. 2014) and Northern Portugal (Oliveira-Santos et al. 2016) and from December to February in Italy (Nenna et al. 2017). Those studies covered the periods of 3, 10, and 10 years, respectively, but the number of RSV patients evaluated in each (128, 367, and 266 patients, respectively) was lower than that in the present study. The largest study was conducted in Athens, covering 12 seasons and 2030 RSV patients (Sirimi et al. 2016), showing that the season lasted from December to April, with the highest incidence between January and March.

Poland is in temperate climate, and thus, relating the results above outlined to Polish patients might not be entirely correct. In this context, two studies from the neighboring Germany seem most relevant for comparison. Weigl et al. (2002) have investigated 384 RSV patients over a 7-year period, failing to notice a fixed RSV seasonal pattern. On the other hand, du Prel et al. (2009), investigating the effect of meteorological conditions on the hospitalization rate of 326 RSV patients over a 3-year period, have reported that the hospitalization rate was the only factor that inversely correlated with outside temperature, indicating increasing RSV frequency with decreasing temperature. Likewise, low temperature correlates with the RSV incidence in the studies by Oliveira-Santos et al. (2016) and Nenna et al. (2017). In the former study, RSV morbidity also is positively associated with wind speed and in the latter with relative humidity. However, the correlations between RSV hospitalizations and relative humidity or wind speed have not been confirmed in multivariable analysis, which may be explicable by the intertwined associations among temperature, relative humidity, and wind speed. In multivariable analysis performed by Sirimi et al. (2016), the average monthly temperature only achieved statistical significance regarding the association with the RSV incidence.

In the present study, we found a strong correlation of RSV hospitalizations with outside temperature, particularly the minimum temperature and with relative humidity. On the other hand, correlation with wind speed (approx. 20%) was rather weak and thus of minor influence on the meteorologically related RSV seasonality. We failed to find any appreciable association between atmospheric pressure and RSV hospitalizations, which is in line with the findings of du Prel et al. (2009). A question arises how lower temperature would facilitate spread of RSV. Studies suggest that the virus is more viable in secretions when temperature drops (Cui et al. 2015; Vandini et al. 2013). Low temperature, in itself, may also promote more indoor activity, while crowding is an essential risk factor for viral human-to-human spread (Jeena et al. 2003).

We also analyzed the possible influence of weather condition 1 week before hospital admission. This approach took into account a 7-day RSV incubation period, which could skew the effect on RSV morbidity and hospitalizations of meteorological factors at admission. In the main, we found that the correlations between RSV-related hospitalization rate and temperature or relative humidity became stronger after the backward meteorological adjustment. Similar approach of the evaluation of weather conditions preceding hospitalization by 7–10 days has been used in other studies (Oliveira-Santos et al. 2016;

Vandini et al. 2013). Likewise, a 14 km distance between the hospital and the meteorological station would rather be unlikely cause of result distortion. The climate and landscape differences are negligible in the city of Warsaw. Therefore, a single-site measurement, in all likelihood, reflects the meteorological condition within a wide perimeter. Moreover, the hospital serves patients from all over the city, as there is no hospital zoning in the current health-care system in Poland, so that a restriction to meteorological stations in the close hospital's vicinity could in fact be a source of bias. A comparably close distance has been reported in other studies (Sirimi et al. 2016).

In the present study, RSV detection was performed using a rapid immunochromatographic assay, and it was confirmed using PCR in some cases; both were qualitative analyses. A recent study by Midgley et al. (2017) has shown that PCR is more relevant regarding the determination of RSV seasonality. Nonetheless, high sensitivity and specificity of about 90–100% of a rapid test speaks in favor of reliability of this method of diagnosis (Mesquita et al. 2017; Peters et al. 2017). In an attempt to circumvent the bias of not including patients with RSV infection due to a defunct test result, the epidemic weeks and months were referred to the total number of confirmed RSV cases in a year and were expressed as a percentage. We also took a substantial leeway in considering what number of RSV hospitalizations would make the epidemic periods, assuming the presence of weeks containing more than one over 52 (*ca* 2%) and months containing more than one over 12 (*ca* 8.5%) of infection cases. Other possible biases were likely minimized in the present study by having over a 500-patient cohort, and the observation period extended to 90 months. Nonetheless, a single center study model could limit the interpretation and generalization of the results obtained. RSV seasonality may also be influenced by other factors, particularly those that are affected by weather condition, e.g., air pollution (Nenna et al. 2017; Ségala et al. 2008), which was not controlled for in the present study.

In conclusion, we believe we have shown that the RSV season starts around Week 50 of a year (late December) and lasts until Week 15 of the following year (half of April), with a peak between Week 4 and Week 10 (February and March). There are annual variations in the length of the epidemic RSV season, but the studies are too scarce to make generalizations to this end. RSV hospitalizations are mostly inversely influenced by the minimum temperature (64% correlation), followed by the relative humidity (30% correlation). Long-term explorations on widespread RSV infections are needed, which would help establish ways of passive and active prophylaxis and thus would much contribute to public health policies to mitigate the spread of virus.

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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 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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Factors Affecting Influenza Vaccination Rate in Adults with Asthma

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Abstract

Asthma is considered one of the most common noncommunicable diseases worldwide, with an incidence of 5.4% in the Polish, adult population. Symptoms of the disease can be triggered or worsened by a variety of factors including viral infection such as influenza, affirming the necessity for prophylactic vaccination. However, there is concern among the general population of the possibility of anaphylactic response to vaccination, which can deter patients with allergic asthma for fear of triggering exacerbation of their condition. The objective of the study was to determine the extent to which patients with asthma adhere to a schedule of recommended vaccinations in Poland. Two hundred fourteen patients were recruited from specialist outpatient clinics in Warsaw to complete voluntarily an anonymous questionnaire created for the purpose of this study. Within the past year, 82% stated having at least one respiratory infection, and 72% of patients were aware of the recommendation for annual vaccination against the flu. Forty-three percent of patients reported receiving the flu vaccine at least once, and only 20% followed through with annual vaccination. The most common sources of information about the importance of annual flu vaccination were from their doctors (47%) and the media (26%). Relatively few asthmatic patients in Warsaw get annual flu vaccinations despite the recommendations. These results were mainly sourced due to uncorrected fears and a lack of information about the importance of prophylaxis in asthma. It is essential to create effective strategies to inform patients with asthma of the importance of annual vaccinations.

Keywords

Adults · Asthma · Education · Flu vaccination · Motivation · Prophylaxis

1 Introduction

Asthma is considered one of the most common noncommunicable diseases worldwide (WHO 2013). In Poland, a study by Liebhart et al. (2007) found the prevalence of asthma to be approximately 5.4% in adults, where family history, sex, and exposure to smoke and traffic-related air pollution were the most significant risk factors.

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The three main pathophysiological characteristics used in the diagnosis of asthma are chronic eosinophilic airway inflammation, airflow limitation, and bronchial hyperreactivity (Walford and Doherty 2014). The symptoms associated with asthma can be triggered or worsened by allergens, tobacco smoke, exercise, temperature, bacterial infections, and especially by viral infections such as influenza (GINA 2015). Viral infections can cause a variety of additional pathophysiological changes, the severity of which depends on the virus. For instance, infection by influenza usually leads to extensive epithelial necrosis (Soo–Kim et al. 2015). The influenza virus is a trigger that causes inflammation in the airways of people with asthma resulting in severe complications, such as exacerbation of asthma, hospitalization, or even death (CDC 2016). The influenza vaccine itself is usually made using egg proteins, which can be seen as a potential trigger for an allergic reaction and consequently exacerbation of asthma. However, not only are these reactions rare, studies have shown there is no significant increase in risk for patients with egg allergies in comparison to those without egg allergies (Kelso 2014). Vaccination, especially in the elderly population, has been shown to reduce rates of mortality and morbidity of influenza in asthmatic patients (Rothbarth et al. 1995). Recently, systematic review and meta-analysis demonstrated that for persons with asthma, influenza vaccination may be effective in both reducing influenza infection and asthma attacks (Vasileiou et al. 2017). Due to the severity of adverse effects attributable to influenza, especially in patients with asthma, global guidelines set out by the Global Initiative for Asthma recommend an annual vaccination against influenza as a preventative measure (GINA 2015). Annual immunization with influenza vaccine of people with asthma is recommended by WHO and many national immunization advisory groups (CDC 2013a; NACI 2013). In Poland, the Ministry of Health recommends vaccination for adults over the age of 55, and in persons at high clinical risk, such as those with asthma. However, only is the cost of vaccine administration paid for by the Polish National insurance scheme, the cost of the vaccine must be covered by the recipient (VENICE 2014).

In a study comparing influenza vaccination rates in 11 EU countries including Poland, it was found that the vaccination coverage rate for those in the high-risk group was lowest in Poland at 11%, whereas the highest coverage was 56% in the UK (Blank et al. 2009). A study by Kroneman and van Essen (2007a) found that patients in Poland were more concerned with the cost of the influenza vaccination than patients in other countries, such as Sweden. A low vaccination rate points to the need for assessment of the patients' understanding of the associated risks of influenza infection on asthma, as well as their source of information. It is important to evaluate whether vaccination rates are affected by cost or misconceptions, such as fear of anaphylaxis or the belief that prophylaxis for influenza will not have a significant effect on the severity of asthma. Another important factor to consider is the information about vaccine recommendations that patients receive from their healthcare providers to create effective strategies to inform patients with asthma of the importance of annual vaccinations. In the USA, a majority of general pediatricians and family physicians reported routinely recommending influenza vaccination for asthma patients (Dombkowski et al. 2008).

The objective of this study was to determine the extent to which patients with asthma adhere to a schedule of recommended vaccinations in Poland. In addition, it assesses the sources of their knowledge and where appropriate, their main motivations for adhering to annual vaccination or equally, what deters them from following recommendations.

2 Methods

The study was based on a questionnaire created specifically for patients with asthma. The questionnaire assessed general information about the patients, including age, sex, and education, as well as information pertaining to their vaccination status. In addition to addressing how often patients were vaccinated, motivations for

Table 1 Characteristics of asthma patients

	Total	Females	Males
Number of patients	214	149	65
Age (yr)	52 ± 17	52 ± 17	52 ± 17
Range of age (yr)	20–91	20–91	20–87
University education (%)	63.6	64.4	61.5
High school education or less (%)	36.5	35.6	38.5
Duration of asthma (yr)	13.1 ± 14.4	13.7 ± 15.0	11.7 ± 12.8
Atopic asthma (%)	51	54	43
Treated by pulmonologist (%)	82.8	83.3	81.6
Treated by allergologist (%)	11.6	10.1	12.6
Treated by family doctor (%)	5.6	5.8	6.6

vaccination and deterring factors were evaluated. The study also enquired from what sources patients received the most information pertaining to the importance of annual vaccination against influenza and more specifically which doctors were most likely to recommend vaccination.

Two hundred fourteen patients were recruited from five specialist outpatient clinics in Warsaw (Warsaw Medical University Hospital, Czerniakowski Hospital, Military Medical Institute, Luxmed Medical Center, and the Independent Public Outpatient Clinic of South Praga). The majority of patients were women (70%) of the mean age of 52 ± 17 years, ranging from 20 to 91 years. Sixty-four percent of respondents had higher education. Half of the patients were atopic. The mean duration of asthma, as declared by patients, amounted to 13.1 ± 14.4 years. The patients were usually treated by a pulmonologist (83%). They were divided into two age groups: (I) below 55 years of age, 116 patients, and (II) 55 years of age and older, 98 patients (Table 1).

Continuous data were expressed as means ±SD and categorical as percentages. A t-test was used to assess the intergroup differences. A p-value of <0.05 was considered to define statistically significant differences. Data was entered into a database using Microsoft Excel 2011.

3 Results

About 51% of patients reported having allergic asthma. The most common triggers of exacerbations were infections (27%), followed by exercise (19%), allergens (17%), weather (12%), and air pollution (12%). Within the preceding year, 82.0% of patients stated having at least one respiratory infection, and 71.5% were aware of the recommendation for annual vaccination against the flu. Annual vaccination was reported by 20% of patients. However, patients younger than 55 years of age were twice as often to be unaware of the need for influenza vaccination when compared to older patients, 38% and 18.4%, respectively. In the younger group, annual vaccinations were also half as often than in the patients 55+ years of age, 13.7% and 26.5%, respectively (Fig. 1). There were no significant gender differences noticed concerning the awareness of recommendations for annual vaccination against the flu or the proportion of patients reporting such vaccination. About 73% of women and 68% of men reported being aware of the recommendation for vaccination, with just 20% of patients of either gender being actually vaccinated (Fig. 2). University education failed to significantly affect the awareness of flu vaccinations when compared to patients having lower educational status, 69.6% and 76.6%, respectively. Nonetheless, patients with university education were more often vaccinated, albeit insignificantly so, than those with lower education (20.6% and 14.1%, respectively (Fig. 3).

Female patients younger than 55 years of age were significantly more aware (81.2%) of the recommendation of annual vaccination against the flu than those 55+ years of age (65%) ($p = 0.02$) (Fig. 4). The younger patients also

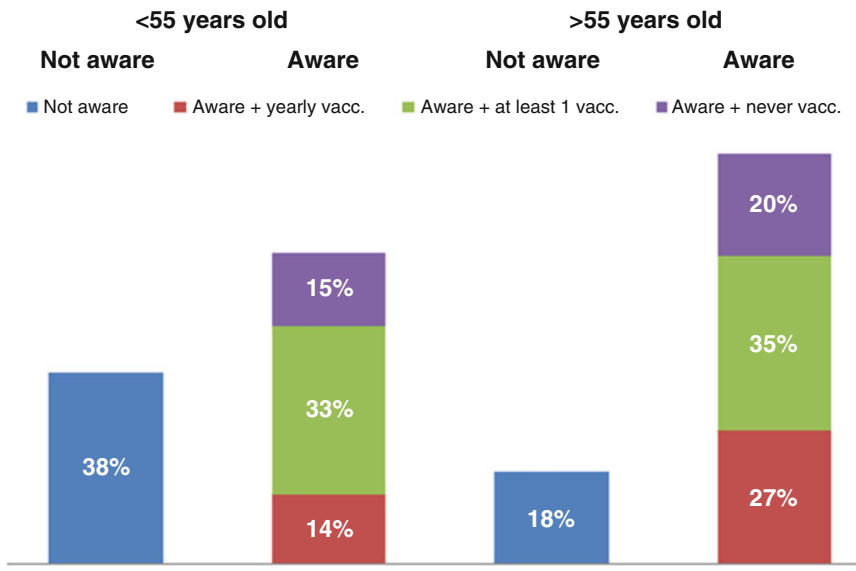

Fig. 1 Awareness of recommendation for vaccination against influenza and vaccination rate by age

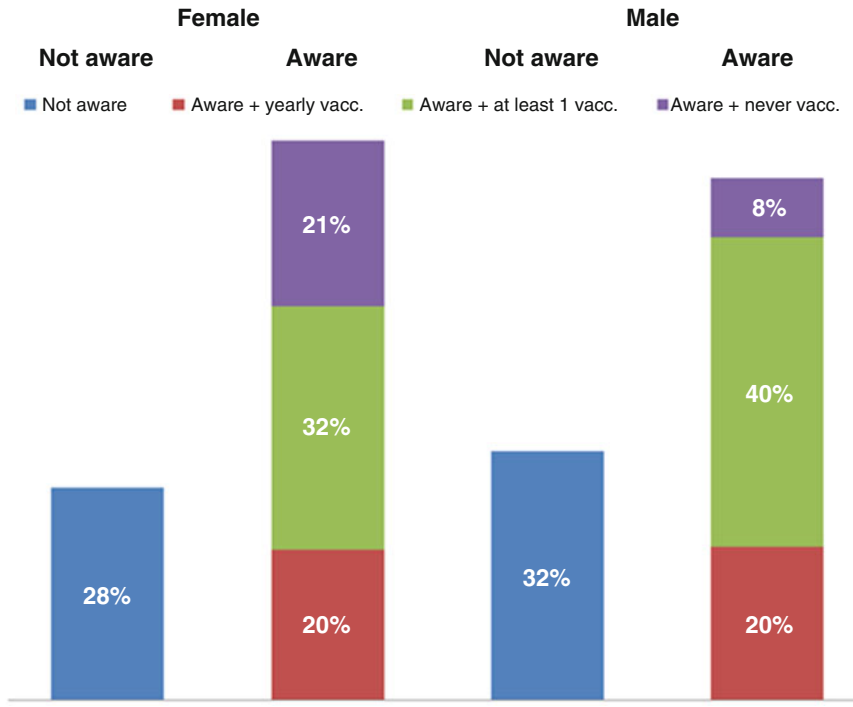

Fig. 2 Awareness of recommendation for vaccination against influenza and vaccination rate by gender

were more likely to have annual vaccination than the older ones; 27% vs. 14%, respectively ($p = 0.08$). There were 75% of women with university education in the younger group and 71.6% of them were aware of the recommendation of annual vaccination, with just scarce 10% following through with it. In contradistinction, out of the remaining 25% of women with lower

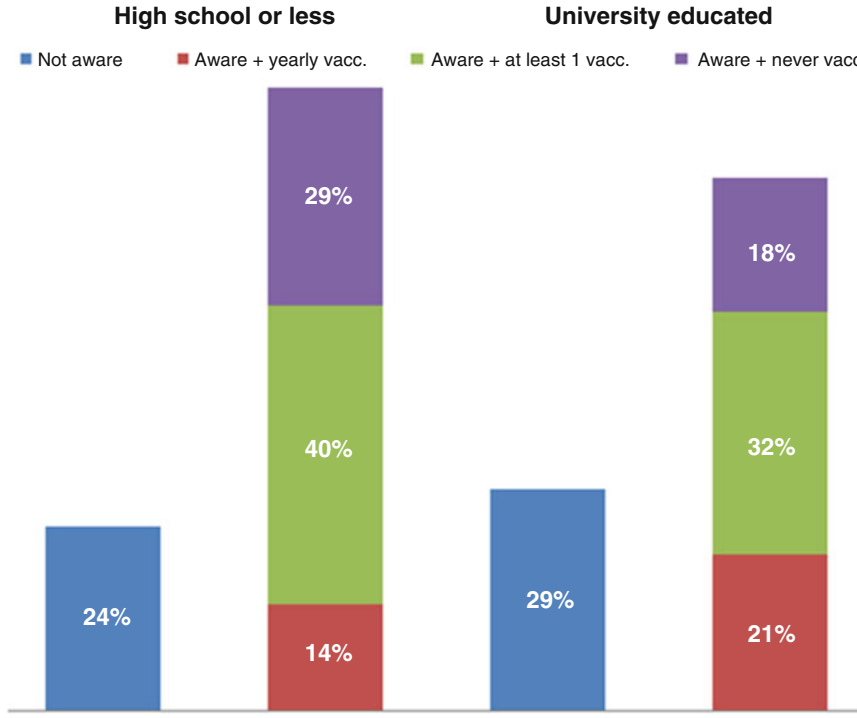

Fig. 3 Awareness of recommendation for vaccination against influenza and vaccination rate by education level

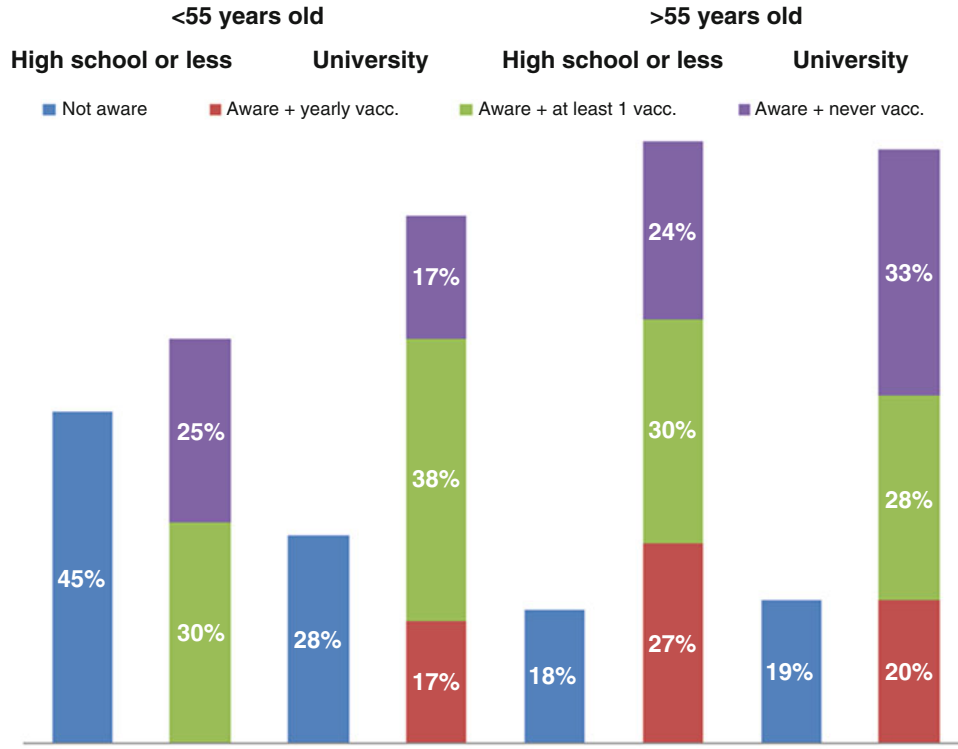

Fig. 4 Awareness of recommendation for vaccination against influenza and vaccination rate in females based on education level and age

educational status in this group only 55% were aware of the recommendation of annual vaccination and none followed through with it. Here, 54.5% of women reported being vaccinated at least once, and the other 45.5% reported never being vaccinated. Such a trend was not noticed in women 55+ years of age, where 80.6% of those with university education and 81.8% of those with lower educational status were aware of the recommendation of annual vaccination, with 24.1% and 29.6% following through it, respectively. Additionally, 34.5% and 41.4% of older women with university education reported vaccination at least once or never being vaccinated, respectively. In those with lower educational status, these figures were 37.1% and 29.6%, respectively (Fig. 4).

Conversely to females, male patients younger than 55 years of age were significantly less aware (55.6%) of the recommendation of annual vaccination against the flu than those 55+ years of age (82.8%) ($p = 0.02$) (Fig. 5). The younger patients also were less likely to have annual vaccination than the older ones (16% vs. 24%, respectively), although the difference failed to reach statistical significance ($p = 0.478$). Male patients' education also appeared to influence the awareness of vaccination. In those 55+ years of age, 72.2% had higher education and one half of them were aware of the recommendation. Out of the remaining 27.8% with a lower educational status, about 70% were aware of the recommendation of annual vaccination. Among male patients aged below 55 years who were aware of the recommendation, those with university education were more likely to follow through with vaccination (38.5%) when compared to those with a lower educational status (14.3%). This trend was unnoticed in male patients aged over 55 years, where of the 48.3% patients with university education, 78.6% were aware of the recommendation but only 24.1% followed through with it. For comparison, in the 51.7% of male patients with a lower educational status, 93.3% were aware of the recommendation, with just 8.1% following through with annual vaccination (Fig. 5).

The most common sources of information about the importance of annual flu vaccination were from doctors (47%) and media (26%) (Fig. 6). Family doctors (24%) and pulmonologist (16%) were the specialists most likely to recommend vaccination, followed by allergologists

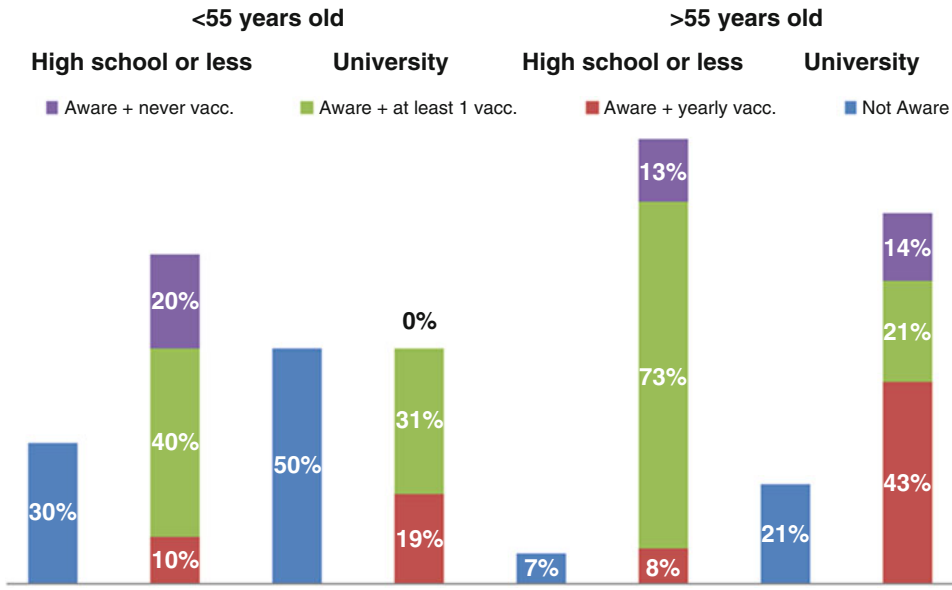

Fig. 5 Awareness of recommendation for vaccination against influenza and vaccination rate in males based on education level and age

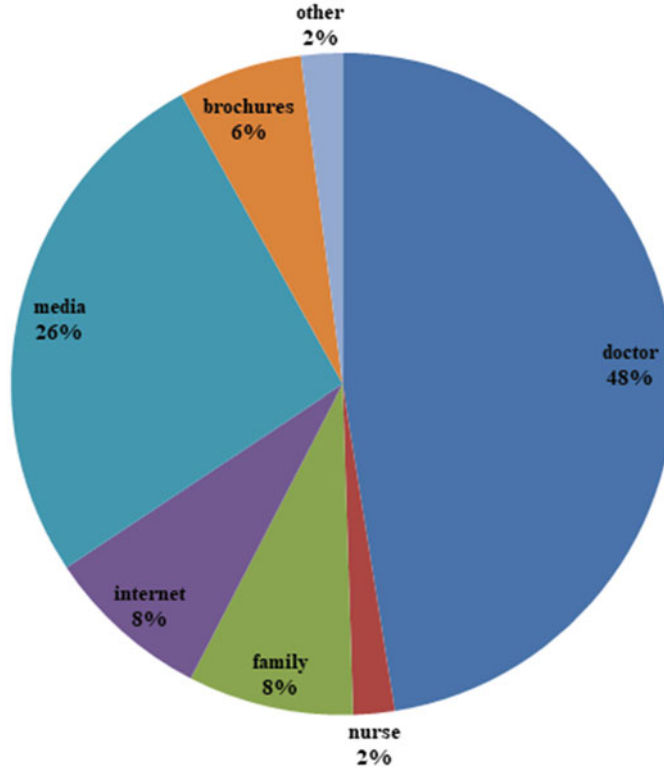

Fig. 6 Factors encouraging for vaccination against influenza

(4%) and others (1%). However, 36% of patients reported getting no recommendation from any doctor, and 19% reported self-requesting the vaccine.

Thirty-eight percent of patients reported never being vaccinated against the flu, which comprised 40% of female and 32% of male participants. The most common reasons for being unvaccinated were the following: disbelief in vaccine effectiveness (29%), fear of adverse effects (18%), lack of information on prophylaxis (16%), and fear of asthma exacerbation (10%). Males were more likely to list disinclination for vaccination, 18% vs. 6% in females ($p = 0.08$), and a lack of awareness, 24% vs. 15% in females ($p = 0.31$). On the other side, females were more likely to list contraindication to vaccination, 8% vs. 0% in males ($p = 0.02$); logistical difficulties, 3% vs. 0% in men ($p = 0.19$); and a lack of faith in vaccine effectiveness, 33% vs. 26% in females ($p = 0.43$) (Fig. 7).

4 Discussion

High-risk patients in Poland are one of the lowest in Europe vaccination rates against influenza of about 3.5% (ECDC 2015). In the present study, annual vaccination rate, declared by asthma patients, stood at 20%. However, this result was obtained from patients treated in university-associated specialist clinics in the capital city of Warsaw, which could, in part, account for a relatively high rate declared. The result may have been substantially lower in provincial centers, where both doctors and patients may not be as aware of the need for vaccination against influenza. A 9% vaccination rate has been reported for chronically ill patients aged below 65 in Poland compared to 13% in Sweden in the years 2003–2005 (Kroneman and van Essen 2007a, b).

We found in this study that there was, overall, no significant gender-related difference in the

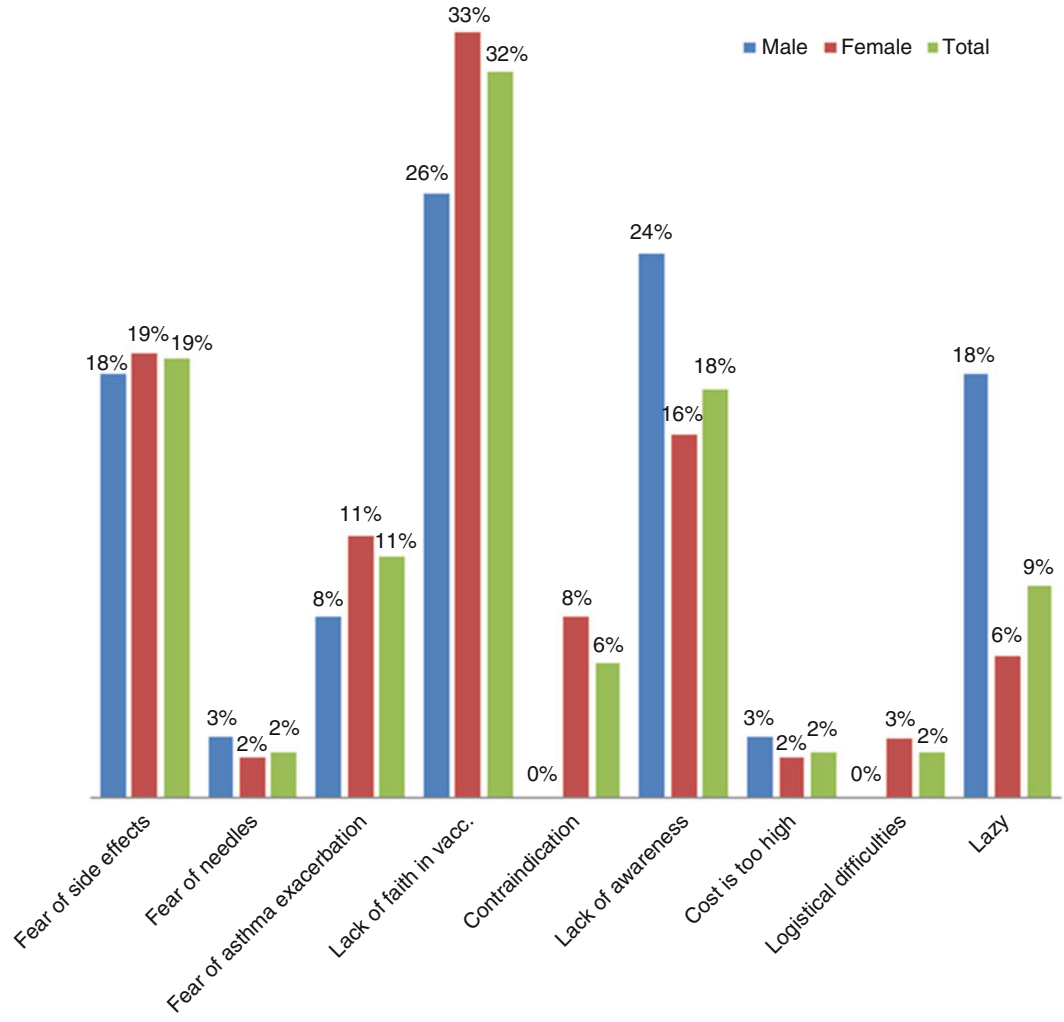

Fig. 7 Factors preventing from vaccination against influenza

vaccination rate (19.5% in women vs. 19.9% in men). A divergent result has been obtained by Kroneman and van Essen (2007a) who have found that vaccination rate is higher in women. We did, however, find that patient had affect on gender-related, asthma-independent differences in the vaccination rate. Younger female patients were more aware of the recommendation for influenza vaccination and were more likely to get the vaccination than the older ones, with the assumed cut-off age limit of 55 years. Interestingly, in male asthma patients, this result was reversed.

In a study by Chung et al. (2017), patients were divided into three age groups, 19–49, 50–64, and 65+, with 28%, 50%, and 82% vaccination rates, respectively. The patients, therefore, had a progressively greater vaccination rate with age, but no gender-related differences are reported. The distribution of those results is grossly akin to the present findings, although the vaccination rate is seemingly higher in the older patients of the study quoted. Likewise, the distribution of results in asthmatic patients in a study of Lu et al. (2009) is in line with the present findings, although the patients aged 18–49 have

a 34% vaccination rate and those aged 50–64 have a 55% rate; the figures are larger than those found in the present study. Those authors have reported enhanced likelihood of vaccination with older age, female gender, health insurance, and in non-smokers. An association between older ages, especially above 65 years, with increased vaccination rate has also been found in an Australian study, which could be due to the eligibility for free immunization as of that age (Dower et al. 2011).

In contradistinction to a dismally low influenza vaccination rate in Poland, some countries such as the Netherlands or the UK exceed the 75% target recommended by the Council of the European Union (CEU 2009). Studies from other countries also show higher vaccination rates in asthmatic patients than those reported in the present study. Lu et al. (2009) have shown a 29% vaccination rate in asthma patients aged 18–64 in the USA. Another US study has reported a 43% rate in asthmatic patients and a greater adherence to vaccination recommendation in older individuals (Mazurek et al. 2014). The vaccination rate has been about 50% in the asthmatic population in the USA in the 2010–2011 epidemic season, an increase by about a dozen of percentage points compared with that 5 years back (CDC 2013b). In Spain, vaccination rate in asthmatic patients has been reported at about 35% and increasing with patient age; this figure was higher than the 22% in non-asthmatic patients (Santos–Sancho et al. 2013). Another Spanish study has reported a vaccination rate of 38% among adult asthmatic patients, the percentage increasing in the elderly, non-smokers, and in those with lower levels of education (Jiménez–García et al. 2010). A study from Australia has reported a vaccination rate of 47% among high-risk patients suffering from asthma, diabetes, or cardiovascular pathologies (Dower et al. 2011). In Canada, 36% of asthmatics have never been vaccinated against influenza (Guthrie et al. 2017). A German study shows that about half of patients aged over 60, particularly those with chronic pathologies, believe the influenza vaccine causes influenza. The most common reasons for not getting vaccinated were mistrust of the vaccine (22%) and the perception that influenza is not a dangerous infection (21%) (Bödeker et al. 2015).

In the present study, misconceptions on influenza were the most commonly reported reasons for not getting vaccinated. The misconceptions comprised the psychological resistance to any vaccination, perceived disqualification to flu vaccination, misjudgment on the severity of influenza infection and thus a notion that preventative measures suffice, and the vaccine cost. We found, however, that the cost played a rather marginal role when compared to the lack of proper education. In Poland, there is a serious shortage of medical staff, both doctors and nurses, with the lowest rates *per* population in the EU. Consequently, medical professionals do not have enough time for patient education, and the findings we herein present show that medical advice is essential in persuading asthmatic patients to get vaccinated. This notion is strengthened by the finding that a monetary incentive of physicians in the UK for providing more extensive medical advice to patients has increased vaccination rates. We show that declared awareness for the need to vaccinate against influenza did not, in most cases, result in vaccination. Therefore, enhanced patient education does not seem to entirely resolve the issue of inadequate vaccination rate. The situation may be further worsened by the contemporary rise in the anti-vaccination movements which may hamper the efforts to improve influenza vaccination rate. As personal beliefs are crucial in the vaccination decision-making process, tailored communication strategies should focus on improving an understanding of health risks and benefits of vaccination in target groups (Bödeker et al. 2015). In Canada, 84.4% of patients with asthma cited perceptual barriers as a reason for not being vaccinated (Guthrie et al. 2017).

A limitation of this study was the reliance on patient declarations, which may have introduce a bias, an inherent feature of self-reported surveys. Another bias, as aforementioned, could be that the study was conducted at university-associated tertiary care institutions, where physicians were well aware of the importance of influenza vaccinations in high-risk groups. Therefore,

further exploration should be needed to confirm the validity of the present findings on larger patient populations at the providers of primary and secondary care.

5 Conclusions

This study shows that influenza vaccination rate in the adult asthmatic patients in Poland currently stands at 20%. This rate is reasonably high when compared to the 3.5% average in the general population, but remains relatively low when compared to other countries. Whether the vaccination rate herein reported is due to improved vaccination coverage or other factors, such as, the tertiary referral centers where the study was conducted, remains to be further explored on larger patient populations. Irrespective of the underlying reason, it seems essential to create effective strategies to inform asthmatic patients on the importance of annual vaccination. The information ought to address the patients' misgivings concerning a lack of value of prophylaxis against influenza, particularly in the presence of chronic pathologies, and the all-too-often notion of a relative no-harm of the infection. Vaccination against influenza also is a cost-effective preventive measure of hospitalization due to post-infection complications associated with asthma exacerbation, which could have added healthcare benefits in countries, such as Poland, with a socialized healthcare system.

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Conflicts Interest No conflicts of interest were declared with relation to this work.

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 Medical University of Warsaw, Poland.

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

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Respiratory Complications in Children Hospitalized with Respiratory Syncytial Virus Infection

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Abstract

The goal of this study was to define the prevalence of respiratory complications, other than bronchiolitis, such as pneumonia, acute otitis media, and conjunctivitis in children treated in a hospital due to respiratory syncytial virus (RSV) infection, with reference to the plausible risk factors. The study included 111 children, aged up to 22 months (median 3 months). Complications were observed in 68 (61%) children, with 32 (29%) children presenting more than one. The most frequent complication was acute otitis media in 53 (48%), pneumonia in 37 (33%), and conjunctivitis in 12 (11%) out of the 111 children. Children with complications were older than those without complications and had fever that lasted for a significantly longer time, both before and during hospitalization, and the fever was stronger. They also presented a significantly lower breathing rate at admission. The age over 3 months was a single risk factor associated with the development of otitis media (OR = 9.8, 95%CI: 3.6–26.7) and pneumonia (OR = 2.8, 95%CI: 1.1–7.3). Other factors such as prematurity, birth weight below 2500 g, exposure to tobacco smoke during pregnancy, and the cessation of breastfeeding below age 6 months were statistically irrelevant to this end. We conclude that complications are very frequent in hospitalized children with RSV infection and their risk increases with the infant age.

Keywords

Bronchiolitis · Infection · Otitis media · Pneumonia · Respiratory syncytial virus

1 Introduction

The respiratory syncytial virus (RSV) is one of the most common infectious agents, responsible for an enormous percentage of acute respiratory tract infections in infants and younger children. The virus causes about 30 m infections each year in low- and middle-income countries and 2.8 m in high-income countries, leading to about 3.2 m hospitalizations. The majority of hospitalizations occur in children below age 5 months, and 45% of

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hospitalizations concerns children in the first 6 months of life (European Medicines Agency 2017). There is a high fatality rate of 6.21 deaths *per* 1000 children below age 5 years, yet the deaths occur mainly in lower-income countries (Stein et al. 2017). The lowest fatality rate is observed in high-income countries where it is estimated to be 0.2%, 0.9%, and 0.7% in children aged 0–5 months, 6–11 months, and 12–-59 months, respectively (Shi et al. 2017). The RSV causes a major health concern and has a significant impact from the medical care and socioeconomic standpoints. In the outpatient setting, acute otitis media (AOM), which is one of the most frequent complications of respiratory tract infections, turns to be a major cost drawing condition. AOM is variably reported in over 20% (Paramore et al. 2004), 47% (Chonmaitree et al. 2008), 57% (Ruuskanen et al. 1989), and 58% (Heikkinen et al. 2017) of children hospitalized due to RSV-associated bronchiolitis and pneumonia. In general, a leading diagnosis in children hospitalized due to RSV infection is bronchiolitis, which concerns about 70–85% of hospitalized, particularly younger children (Hall et al. 2009). Conversely, RSV is also a major etiological factor of bronchiolitis, responsible for about 60–80% of all bronchiolitis cases (Bamberger et al. 2012; Mansbach et al. 2012; Calvo et al. 2010). RSV also plays a causative role in community-acquired pneumonia (CAP) as about 30% of CAP appears RSV positive (Jain et al. 2015; García–García et al. 2012).

RSV is associated with a risk of developing post-infection wheezing/asthma and as allergic rhinoconjunctivitis. A study by Fujishima (2002) has suggested that the risk of rhinoconjunctivitis increases due to the presence of RSV in conjunctival epithelial cells. A meta-analysis by Shi et al. (2015) has shown that the risk factors of a severe RSV infection course, in the decreasing order of odds ratio, are the following: preterm infants, low birthweight, male gender, having siblings, maternal smoking and history of atopy, and a lack of breastfeeding. Regarding mortality, a higher risk is associated with prematurity; severe comorbidities, including chronic lung disease and congenital heart disease; and nosocomial acquisition of infection (Welliver et al. 2010). The goal of this study was to define the prevalence of respiratory tract complications, other than nearly universal bronchiolitis, such as pneumonia, acute otitis media, and conjunctivitis in children hospitalized due to RSV-associated respiratory infections, with reference to the plausible risk factors.

2 Methods

The study included 111 children aged up to 22 months (median 3 months, interquartile range (IQR) 1–16 months), who were treated in a tertiary hospital due to RSV infection in the 2017/2018 season. Clinical and laboratory conditions were assessed at admission and then repeatedly in the course of hospitalization. The following variables were recorded: breathing rate, heart rate, serum C-reactive protein (CRP) and procalcitonin, peripheral capillary blood pH, partial carbon dioxide pressure and oxygen saturation, and hematologic profile consisting of white blood cells count (WBC), absolute neutrophil count (ANC), hemoglobin concentration, and platelet count. In addition, we assessed duration of fever prior to hospitalization, the highest level and duration of in-hospital fever, the number of days on oxygen supplementation and antibiotic treatment, and duration of hospitalization.

Acute otitis media (AOM) was defined as the presence of redness, hemorrhagic or cloudy appearance, and bulging of tympanic membrane. In order not to miss an AOM case, since many viral otitis media runs a self-limiting course, patients were prospectively followed up, and otoscopy was performed in each patient at the following time points, admission and then days 3, 5, and 7 post-admission, depending on the patient's length of hospitalization. The other descriptive definitions in the study were as follows: prematurity, gestational age below 37 weeks; low birth weight, below 2500 g; maternal cigarette smoking, any smoking during pregnancy mentioned by parents surveyed at admission; lack of breastfeeding, cessation of breastfeeding below age 6 months; and crowding

condition at home, 1.5 or more persons *per* one room in a household, which was the median value for the whole group of patients. These definitions are generally in line with those used in previous studies (Shi et al. 2015), with some modifications made for a lack of breastfeeding and crowding. The entire lack of breastfeeding is, in our opinion, impossible to ascertain and may be a misnomer, as even mothers who are unwilling to breastfeed make attempts at the very beginning almost in each case. Breastfeeding up to age 6 months is recommended as a desirable goal by the WHO and the European Society for Pediatric Gastroenterology, Hepatology and Nutrition (WHO 2019; Agostoni et al. 2009). Thus, we considered the lack of breastfeeding as its cessation below age 6 months. Likewise, overcrowding is difficult to ascertain as it may depend on the number of persons *per* room or household, as well as on other poorly controlled for local circumstances. For instance, crowding has been defined as more than 1.5 persons/room (Bruden et al. 2015), $\geq$2 persons/room plus 4 or more children in the household (Bulkow et al. 2002), and $\geq$ 10 persons/household or the presence of $\geq$2 siblings aged 3–5 years sleeping in the same room (Weber et al. 1999). Yet others have set the number of siblings sleeping in the same room at $\geq$3 (Okiro et al. 2008). Shi et al. (2015) have used the definition of crowding of $\geq$7 persons/household in their meta-analysis, excluding the studies that failed to meet this criterion. In view of the arbitrariness and lack of fixed rules for what is crowding, we defined it in this study as the number of inhabitants *per* household exceeding the median value.

Data was presented as means ±SD or medians with lower and upper quartiles. The Shapiro-Wilk test was used to assess normality data distribution. A *t*-test and Mann-Whitney U test were used to compare the independent variables, as appropriate A p-value <0.05 defined statistically significant intergroup differences. Logistic regression analysis was used to calculate the odds ratio (OR) with 95% confidence interval (95% CI). Statistical analysis was performed using a commercial Statistica v13 package (StatSoft; Tulsa, OK).

3 Results

Complications were noticed in 61% of cases (68/111) of RSV-associated respiratory infection, with 29% of children (32/111) presenting more than one complication. There were 30 cases of double complications and 2 cases of the 3 complications above outlined. The most frequent complication was AOM, 48% (53/111), followed by pneumonia, 33% (37/111), and conjunctivitis, 11% (12/111). Referring to the co-occurrence of multiple complications, AOM was accompanied by pneumonia in 25 children and by conjunctivitis in 4 children cases, while in another 3 children, pneumonia and conjunctivitis co-occurred.

At admission, there were 25, 13, and 5 children diagnosed with unilateral, bilateral, and purulent AOM, respectively. On days 3–5, the number increased to 32, 18, and 14 cases, respectively. On day 7, there were 10 unilateral, 7 bilateral, and no purulent AOM case. The peak increases in the number of AOM on days 3–5, when compared to the admission examination, were as follows: unilateral 1.28-fold, bilateral 1.39-fold, and purulent 2.8-fold.

The children with complications, in the main, were older than those without complications (median 6 vs. 2 months, respectively; $p < 0.001$) (Table 1). This age difference between children

Table 1 Baseline characteristics of children with and without complications after respiratory syncytial virus (RSV) infection

Parameter	Complications	No complications	*p*
Calendar age (months)	6 (2–16)	2 (1–3)	0.001
Gestational age (weeks)	39 (38–40)	39 (39–39)	0.600
Birth weight (g)	3,340 (2,835–3,820)	3,400 (3,030–3,760)	0.100
Crowding (persons *per* household room)	1 (1–2)	2 (1–2)	0.120

Results are medians (LQ-UQ)

with and without complications concerned both AOM and pneumonia groups (median 7 vs. 2 months, IQR: 3–16 vs. 1–4 months; $p < 0.010$ and 7 vs. 3 months, IQR: 2–16 vs. 1–6 months, respectively; $p = 0.011$). Fever at admission in these children was higher and lasted longer both before and during hospitalization, and they had a lower breathing rate. Children with complications also required longer antibiotic treatment. All these clinical differences between children with and without complications related to RSV infection were significant as presented in detail in Table 2.

The serum CRP level was distinctly higher in children with RSV-associated complications (median 5.45 vs. 1.00 mg/L; $p < 0.001$; with the upper limit of the norm in the hospital laboratory of 5.00 mg/L). Likewise, neutrophil count was significantly higher in these children (median 4.67 vs. 2.19 k/μL; $p = 0.011$). The other blood and biochemical indices investigated did not differ significantly between children with and without complications (Table 3).

Among the risk factors for RSV-associated complications, only the median age over 3 months correlated with the development of complications (OR = 5.2, 95% CI: 2.0–13.5) in the whole group. The correlation was much stronger for AOM (OR = 9.8, 95% CI: 3.6–26.7) than for pneumonia (OR = 2.8, 95%CI: 1.1–7.3). All the other analyzed risk factors, including prematurity, birth weight below 2,500 g, exposure to tobacco smoke during pregnancy, and the cessation of breastfeeding below age 6 months, were

Table 2 Clinical characteristics of children with and without complications after respiratory syncytial virus (RSV) infection

Parameter	With complications	Without complications	*p*
Fever			
Severity at admission (°C)	37.8 ± 1.0	37.1 ± 0.8	0.001
Total duration (hours)	54.7 ± 55.9	17.8 ± 41.5	0.001
Before hospitalization (hours)	30.0 ± 44.6	11.0 ± 29.5	0.007
During hospitalization (hours)	21.8 ± 27.4	6.2 ± 23.5	0.001
Breathing rate (/min)	50 (40–58)	58 (50–65)	0.031
Heart rate (/min)	138 (130–148)	140 (130–152)	0.064
Oxygen supplementation (hours)	0 (0–36)	0 (0–0)	0.578
Antibiotic therapy (days)	7 (0–10)	0 (0–0)	0.001
Hospitalization (days)	10 (8–13)	8 (7–11)	0.181

Results are means ±SD and medians (LQ-UQ)

Table 3 Laboratory findings in children with and without complications after respiratory syncytial virus (RSV) infection

Parameter	With complications	Without complications	*p*
Hb (g/dL)	11.8 (10.9–12.3)	11.4 (10.8–12.4)	0.721
WBC (k/μL)	11.89 (8.90–13.70)	10.84 (8.49–13.55)	0.317
NEU (k/μL)	4.67 (2.22–6.47)	2.19 (1.12–5.08)	0.011
PLT (k/μL)	379 (311–472)	439 (344–498)	0.133
CRP (mg/L)	5.45 (1.78–14.58)	1.00 (0.49–4.74)	0.001
PCT (ng/mL)	0.13 (0.09–0.38)	0.12 (0.08–0.15)	0.112
ScO_2 (%)	92.2 (90.3–94.0)	90.3 (87.2–93.4)	0.077
$PcCO_2$ (mmHg)	34.9 (30.9–37.5)	35.5 (32.6–40.9)	0.142
pHc	7.42 (7.40–7.44)	7.41 (7.39–7.43)	0.050

Results are medians (LQ-UQ); *Hb* hemoglobin content, *WBC* white blood cell count, *NEU* absolute neutrophil count, *PLT* platelet count, *CRP* C-reactive protein, *PCT* procalcitonin, ScO_2 capillary blood oxygen saturation, $PcCO_2$ capillary blood partial pressure of carbon dioxide, *pHc* capillary blood pH

statistically irrelevant when analyzed for the whole group of patients with complications, as compared to those without complications, as well as in the subgroups of AOM, pneumonia, and conjunctivitis.

4 Discussion

This study demonstrates a substantial 61% (68/111) rate of complications in children up to 22 months of age, hospitalized due to RSV-associated respiratory infection, with about half of those children presenting more than one complication. Acute otitis media (AOM) clearly prevailed at 48% (53/111), followed by pneumonia, 33% (37/111), and conjunctivitis, 11% (12/111). Otitis media paired with pneumonia also prevailed in case of multiple complication in a child. Despite rather severe and frequent complications, there was no fatality in the investigated group. Our findings are grossly in line with other literature reports. Willson et al. (2003) reported a somehow higher 79% prevalence of complications, but that study focused on infants only, and it investigated a broader array of complications, including cardiovascular and electrolyte disorders which took place in 9% and 19% of infants, respectively. On the other side, Gentile et al. (2019) reported a clearly lower 21% prevalence of complications in non-fatal cases, but about 94% in fatal cases, with many patients having more than one complication. Those authors emphasize that the study was conducted in a hospital, and the complications included respiratory distress, atelectasis, sepsis, and the nosocomial infection; the last one not necessarily being RSV-associated.

The present study focused on respiratory complications that constitute a health hazard in an inpatient setting such as AOM, pneumonia, and conjunctivitis. We purposefully excluded bronchiolitis from the array of RSV-associated complications on the ground that it is the most frequent clinical presentation of RSV infection. Had bronchiolitis been considered a complication, the prevalence of complications would have increased to 98% (109/111). For comparison, Hall et al. (2009) have found in a prospective, population-based study that 70% of children below age 5 years, hospitalized due to RSV infection, are diagnosed with bronchiolitis. Thus, bronchiolitis should rather be considered a typical clinical presentation of RSV infection in hospitalized children than a complication.

We found that AOM was the predominating complication affecting 48% of children with RSV-associated infection. Previous studies have shown a similar prevalence of AOM: 47.4% in children aged 6 months to 3 years who were prospectively followed up for 1 year (Chonmaitree et al. 2008); 55.6% in children with bronchiolitis, investigated in a study by Gomaa et al. (2012); and 57% in a study by Ruuskanen et al. (1989). Heikkinen et al. (2017) have evaluated the prevalence of AOM by children's age, showing a peak up to age 1 year. Yet it remained at a high level also in children aged 1, 2, and 3–6 years, amounting to 52%, 58%, and 46%, respectively. Likewise, Vesa et al. (2001) have shown the presence of AOM in 57.7% of children hospitalized due to RSV infection. Further, those authors show the predominating occurrence of AOM in the course of any verified viral infection in children aged 6–11 months. A lower prevalence of AOM has been reported in children hospitalized due to RSV bronchiolitis (20.1%) or pneumonia (21.4%) in a review of the American national databases performed by Paramore et al. (2004). However, that study analyzed the final diagnoses unlike the studies outlined above, including the present one, based on a prospective surveillance of RSV-infected children.

In the present study, prevalence of RSV-associated pneumonia was 33%. This figure seems to reflect well the optimum 32% benchmark of clinical care quality concerning the use of chest radiograph in children hospitalized with bronchiolitis, based on the assumption that each X-ray would yield a positive result (Parikh et al. 2014). In a population-based study by Hall et al. (2009), the highest 51% rate of RSV-associated pneumonia has been in children aged 24–-59 months. In contrast, in a study conducted in more than 15,000 patients in a tertiary hospital

over an 18-year-long surveillance, Gentile et al. (2019) have reported that atelectasis, which closely reflects pneumonia, is seen only in 3.8% of RSV survivors and in 13.4% of fatal cases. Likewise, Heikkinen et al. (2017) have reported pneumonia in 3% of children with RSV infection aged under 13 years. Therefore, there appears to be an essential age-dependent difference in the prevalence of pneumonia in children with bronchiolitis, with much greater vulnerability in infants and small children.

In a study of Souty et al. (2019) in 6000 patients of all ages, conjunctivitis did not associate with RSV infection in children below age 15 years, although it modestly did with influenza infection, the occurrence of both types of infections being alike – 15% and 17%, respectively. Interestingly, in a study of Sigurs et al. (2005), increased frequency of allergic rhinoconjunctivitis has been reported in children aged about 13 years who had been hospitalized years earlier in infancy due to severe RSV infection when compared to non-hospitalized subjects. The underlying pathomechanism of the effect is unclear, but it might have to do with RSV invading the conjunctival epithelial cells during the infection (Fujishima 2002). If the eye is the primary site for RSV invasion, local inflammation could result in persistent rhinoconjunctivitis. In a murine model, RSV is able to replicate in the eye, with subsequent lower respiratory tract infection that is indistinguishable in terms of the disease course from that acquired through the nose. As a consequence of ocular infection, a mix of chemokines and cytokines are produced. Anti-cytokine treatment reduces local inflammation but does not inhibit RSV replication (Bitko et al. 2007). The presence of RSV-associated conjunctivitis might be a prognostic of future allergic rhinoconjunctivitis, considering the persistent ocular inflammatory response subsequent to viral invasion.

In this study we found that age over 3 months was a single risk factor associated with the development of otitis media and pneumonia. Other commonly considered factors such as prematurity, birth underweight, exposure to cigarette smoke during pregnancy, and the cessation of breastfeeding below age 6 months did not appear to associate with any of the complications found. For comparison, Hall et al. (2009) have reported that the duration of breastfeeding for less than 1 month of age is a significant risk factor for the development of infectious complications. However, cessation of breastfeeding between 1 and 6 months of age seems irrelevant to this end. Although the risk factors that concern children who are hospitalized each season are well-known, the practical use of this knowledge seems limited. There is a need to increase awareness of the amenable risk factors of RSV infection in the public at large, the factors that basically concern all kinds of respiratory infection. A case in point may be the issue of cigarette smoking during pregnancy. In this study, 20 (18%) out of the 111 children hospitalized with a severe course of RSV infection were exposed to maternal smoking.

Limitations of this study were a relatively small group of child patients and a preselection bias stemming from the fact that all the patients were hospitalized. We also failed to verify the existence of comorbidities, e.g., congenital heart disease, kidney diseases, and others. The presence or lack of a serious comorbidity was established only the basis of anamnesis taken from the child parents/guardians. Despite these limitations we believe we have conclusively shown that RSV infection is related to a high number of complications, which raises a serious health concern and places a substantial burden on healthcare resources. A risk of complications increases with the infant age. Unfortunately, the known risk factors of a severe RSV course do not exactly reflect the development of complications. Therefore, the ill children need to be carefully followed up in order to discern and treat possible complications in a timely manner.

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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 Center of Postgraduate Medical Education in Warsaw, Poland.

Informed Consent Written informed consent was obtained from the parents or legal guardians of all the child patients of the study.

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