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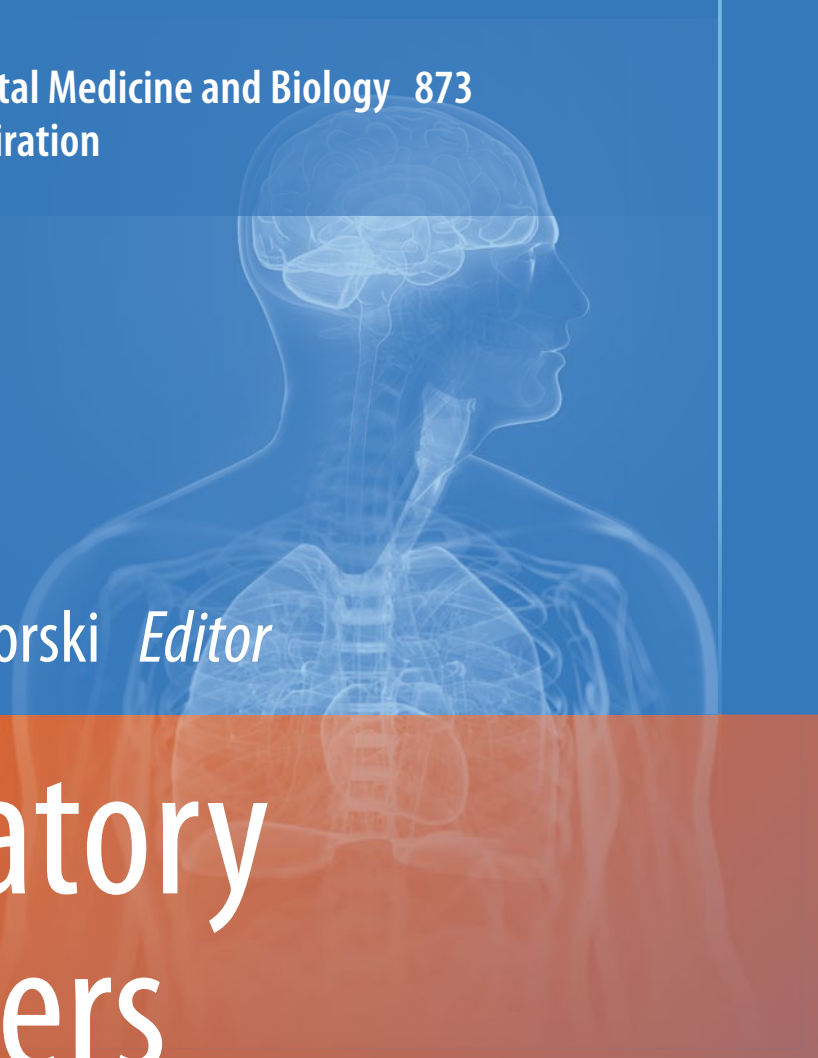

Mieczyslaw Pokorski *Editor*

Ventilatory Disorders

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Ventilatory Disorders

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Preface

The book series *Neuroscience and Respiration* presents contributions by expert researchers and clinicians in the field of pulmonary disorders. The chapters provide timely overviews of contentious issues or recent advances in the diagnosis, classification, and treatment of the entire range of pulmonary disorders, both acute and chronic. The texts are thought as a merger of basic and clinical research dealing with respiratory medicine, neural and chemical regulation of respiration, and the interactive relationship between respiration and other neurobiological systems such as cardiovascular function or the mind-to-body connection. The authors focus on the leading-edge therapeutic concepts, methodologies, and innovative treatments. Pharmacotherapy is always in the focus of respiratory research. The action and pharmacology of existing drugs and the development and evaluation of new agents are the heady area of research. Practical, data-driven options to manage patients will be considered. New research is presented regarding older drugs, performed from a modern perspective or from a different pharmacotherapeutic angle. The introduction of new drugs and treatment approaches in both adults and children also is discussed.

Lung ventilation is ultimately driven by the brain. However, neuropsychological aspects of respiratory disorders are still mostly a matter of conjecture. After decades of misunderstanding and neglect, emotions have been rediscovered as a powerful modifier or even the probable cause of various somatic disorders. Today, the link between stress and respiratory health is undeniable. Scientists accept a powerful psychological connection that can directly affect our quality of life and health span. Psychological approaches, by decreasing stress, can play a major role in the development and therapy of respiratory diseases.

Neuromolecular aspects relating to gene polymorphism and epigenesis, involving both heritable changes in the nucleotide sequence, and functionally relevant changes to the genome that do not involve a change in the nucleotide sequence, leading to respiratory disorders will also be tackled. Clinical advances stemming from molecular and biochemical research are but possible if the research findings are translated into diagnostic tools, therapeutic procedures, and education, effectively reaching physicians and patients. All that cannot be achieved without a multidisciplinary, collaborative, bench-to-bedside approach involving both researchers and clinicians.

The societal and economic burden of respiratory ailments has been on the rise worldwide leading to disabilities and shortening of life span. COPD alone causes more than three million deaths globally each year. Concerted efforts are required to improve this situation, and part of those efforts are gaining insights into the underlying mechanisms of disease and staying abreast with the latest developments in diagnosis and treatment regimens. It is hoped that the books published in this series will assume a leading role in the field of respiratory medicine and research and will become a source of reference and inspiration for future research ideas.

I would like to express my deep gratitude to Mr. Martijn Roelandse and Ms. Tanja Koppejan from Springer's Life Sciences Department for their genuine interest in making this scientific endeavor come through and in the expert management of the production of this novel book series.

Opole, Poland Mieczyslaw Pokorski

Volume 16: Ventilatory Disorders

Respiratory function is a major determinant of the overall quality of health and well-being of an individual. This book runs the gamut of chapters devoted to chronic cough-related conditions in children and adults, health care quality and safety, environmental pollution health effects, efficiency of therapeutic approaches and a mutual dependence of respiratory and non-respiratory illnesses. An integrated approach to the investigation and treatment of sleep disordered breathing as well as the use of new and more efficient diagnostic strategies for pleural tuberculosis are presented. Chapters focus on translating science into practice, with an eye on presymptomatic identification of serious ailments for which there could be more effective therapy, leading to improved general health outcomes. This book includes chapters about disorders which will be of interest to clinicians, family practitioners and medical researchers.

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Gastroesophageal Reflux Disease in Children with Cystic Fibrosis

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Abstract

Previously published studies have indicated that gastroesophageal reflux (GER) disease is common in pediatric patients with cystic fibrosis. The aim of the present study was to get insight into the incidence of GER and to characterize the nature of reflux episodes in children with cystic fibrosis. This was a multicenter, prospective study of children with cystic fibrosis older than 18 months. Forty four consecutive patients (22 boys, mean age 10.4 ± 3.6, range 3.0–17.8 years) were enrolled into the study. All patients underwent 24 h pH-impedance monitoring. GER were classified according to the widely recognized criteria as an acid, weakly acid, weakly alkaline, or proximal. The pH-impedance trace was considered abnormal when acid exposure was >6 %. GER was diagnosed in 24/44 (54.5 %) children. A total of 1585 (median 35, range 7–128) reflux episodes were detected; 1199 (75.6 %) were acidic, 382 (24.1 %) weakly acidic, and 4 (0.3 %) weakly alkaline. Six hundred and ninety-one (43.6 %) reflux episodes reached the proximal esophagus. In 14/44 patients typical GER symptoms were present. We conclude that the incidence of GER in children with cystic fibrosis is very high. In the majority of patients typical GER symptoms are absent. Therefore, diagnostic procedures should be considered, regardless of lacking symptoms. Although acid reflux episodes predominate in children with cystic fibrosis, classical pH-metry may not constitute a sufficient diagnostic method in this population because of a relatively high number of proximal reflux

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episodes. Such episodes also indicate an increased risk for aspiration. The pH-impedance diagnostic measurement is advocated when suspecting GER in children with cystic fibrosis.

Keywords
Children • Cystic fibrosis • Gastroesophageal reflux • Nonacidic reflux • pH-impedance

1 Introduction

The spectrum of symptoms of cystic fibrosis (CF) is broad and extends beyond the respiratory system. It depends mainly on the type of mutation in both *CFTR* gene and modulatory genes (Wilschanski 2008). Other factors, e.g., treatment methods may also bear on the symptoms. Currently, it is believed that as many as several dozens of CF complications are associated with the gastrointestinal system. Gastroesophageal reflux (GER) disease occurs in CF children more frequently (Brodzicki et al. 2002; Walkowiak et al. 2004) than in the healthy pediatric population. The incidence of GER in these children does not tapers off with age and reflux episodes often relapse (Balfour-Lynn and Elborn 2002).

It is still uncertain whether GER in CF is the primary or secondary phenomenon of the disease. Nor is the effect on CF-associated lung pathology of increased frequency of gastroesophageal reflux episodes well understood. However, it is evident that both in children and adults, episodes of acid reflux are associated with the worsening of lung function (Navarro et al. 2001). A negative effect of GER on forced expiratory volume in 1 s (FEV1) and forced vital capacity (FVC) in CF children has been demonstrated (van der Doef et al. 2009). The detriment in lung function could have to do with microaspirations (Mendelson 1946; Boyle et al. 1985) of gastric content into the respiratory tract, irritant pepsin action (Rosen et al. 2012), or reflux-associated bronchospasm (Spaulding et al. 1982). Some available data suggest that GER may also affect nutritional status of CF patients (Dray et al. 2005).

The classic pH-metry has been traditionally used for GER diagnostics in CF patients. However, there are indication that episodes of non-acid reflux, undetectable by this method may provoke respiratory symptoms (Sifrim et al. 2005; Blondeau et al. 2007) and negatively affect FEV1 (Palm et al. 2012). The association of non-acid reflux with bouts of cough has been demonstrated in CF (Blondeau et al. 2008a). Reflux episodes seem to underlie coughing in children with CF, rather than being secondary to cough. These children also are at higher risk of aspiration (Blondeau et al. 2010). Non-acid reflux episodes are detectable by the pH-impedance method (MII-pH). According to the recently published guidelines, MII-pH measurement should be considered in patients with extraesophageal GER symptoms (Wenzl et al. 2012). The goal of the present study was to assess the incidence of GER in children suffering from cystic fibrosis and to characterize the nature of gastroesophageal reflux episodes in these children.

2 Methods

The study protocol was approved by the Ethics Committee of Medical University of Warsaw, Poland. Informed consent was obtained from the patients and/or their parents. This was a multicenter, prospective study. Forty four CF patients (F/M – 22/22) of the mean age of 10.4 ± 3.6 SD years (range 3.0–17.8 years)

Table 1 Characteristics of the study population

Number of patients (M/F)	44 (22/22)
Age, mean ± SD (range)	10.4 ± 3.6 (3.0–17.8)
Symptoms presented (no. of patients)	
Abdominal pain	32
Cough	23
Heartburn	7
Regurgitation	8
Nausea	4
Chest pain	3

were enrolled into the study. CF was diagnosed on the basis of widely recognized criteria (Smyth et al. 2014). The most common symptoms were cough and abdominal pain. Seven patients suffered from heartburn. Detailed characteristics of the study population are shown in Table 1. There were two exclusion criteria: other than CF genetic disorders and treatment with antisecretory medication/prokinetic agents (proton pump inhibitors, H2 blockers, antacids, baclofen, cisapride, metoclopramide, sucralfate, theophylline, anticholinergics, or neuroleptics) used during 2 weeks preceding the study.

All patients underwent 24-h multichannel intraluminal impedance with pH-metry (MII-pH). Two types of MII-pH catheters were used: ZPN-BS-46 M for children younger than 10 years and ZAN-BS-01 M (Sandhill Scientific, Denver, CO) for older children. The location of a pH sensor was controlled by X-ray. The sensor was positioned at the level of the third vertebra above the diaphragm. Data were analyzed with BioView ver. 5.4.3 software (Sandhill Scientific, Denver, CO). There also was a manual check of the data to confirm the accuracy of impedance episodes. The detection and definition of acid and non-acid reflux episodes was based on the criteria described in a consensus report (Sifrim et al. 2004). The total number of reflux events (acid, weakly acid, weakly alkaline, and proximal), the percentage of time with the esophageal pH below 4.0 – called the reflux index, and to the volume of the refluxate were calculated. The exposure to volume was defined as the percentage of time required for the 50 % recovery of the impedance after its 50 % drop from the baseline level for all gastroesophageal reflux episodes detected during the procedure time. The MII-pH tracing was considered abnormal if the acid exposure (AE) exceeded 6 % according to the NASPGHAN guidelines (Rudolph et al. 2001). To assess how different cut-off points would influence the GER diagnosis the total AE of >3 % and AE >7 % were also used (Vandenplas et al. 2009). Results were expressed as median and interquartile range (IQR, 25–75th percentile).

3 Results and Discussion

GER was diagnosed in 54.5 % (24/44) of the patients according to main criteria and in 70.5 % (31/44) and 50.0 % (22/44), when additional criteria were taken into account.

A total number of 1585 gastroesophageal reflux episodes were detected by MII-pH (median 35.0; 20.0–46.3). Of these 1199 (75.6 %) episodes were acidic (median 24.5; 13.0–36.25), 382 (24.1 %) episodes were weakly acidic (median 6.0; 4.0–11.0), and 4 (0.3 %) were weakly alkaline (median 0.0, 0.0–0.0). Neither the patients nor their parents noted any specific symptoms to be mentioned. Thus, symptom correlation parameters could not calculated. There were 691 (43.6 %) proximal gastroesophageal reflux episodes (median 14.0, 6.8–22.3). The median esophageal acid exposure was 6.8 % (2.7–10.2). Median volume exposure was 1.2 % (1.0–1.7).

The main observations in our study regarded a high prevalence of acid and proximal GERs in children with CF. We also confirmed a high frequency of GER in that population (24/44, 54.5 %).

Five studies including the assessment of all types of GER in children with CF have been published so far (Blondeau et al. 2010; Doumit et al. 2012; Palm et al. 2012; Caldaro et al. 2014; Woodley et al. 2014). The population sample of the present study has been larger than those in the studies outlined above. The difference between this and other previous studies also is that all children with CF meeting simple inclusion/exclusion criteria were qualified into the study. In contrast, Palm et al. (2012), Blondeau

et al. (2010); Caldaro et al. (2014), and Woodley et al. (2014) enrolled only the patients with pre-suspected GER into the study, which in our opinion may introduce a selection bias. Only did a single study by Doumit et al. (2012) include GER diagnostics as an element of routine evaluation of all children with CF. However, the population sample of that study was much younger (median age of 12 months) and nearly twice less numerous (n = 20) than that of the present study. Therefore, the present results refer to the general pediatric CF population.

A high proportion of non-acid GERs (49 %) was reported by Palm et al. (2012). That value is twice as high as that reported in the present study. Whereas the population of children investigated by those authors has been similar to ours in terms of the number and age (n = 35, mean age 13.5 ± 5.8 years), nearly all patients of that study (34/35, 97 %) used proton pump inhibitors while being tested with MII-pH. It is worth noting that in other studies, the use of proton pump inhibitors has been an exclusion criterion. Like in the present study, in almost all other studies, acid GERs dominated; however, the observed proportion of acid GERs was slightly lower (*ca*. 65 %) than the 75.6 % of the present study. The difference in the percentage of acid GERs may be explained by different inclusion criteria. The present results support the hypothesis that there is an increased gastric acid secretion and reduced pancreatic bicarbonate secretion in CF (Hallberg et al. 2001). Irrespective of the differences in all the studies discussed, weakly alkaline GERs have been sporadic.

Studies in which a double-channel pH-metric probe has been used indicate that in children with respiratory diseases, other than CF, the proportion of GERs reaching the proximal sensor positively correlates with the pepsin level in the bronchoalveolar lavage (Starosta et al. 2007). It has also been demonstrated that proximal GERs are more often associated with respiratory symptoms that the distal ones (Blondeau et al. 2010). Those observations are not uniformly confirmed in other studies (Cucchiara et al. 1995). Many authors have suggested that aspiration of gastric content into the respiratory tract might have a negative effect on CF patients (Blondeau et al. 2008b). Saliva of as much as 40 % of adults with CF contains bile acids, which could indicate a very high prevalence of gastroesophageal reflux (Blondeau et al. 2008a). The risk of aspiration seems to be higher in adults because of the more advanced CF. There are suggestions that the risk may also be high in children (van der Doef et al. 2009), which has been confirmed by Blondeau et al. (2010) who demonstrated the presence of bile acids in the saliva of 35 % of children with CF while bile acids were absent in a healthy, control group of children. Those authors also demonstrated that, on average, 31 % of all GERs were proximal. In the present study the proportion of GERs was higher than that observed by Blondeau et al. (2010); the finding in line with that of Doumit et al. (2012) or Woodley et al. (2014). Anyhow, all these results seem to exceed the normal values for healthy adult population and point to the potential role of aspiration as the mechanism through which GER could aggravate the course of CF.

Among patients participating in the present study, only 31.8 % (14/44) had typical symptoms of GER, non-associated with CF itself such as heartburn and/or regurgitation. Other symptoms observed, particularly cough and abdominal pain, whereas potentially caused by GER, could also be a sequelae of the underlying disease. When only CF children with diagnosed GER were analyzed, it turned out that typical GER symptoms were presented in less than half of them (42.3 %, 11/26). Observations made by other authors are inconsistent. According to Walkowiak et al. (2004), typical GER symptoms occur in 17.8 % of CF patients (16/90) and in 29.6 % with previously confirmed GER. Blondeau et al. (2010) have reported the respective values as 37.5 % and 56.3 %, which are substantially different. Nonetheless, only slightly over half of CF children with confirmed GER presented typical symptoms of the disease. Therefore, starting GER diagnostics only in the face of the presence of its typical symptoms seems incorrect. In our opinion, classic

pH-metry or MII-pH, if possible, should be considered in CF patients even if their clinical presentation does not clearly suggest the presence of GER.

In the present study, depending on the diagnostic criteria applied, GER was diagnosed in 54.5 % (AE >6 %), 70.5 % (AE >3 %) or 50.0 % (AE >7 %). The difference between the cut-off point (AE >6 %) of the former NASPGHAN guidelines and the AE >7 % of the present NASPGHAN/ESPGHAN guidelines seems to be small. The prevalence of GER in patients with CF is much greater than that in the general population; the frequency of symptoms that could be attributed to GER in healthy children is estimated at not more than 20 % (Nelson et al. 2000). Numerous studies have been published assessing the co-existence of GER and CF, but the results are hardly comparable because of high methodological variability. In many cases, study populations have been small and non-homogenous, both in terms of symptoms and age. The diagnosis of GER was based on several diagnostic methods. In five studies, the diagnosis of GER was based on the AE established by MII-pH. However, different cut-off values have been used by the authors. Most of them considered, MII-pH tracing as abnormal if AE exceeded 6 % according to the former NASPGHAN guidelines. The prevalence of GER observed by us is similar to the values reported by other authors, also when classic pH-metry was used. An interesting observation was made in a study by Palm et al. (2012) in which nearly all patients (97 %) used proton pump inhibitors; the majority of them (67 %) twice daily. Despite the likely intensive suppression of HCl secretion, abnormal values of the reflux index (>6 %) have been noted in as many as 37 % of children. That observation questions the efficacy of that type of GER therapy in CF children. Maybe, long-term use of higher doses of proton pump inhibitors is necessary, which requires alternative study designs possibly combined with a simultaneous measurement of gastric pH.

The present study has limitations. The population sample was relatively small, although the biggest in up-to date pediatric studies on the issue. The analysis of MII-pH traces employed the acid exposure normal values developed for the pH-metric measurement. The absence of standards enabling the unequivocal analysis of MII-pH is a factor that seriously limits the clinical applicability of that diagnostic method. The development of such standards in the foreseeable future does not seem to be real because of ethical reasons. The study is also limited by the absence of analysis of parameters correlating symptoms, mostly related to cough and irritability, with episodes of GER such as symptom index (SI), symptom sensitivity index (SSI) and symptom association probability (SAP). They is strong evidence of a causative relationship between symptoms reported by the patient and GER. Despite our request for recording any acute symptoms during the study, only a few patients did so, which made the statistical analysis impossible.

In conclusion, the present study confirmed a very high incidence of GER in children with CF. However, there is no clear answer to the question of the significance of that phenomenon. The assessment of the efficacy of GER treatment in that group of patients would be interesting in this regard. Considering a low incidence of typical symptoms of GER in pediatric patients with CF, diagnostics of the disease should be considered even in the patients free of symptoms. Acid GER prevails, which suggests that the classic pH-metry may be a sufficient diagnostic option in this group of patients. However, considering a significant frequency of proximal GER, we opine that MII-pH should be a preferred diagnostic tool.

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

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Sleep-Related Breathing Disorders and Bruxism

J. Kostrzewa-Janicka, P. Jurkowski, K. Zycinska, D. Przybyłowska, and E. Mierzwińska-Nastalska

Abstract

Obstructive sleep apnea (OSA) syndrome is a sleep-related breathing disorder, due mainly to peripheral causes, characterized by repeated episodes of obstruction of the upper airways, associated with snoring and arousals. The sleep process fragmentation and oxygen desaturation events lead to the major health problems with numerous pathophysiological consequences. Micro-arousals occurring during sleep are considered to be the main causal factor for night jaw-closing muscles activation called bruxism. Bruxism is characterized by clenching and grinding of the teeth or by bracing or thrusting of the mandible. The causes of bruxism are multifactorial and are mostly of central origin. Among central factors there are secretion disorders of central nervous system neurotransmitters and basal ganglia disorders. Recently, sleep bruxism has started to be regarded as a physiological phenomenon occurring in some parts of the population. In this article we present an evaluation of the relationship between OSA and sleep bruxism. It has been reported that the frequency of apneic episodes and that of teeth clenching positively correlates in OSA. However, clinical findings suggest that further studies are needed to clarify sleep bruxism pathophysiology and to develop new approaches to tailor therapy for individual patients with concomitant sleep bruxism and OSA.

Keywords

Bruxism • Obstructive sleep apnea-hypopnea • Sleep related breathing disorder • Teeth clenching • Upper airways

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1 Introduction

In the adult population two basic physiological states, wakefulness and sleep, occur on a daily basis. Wakefulness is a state of somatic system activity, whereas sleep is the state when this system is at rest and changes in the activity of the cardiovascular, respiratory and digestive systems, as well as in endocrine glands, take place. Along with the body growth and the brain development sleep periods become shorter and wakefulness periods longer. However, too short sleep and sleep disturbances affect the quality of life and the general state of health (Briones et al. 1996).

Sleep is divided into two periods, rapid-eye movement (REM) and non rapid-eye movement (non-REM) that are repeated in cycles every 90–110 min (Peever and McGinty 2007). Sleep begins with non-REM period, comprising light stages 1 and 2 and deep stages 3 and 4. This period constitutes the majority of the sleep process and is considered as the deepest and most restorative level of sleep, when hormonal changes take place. During non-REM sleep spontaneous motor events may occur, causing disruption of the neuronal activity which preserves sleep continuity. However, the most spontaneous motor activity is observed in stages 1 and 2 of non-REM sleep, the period when sleep bruxism, periodic limb movements, or oromandibular myoclonus occur. In deep non-REM stages (3 and 4) approximately 15–20 % of sleep bruxism occurs at the transition time of this sleep into REM sleep. REM sleep lasts about 15 min and shows different properties. During this period an individual is dreaming or hallucinating and displays high brain neurotransmitter-linked activity (acetylcholine, norepinephrine, and serotonin) (Tarokh et al. 2010). In REM sleep, there is the post-synaptic inhibition of spinal motor neurons, causing the body immobilization. This change in muscle tone and activity plays a significant role in breathing and can influence the degree of upper airway obstruction (Lavigne et al. 2003).

2 Sleep Bruxism

Discrimination of motor-related sleep disturbances from physiological reactions to certain stimuli, especially those aimed at decreasing the allostatic overload, is an area of limited understanding. Bruxism is a motor reaction during sleep that remains to be fully elucidated. It is defined as stereotyped movement disorder occurring during sleep and characterized by teeth grinding and clenching (American Academy of Sleep Medicine 2005). Over years, bruxism has been regarded as the activity of major concern to dentists because of its consequences for masticatory structures, such as tooth destruction, dental restoration damages, and induction of temporomandibular disorders as well as headaches, grinding sounds, and myofascial pain (Ramfjord and Ash 1983; Fernandes et al. 2012); the areas involving also neurologists and sleep medicine specialists (Ohayon et al. 2001).

Polysomnography is at present the gold standard for diagnosing sleep bruxism. It serves to discriminate the presence of rhythmic masticatory muscles activity (RMMA), typical of sleep bruxism (Lavigne et al. 1996), from oromotor activities, such as swallowing, coughing, face dubbing, body movements, lip sucking, head movements, chewing-like movements, head rubbing, face scratching, eye opening, and blinking. Clinical and ambulatory electromyographic recordings could include a considerable number of oromotor sleep activities, giving inaccurate diagnostic results (Lobbezoo et al. 2013; Yamaguchi et al. 2012). Bearing this in mind, to identify the relationship between the presence of bruxism and other events during sleep, it is necessary to introduce relevant inclusion and exclusion criteria, to select adequate study and control groups, and to use an appropriate equipment. The mechanisms underlying the pathophysiology of bruxism are multidimensional (Lavigne et al. 2008). However, the results of the most recent studies confirm the hypothesis that this activity is mainly centrally mediated, and the responsiveness of the central nervous system to

the destructive action of peripheral and external factors may be its underlying reason. The relationship between psychological stress biomarkers and more pronounced bruxism has already been evidenced (Tomoeda et al. 2011; Sato and Slavicek 2008). At the same time, the role of bruxism in the allaying of responses to stress has been confirmed. It is suggested that masticatory activity plays a major role in down-regulation of the limbic system, the hypothalamus-pituitary adrenal axis, the autonomic nervous system, and the immune system after their activation due to stressful factors. It has been found that there is a switch from predominating sympathetic activity to parasympathetic enhancement after bruxism episodes (Lavigne et al. 2007), with increased lubrication of oral and esophageal tissues, reduction of obstruction of upper airways, and decrease in heart rate. It is suggested that increased sympathetic activity is essential for initiating sleep bruxism. This type of masticatory muscle activity during sleep is known as the primary type, when no clear medical complications are involved. However, the relationship between bruxism and obstructive sleep apnea/hypopnea (OSA), the condition characterized by sympathetic activity enhancement, has been observed. Bruxism associated with sleep disorders is regarded as the secondary type.

3 Obstructive Sleep Apnea/Hypopnea

Obstructive sleep apnea/hypopnea is characterized by recurrent episodes of partial or complete upper airways collapse during sleep (Kato et al. 2013b). The absence of adequate alveolar ventilation results in the upper airway narrowing, arterial oxygen desaturation, and increase in partial pressure of CO_2. The events are mostly terminated by arousals. Sleep disruption induces daytime sleepiness, anxiety, and difficulties in concentration. Sleep fragmentation and intermittent hypoxia can lead to impaired quality of life, cognitive impairment, and a greater risk for nocturia, hypertension, and cardiovascular complications. The airway narrowing occurs most often in the oropharynx. The mandibular muscles relax and the mandible retrudes. The tongue is based on the pharyngeal wall, so that the posterior pharynx narrows resulting in an increase in negative pressure during breathing, which facilitates the airway collapse. The large tongue, soft palate length, craniofacial morphology, obesity, and neuromuscular components can contribute to narrowing of the upper airway and should also be considered as risk factors for the development of obstructive sleep apnea/hypopnea (Sutherland et al. 2012). Changes associated with breathing lead to arousals in sleep. Those events trigger the muscles of the throat, tongue and mandible, leading to normal respiration. It is known as respiratory effort-related arousals of sleep (Simmons 2012). It seems that the brain has inherent mechanisms utilized to decrease or eliminate the obstruction of the upper airway during sleep. These mechanisms are manifest by preference for an alternative body position during sleep and the sleep bruxing. It is considered that bruxing or clenching is a part of sleep arousals and may occur as a mechanism to prevent airway collapse. What is more, it has been found that the patients free of OSA suffer from sleep bruxism less frequently (Hosoya et al. 2014). However, it should be emphasized that the relationship between OSA and sleep bruxism has not as yet been fully elucidated (Huyuh et al. 2014).

4 Obstructive Sleep Apnea/Hypopnea and Sleep Bruxism

A literature search performed in the English-language PubMed database, using the query ‘Sleep bruxism’ and ‘Obstructive sleep apnea/hypopnea’, yielded 83 articles published up to 2014, of which five were finally included in the present review. Review papers, non-randomized papers, articles without selected control groups and uniform diagnostic methods, which could provide basis for diagnosing bruxism and nocturnal apnea, were excluded.

Hosoya et al. (2014) revealed a positive correlation between the frequencies of OSA and phasic-type sleep bruxism events, based on

polysomnographic recordings analyzed in the experimental group with diagnosed OSA as compared with healthy volunteers. Phasic type of sleep bruxism events occurred during the micro-arousals that ensued from obstructive apnea event. However, they established that sleep bruxism did not affect the sleep time, sleep efficiency, sleep stages, micro-arousal, or snoring events. They also found that sleep bruxism was significantly higher in the OSA than in the control group, although in their study there were no homogenous experimental and control groups regarding age and gender. The results of those studies should be referred to the results reported by Sjöholm et al. (2000) who indicate, also on the basis of polysomnographic examinations, that sleep bruxism is not directly dependent on apneic episodes. However, they cannot rule out that bruxism may occur secondarily to the fragmented sleep pattern. There is evidence that rhythmic masticatory muscle activity is associated with a higher amplitude of respiration within arousal (Khoury et al. 2008). Similar results have also been obtained by Kato et al. (2013a) who show that in patients with OSA the contraction of masseter muscle after respiratory events can be a nonspecific motor phenomena, dependent on the duration of arousals, rather than the occurrence of respiratory events. Those authors demonstrated that the activity of masseter muscles, following arousal episodes due to obstructive sleep apnea, does not differ from the activity of those muscles after spontaneous arousal. However, it has been suggested that bruxism episodes occurring in the close relation with sleep apnea/hypopnea events form a secondary type of bruxism (Saito et al. 2014). The authors diagnosed OSA and sleep bruxism, on the polysomnographic basis, using a threshold apnea-hypopnea index (AHI) defined as >5 events/hour and a threshold sleep bruxism index of >4 events/hour, based on the criteria set by the American Academy of Sleep Medicine (2005). However, the study was performed on a group of only ten men, with no control group, and with only one night analysis in the sleep clinic. The authors found that in patients with concomitant OSA and sleep bruxism, most bruxism events occurred close to sleep apnea/hypopnea events. It has been also shown that all bruxism episodes are secondary to apnea episodes, which suggests a possible parallel occurrence of other factors that regulate the events under investigation.

5 Summary and Conclusions

It is suggested that sleep bruxism is associated with the arousal influenced by sympathetic/parasympathetic fluctuations during sleep. It has been found that sleep bruxism is involved in specific activity in sleep. Before bruxism events, a rise in autonomic sympathetic cardiac activity, along with increases rise in brain activity, heart rate (tachycardia), suprahyoid muscle tone, amplitudes of respiration and masseter and temporalis muscles activity, is observed. It is also known that due to arousals and hypoxia the sympathetic nervous system is activated (Lavigne et al. 2008). Therefore, a hypothesis can be drawn that arousal reactions during OSA intensify the prevalence of bruxism. Moreover, it is observed that bruxism episodes are followed by activation of the parasympathetic system with all related consequences manifested by bradycardia, hypersalivation and increased patency of the upper airways. It could be thought that respiratory disturbances, leading to sleep fragmentation and arousals, trigger the muscles of the masticatory organ to restore normal breathing. Sleep bruxism may reduce the obstruction of the upper airway. So, if bruxing and clenching are considered as part of the arousal process, then also arousal from apnea could provoke bruxism, especially taking into account that treatment of obstructive breathing with continuous positive airway pressure (CPAP) alone reduces sleep bruxism in a high percentage of patients (Oksenberg and Arons 2003). The OSA episodes occur mostly in REM sleep when the inhibition of motor neurons takes place, leading to atonia of the upper airway tissues. This induces snoring and micro-arousals. Hypoxia is responsible for the rise in heart rate and the arousal of the sympathetic nervous system, which along with micro-arousals precede the occurrence of bruxism episodes mostly in non-REM stages 1 & 2. Time

after bruxism episodes coincides with the activation of the parasympathetic nervous system, which becomes dominant as sleep deepens from REM to non-REM periods and bruxism exerts a direct effect on the change in the autonomic nervous system activity from the sympathetic to parasympathetic.

The authors of the literature above outlined point to the limitations of their studies, which should be taken into account when drawing conclusions. These limitations apply to the size of the study and control groups, as well as to the procedures of recognizing and analyzing the observed events. The latter is linked to the need of using polysomnography and recording the data for more than just one night. However, bearing in mind the significance of the medical problem resulting from the night apnea, it seems that the recognition of this relationship will stimulate dentists to early diagnosis of apnea/hypopnea episodes through careful observation of first clinical symptoms of bruxism and thus prevention of the OSA development. Clinical procedures allowing for the diagnosis of bruxism (Lobbezoo et al. 2013) should also include the assessment of concomitant psycho-emotional state of the patient and the effect of stress factors on the prevalence of bruxism and its role in sleep disordered breathing and sleep quality.

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

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Absence of Typical Symptoms and Comorbidities in Patients with Central Sleep Apnea

Josef Yayan and Kurt Rasche

Abstract

Sleep apnea is characterized by pauses in breathing during sleep. There are three forms: central, obstructive, and complex, or mixed sleep apnea. Central sleep apnea, a manifestation of respiratory instability in many clinical conditions and with a variety of causes, is the result of a temporary cessation of breathing in which the inhibitory influences favoring the instability predominate over excitatory influences favoring stable breathing. In contrast to central sleep apnea, according to the published data from previous studies, an association exists between obstructive sleep apnea and various comorbidities, especially chronic obstructive pulmonary disease. This article examines retrospectively the possible association of central sleep apnea with special sleep-related symptoms and various co-morbidities. Data of all patients with different types of central sleep apnea were collected from our hospital charts within the Department of Pneumology, HELIOS Clinic, University of Witten/Herdecke, Wuppertal, Germany, within the study period of January 1, 2011 to September 19, 2014. After clinical examination, all patients underwent polysomnography in our sleep laboratory. We identified a total of 60 (3.5 %) patients with central sleep apnea from 1722 patients with assumed sleep disordered breathing of the mean age of 68.2 ± 13.7 years (44 males – 73.3 %, 95 % CI 0.6–0.9 and 16 females – 26.7 %, 95 % CI 0.2–0.4). Typical symptoms of sleep-disordered breathing were not observed. A relation to co-morbidities was not found. Central sleep apnea was often diagnosed in the elderly. A direct association between central sleep apnea and symptoms of sleep-disordered breathing and various co-morbidities was not detected. This is in direct contrast to the obstructive sleep apnea syndrome.

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Keywords
Central sleep apnea • Comorbidities • Obstructive sleep apnea • Sleepiness • Symptoms • Polysomnography

1 Introduction

Central sleep apnea (CSA) is characterized by respiratory arrest as a result of decreased or missing respiratory drive generated in the respiratory center. Although CSA is seen in less than 5 % of the patients who appear for consultation at the sleep disorders center, the incidence of CSA increases in the presence of congestive heart failure, renal failure, and central nervous system abnormalities (Inönu et al. 2014).

Sleep-disordered breathing events are categorized into obstructive and central events (Mooney et al. 2012). The clear discrimination of central and obstructive hypopneas is highly relevant to avoid misinterpretation and inappropriate treatment of complicated breathing patterns (Randerath et al. 2013). CSA is usually due to instability of the feedback mechanism of the body that controls respiration (Zaharna et al. 2013).

CSA describes a group of conditions in which cessation of air flow occurs without respiratory effort. In contrast, obstructive sleep apnea (OSA) patients have ongoing respiratory effort during respiratory events. However, considerable overlap exists in the pathogenesis and clinical presentation of OSA and CSA. A good working knowledge of the mechanisms underlying CSA is important for optimal clinical care (Malhotra and Owens 2010).

CSA is not often associated with symptoms of insomnia, excessive daytime sleepiness, or frequent awakenings. The prevalence of CSA is dependent on the population being studied, the predominant risk factors are elderly age and comorbid conditions. CSA may be a clinical marker of underlying medical disorders, including cardiac or neurological disease, with consecutive significant morbidity and mortality. Given that the underlying pathogenesis remains poorly understood, therapeutic options are currently limited (Chowdhuri and Badr 2010).

We conducted our investigation for a better understanding of all possible types of CSA. Therefore, using the hospital database at the Department of Pneumology, HELIOS Clinic, University of Witten/Herdecke, in Wuppertal, Germany, we collected data on all patients with various types of CSA (according to the International Classification of Diseases) who were examined for CSA. The study was also used to determine the frequency of various degrees of CSA severity. Since the frequency of CSA is low, the symptoms of CSA can be easily overlooked. Therefore, this study looked for typical symptoms of CSA. The study should also provide clarity on demographic differences in the various forms of CSA and identify comorbidities for possible association with CSA. A proper diagnosis can promote the prompt choice of therapy.

2 Methods

All patients' data were anonymized prior to analysis. The Ethics Committee of the University of Witten-Herdecke in Germany approved the study. Due to the retrospective nature of the study protocol, the Ethics Committee of the University of Witten-Herdecke in Germany waived the need for informed or written informed patient's consent.

This study examined, retrospectively, the clinical correlations in patients with CSA with symptoms and comorbidities using data collected from hospital charts in the Department of Pneumology at the HELIOS Clinic, University of Witten/Herdecke in Wuppertal, Germany, in the study period from January 1, 2011 to

September 19, 2014. The study population was mixed in terms of age. All patients over 18 years of age and who were suspected to have CSA were included in the study. The number of patients and the subdivision into groups are given in the Results section.

The suspected CSA resulted from the patient's history, reports from the patient's partner and the diagnosis of suspected CSA was made primarily by a pronounced daytime sleepiness and by a number of other symptoms and secondary diseases. CSA was defined by a tendency to experience apnea during sleep due to insufficient activity of the respiratory center, weak respiratory muscle activity, and failure of the diaphragm and lungs. A clinical relevant CSA was diagnosed, if more than 55 % of the total number of respiratory events were central (White 1985; AASM 2014). The symptoms of sleep-disordered breathing were classified as breathing pauses, snoring, daytime sleepiness, concentration disturbance, performance degradation, insomnia, dry mouth, headache, and dizziness. The classification of CSA was made in each case, according to the latest edition of the International Classification of Diseases (ICD 2014).

The diagnosis of CSA included various investigations, with a focus on interviewing the participants regarding their sleep habits, daily condition, and medical histories. In addition, physical examinations that included an examination of the ear, nose, and throat were performed. In the present study, a Masimo Radical™ Signal Extraction Pulse Oximeter with Finger Sensor (Masimo Europe Ltd., Puchheim, Germany) was used through the night to measure and record the patients' blood oxygen saturation levels and pulse rates. Polysomnography was performed by standard procedures (Sleep Diagnostic System ALICE 4®, Heinen + Löwenstein, Bad Ems, Germany). The apnea–hypopnea index (AHI), which is used to indicate the severity of CSA, is represented by the number of apnea and hypopnea events per hour of sleep. The apneas must last for at least 10 s and are associated with a decrease in blood oxygenation. Combining AHI and oxygen desaturation gives an overall sleep apnea severity score that evaluates both the number sleep disruptions and the degree of oxygen desaturation. The AHI was calculated by dividing the number of apnea events by the number of hours of sleep. AHI values were categorized as normal: 0–4, mild sleep apnea: 5–14, moderate sleep apnea: 15–29, severe sleep apnea: 30 or more (Thornton et al. 2012). Three study groups were formed according to the calculation by AHI due to the severity of CSA.

The Epworth Sleepiness Scale (ESS) was used to measure daytime sleepiness (Johns 1991). The questionnaire asked the subject to rate the probability of falling asleep on a scale of increasing probability from 0 to 3 for eight different situations that most people engage in during their daily lives, though not necessarily every day. The scores for the eight questions were added together to obtain a single number. A number in the 0–9 range was considered to be normal, while a number in the 10–24 range indicated that expert medical advice should be sought. For instance, scores of 11–15 were shown to indicate the possibility of mild-to-moderate sleep apnea, whereas a score of 16 and above indicated the possibility of severe sleep apnea or narcolepsy. Certain questions in the scale were shown to be better predictors of specific sleep disorders, though further tests were required to provide an accurate diagnosis (ESS 2014).

The influence of the intake of opioids on the CSA was investigated. The possible influence of concomitant diseases was studied in the three study groups. The length of hospital stay was compared between the different forms with CSA.

The data were expressed as proportions and means ± SD wherever appropriate. We calculated 95 % confidence intervals (CIs) for the total number of patients with CSA. Odds ratio was calculated for comparing the sex differences in regarding frequency of CSA. A calculation of chi-squared test for three independent standard normal variables of two probabilities was used to compare associations between sex difference, number of patients with opioid use, clinical symptoms of CSA, cardiovascular risk factors, and comorbidities between the three study groups. One-way analysis of variance ANOVA

for three independent samples was performed to compare the number and severity of CSA cases, age difference, duration of hospital stay, ESS, and AHI among all study groups. The survival rates for all groups were calculated using the Kaplan-Meier method. All tests were expressed as two-tailed, and a p-value of <0.05 was considered to be statistically significant.

3 Results

In the hospital database, we found 79 (4.6 %) patients with CSA out of a total number of 1722 patients with sleep apnea (ICD G47.30–G47.39), who had been treated at the Department of Pneumology, HELIOS Clinic, University of Witten/Herdecke, Wuppertal, Germany, during the study period outlined in the Methods. A total of 60 (3.5 %) patients (95 % CI 0.03–0.04) of the mean age of 68.2 ± 13.7 years (44 [73.3 %, 95 % CI 0.6–0.9] males and 16 [26.7 %, 95 % CI 0.2–0.4] females) with CSA met the inclusion criteria for this trial. The male gender was eight times more likely to suffer from CSA, but that figure was without statistical significance (8.1 odds ratio, 95 % CI, 0.3–208.7; $p = 0.207$). The most numerous group of patients comprised severe CSA cases ($p = 0.002$; Table 1). There was no significant age difference: CSA was diagnosed primarily in older individuals ($p = 0.459$; Table 1). There was no difference in the duration of the hospital stay between the three groups stratified according to the severity of CSA (Table 2). The ESS did not differ between the three groups (Table 2). The intake of opioids showed no causal relation with CSA (Table 1). In contrast, there was a significant difference in AHI between the three groups ($p < 0.0001$, Table 1).

Conspicuously, the clinical symptoms for OSA, which are characterized by respiratory pauses, concentration disturbance, reduced performance, and headache as well as snoring,

Table 1 Demographic and basic data in patients with increasing severity of central sleep apnea

	Mild (%)	Moderate (%)	Severe (%)	p-value
n = 60	6 (10.0)	9 (15.0)	45 (75.0)	**0.002**
Male	3 (50.0)	6 (66.7)	35 (77.8)	0.312
Female	3 (50.0)	3 (33.3)	10 (22.2)	0.312
Age	65.7 ± 12.9	63.6 ± 15.0	69.5 ± 13.8	0.459
Duration of hospital stay in days	3.0 ± 2.5	2.3 ± 1.2	4.4 ± 3.8	0.217
Epworth sleepiness scale	6.3 ± 3.9	11 ± 7.3	8.5 ± 6.3	0.445
Number of patients with opioid use	0	1 (11.1)	7 (15.2)	0.563
Apnea–hypopnea index	7.8 ± 5.5	24.8 ± 3.6	54.7 ± 18.3	**<0.0001**

Values are means ± SD

Table 2 Clinical symptoms of central sleep apnea in patients with increasing severity of central sleep apnea

Symptoms	Mild (n = 6) (%)	Moderate (n = 9) (%)	Severe (n = 45) (%)	p-value
Breathing pauses	0	0	1 (2.2)	0.844
Snoring	4 (66.7)	1 (11.1)	16 (35.6)	0.086
Daytime sleepiness	2 (33.3)	6 (66.7)	18 (40.0)	0.295
Attention deficit	1 (16.7)	1 (11.1)	1 (2.2)	0.206
Performance degradation	1 (16.7)	0	3 (6.7)	0.447
Insomnia	0	3 (33.3)	11 (24.4)	0.307
Dry mouth	2 (33.3)	0	8 (17.8)	0.219
Headache	0	1 (11.1)	3 (6.7)	0.701
Dizziness	0	1 (11.1)	0	0.056

Table 3 Cardiovascular risk factors in patients with increasing severity of central sleep apnea

Risk factors	Mild (n = 6) (%)	Moderate (n = 9) (%)	Severe (n = 45) (%)	p-value
Hypertension	1 (16.7)	5 (55.6)	26 (57.8)	0.164
Diabetes	2 (33.3)	1 (11.1)	9 (20.0)	0.574
Hyperlipidemia	0	0	5 (11.1)	0.403
Obesity	0	1 (11.1)	14 (31.1)	0.148
Smoking	1 (16.7)	2 (22.2)	9 (20.0)	0.966

daytime sleepiness, sleep disturbances, dry mouth, and dizziness were not typical symptoms of CSA (Table 2).

We found no association between cardiovascular risk factors and CSA in the present study (Table 3). Further, this study did not detect a relationship between comorbidities and CSA. Cardiac dysrhythmia was observed mainly in the group of heavy CSA, but most patients with CSA had no cardiac dysrhythmia. No significant correlation was found between CSA and chronic obstructive bronchitis, pulmonary embolism, sarcoidosis, restless legs syndrome, or chronic sinusitis (Table 4). There were no deaths in the three arms of the study; therefore, the survival rate in all the three groups was 100 %.

4 Discussion

In the present study devoted to central sleep apnea we failed to substantiate any association between apneic episodes, sleep related symptoms, and comorbidities, which is in direct contrast to obstructive sleep apnea. In particular, we did not find any link between CSA and chronic obstructive pulmonary disease (COPD). In contrast, OSA and COPD are commonly overlapping, which is reported to cause a more severe condition (Larsson and Lindberg 2008). The coexistence of OSA and COPD occurs in 10–20 % of patients with OSA (Lee and McNicholas 2011). On the other side, some authors have not supported the notion of a relationship between COPD and OSA (Bednarek et al. 2005). Anyhow, patients with OSA and COPD have a higher risk of pulmonary hypertension and worse nocturnal hypoxemia than those with either disease alone (Lee and McNicholas 2011). Such a relationship was not found in the present study between CSA and COPD.

Sleep apnea is manifested in approximately 10 % of adults in the general population. The frequency of sleep apnea can exceed 50 % in some cardiovascular diseases, and in particular those characterized by sodium and water retention (Floras 2014). Although sleep apnea has not yet been associated with official cardiovascular risk assessment algorithms, there is a growing awareness of its significance in the causality or facilitation of hypertension, coronary artery disease, heart failure, atrial arrhythmias, and stroke, and thus, not unexpectedly, as a predictor of early cardiovascular death. However, we found no connection between CSA and hypertension, coronary artery disease, heart failure, atrial arrhythmias, and stroke in the present study. Sleep apnea manifests as two primary forms, both described by respiratory instability. OSA resulting from sleep-related depression of the respiratory drive to the upper airway dilator muscles is superimposed upon a narrow and highly compliant airway that is predisposed to collapse. CSA occurs when the partial pressure of arterial carbon dioxide falls below the apnea threshold, resulting in the withdrawal of the central drive to respiratory muscles (Floras 2014).

The epidemiological data show a noticeable increase in the incidence of sleep disorders in the elderly. Sleep apnea syndrome in the elderly raises serious and differential diagnostic problems (Mihăltan 2005). In the study, we found more instances of CSA in elderly people.

Sleep-related breathing disorders appear in cardiology patients, mostly as OSA or CSA with Cheyne-Stokes respiration. The occurrence is clearly more frequent when compared with the

Table 4 Comorbidities in patients with increasing severity of central sleep apnea

	Mild (n = 6) (%)	Moderate (n = 9) (%)	Severe (n = 45) (%)	p-value
Cardiovascular disease				
Cardiac decompensation	0	0	3 (6.7)	0.592
Cardiac dysrhythmia	0	0	22 (48.9)	**0.003**
Cardiomyopathy	0	0	4 (8.9)	0.489
Coronary artery disease	1 (16.7)	0	13 (28.9)	0.160
Heart failure	0	1 (11.1)	8 (17.8)	0.487
Hypertensive heart disease	0	2 (22.2)	2 (4.4)	0.117
Peripheral arterial occlusive disease	1 (16.7)	0	1 (2.2)	0.150
Sepsis	0	0	1 (2.2)	0.844
Post-myocardial infarction	0	0	4 (8.9)	0.489
Valvular heart disease	0	1 (11.1)	9 (20.0)	0.415
Varicose veins	0	0	1 (2.2)	0.844
Pulmonary disease				
Asthma	0	0	5 (11.1)	0.403
Chronic obstructive bronchitis	2 (33.3)	3 (33.3)	3 (6.7)	**0.031**
Cor pulmonale	0	1 (11.1)	5 (11.1)	0.691
Interstitial lung diseases	0	0	2 (4.4)	0.708
Lung tumor	0	1 (11.1)	0	0.075
Pulmonary embolism	1 (16.7)	0	0	**0.010**
Respiratory failure	0	0	1 (2.2)	0.844
Sarcoidosis	1 (16.7)	0	0	**0.010**
State after pulmonary embolism	0	1 (11.1)	4 (8.9)	0.723
Gastrointestinal diseases				
Diverticulosis	0	0	1 (2.2)	0.844
Reflux esophagitis	1 (16.7)	1 (11.1)	1 (2.2)	0.206
Kidney disease				
Acute urinary tract infection	0	0	1 (2.2)	0.844
Benign prostatic hyperplasia	0	1 (11.1)	0	0.056
Chronic renal failure	0	1 (11.1)	9 (20.0)	0.415
Prostate cancer	0	1 (11.1)	3 (6.7)	0.701
Thyroid disease				
Hyperparathyroidism	0	0	1 (2.2)	0.844
Hyperthyroidism	0	0	1 (2.2)	0.844
Hypothyroidism	0	0	4 (8.9)	0.489
Thyroid autonomy	0	0	1 (2.2)	0.844
Gynecological disorders				
Hysterectomy	0	0	1 (2.2)	0.844
Otorhinolaryngology				
Chronic sinusitis	1 (16.7)	0	0	**0.010**
Orthopedic disorders				
Chronic pain syndrome	0	0	1 (2.2)	0.844
Disc herniation	0	0	1 (2.2)	0.844
Osteoarthritis	1 (16.7)	0	4 (8.9)	0.502
Rheumatism	0	0	1 (2.2)	0.844
Traumatology				
Post-traumatic brain injury	0	0	1 (2.2)	0.844
Nervous system disorders				
Fibromyalgia	0	0	1 (2.2)	0.844

(continued)

Table 4 (continued)

	Mild (n = 6) (%)	Moderate (n = 9) (%)	Severe (n = 45) (%)	p-value
Friedreich's ataxia	0	0	1 (2.2)	0.844
Parkinson's disease	0	0	2 (4.4)	0.708
Polyneuropathy	0	0	1 (2.2)	0.844
Prior stroke	0	1 (11.1)	8 (17.8)	0.487
Restless legs syndrome	1 (16.7)	0	0	**0.010**
Von Hippel Lindau syndrome	1 (16.7)	0	2 (4.4)	0.330
Psychiatric disorders				
Bulimia nervosa	0	0	1 (2.2)	0.844
Confusion	0	0	1 (2.2)	0.844
Dementia	0	1 (11.1)	1 (2.2)	0.355
Depression	0	1 (11.1)	4 (8.9)	0.859
Panic	0	1 (11.1)	0	0.056
Skin disorders				
Allergy	0	1 (11.1)	3 (6.7)	0.701
Eczema	0	0	1 (2.2)	0.844
Lupus erythematosus	0	0	1 (2.2)	0.844
Psoriasis	0	1 (11.11)	0	0.056
Ophthalmological disorders				
Various	0	0	1 (2.2)	0.844

general population (Oldenburg et al. 2014). Depending on the underlying cardiac disease, up to 75 % of patients can have OSA and CSA, and up to 50 % have indications for therapy according to the current guidelines. OSA is considered to be an independent and well treatable risk factor for the development and deterioration of many cardiovascular diseases. This association was described between sleep-related breathing disorders and arterial hypertension, atrial fibrillation, arteriosclerosis with coronary heart disease, myocardial infarction, and heart failure (Floras 2014). Although the role of OSA as a risk factor for the development of these diseases is well documented, CSA is less of a risk factor *per se*, but is considered to mirror an underlying cardiac disease with further negative consequences for this disease (Oldenburg et al. 2014). Although cardiovascular diseases were more common in severe CSA in the present study, a connection between these cardiovascular diseases could not be established, because most of the patients with CSA had no cardiovascular disease.

Sleep apnea can influence cardiac function by facilitating the development of heart failure. Reversely, chronic heart failure increases the risk for sleep apnea. Consequently, in patients with symptomatic chronic heart failure, sleep apnea is a frequent comorbidity, occurring in up to 75 % of cases (Plenge and Müller-Ehmsen 2013). More than half of these patients suffer from CSA, whereas in the general population, conversely, OSA is far more frequent. Both obstructive and central sleep apnea lead to oxygen desaturations during the night, which are followed by increases in serum catecholamines. That is possibly the main reason why the prognosis of patients with symptomatic heart failure and sleep apnea is much worse than that of patients without sleep apnea. Therefore, a screening of all heart failure patients for sleep apnea is mandatory (Plenge and Müller-Ehmsen 2013). Heart failure was more common in severe CSA in the present study, but a connection between both of these diseases could not be confirmed by our results.

While we could not confirm that the use of opioids can develop CSA in the present study, three cases were presented in another study in which significant central sleep apnea developed in patients on opioids used for nonmalignant pain

management (Mogri et al. 2008). In two of those patients, no evidence of sleep-related breathing disorders was evident in polysomnography until after the ingestion of an opioid for treatment of chronic nightly pain when severe central sleep apnea developed. The third patient had an established OSA, but experienced a significant number of central events after the ingestion of an opioid analgesic, which worsened his condition. The ingestion of opioid analgesics can precipitate CSA in patients with chronic pain, who otherwise show no evidence of CSA and have no cardiac or neurologic disease that would predispose them to central sleep apnea (Mogri et al. 2008).

Both OSA and CSA are conditions characterized by repeated breathing pauses during sleep and are associated with sleep fragmentation, intermittent hypoxia, and increased cardiovascular risk. The diagnosis of sleep apnea requires polysomnography, an expensive and time-consuming technique that currently represents the gold-standard method. Several predictive mathematical models have been developed for the prediction of sleep apnea (Lovin et al. 2007). These models combine subjective parameters such as sleepiness, witnessed apneas and snoring, morphometric data, body mass index, neck circumference, and cephalometric measures, associated comorbidities such as hypertension, and oximetry data.

A limitation of the present study was that it examined a small number of patients with CSA over a relatively short period of time. Also, we did not consider a further distinction of CSA in cases of complex or mixed sleep apneas, which could hinder the interpretation of the present findings, but on the other hand was thought to enhance the clarity and homogeneity of the study groups.

In conclusion, we were unable to unravel any link of central sleep apnea with comorbidities, especially with COPD. Based on the data of the present study, we cannot describe a constellation of specific symptoms that could constitute a finger print of central sleep apnea.

Conflicts of Interest The authors report no conflicts of interest related to this work.

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Causes of Chronic Cough in Non-smoking Patients

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Abstract

Chronic cough is a common medical problem. The aim of the study was to analyze chronic cough causes in non-smoking patients and to search for demographic factors associated with different cough reasons. The etiology of cough was determined by medical history, diagnostic tests and response to specific treatment. Patients with significant abnormalities in the chest radiograph or spirometry were not included. The study included 131 non-smoking patients; median age 54 years, 77 % female. The most frequent causes of cough were gastroesophageal reflux disease (GERD) (62 %) and upper airway cough syndrome (UACS) (46 %). Cough variant asthma and non-asthmatic eosinophilic bronchitis (NAEB) were diagnosed in 32 (25 %) and 19 (15 %) patients, respectively. Other cough causes were found in 27 patients (21 %). Asthma was a significantly more common cause of chronic cough in women than in men (31 % *vs.* 3 %, $p = 0.005$). A reverse relationship was demonstrated for UACS (39 % *vs.* 67 %, $p = 0.01$). Patients with chronic cough aged >50 yrs were more likely to be diagnosed with less common cough causes. In conclusion, the most common chronic cough reasons are GERD and UACS. Asthma-related cough is diagnosed more frequently in females, while UACS-related cough is more frequent in males.

Keywords

Asthma • Chronic cough • Gastroesophageal reflux disease • Non-asthmatic eosinophilic bronchitis • Upper airway cough syndrome

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1 Introduction

Chronic cough (lasting over 8 weeks) is a common medical problem with a prevalence ranging from 10 to 20 % in the adult population (Morice et al. 2006). There is a wide variety of chronic cough causes including different pulmonary and extrapulmonary disorders. Some of these entities are relatively easy to diagnose while the others might be a considerable diagnostic challenge. The first include smoking associated chronic bronchitis, which is the most common cause of chronic cough, and some other conditions that can be easily diagnosed by a plain chest radiograph, e.g., lung tumors, tuberculosis, or interstitial lung diseases. Therefore, the chest radiograph plays a pivotal role in the diagnosis of chronic cough. On the other hand, a significant proportion of patients with chronic cough do not have any abnormality in the chest X-ray. In these patients additional diagnostic procedures are usually necessary to identify the underlying cause of cough. The most common causes of chronic cough in adult non-smokers with a normal chest radiograph are: upper airway cough syndrome (UACS), i.e., chronic rhinitis, rhinosinusitis, gastroesophageal reflux disease (GERD), asthma and non-asthmatic eosinophilic bronchitis (NAEB). The prevalence of these conditions shows slight differences related to specific features of the studied population, spectrum of the available diagnostic methods, and interpretation of the diagnostic test results (Levine 2008; Morice et al. 2004, 2006; Irwin et al. 2006; Palombini et al. 1999).

Since there are very few reports concerning the etiology of chronic cough in Polish patients, we undertook a study aimed to define the prevalence of chronic cough causes in a population of adult non-smokers with a normal chest radiograph. The second goal of the study was to search for any demographic factors associated with different cough causes.

2 Methods

2.1 Material

The protocol of the study was approved by the Institutional Review Board of the Medical University of Warsaw, Poland (KB/101/2009). The study was performed in patients with chronic cough, who were referred to the out-patient clinic in the Department of Internal Medicine, Pneumonology and Allergology, Medical University of Warsaw, Poland between 2009 and 2011. Non-smoking (at least for 1 year, with smoking history less than 10 pack-years) adult patients with cough lasting more than 8 weeks were regarded as potential candidates for the study. One hundred forty patients were included in the study. Nine patients had not completed the pre-planned diagnostic procedures and the cause of cough could not be determined. Those patients were excluded from analysis. Finally, the study included 131 non-smoking patients; median age 54 years, range 18–81 years, F/M – 101/30. All patients signed an informed consent.

2.2 Study Design

Pre-enrollment assessment included: chest radiograph (postero-anterior view) and spirometry with reversibility testing when applicable. Patients who were active smokers, patients with significant abnormalities in the chest radiograph (e.g., lung tumor or masses, lung opacities, interstitial changes, or heart failure), and patients with an abnormal ventilatory pattern were excluded from further analysis. The patients with chronic cough who had been previously unsuccessfully treated were included.

Cough etiology was diagnosed based on the detailed medical history, physical examination, and additional investigations. The algorithm of the applied diagnostic work-up (Fig. 1) was based

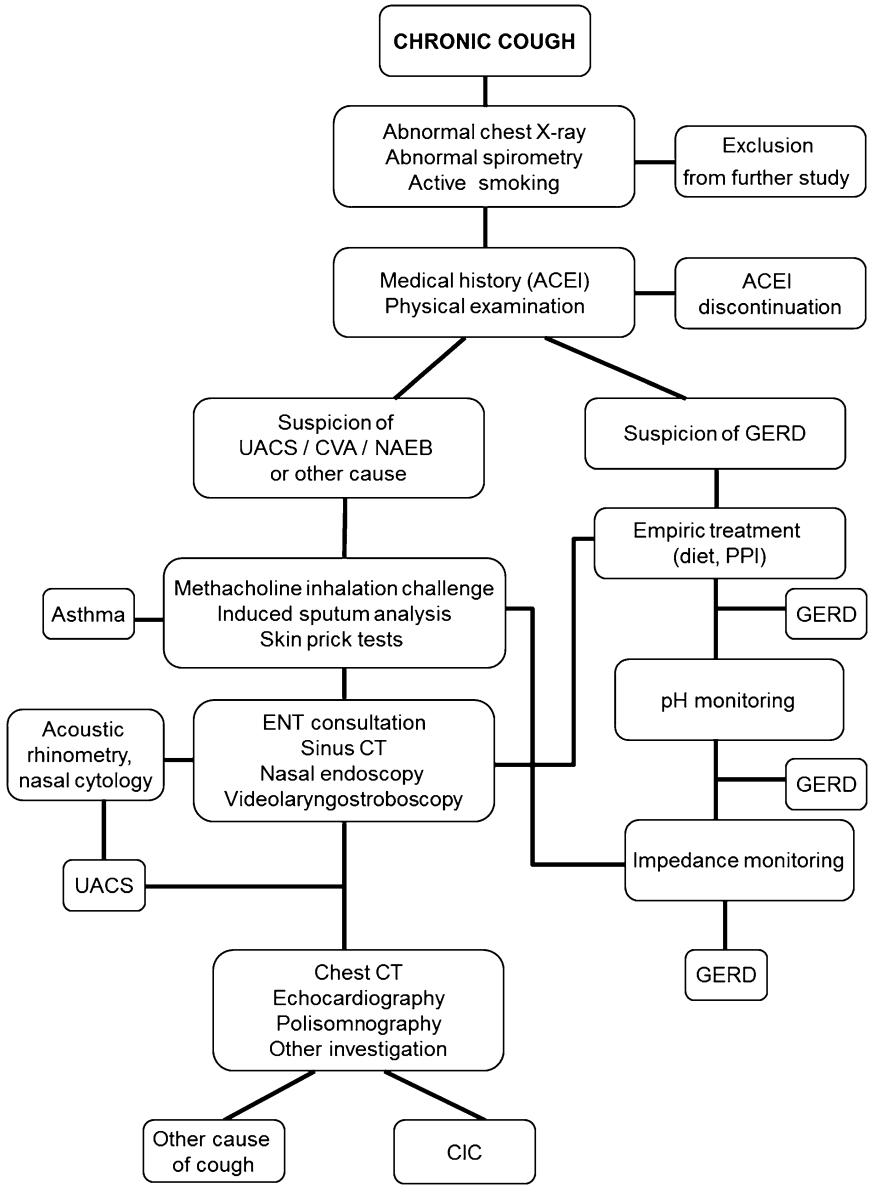

Fig. 1 Flow chart presenting the diagnostic workup designed to diagnose the cause of chronic cough. *ACEI* angiotensin-converting-enzyme inhibitor, *CIC* chronic idiopathic cough, *CVA* cough variant asthma, *GERD* gastroesophageal reflux disease, *PPI* proton pump inhibitor, *NAEB* non-asthmatic eosinophilic bronchitis, *UACS* upper airway cough syndrome

on the international guidelines (Morice et al. 2006; Irwin et al. 2006). Cough etiology was considered as proved when medical history, physical examination, or results of additional tests suggested a specific etiology, and a partial or complete response to specific treatment was confirmed by the patient him- or herself. If specific treatment was unsuccessful, further diagnostic tests were recommended.

2.3 Definitions of the Main Cough Etiologies

The diagnosis of asthma was based on the GINA guidelines (GINA 2014). Cough variant asthma was presumed if spirometry was normal and bronchial hyperresponsiveness was documented in a methacholine inhalation challenge with a provocative concentration of methacholine causing a 20 % drop in FEV_1 (PC_{20}) of less than 4 mg/ml (Lougheed et al. 2012). In case of the negative methacholine challenge, induced sputum eosinophil count was evaluated in order to confirm or exclude NAEB. NAEB was diagnosed when sputum eosinophilia exceeded 3 % of nonsquamous cells (Brightling 2006).

UACS includes chronic rhinitis and rhinosinusitis. The diagnosis of chronic rhinosinusitis required the presence of clinical symptoms defined in the EPOS document and changes in computer tomography sinus scan evaluated according to the Lund and Mackay score (Fokkens et al. 2012). Chronic rhinitis and its etiology were diagnosed on the basis of clinical signs and symptoms and, if suspected by an ear-nose-throat specialist, based on additional examination including nasal endoscopy, acoustic rhinometry or nasal cytology (Pratter 2006).

GERD was diagnosed when improvement after anti-reflux treatment (diet and proton pump inhibitors for 10 weeks) was observed or by results of 24-h esophageal pH or multichannel intraluminal impedance monitoring. The measurements of esophageal pH were performed using two-antimony-pH-electrodes esophageal catheter connected with Digitrapper pH 400 recorder (Synectics Medical; Enfield, UK). A rapid drop of pH below the value of 4 for at least 12 s was necessary to diagnose an acid reflux episode. The diagnosis of GERD-associated cough was made if cough, registered by the patient on the recorder, appeared within 2 min after the reflux episode (Tutuian 2008). Combined multichannel intraluminal impedance–pH monitoring (MII/pH) was performed in patients suspected of weakly acidic or non-acidic reflux. Pathological weakly acidic or non-acidic reflux was diagnosed based on bolus exposure, total reflux percentage time, or median bolus clearance time (Tutuian 2008).

In several patients, other than the above mentioned cough reasons were found. Finally, if none of the known causes of chronic cough could have been diagnosed and no improvement was noted after empiric treatment, chronic idiopathic cough (CIC) was recognized.

2.4 Data Presentation and Statistical Analysis

Data on patient characteristics are shown as median and ranges. Relative contribution of different cough causes was presented in two different ways: as percentage of patients with a particular cough cause and as percentage of all diagnosed causes of cough. The Chi-squared test was used to compare the proportions of patients with various cough etiologies in different groups. A p-value lower than 0.05 was regarded significant.

3 Results

Of the 131 patients, the cause of cough was established in 127 cases (97 %). In four patients the cause of chronic cough was not identified, despite completion of the full diagnostic pathway. Most of the patients (76/131, 58 %) had been unsuccessfully diagnosed or treated because of cough previously.

The median duration of cough was 24 (2.5–360) months. Ninety two patients (70 %) were never smokers, the remaining were ex-smokers (less than 10 pack-years). The median body mass index (BMI) of the whole group was 27.6 kg/m^2. A single cough cause was found in 54 patients (54/127, 42.5 %), two and three coexisting cough causes were diagnosed in 57 (57/127, 45 %) and 16 (16/127, 12.5 %) patients, respectively.

Data on the distribution of specific cough etiologies are presented in Table 1. GERD was

Table 1 Etiology of chronic cough

	Total number (%) of patients with cough etiology	Percentage of all (216) diagnosed causes of cough
GERD	79/127 (62 %)	79/216 (37 %)
UACS	59/127 (46 %)	59/216 (27 %)
Asthma	32/127 (25 %)	32/216 (15 %)
NAEB	19/127 (15 %)	19/216 (9 %)
CIC	4/131 (3 %)	4/216 (2 %)
Other	27/127 (21 %)	27/216 (13 %)

GERD gastroesophageal reflux disease, *UACS* upper airway cough syndrome, *NAEB* non-asthmatic eosinophilic bronchitis, *CIC* chronic idiopathic cough

the most common cough reason and was diagnosed in 79 patients (79/127, 62 % of patients and 37 % of all cough causes). GERD was diagnosed most frequently by cough amelioration after empiric treatment (45 patients). In 28 patients, the diagnosis of GERD was based on the result of pH monitoring and in 6 patients on MII monitoring.

The diagnosis of UACS was established in 59 patients (59/127, 46 %). In this group, rhinosinusitis was found in 9 subjects (9/127, 7 %) while chronic rhinitis in 50 (50/127, 39 %). The most common etiology of UACS was persistent allergic rhinitis (30/127, 24 %). The subgroup of patients with persistent allergic rhinitis included 19 patients allergic to house dust mites, 7 to mold, and 4 to animal dander. In 20 patients non-allergic rhinitis was diagnosed.

Asthma and NAEB were diagnosed in 32 (25 %) and 19 (15 %) patients, respectively. Other cough reasons were found in 27 patients (27/127, 21 %), with the most common being an angiotensin converting enzyme inhibitor (ACEI) treatment (14/127, 11 %). Cough due to bronchiectases was diagnosed in 3 patients, prolonged post-infectious cough in 1 female, airway colonization with *Mycobacterium xenopi* (without radiological signs of pulmonary involvement) in 2 patients, heart failure in 2 patients, mitral valve stenosis in 1, obstructive sleep apnea (OSA) in 3 patients, and pulmonary embolism in 1 patient. Chronic idiopathic cough was diagnosed in 4 (3 %) patients, all of them being never smoking women.

Table 2 Differences in cough etiology in relation to gender

	Female	Male
	n = 101	n = 30
Age (median, range) (years)	59 (18–81)	51 (24–73)
GERD	59 (58 %)	20 (67 %)
UACS	39 (39 %)*	20 (67 %) *
Asthma	31 (31 %)*	1 (3 %) *
NAEB	16 (16 %)	3 (10 %)
CIC	4 (4 %)	0
Other	19 (19 %)	8 (27 %)
Single cough etiology	36 (36 %)	14 (47 %)
Multiple cough etiology	61 (60 %)	16 (53 %)

GERD gastroesophageal reflux disease, *UACS* upper airway cough syndrome, *NAEB* non-asthmatic eosinophilic bronchitis, *CIC* chronic idiopathic cough
*$p < 0.05$

Table 3 Cough etiology in relation to smoking history

	Never smokers	Ex-smokers
	n = 92	n = 39
Age (median, range) (years)	57 (18–81)	53 (19–77)
Female/Male	76/16	25/14
GERD	54 (59 %)	25 (64 %)
UACS	39 (42 %)	20 (51 %)
Asthma	21 (23 %)	11 (28 %)
NAEB	15 (16 %)	4 (10 %)
CIC	4 (4 %)	0
Other	16 (17 %)	11 (28 %)
Single cough etiology	41 (45 %)	13 (33 %)
Multiple cough etiology	47 (51 %)	26 (67 %)

GERD gastroesophageal reflux disease, *UACS* upper airway cough syndrome, *NAEB* non-asthmatic eosinophilic bronchitis, *CIC* chronic idiopathic cough

Asthma was a significantly more common cause of chronic cough in women than in men (31 % *vs*. 3 %, $p = 0.005$). A reverse relationship was demonstrated for UACS (39 % *vs*. 67 %, $p = 0.01$) (Table 2). There were no differences in the proportion of patients with GERD and NAEB-related cough in men and women. Similar results were found for never smokers *vs*. ex-smokers and for patients younger than 50 *vs*. older than 50 years (Tables 3 and 4). The patients with chronic cough aged over 50 were more likely to be diagnosed with less common cough

Table 4 Cough etiology in patients younger and older than 50 years of age

	Age ≤50 years n = 52	Age >50 years n = 79
Age (median, range) (years)	32 (18–50)	63 (51–81)
Female/Male	38/14	63/16
GERD	30 (58 %)	52 (66 %)
UACS	28 (54 %)	32 (40 %)
Asthma	16 (31 %)	19 (24 %)
NAEB	7 (13 %)	12 (15 %)
CIC	1 (2 %)	3 (4 %)
Other	2 (4 %)*	24 (30 %)*
Single cough etiology	24 (46 %)	30 (38 %)
Multiple cough etiology	27 (52 %)	46 (58 %)

GERD gastroesophageal reflux disease, *UACS* upper airway cough syndrome, *NAEB* non-asthmatic eosinophilic bronchitis, *CIC* chronic idiopathic cough
*$p < 0.05$

causes (other than GERD, UACS, asthma, and NEAB, $p = 0.005$) (Table 4).

4 Discussion

The cause of chronic cough depends mainly on the characteristics of studied population and methods used to diagnose different underlying conditions. The majority of authors apply different modifications of the anatomical diagnostic protocol proposed by Irwin (2006), which is based on limited diagnostic tests and empiric trails of treatment. The American College of Chest Physicians (ACCP) and the British Thoracic Society (BTS) recommend a similar diagnostic approach (Morice et al. 2006; Irwin et al. 2006). The value of such protocols is their simplicity and efficacy. The protocols allow recognizing and treating of the most important single cough reason. However, their application relatively often leads to the diagnosis of chronic idiopathic cough. On the other hand, protocols based on numerous diagnostic tests enable to recognize several coexisting cough causes and reduce the number of patients with idiopathic cough (Grabczak et al. 2008; Palombini et al. 1999). The present results are in concordance with those observations (Grabczak et al. 2008; Palombini et al. 1999) as the majority of our patients (57 %) had two or more cough reasons, while the percentage of patients with chronic idiopathic cough was relatively low (3 %).

The most frequent cough reason in the present study was GERD, but the proportion of patients with cough due to GERD in this study was higher than that in other studies; 62 % *vs*. 35–41 % reported by others (Kastelik et al. 2005; Palombini et al. 1999). This may have resulted from using several different diagnostic methods: empiric anti-reflux treatment, 24-h esophageal pH monitoring, and multichannel intraluminal impedance monitoring. Both 24-h esophageal pH and multichannel impedance monitoring are valuable methods in the diagnosis of GERD-related cough, although the interpretation of results is difficult and may vary depending on parameters applied (Kastelik et al. 2005). The sensitivity of pH monitoring in GERD diagnosis is estimated to reach 90 % (Irwin and Madison 2000). As multichannel intraluminal impedance combined with pH monitoring enables to diagnose all types of GERD (acid, weakly acid, and non-acid), it is a notably sensitive method for diagnosing GERD-related cough (Ang et al. 2011; Tutuian 2008).

The upper airway cough syndrome was the second most common cause of chronic cough in the present study (46 % of patients). This is consistent with the results of other authors (Palombini et al. 1999; McGarvey et al. 1998). In our group, chronic rhinitis was a more frequent cause of cough than rhinosinusitis. Persistent allergic rhinitis due to dust mite allergy was the most frequent, followed by allergy to animal coat and mold. It is noteworthy that a thorough ear-nose-throat examination and additional tests resulted in a relatively high recognition of non-allergic chronic rhinitis. Conditions leading to non-allergic rhinitis included structural rhinopathy due to deviation of the nasal septum or conchae hypertrophy. In six patients with chronic non-allergic rhinitis, we observed neutrophilia in nasal cytology (>10 %), despite no evident infection or structural abnormalities.

The significance of this observation is unclear. Rhinosinusitis was relatively rare in patients with upper airway cough syndrome, similarly to the observation of Watalet et al. (2010). Interestingly, in the present study cough related to this syndrome was significantly more common in men than in women. To our knowledge, such observation has not previously been reported. There have been reports that the frequency of allergic rhinitis is comparable in males and females, while chronic rhinosinusitis is slightly more frequent in females (Hastan et al. 2011; Bousquet et al. 2008).

Asthma is estimated to be the cause of chronic cough in 24–29 % of adult non-smokers (Dicpinigaitis 2006). The results of the present study correspond with these reports, as we recognized asthma in 25 % of the patients. Due to the inclusion criteria applied, only cough variant asthma was diagnosed in patients participating in this study. We might speculate, that had we not applied clinical and spirometric criteria excluding the majority of patients with asthma, the proportion of asthmatics in our study group would have been even higher. Cough variant asthma was diagnosed more often in females, what corresponds to a higher prevalence of asthma in adult women (GINA 2014).

According to the literature, NAEB is diagnosed in 10–30 % of patients with chronic cough (Brightling 2006). In the present study, NAEB was diagnosed in 15 % of patients. NAEB is an entity described quite recently, with an unclear prognosis. Most authors agree that NAEB responds well to treatment with inhaled steroids (Brightling 2006). However, there are studies suggesting that NAEB may lead to COPD or asthma. In an observational study by Berry et al. (2005) induced sputum eosinophilia and cough persisted in some patients with NAEB despite treatment with inhaled corticosteroids. Moreover, 25 % of these patients eventually developed airway obstruction (COPD in 16 % and asthma in 9 %).

In the present study, the cause of cough other than GERD, UACS, asthma, or NAEB was diagnosed relatively often. Despite the fact that 60 % of our patients had already been diagnosed or treated because of chronic cough previously, in as many as 14 patients (11 %) cough due to ACEI was diagnosed. That indicates that physicians should verify the patients' medication not only at the beginning, but also in the course of the diagnostic work-up. It also is worth noting that in another five patients we found cardiovascular diseases such as heart failure, mitral stenosis, and pulmonary embolism responsible for chronic cough. Those findings may have resulted from including patients up to the age of 80 years (median age was 54 years), which would be consistent with the observation that cough causes other than the four main entities discussed above are found more often in older patients. Among other cough reasons, we found OSA as one of comorbidities inducing cough in three patients. Recently, OSA has been suggested to be a cause of chronic unexplained cough in certain patients and treatment with continuous positive airway pressure leads to a decrease in the severity of OSA-related cough (Lee and Birring 2010).

If the cause of cough could not have been determined despite a thorough diagnostic work-up and empiric treatment – chronic unexplained or idiopathic cough was diagnosed (McGarvey et al. 2008). In the present study, the percentage of patients with CIC was merely 3 %. It probably resulted from the numerous additional tests included in the study protocol. The incidence of CIC depends on the study population and protocol, but according to certain authors it may reach up to 42 % (Haque et al. 2005). The etiology of CIC is unknown. However, hyperreactivity of the cough reflex in these patients has been found (Birring 2011). In some patients with CIC, autoimmunological disease, predominantly of the thyroid gland, and lymphocytic airway inflammation have been observed, but the importance of this phenomenon is unclear (Birring et al. 2003). CIC is more likely to occur in women (Haque et al. 2005; Birring et al. 2003). Further studies on the pathomechanism and treatment of CIC are needed.

The diagnosis of the underlying reason of cough requires not only the identification of the underlying disease, but also obtaining a decrease

in cough after specific treatment (Irwin et al. 2006). A limitation of the present study is using only subjective methods of assessment of the response of cough to treatment, based solely on the patient's opinion. Recent recommendations concerning chronic cough suggest using not only subjective but also objective methods of measuring cough severity (provocation tests with capsaicin or citric acid or monitor the number of cough episodes by using cough monitors) (Birring 2011). Another limitation may be a potential selection bias. Since as much as 60 % of our patients had earlier been diagnosed or treated because of cough, we may suppose that the patients might have particularly 'difficult to treat' cough. Besides, we excluded smokers or patients with abnormalities in chest X-ray and abnormal ventilatory pattern in spirometry. Thus, we had no patients diagnosed with COPD.

The thorough approach to diagnosing the cough reason requires cooperation of different specialists, such as pulmonologists, ear-nose-throat specialist, gastroenterologist, and others. Therefore, cough clinics are set up in many countries, which assemble different specialists in order to better diagnose and treat patients with chronic cough (Dicipingaitis 2012; Dettmar et al. 2009).

5 Conclusions

In conclusion, the most common reasons of chronic cough found in the present study were GERD and UACS. There are some differences in cough etiology between men and women: underlying asthma is are more frequently diagnosed more frequently in females, while UACS in males. Cough causes other than GERD, UACS, asthma, and NAEB are more frequently diagnosable in patients above the age of 50 years. Using a protocol with numerous diagnostic tests enables to find several coexisting cough causes and to reduce the number of patients with unexplained or idiopathic cough.

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

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Diseases of the Upper Respiratory Tract in Preschool and School Age Children in Ambulatory Ear Nose Throat Practice

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Abstract

The most common diseases of the upper respiratory tract in children treated by ear-nose-throat (ENT) specialists in ambulatory practice are infections, such as colds, rhinitis, sinusitis and pharyngitis, very frequently accompanied and promoted by chronic nasal obstructions of various etiology. These diseases, when treated incorrectly or for too long, cause frequent school absenteeism and may also lead to hearing disorders linked with acute or suppurative otitis. They may also habitually perpetuate abnormal breathing and result in occlusal disorders. The aim of this study was to assess the incidence and type of upper respiratory tract diseases in children, depending on age and sex of patients and on the seasons. We also discussed the role of the ENT specialist in the diagnosis and treatment of certain diseases. In the study we analyzed the medical records of patients of preschool and school age treated in the ENT outpatient clinic over one calendar year. It was found that the largest group of patients comprised children of 3–7 years of age, and most children visited the outpatient clinic in the period March-May. The most common main disorder, according to ICD-10, was acute nasopharyngitis (J00) and vasomotor and allergic rhinitis (J30). Among the comorbid disorders H65 and H66 were the most frequent. No significant gender differences were noted in the frequency of particular types of disease.

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Keywords
Comorbid diseases • Infection • Nasopharyngitis • Otolaryngology • Pediatric patients • Rhinitis • Seasonal frequency

1 Introduction

Upper respiratory tract infections (URTI) are the most common diseases in children treated in outpatient clinics. This is probably associated with the anatomical differences between pediatric and adult patients. The airway in children is shorter and narrower, and the mucous membrane is very rich in blood and lymph vessels and is prone to congestion and swelling. The short distance between the individual airway segments facilitates the spread of infection. In addition, the shorter respiratory tract does not ensure the adequate humidity, filtration, and temperature of inhaled air. As a consequence, cold air irritates the mucosa, triggers increased production of mucus, and stimulates bronchial hyperresponsiveness. In young children the overstimulation and impaired inhibition process frequently leads to hypercontractility in the respiratory tract, which is attributed to insufficient regulatory activity of the cerebral cortex. Thus, bronchospasm and asthma may develop in pediatric patients. Coughing is also a common and troublesome symptom of URTI (De Blasio et al. 2012).

The role of the nose as the gateway opening the respiratory tract is extremely important. In the nose inhaled air is heated and brought to body temperature. The relatively small nose in children is unable to fully perform this function, and in many cases its patency is impaired due to hyperplasia of the lymphoid tissue in the nasopharynx or oedema. The child begins to breathe through the mouth, which can then lead to inflammation of the oropharynx and reactive lymphoid hyperplasia of palatine tonsils or, less frequently, the lingual tonsil. Recurrent infections aggravate this problem, Waldeyer's tonsillar ring continues to enlarge, and instead of fulfilling a protective function it creates a mechanical barrier to breathing, which is especially perceptible during sleep (Dzięciołowska-Baran et al. 2013). Children begin snoring and eventually develop sleep apnea. Even a transient obstruction can cause coughing in children. This affects the comfort of sleep, and both children and their parents do not get enough rest. The activity of children at school and during sports exercise becomes diminished (De Blasio et al. 2012). The common cold, i.e. inflammation within the throat and nose with persistent rhinitis or only inflammatory edema of the nasal mucosa, often leads to acute otitis media with pain and fever, while long-term obstruction of the Eustachian tubes' ostia results in suppurative otitis media and conductive hearing loss (Chontmaitree et al. 2008). Obstruction in the respiratory tract leads to malocclusion and speech problems. Delay in treatment can lead to the development of habits which are difficult to eliminate, and children continue breathing through the mouth despite the complete or partial resolution of obstructive respiratory tract disorders.

Upper respiratory tract infections are particularly common in autumn and winter. This is promoted by weather conditions, including low temperatures and insufficient exposure to natural light, creating favorable conditions for the incubation of pathogens. It is also important that during this season children begin preschool or nursery, and when confronted with peers, especially those who previously stayed mainly at home and had contact only with siblings, a high risk of contracting the disease is created. The immune response in children is less effective than in adults, because certain processes involved in the regulation of immunity are still being formed. The immune system reaches full maturity in adolescents, and non-specific and specific immunity develop slowly in response to

pathogens. The first exposure to a pathogen triggers a cascade of reactions targeted at its destruction, and the development of immunological memory.

The aim of this study was to analyze the incidence of upper respiratory tract diseases in preschool and school children depending on the age and sex and the season. We analyzed the incidence of disorders which are most frequently comorbid with upper respiratory tract diseases, and their etiological factors. We also analyzed the need to treat patients with selected diseases in ear-nose-throat (ENT) outpatient clinics *versus* general health care centers.

2 Methods

This study was conducted in accordance with the principles of the Declaration of Helsinki for Human Experimentation. For the purpose of the study we used data from medical records – medical files from the ENT outpatient clinic regarding children aged 1–13 years (n = 294) treated over the calendar year 2013. These children accounted for 16 % of all the patients treated at the ENT clinic. The analyzed group comprised boys (57 %, n = 168) and girls (43 %, n = 126). All the children were examined by the same ENT specialist, and diseases were classified according to the ICD-10 system.

The significance level for results was adopted at $\alpha = 0.05$. The difference in the proportion of boys and girls was estimated using a test for difference between two proportions. The chi-square (χ^2) test of independence was used to analyze qualitative features. Quantitative features were analyzed with the nonparametric Kruskal-Wallis test for multiple comparisons. Statistica 10 software, Polish version, was used for data analysis.

3 Results

Children treated at the ENT outpatient clinic were diagnosed with the following upper respiratory tract diseases: acute nasopharyngitis (J00), acute sinusitis (J01), acute pharyngitis (J02), acute tonsillitis (J01), acute laryngitis and tracheitis (J04), acute upper respiratory infections of multiple and unspecified sites (J39), vasomotor and allergic rhinitis (J30), chronic rhinitis, nasopharyngitis and pharyngitis (J31), chronic disease of tonsils and adenoids (J35), and peritonsillar abscess (J36). The most frequent diseases were the following: J00 (37 %), J30 (23 %), J35 (14 %), and J01 (14 %). There were no statistically significant differences between boys and girls in the number of individual diseases (Table 1, Fig. 1).

In many patients, respiratory disease was co-morbid with other medical conditions, being the consequence of, or closely related with the main condition such as non-suppurative otitis or suppurative otitis media (H65 and H66), Eustachian salpingitis with obstruction (H68), otalgia and effusion of ear (H92), stomatits and related lesions (K12), and diseases of the tongue (K14). The most frequent co-morbidity was found for vasomotor and allergic rhinitis (J30) with Eustachian salpingitis and obstruction (H68) (23 cases), chronic disease of tonsils and adenoids (J35) with non-suppurative otitis media (15 cases), acute nasopharyngitis (J00) with suppurative otitis media (H66) (13 cases), acute sinusitis (J01) with vasomotor and allergic rhinitis (J30) (12 cases), and acute nasopharyngitis

Table 1 The number of cases of different types of disease in male and female groups

ICD-10	J30		J35		J00		J01		J02		J04		J06		J31		J39		J03		J36	
Gender	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
n	34	35	25	15	59	50	22	18	5	6	5	1	5	1	3	–	1	–	5	–	1	–
Total	69		40		109		40		11		6		6		3		1		5		1	

M male, *F* female

See first paragraph of Results for the explanation of disease acronyms

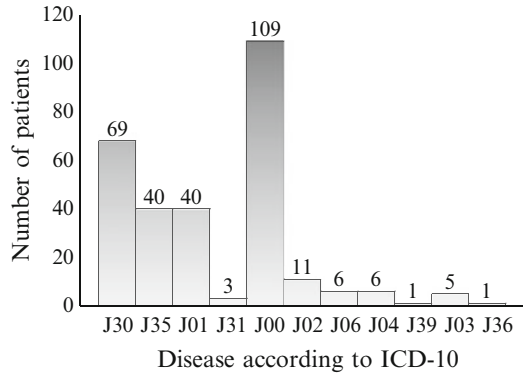

Fig. 1 The number of cases of different types of disease (ICD-10). See first paragraph of Results for the explanation of disease acronyms

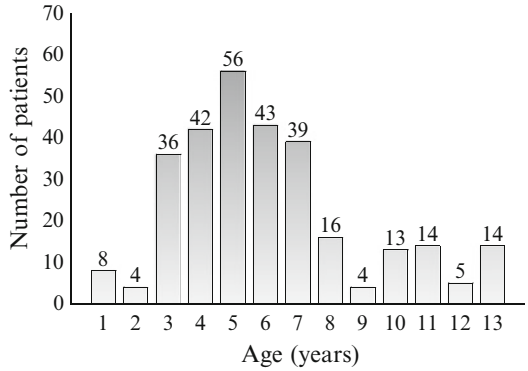

Fig. 2 The number of children in age-groups

(J00) with vasomotor and allergic rhinitis (J30) (10 cases). There was a significant correlation between the incidence of co-morbid disorders and the main condition (χ^2 test; $p = 0.02$).

The mean age of all treated children was 6.1 years. The quantitative analysis of children, focused on individual age groups, demonstrated the strong dominance of preschool age children. Most children were aged 3–7 years ($n = 215$; 73 % of the study group), and the largest group treated at the clinic were children aged 5 years (Fig. 2).

Concerning the association of children's age and proneness to morbidity in consecutive months of the year, it may be seen that the youngest children visited the clinic in November and December. The mean age of children treated in November was significantly lower than that of children treated in March ($p = 0.03$) or May ($p = 0.009$) (Table 2).

Table 2 The mean age of children in calendar months

Month	n	Mean age (year)
January	29	6.2 ± 2.5
February	27	6.4 ± 3.3
March	32	6.8 ± 2.8
April	29	6.9 ± 3.4
May	35	7.1 ± 2.8
June	28	6.2 ± 2.8
July	16	5.8 ± 2.5
August	24	6.0 ± 3.1
September	9	6.7 ± 3.8
October	25	5.1 ± 2.1
November	27	4.5 ± 1.8
December	13	4.2 ± 1.7

Concerning, however, the sheer number of pediatric patients in relation to particular months of the year, the results were quite surprising results. Most children visited the clinic in May and March (Fig. 3), with the lowest numbers in September, December, and June. The total proportion of boys treated at the clinic during the whole year (57 %) was significantly greater than that of girls (43 %) ($p = 0.015$).

4 Discussion

Upper respiratory tract infections in children are a frequent and troublesome problem. Although URTI are rarely fatal, unlike diseases of the lower respiratory tract, they are a source of significant morbidity and are associated with a considerable economic burden. This results from treatment costs and prescription of antibiotics, despite the fact that most of these diseases have a viral etiology (Simoes et al. 2006). In many cases, infections are resolved spontaneously and do not require any specific therapeutic procedure. Nevertheless, costly examinations are carried out and patients are referred to specialists for consultation (Chow et al. 2012; Van Der Gaag 2012). Another problem is the absenteeism of children from school or preschool and, most importantly, the absenteeism of parents/guardians from work.

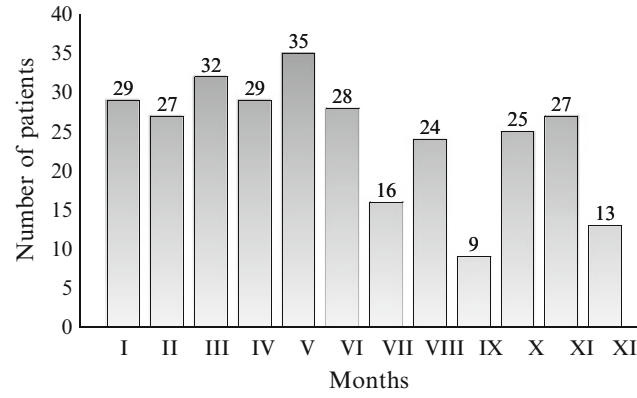

Fig. 3 The number of children in particular months of the year

The analysis of disease incidence among children clearly indicates an increase in morbidity and a reduction in the age of patients during the autumn and winter seasons, which in Poland are associated with a rapid fall in temperature, change of diet, consisting of lower intake of fruit and vegetables, and reduced levels of physical activity in the open air. Additionally, these factors coincide with the beginning of the school/preschool year and the stay of children in places highly populated by peers (Covington et al. 2004). Infections are particularly frequent in children who have just begun going to nursery of preschool after being brought up in almost sterile conditions, often separated from the environment creating the potential risk of infection. After the unproblematic first years of life, children often fall ill as soon as they start attending preschool (Roberts et al. 2000). In the youngest children, the beginning of a more social life is also an important stressor. Another important issue is the fact that ill or incompletely recovered children are sent back to preschool or nursery. There are, however, attempts to curb this problem by requesting from parents a relevant health certificate to show that child's health is in good order, and this enables them to return to preschool.

Another problem is created by the negative effect of antibiotics, which are overused in the treatment of viral infections, suppressing the maturation of the immune system (Chow et al. 2012). This results from the direct activity of antibiotics on the competent immune cells and the reduced time of exposure of the immune system to antigens. In most children of preschool age the problem of recurrent diseases is resolved when they begin school education, because the immune system, programed during the preschool period by repeated exposure to antigens, is more functionally mature and allows for the efficient control of the infection process. Usually, upper respiratory tract infections, especially those of a viral etiology, do not require intensive treatment and resolve spontaneously in many cases.

Thus, it is worth considering the problems associated with the treatment of upper respiratory tract diseases in pediatric patients, i.e., who should be involved in it and when. Should cases of common cold be treated at all by a doctor, and what symptoms should raise parents' concerns? At what point is the involvement of an ENT specialist or allergologist necessary in treatment and diagnosis? We are inclined to propose that some infections, especially colds, do not require a visit to an outpatient clinic. Instead, pediatric patients should stay at home, have enough rest, and be separated from their peers, and sometimes be given medications to resolve symptoms. However, parents may need time off from work, and here a visit to a doctor is necessary. It seems that most common upper respiratory tract infections in children can be successfully treated by general practitioners. In some cases, a consultation with a pediatrician may be helpful, especially in infants or children from risk groups, e.g., prematurely born, with low birth weight, or immunodeficient (Wald et al. 1991). In Poland, currently, the

treatment of patients in specialist outpatient clinics, e.g. ENT or allergology, financed by the National Health Care Service, is only possible for patients with a referral from a family doctor or another specialist. Thus, pediatric patients are seen by a specialist after being examined by a general practitioner, who decides on referral under pressure from parents who are concerned by recurrent infections in their children and attribute this problem to the malpractice of a family doctor. However, most of these diseases are caused by viral factors and are common colds.

Respiratory tract infections in children of preschool age, facilitated by frequent exposure to pathogens, may in many cases be caused by the slow or abnormal maturation of the immune system. Some infections tend to be chronic, recurrent, and some require hospitalization (Chow et al. 2012). Appropriate analysis of the problem should be focused on the severity, duration and potential complications of infections, recurrence-free periods between infections, and environmental conditions (West 2002). In cases when measures taken by parents or doctors to improve a child's health do not provide the expected results, recurrent infections may be associated with more serious disorders and may require specialist diagnosis and treatment.

The onset of symptoms such as otalgia and discharge from the ears, hearing loss, severe dry cough, particularly of barking type, snoring, sleep apnoea, or chronic nasal obstruction are an indication for ENT consultation. Most family doctors do not have appropriate equipment to diagnose these symptoms. This is particularly important in patients with impaired hearing or recurrent otitis. Acute otitis media with fever and rhinitis is usually treated with antibiotics, while in fact only some patients require such medication. Acute otitis media frequently coexists with, or is a consequence of, acute nasopharyngitis (J00), caused by viral factors, while otitis media with effusion is often asymptomatic, and when inappropriately treated may lead to conductive hearing loss. In children, it is often comorbid with upper respiratory tract infections (Chonmaitree et al. 2008). However, our observations revealed that otitis media with effusion is comorbid with hyperplasia of lymphoid tissue, particularly the palatine tonsil and tubal tonsil (Simoes et al. 2006). The second identified correlation is between nasal obstructive disorders of allergic etiology and Eustachian tube dysfunction, manifested by a feeling of fullness in the ear, transient conductive hearing loss, discomfort, and even otalgia associated with negative pressure in the tympanic cavity. At this point it should be emphasized that in children allergy is extremely difficult to confirm by relevant tests (Sukumaran 2011). The introduction of antihistamine or anti-inflammatory drugs acting topically and reducing oedema improves therapeutic outcomes.

There are a number of measures that can be used to prevent infections or support the recovery of children from infections. The therapeutic procedure in patients with infections should include the treatment of acute disease, avoiding antibiotics whenever possible, and the assessment of complications and recovery from disease (Pitrez and Pitrez 2003). Measures to prevent recurrent infections should include supplementary immunization as well as traditional well-tested methods, such as climatic therapy, education in proper hygiene, sufficient physical exercise, boosting endurance (outdoor activities, appropriate clothing) and the elimination of negative environmental factors. Proper nutrition in autumn and winter is important to supply the body with vitamins, and major and trace minerals, as well as popular traditional remedies in Poland used in reasonable doses, such as cod liver oil, aloe, honey, garlic, and onion juice. Prebiotics and probiotics, being natural stimulants of the immune system and alimentary tract functions, contained in dairy products known as functional foods, may also be useful. Other very important products are immunostimulants or immunomodulators (e.g., bacterial, fungal, synthetic and plant-derived, or bacterial vaccines), which are designed to enhance immunity. However, the efficacy of these products, especially those taken without consulting specialists, is often questioned, and they should be used with care in children because of the maturing immune system.

5 Conclusions

Boys are significantly more frequently treated at the ENT outpatient clinic than girls. This indicates a greater susceptibility of male children to negative environmental factors responsible for upper respiratory tract infections. Diseases are most frequent in children of preschool and early school age (3–7 years of age). In autumn, the mean age of patients decreases significantly, and it coincides with the beginning of the school year and the poor weather season. The most frequent comorbidity was found for vasomotor and allergic rhinitis (J30) and Eustachian salpingitis with obstruction (H68), chronic disease of tonsils and adenoids (J35) and non-suppurative otitis media (H65), as well as acute nasopharyngitis (J00) and suppurative otitis media (H66).

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

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Assessment of Air Pollution Effects on the Respiratory System Based on Pulmonary Function Tests Performed During Spirometry Days

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Abstract

The Polish Spirometry Day is an initiative aimed at increasing awareness of the causes, symptoms, course, and effects that accompany respiratory diseases, especially asthma and chronic obstructive pulmonary disease (COPD). In 2013, the second edition of the Spirometry Day was held. It gathered 180 medical centers and other institution. The final analysis encompassed a total of 1187 persons from 26 different locations, including rural areas, and smaller and larger city agglomerations. Of this total, 755 persons (63.6 %) completed their spirometry tests for the first time in life. Each person fulfilled a questionnaire regarding the personal information, respiratory diseases, symptoms, lifestyle, and a place of residence. In the total group, 234 (19.7 %) cases of bronchial obstruction were diagnosed. A hundred and thirty four persons with obstruction, among those tested for the first time in life (17.8 %), were unaware of their disease. The lowest values of FEV_1 and FEF_1/FVC, corresponding to the highest percentage of persons with obstruction (27.9 %) were observed in small and medium cities (100,000–500,000 inhabitants). There were differences in the prevalence of obstruction depending on the distance of the place of residence from a busy traffic road. A significant decrease of both spirometric variables was observed among people living in cities above 100,000 inhabitants within a distance lower than 50 m from roads.

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In general, better spirometry results were observed among inhabitants living more than 150 m from main roads.

Keywords
Asthma • Bronchial obstruction • COPD • Pulmonary function test • Respiratory disease • Screening

1 Introduction

According to the Global Burden of Disease Study (Murray et al. 2012a), chronic obstructive pulmonary disease (COPD) is the third most frequent cause of deaths in the world and comes in the ninth position in the rank based on the disability adjusted life-years (DALY) index (Lozano et al. 2012; Murray et al. 2012b). COPD is characterized by reduced airway flow. The basic test used to diagnose and assess the progress of the disease is spirometry. The stage of the disease is defined with the forced expiratory volume in 1 s (FEV_1) index, expressed with the percentage of predicted value (Vestbo et al. 2013), and based on the dyspnea progress scale and the number of exacerbations within 1 year as defined by the guidelines of the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2015).

The tests carried out within Spirometry Days are aimed mainly at drawing public attention to the causes, course, and consequences of respiratory system diseases. A major problem related to the respiratory system diseases is their late diagnosis (Mannino et al. 2000). The progression of the disease may be contributed by the lack of public awareness of the underlying etiology. The risk factors of COPD are both host (genotype) and environmental related. The development of the disease is accelerated by smoking cigarettes (being the key prerequisite for the morbidity), but the risk-increasing factor may also be an increased exposure to air pollution. It should be noted that although smoking cigarettes is the most significant among the risk factors for COPD, the disease affects also non-smokers (Kohansal et al. 2009). It is estimated that the non-smokers are 25–45 % of all cases of patients suffering from COPD all over the world (Salvi and Barnes 2009). Therefore, it is so important to take all potential risk factors into account in epidemiological studies. The results of numerous studies show that exposure to pollution, including the one generated by road transport, affects the health of people causing, *inter alia*, respiratory system disorders (Anderson et al. 2011; de Marco et al. 2011; Canova et al. 2012; Colais et al. 2012; Sousa et al. 2012). Population-based studies show that – depending on a country – 8–10 % of people aged over 30 are affected with COPD, which is also confirmed by the studies completed in Poland, where 5–20 % of city residents demonstrate features of bronchial obstruction (Zieliński et al. 2006). It has also been shown that significantly lower values of spirometric variables occur in the group of inhabitants of cities compared to the residents of the areas located outside cities, characterized by appreciably lower concentrations of air pollutants (Badyda et al. 2015). An example of studies showing that living near busy roads has a direct impact on COPD morbidity are the cohort studies completed in Denmark. These studies were aimed at the assessment of impact of automotive pollution within the period of 35 years on the occurrence of COPD. Data of more than 57,000 persons hospitalized in the years 1993–2006, as a result of suffering from COPD, have been reviewed. Concentration of pollutants (NO_2 and NOx) near the places where the subjects were living was also analyzed. With the use of regression analysis, an impact of exposure to air pollution on the occurrence of COPD has been assessed for the whole group of subjects and

separately for the groups with or without comorbid conditions such as asthma, diabetes, or cardiovascular diseases. The prevalence of COPD was connected with a 35-year long average concentration of NO_2 (hazard ratio – HZ: 1.08, 95 % confidence intervals – CI: 1.02–1.14, and interquartile range: 5.8 μg/m^3), and it was greater in case of patients suffering from diabetes or asthma (Andersen et al. 2011).

The research conducted in 36 cities in the United States assessed the impact of ozone and PM_{10} particulate matter on the number of hospitalized due to respiratory system diseases. Regression models adjusted to the specifics of each city were selected for meta-analysis, taking into account data of the years from 1986 to 1999. In the warm time, the effect of ozone concentration increase (5 ppb), cumulated within 2 days, resulted in an increase of 0.27 % (95 % CI: 0.08–0.47) in admission of patients suffering from COPD and an increase of 0.41 % (95 % CI: 0.26–0.57) in admission of patients suffering from pneumonia. Similarly, the increase of PM_{10} concentration by 10 μg/m^3 in the warm time led to an increase in admission of patients with COPD by 1.47 % (95 % CI: 0.93–2.01), and with pneumonia by 0.84 % (95 % CI: 0.50–1.19). The percentage of households with central air conditioning-supported reduction of exposure to air pollution and temperature fluctuations in the summer time limited the ozone impact on the COPD morbidity. The studies performed on a large sample of cities confirmed that the exposure to ozone and particulate matter is connected with hospitalization of people with respiratory problems and proved that the impact of air pollutants is modified with some urban features (Medina-Ramón et al. 2006).

Risk of development of COPD and its health effects was one of the bases for founding the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2015). One of the aims of founding GOLD was preparation of a global COPD diagnostic strategy for better management and more effective prevention of the disease development. Romain et al. (2011) pointed out that education of patients and doctors is an important aspect of the COPD-related public health care. Making both of these groups aware that dyspnea, chronic cough, and expectoration are possible symptoms of the disease and should be diagnosed.

The International COPD Coalition (ICC) conducts the activity and research in the field of raising awareness of COPD occurrence amongst the society. Grouse and Nonikov (2014) have summed up the ICC activities to-date. The first exploratory results from 2001 have shown that a global awareness of COPD is very low and comes to 4 % for Brazil (the lowest awareness) and 10 % for Germany (the highest awareness). Currently, many countries belong to the Coalition and perform questionnaire surveys systematically. The results of the last questionnaire completed in 41 countries (26 developed and 15 developing countries) indicate a significant progress. For almost 37 % of countries public awareness of COPD is 20 % or more. For 31 % of the developed countries the awareness exceeds the level of 40 %. Unfortunately, such high results were not reached in any of the developing countries participating in the study. It should be pointed out that the research completed in Poland at the request of the Polish Society of Lung Diseases indicates that merely 3 % of the population is aware of COPD (Śliwiński and Puchalski 2015).

Interesting results of the study on social awareness regarding COPD were obtained in Norway, where such studies are being conducted for many years (Gulsvik et al. 2007). The percentage of the aware of COPD existence came to 78 % in the last study. The success in fostering public awareness was achieved along with the Norwegian Heart and Lung Patient Organization (LHL) and the Norwegian Medical Association's COPD Strategy Group. The activities of these organizations have intensified in the course of the annual Spirometry Days. In Norway, a national strategy for protection against COPD was implemented for years 2006–2011.

The World Spirometry Day in many countries is one of the important opportunities to undertake awareness-raising activities. One of the examples is also Portugal, where COPD is the cause of 14 % of deaths. The activities conducted within

Spirometry Days in Portugal in 2012 included cross-sectional studies consisting of a questionnaire and spirometric test. A hundred and sixty cases were examined. Sixty two and a half percent of people among this group answered that they had been aware of the existence of chronic pulmonary disease, but only 29.4 % knew what spirometry consists in (Pacheco et al. 2013).

2 Methods

2.1 Subject of Research

Almost 5000 pulmonary function tests were performed in June 2013 as part of the Second Polish Spirometry Day. Due to some key data missing from the resultant questionnaires delivered by research centers or failure to meet the requirements concerning the proper quality of research (A–C category) specified by European Respiratory Society (Quanjer et al. 1993) and American Thoracic Society (ATS) only approximately 25 % of them were taken into account in the final analyses. Therefore, the analysis of the study material was limited to 1187 results of spirometric tests meeting the quality requirements and completeness of the questionnaire constituting an integral part of the test result.

2.2 Implementation of Research

The pulmonary function tests were performed in 26 urban areas (in 180 institutions) all over Poland. Physical examination was accompanied by taking medical history based on a detailed questionnaire prepared especially for this purpose. It served collecting the necessary information, including such information as: occurrence of chronic respiratory system diseases, occurrence of chronic symptoms of respiration disorders, allergies, residence and living conditions, lifestyle (including smoking cigarettes, physical activity, and type of work), and others.

Spirometric test is one of the most commonly performed functional tests in medical treatment and one of the conditions for diagnosing and proper treatment of patients suffering from COPD and bronchial asthma, the diseases more and more common worldwide (Miller et al. 2005). The following are mentioned among the recommendations for performing the above mentioned test: smoking cigarettes, chronic cough and expectoration, passive exposure to tobacco smoke, and environmental factors of respiratory system exposure to air pollution (Saad et al. 2014).

Each test consisted of two parts:

1. medical history (questionnaire), the main objective of which was obtaining information on the possible aforementioned factors of exposure to respiratory system diseases;
2. physical examination, i.e., pulmonary function test performed in sitting position (in exceptional cases in the standing position) in the upright posture and after providing instructions on the proper performance of spirometric tests. The test was repeated at least thrice with recording the flow-volume curves until the repeatability of the results meeting the criteria of ATS and ERS are achieved (the results of the individual tests which do not differ by more than 5 %). The test result included the following variables:
   - FVC (forced vital capacity);
   - FEV_1 (forced expiratory volume in 1 s);
   - PEF (peak expiratory flow);
   - FEV_1/FVC – the proportion of a person's vital capacity that he is able to expire in the first second of expiration, the so-called pseudo-Tiffeneau index.

The elementary diagnostic criterion for obstruction is a reduction of the FEV_1/FVC index below the lower limit of normal (LLN) or 5th percentile of predicted values. Due to the age of the patients, a criterion of lowering the FEV_1/FVC index below 0.7 was applied taking into account the risk of overdiagnosing obstruction in persons aged over 60. The level of bronchial obstruction is assessed as based on FEV_1 value expressed as a percentage of predicted value. The

measurement of FEV_1 helps establish the stadium of COPD (GOLD 2015).

The obtained values of indexes expressed in dm^3 were standardized with the use of the ERS/ECCS guidelines (Quanjer et al. 1993). The statistical analyses were performed with the use of Statistica 10 software.

3 Results

Of a total of 1187 persons examined (mean age of 54.7 ± 16.5 years), 755 persons (63.6 %) completed their spirometry tests for the first time in life. There were 234 (19.7 %) cases of bronchial obstruction, including 96 mild, 116 moderate, 16 severe, and 6 results indicating very severe obstruction. A hundred and thirty four persons with obstruction, among those tested for the first time in life (17.8 %), were unaware of their disease. The value of the pseudo-Tiffeneau indicator averaged for the whole group (77.0 ± 11.4 %) turned out to be relatively low. However, the average percentages of predicted values of FEV_1 and FVC (Table 1) remain at a noticeably higher level.

The tests showed the existence of noticeable discrepancies between the declarations of the subjects and the actual results of their pulmonary function tests. A hundred and ten of the subjects declared that they had been diagnosed with asthma and/or COPD before. The occurrence of obstruction was confirmed only in 41 persons declaring COPD and in 24 persons declaring asthma, taking account of the fact that obstruction is not an immanent feature of bronchial asthma. Conversely, among persons declaring not being diagnosed with COPD or asthma before (n = 1067), 197 cases of obstruction were found.

The occurrence of chronic (over 12 weeks-long) symptoms of respiratory system disorders was taken into account in the analyses. The patients proved to have bronchial obstruction significantly more often ($p < 0.05$) declared a long-term occurrence of symptoms indicating breathing disorders. However, in the group of persons, who did not show any features of obstruction in spirometric tests (n = 953), numerous cases of long-term occurrence of such symptoms were also shown (Table 2). Furthermore, it should be emphasized that a considerable part of persons showing the above-mentioned symptoms, which is the indication for performing the spirometric test, has never had such test performed.

Having regard to the earlier studies (Badyda et al. 2013), indicating occurrence of statistically significantly lower values of spirometric variables among inhabitants of the selected urban areas characterized by significantly higher concentrations of air pollutants against the background of rural areas, the analyses also were performed taking into account the residence of the subjects. Only non-smokers were selected of the whole examined group and allocated to 5 types of areas: rural areas (9.3 % of the subjects), cities with population below 100,000 inhabitants (35.4 % of the subjects), cities with population between 100,000 and 250,000 inhabitants (34 %), cities with population

Table 1 Percentage of predicted values of basic spirometric variables for the analyzed group

Variable	Mean ± SD	Median	Lower/upper quartile
FEV_1 (%)	92.6 ± 22.0	94	80/106
FVC (%)	100.0 ± 23.0	100	86/112
FEV_1/FVC (%)	77.0 ± 11.4	79	72/84

FEV1 forced expired volume in 1 s, *FVC* forced vital capacity, *FEV1/FVC* the proportion of a person's vital capacity that he is able to expire in the 1st sec of expiration – pseudo-Tiffeneau index; data are means ± SD

Table 2 Declaration of long-term occurrence of chronic pulmonary symptoms in persons with and without diagnosed bronchi obstruction

	People with obstruction (*n = 234*)	People without obstruction (*n = 953*)
Symptoms	n (%)	n (%)
Dyspnea	93 (39.7)	307 (32.2)
Cough	55 (23.5)	230 (24.1)
Cough with expectoration	91 (38.9)	285 (29.9)
Wheezing	53 (27.7)	159 (16.7)

between 250,000 and 500,000 inhabitants (5.7 %), and cities with population above 500,000 inhabitants (15.4 %). Occurrence of statistically significant differences between the values of FEV_1 and FEV_1/FVC was observed among persons living in areas with a different population number. The best breathing efficiency was found in the inhabitants of rural areas and the weakest spirometric variables were found in persons living in small and average-sized cities (from 100,000 to 500,000 inhabitants) (Table 3). These relations are similar to the connections observed in the previous studies conducted within Spirometry Days (Dąbrowiecki et al. 2013). In terms of percentages of the subjects showing features of bronchial obstruction, the most disadvantageous is the situation in small and average-sized cities (Fig. 1). It could partly be connected with the relatively high air pollution problem in such cities, caused mainly by low-stack emission (from commercial and household sectors using low-quality coal, wood, and some other fuels of unknown origin) and traffic-related emission. It may also be due to specific dispersion conditions of air pollutants, as well as fairly lower life standard or weaker access to health care system as compared to larger cities.

The assessment covered also the variability of the value of spirometric variables depending on the distance of the place of residence of the subjects (non-smoking only) from the nearest busy road. The results show that breathing efficiency as well as the percentage of persons showing the features of obstruction depend on the distance of the place of residence from a busy

Table 3 Percentage of predicted values of basic spirometric variables

	Rural areas	Cities <100,000 inhabitants	Cities 100,000–250,000 inhabitants	Cities 250,000–500,000 inhabitants	Cities >500,000 inhabitants
FEV_1 (%)	95.5 ± 20.3	93.2 ± 21.5	93.2 ± 19.9	86.8 ± 26.2	90.0 ± 22.4
FEV_1/FVC (%)	80.1 ± 8.9	77.4 ± 11.1	76.2 ± 11.5	71.7 ± 13.9	77.9 ± 10.8

FEV1 forced expired volume in 1 s, *FEV1/FVC* the proportion of a person's vital capacity that he is able to expire in the 1st sec of expiration – pseudo-Tiffeneau index; data are means ± SD

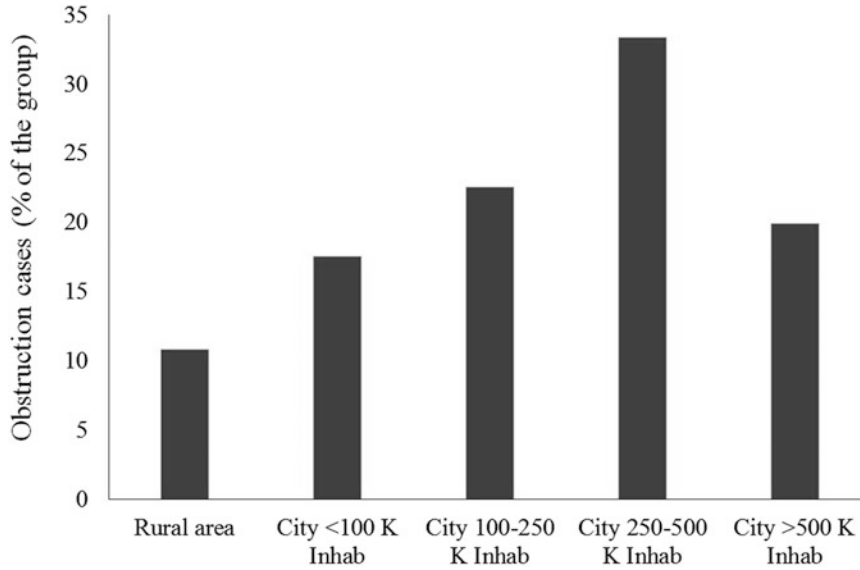

Fig. 1 **Percentage of people with diagnosed bronchial obstruction in different places of residence;** *K Inhab*, thousands of inhabitants

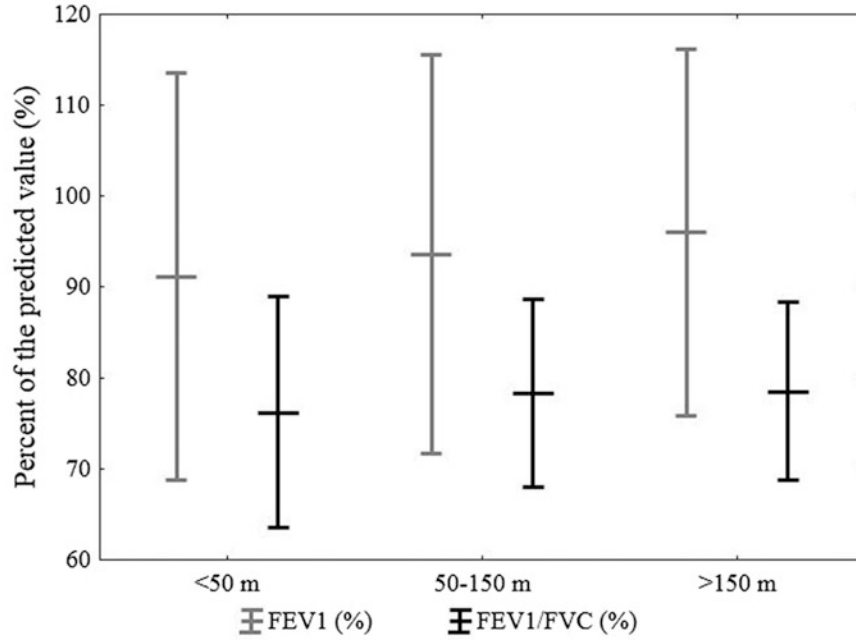

Fig. 2 Spirometric variables depending on the distance of place of residence from the nearest busy road; data are means ± SD

road. It may be a consequence of increased exposure to traffic-related air pollutants among the persons living closer to the roads.

The observations indicate that the percentages of predicted values of spirometric variables have lower values among persons, whose places of residence are located at a distance of 50 m or less from a road and the highest values among persons living further than 150 m from a road (Fig. 2).

There was a clear influence of the distance from a road of the place of residence on the frequency of bronchial obstruction in persons living less than 50 m from a road compared to those living further away. A limit of 50 m from a road seems to be quite significant, given that the differences in obstruction frequency were much smaller when this limit was increased to 100 m. In both cases, more disadvantageous is the situation among persons inhabiting cities with population of 100,000 and more, compared to the inhabitants of smaller urban areas. The highest percentage of persons showing features of bronchial obstruction was found in persons inhabiting larger cities (above 100,000 inhabitants) at distances of under 50 m from busy roads – obstruction was found in 37 of 131 persons in this group (28.2 %). On the other hand, the lowest percentages of persons with obstruction were found in the inhabitants of smaller urban areas living more than 50 m (14.6 %) or 100 m (15.9 %) from a busy road (Table 4).

Table 4 Percentages of persons with obstruction in non-smokers according to the size of the city and distance of the place of residence to the nearest road

Distance from road	Cities <100,000 inhabitants	Cities ≥100,000 inhabitants
<50 m	18.6	28.2
≥50 m	14.6	19.7
<100 m	16.8	25.9
≥100 m	15.9	20.4

4 Discussion

The present study indicates that a significant percentage of persons with symptoms of respiratory system disorders were persons who have never had a spirometric test performed before,

even though there were medical reasons for conducting a pulmonary function test. This percentage ranged from 15.3 % in case of the subjects declaring a long-term occurrence of wheezing to 33.1 % of persons showing chronic dyspnea.

A significant part of the subjects declared also suffering from COPD as based on the previous diagnosis. However, obstruction was substantiated only in 24.4 % of the persons declaring COPD. This means that over 75 % of persons did not realize what the chronic obstructive pulmonary disease is, does not remember their actual diagnosis, or have been misdiagnosed without conducting the necessary functional test. Such a significant percentage of discrepancy undoubtedly indicates that there are good reasons for implementation of wide-ranging informative and educational campaigns combined with diagnostic tests, such as the ones performed within the national and world Spirometry Days or Asthma and COPD Days.

Apart from tobacco smoking, the key risk factor related to obstructive disease morbidity, particularly to COPD morbidity, another significant factor is exposure to air pollutants which contribute to the development of chronic bronchitis (Hoek et al. 2002; Tager et al. 2005; Yang et al. 2005). These pollutants come from household and municipal sources. A major source emitting air pollutants in urban areas is road transport. Its contribution is particularly important in the large urban centers. Therefore, proximity of the place of residence to busy traffic routes is of vital significance.

In the subject-related literature, the factor of the distance from the place of residence to a busy road is sometimes compared in the assessment of risk of health related consequences (mostly asthma and COPD). Usually, the utilized limit value is 50, 100, or 150 m (Lindgren et al. 2009; Schikowski et al. 2005b; Venn et al. 2001). For example, in a study carried out in Germany in the years 1985–1994, the influence of exposure to air pollutants on the breathing efficiency and COPD morbidity was assessed in 4757 women (Schikowski et al. 2005a). It has been shown that living at a distance of less than 100 m from a busy road contributes to the decrease of breathing variables and increases the risk of COPD morbidity by 1.79 times (95 % CI: 1.06–3.02) compared to persons living at larger distances. The studies carried out in Sweden showed that living less than 100 m from a busy road, with the traffic intensity of over 10 vehicles/min, causes an increase in the number of diagnosed asthma cases (OR: 1.40, 95 % CI: 1.04–1.89) and COPD (OR: 1.64, 95 % CI: 1.11–2.40) (Lindgren et al. 2009). The influence of living in the neighborhood of a busy road on the occurrence of wheezing in children has also been studied in the UK. The number of the subjects was nearly 10,000 children in two age-groups (4–11 and 11–16 years). Among the subjects living at a distance of less than 150 m from a road, the risk of wheeze increased with increasing proximity to the road by the OR of 1.08 (95 % CI: 1.00–1.16) per 30 m increment in the younger group of children and 1.16 (95 % CI: 1.02–1.32) in the older children. A significantly higher risk of wheezing concerns children living at a distance of less than 90 m from a road (Venn et al. 2001). In the studies outlined above, a significant influence of residence within 50 m from a road on the incidence of bronchial obstruction has also been observed. When comparing the persons living within 100 m from a road and the ones living at a distance of 100 m and more, some differences in the frequency of obstruction have also been observed. Residents of cities with population over 100,000 are more disadvantaged (even over 28 % of the subjects with proven obstruction) compared to the persons living in smaller cities. A decrease in breathing efficiency, as assessed from FEV_1 and FEV_1/FVC, has been observed in the whole group (excluding the smokers) in parallel with decreasing distance of the place of residence from roads. The highest percentages of the variables are found in persons living more than 150 m from a road and the lowest in those living less than 50 m from a road.

The results of studies obtained during the Second Polish Spirometry Day indicate that almost 20 % of the subjects showed obstruction, which is similar to the results of the previous events of the type. In the current Spirometry Days, the

percentage of persons who showed the features of bronchial obstruction fluctuated from 12.4 to 19.7 %. On the other hand, among the persons who performed a functional test for the first time 10.5–17.8 % of them showed obstruction. Such a high percentage of persons with disorders that obstruct bronchial airflow demonstrates the necessity to make pulmonary function tests routinely available at the lowest possible level of medical care. However, a serious lingering problem, as also indicated by the results of the studies gathered within the Second Polish Spirometry Day, is the quality of the performed tests. The training efficacy of the personnel carrying out tests remains to be overviewed. Every year a significant percentage of test trials is unacceptable in terms of quality (a category lower than C).

The initiatives such as National or World Spirometry Days foster public awareness of respiratory system diseases, their causes, risk factors for developing the disease, the course of disease, and remote health effects, including reduced quality of life. This awareness is still very low, taking into account a high percentage of persons declaring suffering from a disease, while they objectively do not, but also a significant percentage of persons having the spirometry performed for the first time, who were proven to have bronchial obstruction not diagnosed before (its almost 18 % of this group). On the other hand, a rather large number of persons, who have had the spirometry performed for the first time during the Second Polish Spirometry Day, almost 65 % of all subjects, demonstrates the need for carrying out such tests. Nonetheless, the results of Spirometry Days are not representative for the whole population. It should be pointed out that there is a large group of people who are entirely unaware of their health problems, which indicates that there are good reasons or even the necessity of conducting pulmonary function tests. Thus, the assessment of pulmonary function should become part of basic medical screening.

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

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Development and Evaluation of the New Predictive Models in Tuberculous Pleuritis

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Abstract

Different pleural fluid biomarkers have been found useful in the discrimination between tuberculous pleural effusion (TPE) and non-TPE, with interferon gamma (IFN-γ) showing the highest single marker diagnostic accuracy. The aim of the present study was to develop predictive models based on clinical data and pleural fluid biomarkers, other than IFN-γ, which could be applied in differentiating TPE and non-TPE. Two hundred and forty two patients with newly diagnosed pleural effusion were prospectively enrolled. Upon completion of the diagnostic procedures, the underlying disease was identified in 203 patients (117 men and 86 women, median age 65 years; 44 patients with TPE and 159 with non-TPE) who formed the proper study group. Pleural fluid level of ADA, IFN-γ, IL-2, IL-2sRα, IL-12p40, IL-18, IL-23, IP-10, Fas-ligand, MDC, and TNF-α was measured and then ROC analysis and multivariate logistic regression were used to construct the predictive models. Two predictive models with very high diagnostic accuracy (AUC $>$ 0.95) were developed. The first model included body temperature, white blood cell count, pleural fluid ADA and IP-10. The second model was based on age, sex, body temperature, white blood cell count, pleural fluid lymphocyte percentage, and IP-10 level. We conclude that two new predictive models based on clinical and laboratory data demonstrate very high diagnostic performance and can be potentially used in clinical practice to differentiate between TPE and non-TPE.

Keywords

Adenosine deaminase • Biological markers • Interferon gamma • Pleural fluid • Pleural tuberculosis • Tuberculous pleurisy • Tuberculous pleural effusion

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1 Introduction

Pleural effusion is a common medical condition affecting both children and adults. There is a number of underlying diseases that may result in pleural fluid accumulation. Thus, the causative diagnosis of pleural effusion might be challenging. In some patients the etiology of pleural effusion can be suspected on the basis of typical signs and symptoms, the results of chest radiography, and laboratory tests. In other patients, the diagnosis of underlying disease requires an extensive diagnostic work-up, which includes thoracic imaging, thoracentesis, pleural fluid and blood analysis, microbiological and cytological studies, and pleural biopsy. Differentiation between pleural transudates and exudates is a primary point in the diagnostic approach to patients with pleural effusion (Light 2002, 2011).

Pulmonary and pleural infections are one of the major causes of exudative pleural effusions. These mainly include bacterial infections that usually present as a parapneumonic effusion or pleural empyema and infections caused by *Mycobacterium tuberculosis* that are known as tuberculous pleurisy or tuberculous pleural effusion (TPE). Since both conditions may be successfully treated, proper diagnosis enables adequate treatment and minimizes the risk of disease-related complications. While the diagnosis of parapneumonic effusion and empyema is relatively simple (e.g., acute febrile disease, or pus withdrawn during thoracentesis), the clinical course of tuberculous pleurisy may be obscure. Hence, the correct diagnosis can be difficult or delayed. This is because TPE usually contains only few mycobacteria and, in consequence, the diagnostic sensitivity of conventional microbiological methods (direct microscopy and pleural fluid cultures) is relatively low. Better results were reported with nucleic acid amplification tests (NAATs), but these test are costly and technically demanding (Safianowska et al. 2012; Pai et al. 2004) To solve this problem a variety of biological markers measured in pleural fluid have been proposed to help in the diagnosis of TPE (Trajman et al. 2008). Previously, we have shown that pleural fluid adenosine deaminase (ADA) and interferon gamma (IFN-γ) are sensitive and specific markers that can be successfully used to differentiate between tuberculous *vs*. non-tuberculous pleural effusion (Krenke and Korczyński 2010; Krenke et al. 2008).

The goal of the present study was to develop predictive models for patients with pleural effusion, which would enhance accurate diagnosis of TPE. Two assumptions were made: (1) the model should be based on clinical and laboratory data, including pleural fluid biomarkers with which our institution has adequate experience; and (2) pleural fluid IFN-γ should not be included, as IFN-γ itself has been shown to have very high diagnostic accuracy, and if so, we could not expect that the combination of pleural fluid IFN-γ and other clinical or laboratory variables would significantly increase the very high diagnostic performance of IFN-γ *per se*.

2 Methods

The protocol of the study was approved by the Institutional Review Board of the Medical University of Warsaw, Poland. This was a prospective study which enrolled 242 adult patients (aged 18–85) with newly diagnosed pleural effusion who were admitted to the Department of Internal Medicine, Pneumology and Allergology, Medical University of Warsaw between 2007 and 2012. In all patients, appropriate diagnostic approach was applied, aimed at making the causative diagnosis of pleural effusion. Pleural fluid samples were collected and stored for subsequent assessment of selected biomarkers associated with *M. tuberculosis* infection. Then, the levels of various biomarkers were measured and assigned to different underlying diseases. Finally, statistical analysis was performed with predictive models development and testing.

The current study is complimentary to a previously published paper on the diagnostic performance of an array of pleural fluid biomarkers in distinguishing between TPE and non-TPE (Klimiuk et al. 2015). Both studies were

conducted in the same population of patients with pleural effusion. However, the development of predictive models based on clinical data and pleural fluid biomarkers, which could be useful in differentiating TPE from non-TPE was considered an independent research. Thus, it was herein described as a separate entity.

2.1 Diagnostic Work-up

The patients were diagnosed according to the general algorithm presented in Table 1. Briefly, all patients underwent thoracentesis and routine pleural fluid analysis, including the assessment of its biochemical properties and total and differential cell count. Light's criteria were used to differentiate between transudates and exudates (Light 1997). In the majority of patients, cytological and microbiological examinations of pleural fluid were also performed. The methods for detecting acid-fast bacilli in pleural fluid included direct microscopy of Ziehl-Neelsen stained slides and Löwenstein-Jensen culture. In some pleural fluid samples, nucleic acid amplification tests: Amplicor MTB PCR assay (Roche Diagnostics, Branchburg, NJ) or GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA) were performed to identify specific *M. tuberculosis complex* DNA sequences (Safianowska et al. 2012). Closed pleural biopsy and/or medical thoracoscopy were carried out in patients with exudative pleural effusion in whom the cause of pleural fluid could not have been diagnosed based on pleural fluid analysis, blood laboratory tests, and basic imaging studies. Additional diagnostic procedures were undertaken, whenever necessary, to further evaluate pleural fluid etiology.

Ultimately, the cause of pleural effusion was diagnosed in 203 of the 242 patients initially enrolled. These patients constituted the proper study group. This group included 117 men and 86 women; median age 65 (IQR 55–77 years). The patients were subclassified according to the cause of pleural effusion: tuberculous pleurisy (n = 44), malignant pleural effusion (MPE)

Table 1 Diagnostic criteria for the causative diagnosis of pleural effusion (Light 1997, 2010; Udwadia and Zen 2010; Porcel 2009; Krenke et al. 2008; Sahn 1997)

Etiology/type of pleural effusion	Definition
Tuberculous pleural effusion	Positive culture for *M. tuberculosis* in pleural fluid, pleural biopsy, fibrinous adhesions collected during thoracoscopy or in respiratory samples (sputum, bronchial washing, bronchoalveolar lavage fluid), and/or
	Positive smear of pleural fluid or sputum and positive result of NAAT for *M. tuberculosis complex*, and/or
	Caseating granulomas in pleural biopsy samples in patients with typical clinical and radiological symptoms and signs
Malignant pleural effusion	Positive pleural fluid cytology, and/or
	Positive histology of pleural biopsy, and/or
	Known malignant disease, after the exclusion of alternative causes of pleural effusion
Parapneumonic effusion and empyema	Typical signs and symptoms (acute illness with fever and chest pain) with characteristic features of pleural fluid (elevated white blood cell count with neutrophil predominance, low pH, low glucose, high lactate dehydrogenase) and regression after antimicrobial treatment and/or pleural drainage treatment, and/or
	Pus or positive pleural fluid culture
Miscellaneous effusions	Positive Light's criteria for exudate, and
	Exclusion of malignant, tuberculous or bacterial etiology, and
	Diagnosis of other disease that can be associated with pleural effusion
Transudate	Positive Light's criteria for transudate, and
	Clinical features consistent with or known heart failure, hepatic cirrhosis or nephrotic syndrome

(n = 88), parapneumonic effusion (PPE) and empyema (n = 35), pleural transudates (n = 30), and miscellaneous pleural effusions (n = 6).

2.2 Definitions

The diagnostic criteria for specific causes of pleural effusion applied in our study are presented in Table 1.

2.3 Pleural Fluid Biomarkers Associated with *M. Tuberculosis* Infection

Pleural fluid samples for subsequent assessment of biomarkers associated with *M. tuberculosis* infection (mean pleural fluid volume 100 ml) were collected during the first or second diagnostic thoracentesis. The samples were centrifuged at 2000 rpm for 10 min and the supernatant was frozen at −70 °C. The following biomarkers were measured: adenosine deaminase (ADA) activity, concentrations of interferon gamma-induced protein 10 kDa (IP-10), soluble Fas ligand (FAS ligand), tumor necrosis factor alfa (TNF-α), sub-unit p40 of interleukin 12 (IL-12p40), human macrophage-derived chemokine (MDC), interleukin 23 (IL-23), soluble IL-2 receptor (IL-2sRα), and interleukin 18 (IL-18). ADA activity was determined with colorimetric method by Giusti (1974), while the concentrations of the remaining cytokines were measured with respective ELISA kits (R & D System, Minneapolis, MN) according to the manufacturer's recommendations.

2.4 Statistical Analysis and Algorithm Development and Testing

Data were presented as median and interquartile range (IQR) (continuous variables) or percentages (categorical variables). Shapiro-Wilk test and Q-Q plot test were used to assess data distribution. A *t*-test for unpaired samples and analysis of variance were used to test the differences between various groups. Whenever the variables did not show a normal distribution, nonparametric tests (Mann–Whitney U test or Kruskal-Wallis test) were applied. Depending on the sample size, the Chi-squared and Fisher-exact tests were used to assess the proportions of patients with different characteristics.

To search for the classification rules consistent with the diagnostic codes, ROC analysis based on the multivariate logistic regression model was performed. The AIC test was used to choose the appropriate multivariate logistic model. Two additional variables were introduced to the models (age and sex) as variables that may disrupt relationships (confounding factors). Goodness-of-fit of the logistic regression models was tested using changes in the information measure AIC and the coefficient of determination R^2 (Cox and Snell 1989). To present the array of attributes measured in patients with different identification codes, the multidimensional scaling technique (MDS) was used to provide a visual representation of attributes in a two-dimensional plane. In this plane, the scatter-plot of all patients with a diagnostic code was drawn. The multidimensional scaling is a method of reducing the dimensionality in such a way that the distances between observations in the reduced space are as close as possible to the original.

The efficacy of the developed models was tested in two clinical scenarios. The first scenario assumed the calculation of the probability of TPE in the entire group of patients with pleural effusion (differentiation between TPE and non-TPE). In the second clinical scenario (scenario 2) only patients with exudative pleural effusion were analyzed, i.e., the probability of TPE in patients with pleural exudates was evaluated. The diagnostic accuracy of the predictive models was assessed with ROC analysis that included calculation of the sensitivity, specificity, positive predicted value (PPV), and negative predicted value (NPV). A good diagnostic efficacy was

defined as the area under ROC curve (AUC) >0.90. The statistical hypotheses were verified by two-tailed tests, assuming the significance level of $p < 0.05$. Statistical analysis was performed using a commercial SAS 9.3 software package.

3 Results

The characteristics of patients with pleural effusions caused by different underlying diseases is presented in Table 2. Pleural fluid characteristics and the results of selected blood tests in patients with different causes of pleural effusion are demonstrated in Table 3.

The use of multivariate analysis resulted in the development of two predictive models for tuberculous pleurisy. The first model included four variables: body temperature (fever), peripheral blood WBC count, and the two pleural fluid biomarkers ADA and IP-10. Figure 1 presents ROC curves expressing the probability of TPE when applying the following equations for the two clinical scenarios outlined in the Methods section:

$$\text{Scenario 1}: Y = 0.1466 \cdot \text{ADA} + 0.0004 \cdot \text{IP10} + (-2.1643) \cdot \text{WBC} + 2.1648 \cdot (\text{fever}) + (-77.9391)$$

$$\text{Scenario 2}: Y = 0.1438 \cdot \text{ADA} + 0.0004 \cdot \text{IP10} + (-2.1278) \cdot \text{WBC} + 2.1076 \cdot (\text{fever}) + (-75.8152)$$

In both scenarios, the sensitivity of the 4-component predictive model was 97.6 %, specificity ranged between 98.3 and 98.6 %, and PPV was 95.3 % (Fig. 1). NPV was found to be equal or higher than 99 %, while AUC was 0.997 in both scenarios.

The cut-off value of Y for the diagnosis of TPE was −0.360 in Scenario 1 and −0.350 in Scenario 2. The probability values for cut-off value were 0.570 and 0.577 in Scenario 1 and Scenario 2, respectively.

The second proposed predictive model was based on six variables: three clinical parameters (age, sex, and body temperature), two laboratory parameters (blood WBC count and pleural fluid lymphocyte percentage), and one pleural fluid biomarker (IP-10). Figure 2 shows ROC curves demonstrating the probability of TPE after the applying of the following equations for the two clinical scenarios:

Table 2 Clinical characteristics of patients with pleural effusion caused by different underlying diseases

	Tuberculous pleural effusion	Malignant effusion	Parapneumonic effusion/empyema	Transudative effusion	Miscellaneous pleural effusions	P value
Number of patients	44	88	35	30	6	NA
Age (years)*	51.5 (35.5–71.5)	69 (60–76.5)	60 (50–71)	78 (58.5–83)	60 (53–66)	<0.0001
Sex F/M	11/33	51/37	11/24	11/19	2/4	0.002
Pleural fluid site: left/right/bilateral	23/21/0	42/46/0	14/20/1	3/25/2	3/3/0	0.001
Previous history of TB (% of entire group)	2.5 %	2.5 %	2.5 %	2.0 %	1.0 %	0.202
Chest pain	15 (34.1 %)	42 (47.7 %)	25 (71.4 %)	4 (13.3 %)	3 (50.0 %)	<0.0001
Dyspnea	19 (43.2 %)	74 (84.1 %)	19 (54.3 %)	26 (86.7 %)	4 (66.7 %)	<0.0001
Fever ≥ 38 °C	28 (63.6 %)	24 (27.3 %)	25 (71.4 %)	1 (3.33 %)	2 (33.3 %)	<0.0001
Cough	32 (72.7 %)	58 (65.9 %)	21 (60.0 %)	17 (56.7 %)	3 (50.0 %)	0.530
Weakness	31 (70.5 %)	69 (78.4 %)	28 (80.0 %)	23 (76.7 %)	3 (50.0 %)	0.457
Weight loss	19 (43.2 %)	41 (46.6 %)	10 (28.6 %)	11 (36.7 %)	0 (0.0 %)	0.957

*Data are expressed as a median (IQR); *LDH* lactate dehydrogenase, *TB* tuberculosis, *NA* not applicable

Table 3 Pleural fluid characteristics and the results of selected blood tests in relation to pleural fluid etiology

	Tuberculous pleural effusion (n = 44)	Malignant pleural effusion (n = 88)	Parapneumonic effusion/ empyema (n = 35)	Transudative effusion (n = 30)	Miscellaneous pleural effusions (n = 6)	P value
pH	7.4 (7.3–7.4)	7.4 (7.3–7.4)	7.2 (6.9–7.4)	7.5 (7.4–7.5)	7.4 (7.4–7.5)	<0.0001
Pleural fluid protein (g/dL)	5.0 (4.6–5.6)	4.3 (4.4–7.0)	4.2 (3.7–5.1)	1.8 (1.3–2.3)	4.5 (4.5–5.6)	<0.0001
Pleural fluid protein/serum protein ratio	0.7 (0.7–0.8)	0.6 (0.6–0.7)	0.7 (0.6–0.7)	0.3 (0.2–0.3)	0.7 (0.6–0.7)	<0.0001
Pleural fluid LDH (U/L)	1078 (604–2070)	830 (429–1511)	3722 (920–14270)	144 (117–238)	1039 (667–1666)	<0.0001
Pleural fluid LDH/serum LDH ratio	2.5 (1.5–3.8)	1.4 (0.8–2.6)	8.1 (1.9–34.8)	0.3 (0.2–0.4)	1.7 (1.2–2.5)	<0.0001
Pleural fluid glucose (g/dL)	77 (60–93)	101 (76–112)	61 (10–95)	118 (110–131)	97 (94–102)	<0.0001
Pleural fluid total cell count (cell/mm^3)	1700 (1200–3300)	1300 (590–2450)	5905 (2190–10140)	315 (205–423)	2650 (1375–4263)	<0.0001
Lymphocytes (%)	93 (81–96)	70 (51–82)	20 (4–48)	70 (50–24)	53 (21.3–76.3)	<0.0001
Macrophages (%)	4.0 (2.0–7.0)	8.5 (5.0–18.0)	3.3 (2.0–13.0)	13.0 (6.4–24.4)	4.8 (2.8–7.5)	<0.0001
Neutrophils (%)	2.5 (1.0–8.5)	7.0 (2.0–20.5)	74.0 (21.0–94.0)	8.5 (4.6–15.0)	10.5 (7.6–13.6)	<0.0001
Eosinophils (%)	0.0	0.0 (0.0–2.0)	0.0 (0.0–1.0)	0.0	11.8 (4.5–39.6)	<0.0001
Mesothelial cells (%)	0.0	1.0 (0.0–3.0)	0.5 (0.0–3.0)	3.5 (1.5–5.4)	1.0 (0.6–2.5)	<0.0001
CRP (mg/dL)	74.6 (33.3–165.5)	44.4 (16.9–76.3)	174.5 (63.8–279.1)	11.7 (6.7–19.1)	41.3 (35.4–66.4)	<0.0001
WBC (10^9/L)	6.8 (5.3–8.7)	9.6 (7.1–11.8)	14.3 (11.5–19.4)	7.3 (5.4–8.2)	7.3 (6.7–8.3)	<0.0001
Hb (g/dL)	12.0 (11.0–13.4)	12.7 (11.5–14.2)	12.2 (11.0–13.3)	13.1 (12.0–13.6)	12.9 (12.7–13.6)	0.2246

Data were expressed as median (IQR); *LDH* lactate dehydrogenase, *TB* tuberculosis, *CRP* C-reactive protein, *WBC* white blood cells

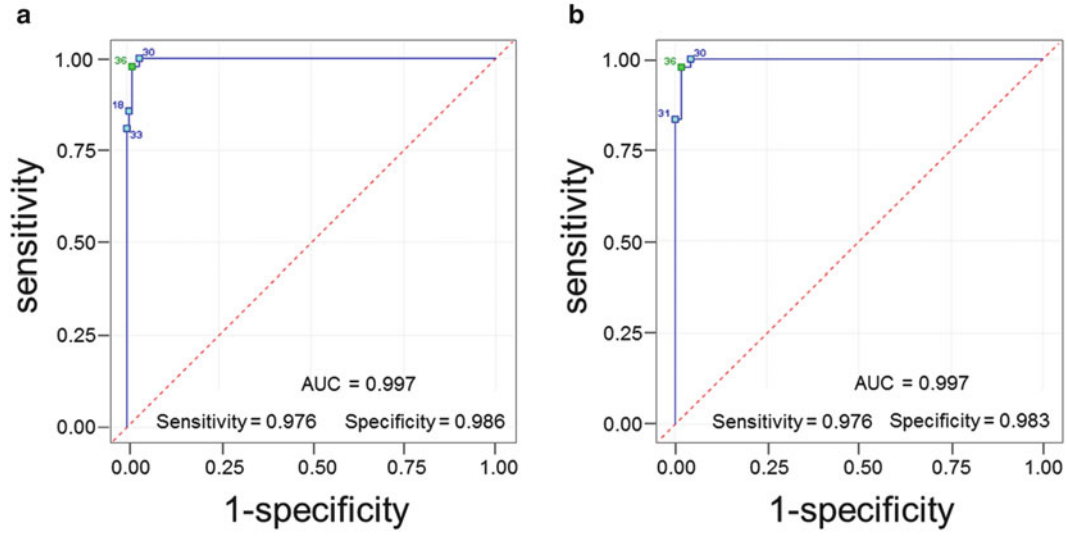

Fig. 1 Diagnostic accuracy of predictive Model 1 that included data on fever, blood WBC, pleural fluid ADA, and pleural fluid IP-10. (**a**) ROC curve constructed in clinical Scenario 1 and (**b**) ROC curve constructed in clinical Scenario 2 (see results for details)

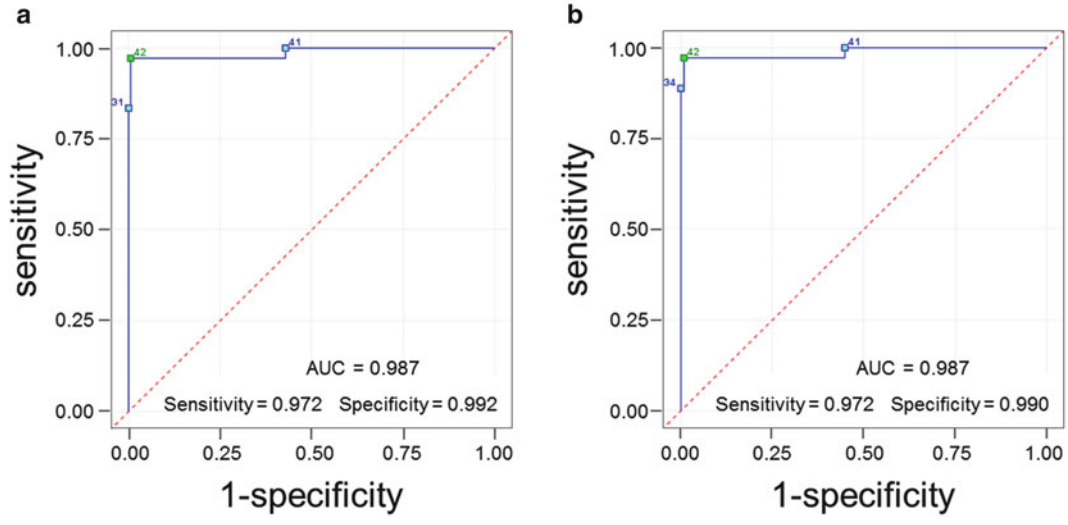

Fig. 2 Diagnostic accuracy of predictive Model 2 that included data on age, sex, fever, blood WBC, pleural fluid lymphocyte percentage, and pleural fluid IP-10. (**a**) ROC curve constructed in clinical Scenario 1 and (**b**) ROC curve constructed in clinical Scenario 2 (see results for details)

$$\text{Scenario 1}: \quad Y = 0.0003 \cdot \text{IP10} + (-0.4124) \cdot \text{WBC} + 1.2706 \cdot \text{fever} + 0.0972 \cdot \text{pleural fluid lymphocyte percentage} + (-0.0159) \cdot \text{age} + 3.0679 \cdot \text{sex} + (-57.2065)$$

$$\text{Scenario 2}: \quad Y = 0.0030 \cdot \text{IP10} + (-0.4463) \cdot \text{WBC} + 0.9985 \cdot \text{fever} + 0.0825 \cdot \text{pleural fluid lymphocyte percentage} + (-0.0226) \cdot \text{age} + 3.068 \cdot \text{sex} + (-44.7082);$$

where sex is 0 for female and 1 for male.

In both clinical scenarios the sensitivity of the 6-component predictive model was 97.2 %, specificity ranged between 99.0 and 99.2 %, PPV was 97.2 %, NPV ranged between 99.0 and 99.2 %, and AUC was 0.987 (Fig. 2).

The cut-off value of Y for the diagnosis of TPE was −0.4398 in Scenario 1 and −0.398 in Scenario 2. The probability values for cut-off value were 0.458 and 0.516 for Scenario 1 and Scenario 2, respectively.

4 Discussion

The present study demonstrated that the use of predictive models based on clinical data, the results of blood laboratory tests, and pleural fluid biomarkers can result in a highly accurate diagnosis of tuberculous pleural effusion. Two predictive models were developed. The first one uses data on body temperature, pleural fluid ADA, pleural fluid IP-10, and the WBC count in the peripheral blood. The second model was based on three clinical variables (age, sex, and body temperature), two laboratory parameters (blood WBC count and pleural fluid lymphocyte percentage), and one pleural fluid biomarker (IP-10). The development of these predictive models is a major advantage of our study. It is noteworthy that our prediction models were based on numerous clinical and biochemical parameters (including ten different pleural fluid biomarkers) which were tested in a relatively large group of patients with diverse pleural fluid etiologies. Both models were tested in different clinical scenarios which included differentiation between tuberculous and non-tuberculous pleural effusions and discrimination between tuberculous and non-tuberculous etiology of pleural exudates. In these settings both models showed very high sensitivity, specificity, PPV, and NPV. Thus, we believe the results of this study are reliable and may impact clinical practice. In this context, one assumption we had made before we constructed the predictive models should be stressed. Our previous studies have shown very high diagnostic accuracy of pleural fluid IFN-γ in diagnosing TPE. The sensitivity and specificity of pleural fluid IFN-γ ranged between 97–100 % and 98–99 % in those studies (Krenke et al. 2008; Klimiuk et al. 2015). We decided not to use this biomarker in our predictive models of TPE in the present study because we could easily predict that the combination of pleural fluid IFN-γ and other pleural fluid biomarkers would not significantly increase the very high diagnostic performance of IFN-γ itself. On the other hand, our intention was to evaluate whether any predictive model, which was not based on pleural fluid IFN-γ, could have a similarly high diagnostic performance.

Our multivariate analysis showed that pleural fluid biomarkers ADA and IP-10 could be the most valuable components of predictive models. While ADA is a well established biomarker of TPE, IP-10 is a relatively new biomarker, which had been tested in considerably fewer studies. IP-10, an interferon gamma induced 10 kDa protein, also known as CXCL-10, is a small particle chemokine that is produced and secreted by different cells (e.g., monocytes or neutrophils) in response to interferon or lipopolysaccharide stimulation (Guo et al. 2014; Liu et al. 2011). Our data on the diagnostic performance of IP-10, as compared with several other biomarkers of TPE, have been recently reported (Klimiuk et al. 2015). Although some individual pleural fluid biomarkers demonstrate high diagnostic accuracy in the differentiation between TPE and non-TPE, there is still a room for predictive models that use not only pleural fluid biomarkers but also relatively simple and easily available clinical parameters. This approach is well-perceived by the clinicians and allow to maintain a high accuracy of TPE diagnosis at a reasonable reduction of costs.

Several predictive models have been earlier proposed by other authors. A relatively simple solution aimed at the improvement of the diagnostic specificity of pleural fluid ADA in differentiation between TPE and non-TPE has been presented by Burgess et al. (1996). The authors found that a combination of pleural fluid ADA level with pleural fluid lymphocyte/neutrophil

ratio equal or greater than 0.75 resulted in a significant increase in the diagnostic specificity of ADA alone (95 *vs*. 81 %) and PPV (95 *vs*. 84 %). This approach did not adversely affect the sensitivity (91 *vs*. 88 %) and NPV (89 *vs*. 88 %). Since then, a high proportion of pleural fluid lymphocytes to neutrophils has been accepted as a parameter increasing the diagnostic accuracy of pleural fluid ADA in patients with TPE (Porcel 2009).

Porcel and Vives (2003) have proposed a scoring system and two diagnostic models for patients with tuberculous pleurisy. The scoring system included seven parameters; pleural fluid ADA $\geq$ 40 U/L, age $<$ 35 years, body temperature $\geq$37.8 °C, pleural fluid red blood cell count (RBC) $< 5 \times 10^9$ L, no history of malignancy, pleural fluid protein $\geq$50 g/L, and pleural fluid to serum lactate dehydrogenase ratio $\geq$2.2. Each parameter above or below the cut-off threshold scored 1–5 points. A ten-point scale was built for both prediction models and the probability of TPE was estimated according to the number of points. Four parameters were used in the first model (ADA, age, body temperature, and pleural fluid RBC) A score of $\geq$5 points allowed discriminating TPE from MPE with 95 % sensitivity and 94 % specificity. Six parameters were incorporated to the second model (all parameters of the scoring system except pleural fluid ADA). Six points or more were associated with 97 % sensitivity and 91 % specificity in differentiating TPE from MPE. That study clearly showed that a combination of clinical parameters and pleural fluid chemistry in a score-based model can help distinguish TPE and MPE. As only patients with MPE and TPE were included in that study, the applicability of these models is limited to the differentiation between tuberculous and malignant pleural effusion. It should be underlined that even though the materials and methods used in the above study were different from those used in the present study, both studies demonstrated a high diagnostic yield of pleural fluid ADA, patient's age, and body temperature in distinguishing TPE from non-TPE. The discriminative value of those parameters has also been reported by other authors (Villegas 2000).

A study aimed at the development of predictive models discriminating TPE and MPE was also undertaken by Sales et al. (2009). Different biochemical and cytological parameters of pleural fluid were measured and tested with the use of ROC analysis. Variables with an area under the curve of $\geq$0.5 were included in the logistic regression. Ultimately, the authors proposed two models for the diagnosis of tuberculous pleurisy and two other for MPE. Both models for tuberculosis had similar performance (accuracy of 97.7 % and 96.6 %). The first model which included pleural fluid ADA and globulin levels as well as negative pleural fluid cytology showed a sensitivity and specificity of 99.4 % and 96.0 %, respectively. In the second model, negative pleural fluid cytology was replaced with nonhaemorrhagic fluid appearance, without significant decrease in sensitivity and specificity (95.8 % and 97.4 %, respectively). Interestingly, to promote the clinical application of these models, the authors have introduced a numerical score similar to that described by Porcel and Vives (2003). Negative pleural fluid cytology and high pleural fluid ADA level (>46.5 U/L) scored highest.

A Brasilian study by Neves et al. (2007) shares similar characteristics to present study. That study included 215 patients with five different categories of pleural effusion. After identification of the discriminative power of individual parameters, eight parameters were included in a multivariate analysis. The best predictive model of TB pleural effusion included five variables: ADA, protein, WBC, and lymphocyte percentage in pleural fluid as well as duration of disease. The model resulted in a high sensitivity and specificity (both exceeding 95 %) and area under ROC curve 0.991. The authors have also shown that a regression equation which includes only three variables (pleural fluid ADA, protein, and WBC count) may have a similar performance in clinical practice. In the present study the two predictive models that included four and six variables showed similar and very high diagnostic accuracy, with an area under ROC curve discriminating between TPE and non-TPE of 0.997 and 0.987, respectively. In earlier models, ADA has

invariably been one of the most powerful variables used to predict the probability of tuberculous pleurisy. As our study tested more pleural fluid biomarkers than were tested in the majority of other studies, the new markers could have been incorporated into the predictive models. To our knowledge, our regression equations are the first models in which IP-10 was successfully used to discriminate TPE and non-TPE.

Seven different pleural fluid biomarkers were evaluated by Daniil et al. (2007) in terms of their discriminative properties between MPE, PPE, and TPE. However, the study group included only 12 patients with TPE. Moreover, only two of the assessed markers were typical markers of tuberculous pleurisy (ADA and IFN-γ), while the other markers were selected to diagnose PPE and MPE. Only ADA and C-reactive protein were of significant importance after fitting the logit model. Patients with pleural fluid ADA level >45 U/L and CRP < 4 mg/dL were more likely to have TPE than MPE or PPE. The overall proportion of misclassified individuals was 11 %, with one individual with TPE wrongly classified as having PPE, and one individual with PPE improperly classified as TPE. Although in the context of diagnosis of tuberculous pleurisy, the results of the study seem not particularly useful, the authors conclude that the combination of pleural fluid ADA and CRP might be sufficient for discriminating between TPE, PPE, and MPE.

We believe that the present study adds new data on the predictive models used in the differentiation between tuberculous and non-tuberculous pleural effusion, despite some potential limitations. There was a significant difference in the number of patients with various pleural fluid etiologies, e.g., 88 patients with MPE but only 6 patients classified as miscellaneous causes of pleural effusion. This could be a confounding factor influencing our results. The models developed were not validated in an independent group of patients with undiagnosed pleural effusion. To confirm the diagnostic performance of the models, an internal and external validation of our findings should be performed. Finally, it should be underlined that the NPV and PPV of the predictive models depend on the prevalence of TPE in a population, and both parameters can differ in populations with different prevalence of TB. Therefore, positive and negative likelihood ratio might better characterize the clinical reliability of a model when the prevalence of TB is unknown.

We conclude that tuberculous pleural effusion can be reliably discriminated from non-tuberculous pleural effusion with the use of two newly developed predictive models. Although a high diagnostic performance was found for both these models, it requires external validation.

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

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The Influence of Asthma Exacerbations on Health-Related Quality of Life

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Abstract

The purpose of this study was to determine the influence of asthma on the quality of life (QoL) of patients hospitalized for an exacerbation of the disease and those with controlled asthma receiving outpatient treatment, and to establish the patients' somatic status and the level of health care utilization. This study involved 239 adults with asthma (123 hospitalized patients and 116 outpatients of family physicians). The authors used: WHOQOL-BREF questionnaire and a questionnaire measuring health care utilization. There were no differences in QoL levels between the patients with severe and controlled asthma. The psychological domain was assessed higher by hospitalized patients ($p = 0.02$). QoL levels correlated negatively with age, place of residence, and marital status, and positively with education. The general QoL level was most strongly influenced by gender, age, education, the number of home visits and interventions of a district nurse, and the somatic index ($p < 0.05$). Somatic symptoms were more severe in hospitalized patients. The QoL assessment of asthma patients in relation to somatic symptoms, health care services and socio-demographic variables allows better understand-

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ing of the complex health situation of patients at various stages of the disease, and tailoring the therapy to individual needs. Patients receiving outpatient treatment require professional psychotherapeutic support.

Keywords
Asthma • Health care services • Health education • Somatic symptoms • WHOQOL-BREF

1 Introduction

The increasing incidence and morbidity rates of asthma make it a serious public health problem. According to the Global Initiative for Asthma (GINA 2013) around 300 million people in the world, including 30 million in Europe, suffer from asthma. It is estimated that in Poland asthma affects approximately 4.5–6 % of the population (Kokot et al. 2004), but in some countries it is as many as 20 %. Asthma accounts for about 1 % of all potentially lost years of life, which reflects the severity of this disease. The number of disability-adjusted life years (DALYs) lost due to asthma is comparable with that for diabetes, liver cirrhosis, and schizophrenia.

Asthma is believed to affect mainly young people of school and working age (Kokot et al. 2004). Furthermore, some researchers claim that the knowledge of asthma in the post-working age population is insufficient. De Marco et al. (2013) asserts that diagnosing asthma in advanced age people can be difficult as asthma can coexist with chronic obstructive pulmonary disease (COPD). It has been found that the clinical course of asthma in advanced age is often similar to that in younger patients. However, co-existing diseases and psychosocial consequences of the aging process can considerably influence the diagnosis, clinical features, and therapy for asthma in this population (Hanania et al. 2011). Most patients are diagnosed as having mild or moderate asthma with periods of severe symptoms, but in family physician practice there are patients who – probably unaware of the disease – accept mild asthma symptoms (Ehrs et al. 2001). The efficiency of asthma treatment is usually evaluated within ambulatory care through the measurement of pulmonary function and clinical symptoms. Nonetheless, the correlation between changes in forced expiratory volume in one second (FEV1) and peak expiratory pressure (PEF) and the changes in Qol level is weak, which means that there is not always a direct relationship between QoL level and the clinical condition of a patient (GINA 2013; Dean et al. 2009; Bacon et al. 2009; Ehrs et al. 2001).

QoL is a subjective concept based on human perception of impact that various events and life experiences have on key spheres of life (ATS 2004). Particular life domains are of different significance for different individuals, depending on their social and cultural origin. Even so, five domains: physical, psychological, social, economic, and spiritual are commonly regarded as crucial for QoL levels irrespective of life situation, health status, and other non-medical aspects of life (Juniper et al. 1993). The QoL concept includes not only factors related to health or emotional, physical and intellectual well-being, but also non-medical issues, such as: work, family, friends, and other life circumstances (ATS 2004).

Considering its chronic character and bouts of severe symptoms and remissions, which may feel life-threatening, asthma has profound effects on the QoL level, and satisfaction with QoL and health status. Studies show that people with asthma report negative effects of this disease within the physical, psychological and social QoL domains and complain of worse social functioning (Dean et al. 2009; Bacon et al. 2009), which may potentially worsen their somatic status and lead to higher health care utilization. The purpose of the present study was to determine the influence of asthma on quality of life in respect of sociodemographic variables, somatic status, and the level of health care utilization.

2 Methods

The study was approved by the Bioethical Commission of Wroclaw Medical University (no. KB 608/2011). The director of the hospital in Chodziez, Poland, gave written consent for the authors to conduct the study among hospitalized patients.

The study was carried out between the years 2011 and 2013 in a group of 239 adults with asthma diagnosed by a family doctor, and confirmed by spirometry tests (ATS 2004). The study involved patients hospitalized in the city of Chodziez during severe symptoms of asthma – Group 1 (123 patients), and patients of cared for by 78 family doctors – Group 2 (116 patients). There were 128 (53.6 %) women and 111 (48.5 %) men. Sociodemographic data of the patients are shown in Table 1. The criteria for inclusion in the study were: patients aged 18 years or more, and asthma diagnosed at least 36 months prior to the study. The exclusion criteria were: pregnancy or mental disease (including alcoholism).

The QoL was evaluated with the WHOQOL-BREF questionnaire adapted by Jaracz

Table 1 Sociodemographic data of the patients with asthma

	Hospitalized		Family practice		
	n	%	n	%	Pearson's χ^2 test
Gender					$\chi^2 = 0.127$
Women	64	52.0	64	55.2	p = 0.721
Men	59	48.0	52	44.8	
Age					$\chi^2 = 22.658$
24 and below	7	5.7	6	5.2	p = 0.0002
25–44	9	7.3	13	11.2	
45–64	73	59.4	37	31.9	
65–84	33	26.8	51	44.0	
85 and above	1	0.8	9	7.8	
Place of residence[b]					$\chi^2 = 12.573$
Village	44	35.8	47	40.9	p = 0.127
Below 5,000[a]	18	14.6	9	7.8	
5,000–10,000[a]	6	4.9	7	6.1	
10,000–50,000[a]	31	25.2	27	23.5	
50,000–100,000[a]	13	10.6	8	7.0	
100,000–200,000[a]	10	8.1	7	6.1	
Over 200,000[a]	1	0.8	10	8.7	
Education[c]					$\chi^2 = 10.330$
Incomplete primary	1	0.8	1	0.9	p = 0.243
Primary	10	8.1	23	20.2	
Vocational	51	41.5	32	28.1	
Secondary	31	25.2	31	27.2	
Post-secondary	15	12.2	15	13.2	
Higher	15	12.2	12	10.5	
Marital status[d]					$\chi^2 = 8.409$
Single	13	10.6	18	15.7	p = 0.078
Married	83	67.5	68	59.1	
Separated	3	2.4	1	0.9	
Divorced	6	4.9	1	0.9	
Widowed	18	14.6	27	23.5	

[a]City/town population. In the column 'Family practice' there are missing data, not provided by respondents:
[b]one case
[c]two case
[d]one case

et al. (2006), which measures the QoL of chronically ill patients across different conditions and cultures. WHOQOL-BREF is a well-validated questionnaire, widely used for the clinical health and QoL measurement in chronically ill patients. It includes 26 questions, and analyzes four QoL domains: D1-Physical, D2-Psychological, D3-Social relationships, and D4-Environmental. All answers were rated on a five-point Likert-type scale. The score in each domain, after calculating according to the algorithm for WHOQOL-BREF, ranged from 4 to 20 points: the higher the score, the better the QoL in the analyzed domain. The general QoL score is the sum of the scores obtained in all domains. The questionnaire also contains two separately analyzed questions about satisfaction with quality of life (Question 1) and satisfaction with health (Question 2). The score range for each of these two questions is 1–5; an average score below 3.0 reflects dissatisfaction with QoL or health status. The reliability of the Polish version of the WHOQOL-BREF questionnaire measured with the Cronbach alpha coefficient is high (results from 0.81 to 0.69 for particular domains, and 0.90 for the general QoL measurement) (Bousquet et al. 1994; Jaracz et al. 2006).

WHOQOL-BREF was complemented with questions concerning socio-demographic data (age, gender, education, place of residence, and marital status), questionnaire for measuring patients' somatic status (the last spirometry results and complaints: blood pressure >140/90, pain in the chest, dyspnea, and improvement in well-being during the last 12 months), and the level of health care utilization (the number of: visits to a family doctor, home visits, consultations by phone, and interventions of a district nurse at a patient's home due to the worsening health status during 12 months). In order to unify the results, a somatic index was used in the study. Somatic symptoms reported by patients were assigned values from one (symptoms occurring once a year) to seven (permanent symptoms). The index was calculated by summing up values assigned to somatic symptoms, and then dividing this sum by 49 (the highest possible score).

2.1 Statistical Analyses

A statistical analysis was performed using R 2.10.1 (for Mac OS X Cocoa GUI) by which the type of distribution for all variables was determined. The overall results of the variables were not normally distributed, which was confirmed by the Shapiro-Wilk normality test. The Wilcoxon rank test and the Welch Two Sample *t*-test were used to check that the distributions of x and y differ in the location shift.

Arithmetic means, standard deviations, medians, as well as the range of variability (extremes) were calculated for measurable (quantitative) variables, while for qualitative variables, the frequency (percentage) was determined.

An analysis of logistic regression was used in order to examine the impact of explanatory variables on the odds ratio (OR) of quality of life. 'Chance' was defined as the ratio of the probability of a certain event to the probability of the opposite event. The 'chance quotient' was defined as the ratio of the probability of a certain event happening in one group to the probability of it happening in another group. A 95 % confidence interval was established for both the risk and chance quotient.

Logistic regression was performed using a method of an in-depth analysis of all ten models with two explanatory variables always selected from a set of five variables: gender, age, education, marital status, and place of residence. The authors obtained seven models with two well-matched variables, and one model with three well-matched variables. The critical level of significance was assumed at $p < 0.05$.

3 Results

In the study group, the disease was present in the patients' lives for 11.6 ± 7.7 years on average (Q1–Q3 = 5.0–15.0). Based on the results of spirometry tests performed in 186 patients (77.8 % of all analyzed patients), chronic moderate asthma (II) was diagnosed in 88 (47.3 %)

patients, chronic severe asthma (III) in 16 (9.6 %) patients, and intermittent and chronic mild asthma in 82 (44.0 %) patients. Higher FEV1% values were observed in the group of patients hospitalized due to exacerbation of the disease; the differences were statistically significant ($p = 0.01$). Asthma coexisted with COPD in 14 (5.8 %) patients aged 55–85 years; the mean age was 69.9 ± 9.5 years.

Most patients (63, 26.4 %) had two coexisting diseases, including 38 (32.8 %) family practice patients (Group 2) and 25 (20.3 %) patients hospitalized due to exacerbation of asthma (Group 1). The average number of chronic diseases in the analyzed patients was 3.0 ± 2.0. In both groups, asthma most often coexisted with: hypertension (98, 41.0 %), gastrointestinal diseases (76, 31.8 %), cardiovascular diseases (48, 20.8 %), and diabetes (36, 15.1 %).

In the 3-year period prior to the study, the average number of hospitalizations in the analyzed patients was 2.3 ± 3.8. A higher number of hospitalizations was noted among patients (both women and men) from Group 1 than Group 2 ($Chi^2 = 53.2$, $p = 0.001$), namely: married patients and widows/widowers from Group 1 ($Chi^2 = 12.4$, $p = 0.006$), residents of rural areas from Group 1 and residents of towns/cities with a population of 20,000–50,000 in Group 1 ($Chi^2 = 74.8$, $p = 0.0001$), and patients aged 45–84 and over 85 years in Group 1 ($Chi^2 = 26.7$, $p = 0.002$).

3.1 Quality of Life

The patients with asthma were dissatisfied with their health; the average score was 2.8 ± 0.9. There were no differences in the scores between the groups Group 1 and Group 2 ($p = 0.50$). In the group of patients hospitalized due to exacerbation of asthma (Group 1), 46 (37.4 %) subjects were dissatisfied with their health. Similar percentage (42, 34.2 %) was neither satisfied nor dissatisfied. Nobody in Group 1 marked the answer 'very satisfied' (five points). Satisfaction with QoL in the analyzed patients was moderate (3.4 ± 0.9) and average scores in Group 1 and Group 2 were similar: Group 1 – 3.4 ± 0.9 and Group 2 – 3.4 ± 0.8 ($p = 0.91$).

The average QoL score in the analyzed patients was 53.7 ± 10.1; there were no statistically significant differences between the QoL scores obtained by patients hospitalized due to exacerbation of asthma (Group 1), and family practice patients (Group 2), $p = 0.309$ (Table 2). Statistically significant differences were found in the psychological domain: patients in Group 1 rated QoL in the psychological domain higher than patients in Group 2 ($p = 0.02$). Average scores in other WHOQOL-BREF domains were similar in both groups.

3.2 Statistically Significant Correlations with Reference to Sociodemographic Data

The general QoL score (arithmetic sum of the scores obtained in the four WHOQOL-BREF domains) correlated negatively with marital status, place of residence, and age. The QoL was evaluated lower by patients living alone than married patients ($r_s = -0.30$, $p < 0.001$), by widows/widowers than unmarried women/men ($r_s = -0.3$, $p < 0.001$), patients from rural areas than those from towns/cities with a population of over 200,000 ($r_s = -0.13$, $p = 0.049$) and by older than younger patients ($r_s = -0.50$, $p < 0.001$). Patients with higher and graduate education rated QoL higher than those with primary and incomplete primary education ($r_s = 0.39$, $p < 0.001$). No statistically significant differences in QoL levels between the groups of smokers and non-smokers were found ($p = 0.31$). There were no statistically significant correlations between gender and the general QoL score ($r_s = 0.08$, $p = 0.08$).

The general QoL score correlated positively with all four domains; the correlations were statistically significant (Table 3). We attempted to answer the question: which of the analyzed WHOQOL-BREF domains contributes most to the QoL level in patients with asthma. The analysis of variance demonstrated the highest contribution of the domain D3 – 'Social

Table 2 Quality of life results according to WHOQOL-BREF – patients hospitalized due to asthma (Group 1) *vs.* family practice patients (Group 2)

QoL – domains		M	SD	Q.25 %	Q.50 %	Q.75 %	Min	Max	W	p
Satisfaction with QoL	Group 1	3.4	0.9	3.0	4.0	4.0	1.0	5.0	7190	0.911
	Group 2	3.4	0.9	3.0	4.0	4.0	1.0	5.0		
	Total	3.4	0.9	3.0	4.0	4.0	1.0	5.0		
Satisfaction with health status	Group 1	2.8	0.9	2.0	3.0	3.5	1.0	4.0	7349	0.502
	Group 2	2.9	0.9	2.0	3.0	4.0	1.0	5.0		
	Total	2.8	0.9	2.0	3.0	4.0	1.0	5.0		
D1. Physical domain	Group 1	13.1	3.2	10.8	13.1	15.4	5.1	19.4	7022	0.834
	Group 2	12.9	2.9	10.8	13.1	14.8	4.0	19.4		
	Total	13.0	3.1	10.8	13.1	15.4	4.0	19.4		
D2. Psychological domain	Group 1	13.6	3.1	11.3	14.0	16.0	6.7	20.0	5906.5	0.021*
	Group 2	12.7	2.8	10.7	12.7	14.7	4.0	18.7		
	Total	13.2	2.9	11.3	13.3	15.3	4.0	20.0		
D3. Social relationships domain	Group 1	14.2	3.2	13.3	14.7	16.0	6.7	20.0	6548	0.268
	Group 2	13.8	2.9	12.0	14.7	16.0	4.0	20.0		
	Total	14.0	3.1	12.0	14.7	16.0	4.0	20.0		
D4. Environmental domain	Group 1	13.6	2.6	12.0	14.0	15.5	7.5	18.5	6686	0.401
	Group 2	13.4	2.1	12.0	13.5	14.5	7.5	19.5		
	Total	13.5	2.4	12.0	14.0	15.0	7.5	19.5		
General QoL	Group 1	54.5	10.9	45.3	54.4	63.6	31.3	76.2	6578.5	0.309
	Group 2	52.8	9.1	47.2	52.8	59.3	19.5	75.1		
	Total	53.7	10.1	46.7	54.1	60.4	19.5	76.2		

M mean, *SD* standard deviation, *Q* quartile, *Min* minimum, *Max* maximum, *W* Wilcoxon rank sum test with continuity correction, *p the level of significance below 0.05

Table 3 Correlation coefficients for the four QoL domains with reference to sociodemographic variables

Variables		D1	D2	D3	D4	Gender	Marital status	Education	Place of residence	Age
D1. Physical domain	r_s	1.00								
	p	0.0001								
D2. Psychological domain	r_s	0.77	1.00							
	p	<0.001	0.0001							
D3. Social relationships domain	r_s	0.62	0.69	1.00						
	p	<0.001	<0.001	0.0001						
D4. Environmental domain	r_s	0.70	0.80	0.71	1.00					
	p	<0.001	<0.001	<0.001	0.0001					
Gender	r_s	0.03	0.09	0.07	0.04	1.00				
	p	0.65	0.18	0.26	0.52	0.0001				
Marital status	r_s	−0.34	−0.21	−0.30	−0.19	0.06	1.00			
	p	<0.001	<0.001	<0.001	<0.001	0.35	0.0001			
Education	r_s	0.35	0.37	0.35	0.32	0.07	−0.24	1.00		
	p	<0.001	<0.001	<0.001	<0.001	0.25	<0.001	0.0001		
Place of residence	r_s	−0.11	−0.11	−0.11	−0.11	−0.13	0.10	−0.36	1.00	
	p	0.10	0.08	0.07	0.08	0.05	0.11	<0.001	0.0001	
Age	r_s	−0.55	−0.41	−0.44	−0.38	0.04	0.51	−0.46	0.15	1.00
	p	<0.001	<0.001	<0.001	<0.001	0.52	<0.001	<0.001	0.02	0.0001
Quality of life	r_s	0.87	0.91	0.86	0.89	0.08	−0.30	0.39	−0.13	−0.50
	p	<0.001	<0.001	<0.001	<0.001	0.22	<0.001	<0.001	<0.001	<0.001

r_s Spearman's correlation coefficient, p the level of significance

relationships' where the average score was 14.0 ± 3.0 (Q_1–Q_3 = 12.0–16.0, $Chi^2 = 19.08$, $p < 0.001$).

3.3 Somatic Index with Reference to Sociodemographic Variables

The somatic index for the analyzed patients was 0.4 ± 0.2. It was higher in the group of hospitalized patients (Group 1: 0.5 ± 0.2 *vs.* Group 2: 0.4 ± 0.2) where the difference was statistically significant ($p = 0.011$).

A somatic improvement during 12 months prior to the study was reported by 118 (65.9 %) patients, and a psychological improvement by 111 (62.4 %) patients. Patients from Group 1 more often than those from Group 2 reported the improvement in both spheres: somatic and psychological ($Chi^2 = 4.2$, $p = 0.04$ *vs.* $Chi^2 = 6.3$, $p = 0.01$).

The most common symptoms occurring very often (permanently, several times a week, 1–2 times a week) were: dyspnea – 166 (69.4 %), pain in the chest – 107 (44.8 %) and blood pressure above 140/90 – 38.4 % (92). No statistically significant differences in the occurrence of these symptoms were found between the groups ($Chi^2 = 1.0$, $p = 0.3$). There were statistically significant differences in the frequency of dyspnea and pain in the chest between Group 1 and Group 2, depending on gender, age, the place of residence, and marital status.

Dyspnea was more common among women in Group 1 (38, 55.1 %) than Group 2 (36, 37.1 %), men in Group 1 (23, 33.3 %) than Group 2 (27, 27.8 %) ($Chi^2 = 13.6$, $p = 0.003$), married patients (37, 53.6 %) and widows/widowers (13, 18.8 %) in Group 1 ($Chi^2 = 18.5$, $p = 0.02$), residents of rural areas in Group 2 (26, 26.8 %); residents of towns/cities with a population of 10,000–100,000 in Group 1 (33, 47.8 %) ($Chi^2 = 38.5$, $p = 0.002$). Additionally, dyspnea in Group 1 occurred more often in patients at the age of 45–64 (29, 42.0 %) and below 24 years (5, 7.2 %), but in Group 2 in patients between 65 and 84 (31, 31.9 %) ($Chi^2 = 23.9$, $p = 0.004$).

Chest pain was more frequent among patients (both women and men) in Group 1 (35, 77.8 %) than Group 2 (22, 35.4 %) ($Chi^2 = 19.9$, $p = 0.0002$); they were mainly patients aged 45–84 (32, 46.4 %) in Group 1 *vs.* (19, 19.6 %) in Group 2 ($Chi^2 = 26.4$, $p = 0.002$).

3.4 Medical Services in Respect of Sociodemographic Variables

During the last 12 months prior to the completion of the questionnaire, the average number of visits to a family doctor was higher in Group 2 (6.6 ± 6.2) than in Group 1 (3.1 ± 2.7) ($p < 0.001$). More visits to a family doctor were paid by women and men in Group 2 than in Group 1 ($Chi^2 = 21.2$, $p < 0.001$) and by married patients, widows/widowers, and those separated from their husbands/wives in Group 2 than in Group 1 ($Chi^2 = 26.2$, $p < 0.002$). Residents of rural areas with a population of 5,000–10,000 in Group 2 and with a population of 20,000–40,000 in Group 1 ($Chi^2 = 50.9$, $p < 0.0001$) and patients of all ages paid more visits to family doctors in Group 2 than in Group 1 ($Chi^2 = 32.7$, $p < 0.0001$).

Interventions of a district nurse were more frequent among Group 2 patients: 4.5 ± 18.1 (range 0–150) than among Group 1 patients: 0.7 ± 2.5 (range 0–16, $p = 0.002$). Such interventions were significantly more common among: men and women in Group 2 than in Group 1 ($Chi^2 = 12.4$, $p = 0.006$), residents of rural areas and towns/cities with a population of 50,000–5,000 in Group 2 and residents of towns/cities with a population below 5,000 in Group 1 ($Chi^2 = 27.2$, $p < 0.0001$).

The average number of home visits was 0.3 ± 0.7 in Group 1 and 3.7 ± 33.8 in Group 2. The differences were statistically insignificant ($p = 0.18$). The average number of consultations by phone was in Group 1: 2.3 ± 4.7 and in Group 2: 2.7 ± 14.3 ($p = 0.73$). This form of medical consultations was mostly used by patients aged 45–64 years in Group 2, aged 65–84 years in Group 1, and older than 85 years in Group 1; the differences were significant

($Chi^2 = 11.8$, $p = 0.02$). Gender, the place of residence, marital status, and education had no effects on the number of consultations by phone.

186 (77.8 %) patients confirmed that they had spirometry tests during the last 12 months. In most cases spirometry was performed once a year – 92 (49.6 %), in 51 (27.3 %) patients – twice a year, and in 43 (23.1 %) – three and more times a year. The average number of spirometry tests performed in patients analyzed during the last 12 months was 1.2 ± 1.6 (the range of 0–8). Spirometry tests were more often performed in Group 1: 2.8 ± 1.5, than in Group 2: 0.3 ± 0.8; the difference between the groups was significant ($p < 0.0001$). Spirometry was more often performed in women in Group 1 than in Group 2 ($Chi^2 = 17.8$, $p = 0.001$); patients separated from their husbands/wives in Group 1 than Group 2 ($Chi^2 = 130.1$, $p = 0.0001$); residents of: rural areas, towns/cities with a population below 2,000, and towns/cities with a population of 20,000–49,999 in Group 1 than in Group 2 ($Chi^2 = 148.3$, $p = 0.0001$).

Patients were asked how many times they received health services over the last year including: blood pressure measurement, spirometry tests, ECG, the issue of medical certificates, referrals to specialists, laboratory requests and prescriptions, and health education in family practice. The number of blood pressure measurements during the last 12 months ranged from 0 to 720 (mean 76.6 ± 155.2). Blood pressure was more often measured in Group 1 (183.8 ± 208.6) than in Group 2 (10.8 ± 30.8); the difference was statistically significant ($p < 0.0001$).

Medical certificates were rarely issued (0.2 ± 0.5), but more often in Group 1 (0.3 ± 0.5) than in Group 2 (0.2 ± 0.5) ($p = 0.03$). Prescriptions were issued more frequently (15.1 ± 52.8) – the range of 0–720 during the last 12 months and more often in the group of patients hospitalized due to exacerbation of asthma in Group 1 (25.6 ± 85.2) than in Group 2 (8.7 ± 5.6) ($p = 0.001$). The average number of referrals to specialists during 12 months was 1.6 ± 1.7 (the highest number of referrals received by one patient was 12). The differences between the groups were significant (Group 2: 1.6 ± 2.0 *vs.* Group 1: 1.6 ± 1.2) ($p = 0.04$). Referrals to laboratory were more often given to patients in Group 1 (2.4 ± 2.7) than in Group 2 (1.7 ± 2.2). The differences were significant ($p = 0.003$). ECG was more often performed in family practice patients (Group 2: 2.6 ± 9.6, range 2–100 measurements in one patient *vs.* Group 1: 2.1 ± 1.5, range: 0–9); the differences was significant ($p = 0.0008$).

Health education was implemented according to 52 (21.8 %) patients. The average number of educational interventions was 1.3 ± 3.5 (range of 0–36). In Group 1, the average number of educational interventions was 0.8 ± 1.9 (range of 0–13) and in Group 2 – 1.6 ± 4.1 (range of 0–30), the difference was insignificant ($p = 0.08$). The influence of gender, education, marital status, and age on health education during visits to a family doctor was not demonstrated. A variable which had effects on health education was the place of residence; patients from rural areas and towns/cities with a population over 200,000 patients in Group 2 received health education more often than patients in Group 1; the difference was significant ($Chi^2 = 33.9$, $p = 0.008$).

Gender had a significant influence on obtaining medical certificates and referrals to the hospital; women (0.3 ± 0.6) received them more often than men (0.1 ± 0.3) ($Chi^2 = 6.8$, $p = 0.008$; $Chi^2 = 4.9$, $p = 0.02$, respectively). Blood pressure measurement was more often performed in patients aged 45–64 years 90.3 ± 170.3 ($Chi^2 = 13.8$, $p = 0.008$), widows/widowers (117.5 ± 194.1), divorcees (94.8 ± 176.8), and those separated from their husbands/wives (125.6 ± 203.0) ($Chi^2 = 12.4$, $p = 0.01$), and in patients with vocational (117.7 ± 192.4) and incomplete higher education (152.7 ± 276.4) ($Chi^2 = 16.8$, $p = 0.03$). Prescriptions were more often given to widows/widowers (13.4 ± 10.4), married patients (17.8 ± 67.6), and those separated from their husbands/wives 11.3 ± 5.0 ($Chi^2 = 10.6$, $p = 0.03$), and patients with vocational

(24.2 ± 91.1), primary (13.4 ± 8.4), and incomplete higher education (12.3 ± 9.2) ($Chi^2 = 16.6$, $p = 0.03$). Patients aged over 45 received prescriptions (20.4 ± 85.2) ($Chi^2 = 9.6$, $p = 0.04$), and had ECG performed more often (3.2 ± 12.0) ($Chi^2 = 20.2$, $p = 0.0004$).

3.5 Odds Ratio

The results of the logistic regression are shown in Table 4. The general QoL level was most strongly influenced by gender, age and education, the number of home visits, interventions of a district nurse, and the somatic index.

The odds ratio (OR) of high QoL levels was twice greater in women than men (OR = 1.9, 95 % CI: 1.2–3.0) and 12 times higher in patients with higher education than those with primary and incomplete primary education (OR = 12.5, 95 % CI: 6.6–25.2). OR of high QoL levels in patients aged 50 years was twice greater than in patients aged 70 years (OR = 2.2, 95 % CI: 1.1–4.6), and in married patients and 2.4 times higher than in widows/widowers (OR = 2.4, 95 % CI: 1.4–3.8). Patients who had never had home visits had OR 1.7 times higher than patients who had one home visit during the last 12 months (OR = 1.68, 95 % CI: 1.1–2.6). Furthermore, patients who did not need interventions of a district nurse, had an OR 1.6 times higher than patients who had four such interventions (OR = 1.5, 95 %, CI: 1.0–2.4). OR of high QoL levels was 5.6 times higher in patients without somatic complaints than in patients who had had such complaints throughout the previous 12 months (OR = 5.6, 95 %, CI: 3.4–9.6).

4 Discussion

The results of QoL research performed with reference to socio-demographic and medical variables significantly contributes to the assessment of treatment efficiency. What is more, QoL research allows estimating the influence of the disease and therapy on patients' functioning in various domains of life. On the other hand, effects of asthma on satisfaction with life, health status, and QoL may vary in different patients

Table 4 Results of logistic regression ($n = 239$)

i	Constant/Variables	Estimate β_i	Std. Error	z value	Pr(>|z|)	AIC
Model 1						
1	Gender	0.61	0.16	3.68	<0.001	315.3
2	Marital status	−0.40	0.09	−4.04	<0.001	
Model 2						
1	Gender	−0.43	0.18	−2.45	0.010	323.6
3	Education	0.19	0.06	2.86	0.004	
Model 3						
1	Gender	1.14	0.24	4.81	<0.001	305.6
5	Age	−0.03	0.01	−5.04	<0.001	
Model 4						
2	Marital status	−0.42	0.08	−4.72	<0.001	301.7
3	Education	0.26	0.06	4.64	<0.001	
Model 5						
3	Education	0.18	0.05	3.30	0.001	318.8
4	Place of residence	−0.10	0.03	−3.06	0.002	
Model 6						
3	Education	0.40	0.08	5.32	<0.001	291.6
5	Age	−0.02	0.01	−5.58	<0.001	
Model 7						
4	Place of residence	0.10	0.04	2.26	0.020	325.1
5	Age	−0.01	0.01	−2.81	0.005	

(continued)

Table 4 (continued)

i	Constant/Variables	Estimate β_i	Std. Error	z value	Pr(>|z|)	AIC
Model 8						
1	Gender	0.67	0.26	2.50	0.010	287.1
3	Education	0.32	0.08	3.95	<0.001	
5	Age	−0.04	0.01	−5.56	<0.001	
Model 9						
6	Home visits	−0.52	0.20	−2.54	0.010	239.6
1	Gender	0.52	0.18	2.85	0.004	
2	Marital status	−0.28	0.11	−2.61	0.008	
Model 10						
6	Home visits	−0.63	0.20	−3.04	0.002	230.5
1	Gender	−0.56	0.21	−2.65	0.008	
3	Education	0.30	0.08	3.54	<0.001	
Model 11						
6	Home visits	−0.44	0.20	−2.12	0.030	236.2
1	Gender	0.92	0.26	3.50	<0.001	
5	Age	−0.02	0.01	−3.31	<0.001	
Model 12						
6	Home visits	−0.44	0.21	−2.11	0.030	240.6
5	Gender	0.60	0.21	2.92	0.003	
7	Somatic index	−2.18	0.82	−2.67	0.008	
Model 13						
6	Home visits	−0.50	0.21	−2.46	0.013	220.8
2	Marital status	0.38	0.10	−3.75	<0.001	
3	Education	0.30	0.06	4.50	<0.001	
Model 14						
6	Home visits	−0.62	0.21	−2.92	0.003	228.6
3	Education	0.24	0.06	3.74	<0.001	
4	Place of residence	−0.12	0.04	−2.68	0.007	
Model 15						
8	Interventions of a district nurse	−0.11	0.05	−2.04	0.040	238.8
1	Gender	−0.46	0.20	0.02	0.020	
3	Education	0.24	0.08	0.002	0.002	
Model 16						
1	Gender	0.88	0.23	3.80	<0.001	241.4
2	Marital status	−0.23	0.12	−2.04	0.040	
9	Psychological well-being	−2.30	0.81	−2.83	0.004	
Model 17						
1	Gender	1.15	0.27	4.20	<0.001	239.0
5	Age	−0.02	0.01	−2.68	0.007	
7	Somatic index	−1.72	0.87	−1.97	0.040	

Response variable: general QoL score
AIC Akaike information criterion; Models 1–7 – models with two explanatory variables; Models 8 – 17 – models with three explanatory variables

depending on their gender, age, education, marital status, the place of residence, and the stage of the disease (Dean et al. 2009).

In the present study, the patients with asthma were dissatisfied with their health, but their satisfaction with QoL was higher. Both patients hospitalized due to acute exacerbation of the disease and family practice patients assessed their health negatively. This could be caused by severe somatic symptoms, such as: dyspnea,

chest pain, blood pressure above 140/90 mm Hg, and hospitalization itself. These results considerably diverge from those obtained in those population studies in which health was assessed as below 'good' merely by just one third of adult Poles.

In the present study, the QoL score was similar to the norm for the general population (53.7 ± 10.1) and there were no differences between the groups. QoL was rated lower by patients living alone (widows/widowers, divorcees, and those separated from their husbands/wives), residents of rural areas, patients of older age, and with low education levels. The analysis of particular QoL domains described in the WHOQOL-BREF questionnaire demonstrated that the QoL assessment was most strongly influenced by the domain D3 – 'social relationships'. The QoL scores in other domains were slightly higher in the group of hospitalized patients.

The QoL of asthma patients is significantly influenced by sociodemographic factors. However, in the study, the correlations between the scores in the four WHOQOL-BREF domains and such variables as gender and the place of residence were not observed. The QoL scores in four domains significantly correlated with education, age, and marital status. Similar results concerning the influence of age and education on the QoL scores were obtained by Oguzturk et al. (2005), Hazell et al. (2003), Ferreira et al. (2010) and Laforest et al. (2005). Nevertheless, the analysis of the logistic regression revealed that women had twice greater chances for a positive QoL assessment than men. Lower QoL scores obtained by men may suggest poor control of asthma symptoms. Dean et al. (2009) indicate that uncontrolled or badly controlled asthma has far-reaching consequences not only for the QoL of asthma patients but also their caregivers. Powell and Gibson (2003) demonstrated that the preparation of an asthma patient for the implementation of an individual plan of the therapy reduces by one third the number of hospitalizations and other events undesirable for health (night attacks, fatigue during a day, or anxiety), as well as social and economic reasons (visits to the emergency unit, absence from work, and unscheduled visits to a family doctor). According to Frish (1998) positive QoL assessment is a crucial health care result, which prevents lowering of patients' satisfaction with this care. Doz et al. (2013) compared health care systems for patients with asthma in France and Spain and showed that in both countries the costs of asthma treatment depended on whether asthma was controlled, partially controlled or uncontrolled, and found that the worse asthma was controlled, the higher the costs of its treatment, and the lower HRQOL ($p < 0.001$).

Ståhl et al. (2005) provided evidence that individuals with severe and moderate asthma rated QoL in the sphere of physical and mental functioning considerably lower. In the present study, on the other hand, patients hospitalized due to exacerbation of the disease assessed their QoL in D2 and rated their psychological domain higher than family practice patients. It can be assumed that the assessment was associated with the feeling of safety provided by the stay in hospital and the presence of medical staff. It was shown that exacerbation of asthma symptoms was affected by age as the worsening of health status more often happened among patients of over 65 years of age.

In the study presented, lower QoL in the asthma patients were accompanied by a higher somatic index and a higher index of medical services. Ferreira et al. (2010) noticed that the exacerbation of the disease had a negative influence on the general QoL assessment. They demonstrated that the severity of asthma and exacerbation of symptoms led to the increase in medical services, such as a higher frequency of hospitalizations during the last 12 months (OR = 0.9–6.1), visits to the emergency unit (OR = 1.8–17.2) and scheduled visits to a family doctor. In line with the above outlined, a multi-factor analysis performed by Laforest et al. (2005) revealed that main predictors of the lower HRQoL scores among asthma patients are asthma attacks and the frequency of medical contacts during the last 12 months. The increase in medical services among asthma patients with

lower QoL levels and worse control of the disease has also been observed in other studies (Hutter et al. 2011; Maio et al. 2012).

In the present study, the patients in whom asthma coexisted with COPD and/or other diseases were aged over 56. This group (14, 5.8 %) was too small to conclude about the influence of the coexistence of asthma and COPD and other diseases on the QoL level. Likewise, de Marco et al. (2013) found that asthma and COPD more often occurred together in people older than 65 years. Further, we demonstrate the influence of the somatic index on QoL in asthma patients and show that dyspnea, chest pain, and high blood pressure could contribute to the lowering of the QoL levels.

Based on the patients' statements, neither hospitalized patients nor family practice patients involved in this study received sufficient health education. Only every fifth patient was once or twice informed by a doctor about the disease management. As stated by Urek et al. (2005), the participation of patients in health education programs and receiving oral instructions during visits to family doctors may improve control of asthma and QoL levels, and reduce the costs of treatment. These researchers analyzed how various education programs contributed to the better control of asthma and higher QoL levels in 60 adult patients with chronic moderate asthma. They concluded that asthma education had the greatest input to the QoL improvement, while oral instructions resulted in the better control of asthma, including peak expiratory flow (PEF) and the use of emergency drugs, and also increased QoL levels.

5 Conclusions

The QoL scores, satisfaction with QoL, and health status of patients hospitalized due to exacerbation of asthma symptoms do not significantly differ from those observed among family practice patients. Hospitalized patients evaluated QoL higher in the psychological domain than family practice patients. The analyzed sociodemographic variables were shown to influence the QoL, and factors which had the strongest positive impact were: youth, higher education, marriage, lack of somatic symptoms, and low health care utilization.

The results demonstrate that the QoL measurement in asthma patients with reference to socio-demographic variables, somatic symptoms, and health care services, enables to understand the complex bio-psychosocial situation of patients at different stages of the disease, and to tailor therapy to individual needs. Patients with lower QoL levels require health education which can alleviate somatic symptoms, and thus reduce the use of medical services.

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Increased Serum IgA in Children with IgA Nephropathy, Severity of Kidney Biopsy Findings and Long-Term Outcomes

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Abstract

The aim of the study was to determine whether an elevated IgA level at the time of the diagnosis of IgA nephropathy has an effect on the severity of kidney biopsy findings and long-term outcomes in children. We retrospectively studied 89 children with IgA nephropathy who were stratified into Group 1- elevated serum IgA and Group 2 – normal serum IgA at baseline. The level of IgA, proteinuria, hematuria, glomerular filtration rate (GFR) and hypertension (HTN) were compared at baseline and after the end of the follow-up period of 4.0 ± 3.1 years. Kidney biopsy findings were

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evaluated using the Oxford classification. The evaluation of treatment included immunosuppressive therapy and renoprotection with angiotensin converting-enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB), or no treatment. The elevated serum IgA was found in 46 (52 %) patients and normal serum IgA level was found in 43 (48 %) patients. No differences were found between the two groups regarding the mean age of patients, proteinuria, and the number of patients with reduced GFR or HTN at baseline. In kidney biopsy, mesangial proliferation and segmental sclerosis were significantly more common in Group 1 compared with Group 2 ($p < 0.05$). Immunosuppressive therapy was used in 67 % children in Group 1 and 75 % children in Group 2. The Kaplan-Meier survival curves for renal function (with normal GFR) and persistent proteinuria did not differ significantly depending on the serum IgA level at baseline. We conclude that in IgA nephropathy the elevated serum IgA at baseline may be associated with mesangial proliferation and segmental sclerosis contribute to glomerulosclerosis, but has no effect on the presence of proteinuria or on the worsening of kidney function during several years of disease course.

Keywords
Biopsy • Glomerulosclerosis • Kidney • Nephropathy • Proteinuria • Serum IgA • Introduction

1 Introduction

IgA nephropathy (IgAN) is a form of chronic glomerulonephritis with predominant IgA deposits in kidney biopsy findings. It is a chronic kidney disease that develops in 5–30 % of patients at 10 years of age and in 25–50 % of patients at 20 years of age (Coppo and D'Amico 2005; Yoshikava et al. 2001). Complete remission of IgAN has been reported in only 3–30 % of cases (Coppo and D'Amico 2005).

IgA may be present in a monomeric form or may polymerise (pIgA) due to the J-chain. In healthy subjects, most pIgA is produced by the mucosal immune system, while patients with IgAN show reduced mucosal pIgA1 production and increased pIgA1 production in the bone marrow (Van der Boog et al. 2005). Different studies show that pIgA1 is the major component of glomerular deposits. IgA glomerular deposits sustain over a long-term follow-up in patients with persisting urinary changes and disappear in the course of clinical remission.

IgA1 in patients with IgAN shows an increased ability to form larger conglomerates as a result of abnormal galactosilation within the O-glycosylation hinge region. A defect of β1,3-galactose transferase, responsible for IgA galactosilation, has been found in B cells of patients with IgAN. Normally, IgA1 is catabolised in the liver by binding with an asialoglycoprotein receptor (ASGPR), and O-glycosylation results in a significant decrease of hepatic IgA1 clearance. IgA1 O-glycoforms that are deficient in galactose have an increased immunogenic potential attributable to hinge region O-glycans protecting IgA1 from the immunerecognition (Suzuki et al. 2009). IgA1 molecules with reduced galactose content form immunologic complexes that attach to mesangial fibronectin, laminin, and collagen type IV, and activate C3 further promoting nonspecific inflammatory responses within the glomeruli (Silva and Hogg 1989). Japanese studies have shown that an elevated serum IgA level is observed in 50–70 % of the adults and 16 % of children with IgAN (Yoshikava et al. 2001).

The aim of the present study was to determine whether an elevated IgA level at the time of the diagnosis of IgAN in children is related to the severity of kidney biopsy findings and long-term treatment outcomes.

2 Methods

We retrospectively studied 89 children from 8 pediatric nephrology centers, including 56 (63 %) boys and 33 (37 %) girls aged 11 ± 4 years, in whom IgAN was diagnosed based on kidney biopsy findings. The patients were divided into two groups based on the baseline serum IgA level: Group 1 included patients with elevated serum IgA and Group 2 included patients with normal serum IgA level. The analysis consisted of the assessment of the severity of urinary changes at baseline, kidney biopsy findings, applied therapy, and the long-term outcomes in both compared groups.

The baseline evaluation included the degree of proteinuria, hematuria, serum albumin, creatinine and IgA levels, GFR – the estimation using the Schwartz formula (Schwartz et al. 2009), and the blood pressure measurements. Proteinuria was determined using the turbidimetric method of Exton in a 24-h urine collection and expressed in mg/kg/day. The nephrotic proteinuria was recognized if greater than 50 mg/kg/day. The nephrotic syndrome was diagnosed if the nephrotic proteinuria was accompanied by hypoalbuminemia of less than 2.5 g/dl and hyperlipidemia.

Erythrocyturia was defined as more than 5 erythrocytes per field of view at the magnification of 400×. Hematuria was defined as a visible change of urine color. Serum IgA was determined using immunonephelometric or immunoturbidimetric method and related to the age-specific reference ranges in a given center. Blood pressure was measured using the Korotkoff method. Hypertension was diagnosed if blood pressure exceeded the 95th percentile for height, age, and gender in three independent measurements (National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents 2005).

Kidney biopsy was performed in all children at 1.4 ± 2.2 years (median 0.5; range 0.1–13 years) since the disease onset. The specimens were evaluated using the Oxford classification (1 – changes present, 0 – absent: M – mesangial proliferation; E – endocapillary hypercellularity; S – segmental sclerosis; T – tubular atrophy/interstitial fibrosis T0: 0–25 %, T1: 26–50 %, and T2: >50 %) (Roberts et al. 2009). In addition, the absence or presence of crescents (c0/c1) was indicated in the kidney biopsy report. Histopathological evaluation was performed locally and verified at the Department of Pathology, Medical University of Warsaw in Poland.

The treatment modalities included supportive therapy with angiotensin converting-enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) agents, IS – immunosuppressive drugs (prednisone, azathioprine, cyclophosphamide, CsA/Tac – cyclosporin A or tacrolimus, MMF – mycophenolate mofetil). The follow-up period ranged from 1 to 14 years (mean 4.0 ± 3.1 years). The severity of proteinuria and erythrocyturia, GFR, and the presence of hypertension were evaluated in the course of the long-term follow-up. Disease remission was defined as resolution of urinary changes with normal GFR and blood pressure values.

Results were expressed as means ± SD, median, and minimum-maximum values. Parameters were compared using the Wilcoxon test and the *t*-test, and frequencies were compared using a proportion test. Linear regression analysis, chi-squared test, Pearson's correlation coefficient with the exact Fisher test, and Spearman's rank correlation test were used to evaluate relations between studied parameters at the end of the follow-up in relation to the baseline serum IgA level. Kaplan-Meier curves were plotted for the two study groups.

P values of <0.05 were considered statistically significant. Statistical analyses were performed using the Statistica ver. 10 software package.

3 Results

In all children, IgAN was diagnosed on the basis of kidney biopsy findings. The nephrotic range of proteinuria with erythrocyturia was present in 19 (21 %) children (including 6 with nephrotic syndrome). Non-nephrotic range of proteinuria was found in 48 (54 %) patients, and isolated erythrocyturia/hematuria in 22 (25 %) patients. 19 (21 %) had intermittent gross hematuria. At baseline, GFR <90 mL/min was noted in 35 (39 %) children and hypertension in 18 (20 %) children. Elevated serum IgA level was found in 46 (52 %) out of the 89 patients (Group 1), and the normal serum IgA level was found in 43 (48 %) patients (Group 2). Patient characteristics at baseline are shown in Table 1. In Group 1, proteinuria was less frequent at baseline, although the difference between the groups was insignificant, while the nephrotic range of proteinuria was significantly more common ($p < 0.05$) compared with the Group 2. No significant differences were found between both groups regarding the patients' age, protein loss, decreased GFR, and the presence of hypertension at baseline.

Kidney biopsy was performed after 1.2 ± 2.0 years in Group 1 and after 1.6 ± 2.4 years in Group 2 from the disease onset. The timing of kidney biopsy in relation to initial symptoms of the disease did not differ significantly between the two groups. The most common finding in both groups was mesangial proliferation. Both mesangial proliferation (M1) and segmental sclerosis (S1) were significantly more common in Group 1 compared with Group 2 (89 % *vs.* 72 % and 41 % *vs.* 21 %, $p < 0.05$, respectively), while the rates of endocapillary hypercellularity (E1), tubular atrophy/interstitial fibrosis (T1), and the presence of crescents (c1) did not differ between the groups. The biopsy findings in the two groups are shown in Table 2.

Supportive therapy or watchful waiting was applied in 28 (31 %) children, including 16 (35 %) in Group 1 and 12 (28 %) in Group 2; the difference between the two groups was insignificant. Immunosuppressive therapy with prednisone, azathioprine, cyclophosphamide, cyclosporin A, and mycophenolate mofetil was used as a first-choice treatment in 61 (68 %) children, including 33 (72 %) in Group 1 and 28 (65 %) in Group 2 (insignificant difference). The indications for immunosuppressive therapy included severe urinary changes (mainly nephrotic range of proteinuria), reduced GFR and/or histopathological findings of poor prognosis (M, E, S, and T). In Group 1, azathioprine with prednisone were the most frequent first-

Table 1 Clinical parameters in patients with IgA nephropathy in relation to baseline serum IgA level

	Group 1 ($n = 46$)	Group 2 ($n = 43$)	p
Gender (M/F)	30/16	26/17	ns
Age at onset of IgAN (years)	11.4 ± 4.2	11.2 ± 4.1	ns
Time to renal biopsy (years)	1.2 ± 2.0	1.6 ± 2.4	ns
Proteinuria (mean, median, range) (mg/kg/day)	20.6 ± 35.0	66.9 ± 163.9	ns
	12 (0–202)	15 (0–967)	
No. of patients with proteinuria	32 (69 %)	35 (76 %)	ns
No. of patients with nephrotic range proteinuria	6 (13 %)	13 (30 %)	$p < 0.05$
GFR (mL/min)	97.4 ± 27.4	94.7 ± 36.1	ns
No. of patients with GFR <90 mL/min	17 (37 %)	18 (42 %)	ns
No. of patients with HTN	8 (17 %)	10 (23 %)	ns

Group 1 – elevated serum IgA1; Group 2 – normal serum IgA1
IgAN IgA nephropathy, *GFR* glomerular filtration rate, *HTN* hypertension, *ns* non-significant

choice treatment used significantly more frequently compared with Group 2 ($p < 0.05$). A repeated course of immunosuppressive therapy due to disease recurrence or persisting proteinuria was used in 16 children (18 %), including 10 in Group 1 and 6 in Group 2; insignificant difference. The duration of a follow-up was similar in both groups and ranged from 1 to 14.3 years (mean 4.0 ± 3.1 years in Group 1 and 3.7 ± 2.6 years in Group 2). Treatment modalities in both examined groups are summarized in Table 3.

Comparison of long-term outcomes in relation to the serum IgA level at baseline is shown in Table 4. The number of patients with reduced GFR (<90 mL/min) and the rate of persisting proteinuria did not differ between the two groups examined. We failed to find any relation between the elevated serum IgA level at baseline and reduced GFR (chi-squared test) and/or persisting proteinuria (Spearman's rank correlation test) at the end of the follow-up.

The Kaplan-Meier analysis for survival probability in children with normal GFR and with persisting proteinuria in the two groups did not confirm the presence of a correlation between these parameters and the serum IgA level at baseline (Figs. 1 and 2). However, the baseline GFR <90 mL/min, as compared with that ≥90 mL/min, appeared a poor prognostic indicator of survival ($p < 0.001$) (Fig. 3).

Table 2 Number of children with various kidney biopsy findings (M1, E1, S1, T1) according to Oxford classification in Group 1 – elevated serum IgA1 and Group 2 – normal serum IgA1

	Group 1 ($n = 46$)	Group 2 ($n = 43$)	p
M1	41 (89 %)	31 (72 %)	<0.05
E1	6 (13 %)	11 (25 %)	ns
S1	19 (41 %)	9 (21 %)	<0.05
T1	7 (15 %)	5 (12 %)	ns
C1	10 (22 %)	7 (16 %)	ns

M mesangial hypercellularity, *E* endocapillary hypercellularity, *S* segmental sclerosis, *T* tubular atrophy/interstitial fibrosis, *c* crescents, *ns* non-significant

4 Discussion

A defect underlying IgAN is the production of abnormal, galactose-deficient glycosylated IgA1 that forms circulating immune complexes deposited in the glomeruli (Tanaka et al. 2011). An increase in IgA1 production by bone marrow plasmocytes is visible as an enhanced IgA serum level (Galla 1995), whose prevalence may be as high as 41 % in children (Bulut et al. 2012). In the present study we found an elevated IgA in the serum samples from 46 out of the 89 (52 %) children. In adult patients, IgA >315 mg/dL is considered diagnostic of IgAN, although the IgA level has not been identified as a valuable prognostic factor (Tomino et al. 2000).

Episodes of overt hematuria in the course of IgAN are less frequent in adults compared with children. Such episodes have been found in 43 % (Emancipator et al. 1995) and 18–32 %

Table 3 Treatment used in Group 1 patients – elevated serum IgA1 and Group 2 patients – normal serum IgA1[a]

	First choice treatment		p	Second choice treatment		p
	Group 1	Group 2		Group 1	Group 2	
IS	33 (72 %)	28 (65 %)	ns	10 (23 %)	6 (14 %)	ns
Corticosteroids+ACEI/ARB	11 (24 %)	10 (23 %)	ns	4 (9 %)	0	–
ACEI/ARB/0	16 (35 %)	12 (28 %)	ns	3 (6 %)	2 (5 %)	ns
Azathioprine	21 (46 %)	10 (23 %)	<0.05	4 (9 %)	2 (5 %)	ns
Cyclophosphamide	0	5 (11 %)	–	1 (2 %)	3 (7 %)	ns
CsA/Tac	1 (2 %)	2 (5 %)	ns	0	1 (2 %)	–
MMF	0	1 (2 %)	ns	1 (2 %)	0	–

[a]Patients may have received more than one treatment modality

ACEI/ARB/0 angiotensin converting-enzyme inhibitor or angiotensin II receptor blocker or no treatment, *IS* immunosuppressive drugs, *CsA/Tac* cyclosporin A or tacrolimus, *MMF* mycophenolate mofetil, *ns* nonsignificant

Table 4 Long-term outcomes in Group 1 patients – elevated serum IgA1 and Group 2 patients – normal serum IgA1

	Group 1 (n = 46)	Group 2 (n = 43)	p
Follow-up (years)	4.0 ± 3.1	3.7 ± 2.6	ns
GFR (mL/min)	99.4 ± 23.2	108.0 ± 24.7	ns
No. of patients with GFR <90 mL/min	14 (30 %)	6 (14 %)	ns
Proteinuria (mean, median, range) (mg/kg/day)	3.2 ± 6.4	12.2 ± 23.2	ns
	Median 0 (0–22)	Median 0 (0–97)	
No. of patients with proteinuria	14 (30 %)	19 (44 %)	ns
No. of patients with nephrotic range proteinuria	1 (2 %)	4 (9 %)	ns
No. of patients with complete disease remission	11 (24 %)	9 (21 %)	ns

ns nonsignificant

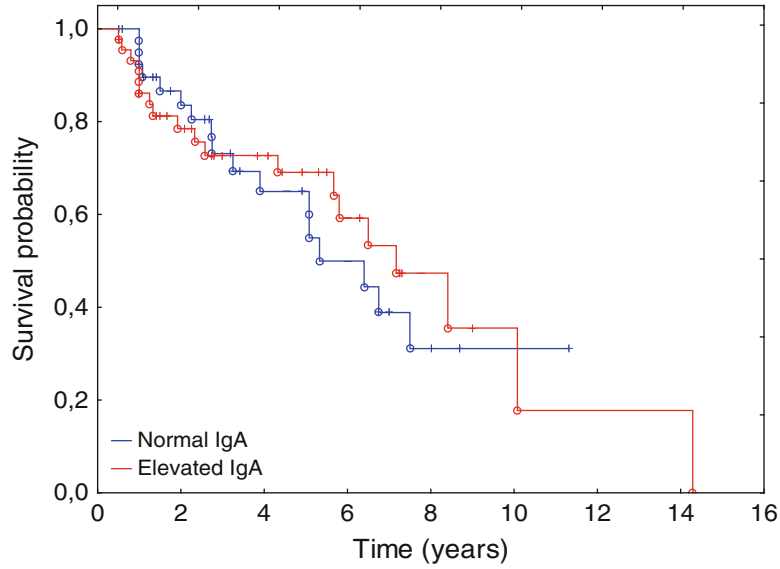

Fig. 1 Kaplan-Meier survival curves regarding renal function in relation to the baseline serum IgA level

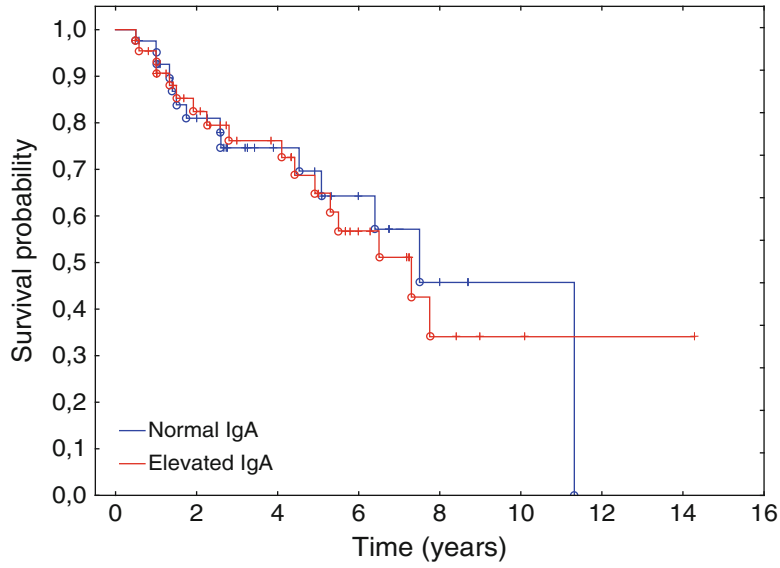

Fig. 2 Kaplan-Meier survival curves regarding persistence of proteinuria in relation to the baseline serum IgA level

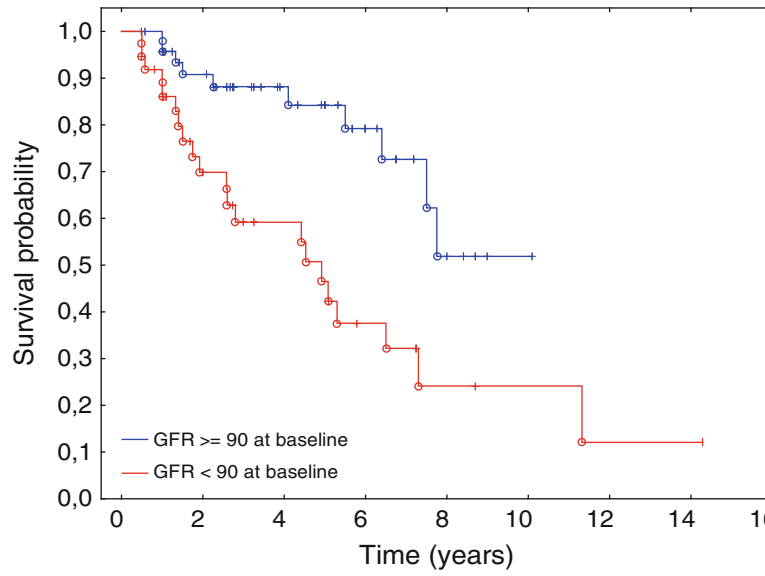

Fig. 3 Kaplan-Meier survival curves regarding renal function in relation to baseline GFR

(Yoshikava et al. 2001) of adult patients. Symptoms may also include chronic erythrocyturia or proteinuria of varying severity, acute nephritic syndrome with hypertension, or acute renal failure with nephrotic syndrome. In children, erythrocyturia or asymptomatic proteinuria has been found in 62 %, hematuria in 26 %, and nephritic syndrome in 12 % of patients (Yoshikava et al. 2001).

In line with those results, in the present study we found hematuria in 21 % of children, non-nephrotic range proteinuria in 54 %, and hypertension in 20 % of patients. Acute kidney injury, observed in 39 % of patients, was frequently associated with nephritic syndrome. The severity of initial symptoms was unrelated to the serum IgA level, and eventfully the nephrotic range of proteinuria was found to be more frequent among children with the normal serum IgA level.

Kidney biopsy showed mesangial cell proliferation and segmental sclerosis to be significantly more common in the group with elevated Serum IgA level, which may be attributable to glomerular injury triggered by circulating immune complexes, in particular those containing aberrantly glycosylated IgA1 (Tanaka et al. 2011). The *in vitro* studies by Novak et al. (2005) have demonstrated that circulating immune complexes with galactose-deficient IgA1 from the sera of IgAN patients stimulate mesangial cell proliferation more effectively than non-complexed IgA1 or immune complexes isolated from healthy persons.

In view of the lack of standards of IgAN therapy in children, various medical centers use different treatment modalities guided by the severity of urinary changes and GFR values. Supportive therapy was comparable in both groups analysed in the present study. Immunosuppressive treatment was applied more frequently than renoprotection alone in both groups, but the immunosuppression was different in the two groups: azathioprine combined with corticosteroids was used more frequently in the group with elevated Serum IgA level. Pozzi et al. (2010) have failed to demonstrate an additional benefit of low-dose azathioprine added to corticosteroids in patients with IgA nephropathy. However, an important limitation of all studies on the issue of therapy is a short observation period taking into account a slowly progressing nature of this disease (Tanaka et al. 2011).

The mean follow-up time in the present study, amounting to around 4 years, also cannot truly reflect the effects of the long-lasting course of the disease. The Kaplan-Meyer analysis failed to substantiate a relation between a decline in

GFR in the follow-up period and a baseline serum IgA level. That, however, does not eliminate the possibility that a pathological IgA contributes to the pathogenesis of the disease, but the association between the renal function and humoral pathology is not straightforward. In contrast, reduced baseline GFR seems a poor prognostic factor, as shown in previous studies (Coppo and D'Amico 2005).

In view of the data suggesting a key role of galactose-deficient IgA in the pathogenesis of IgAN, it seems that the serum level of galactose-deficient IgA1 may be a prognostic factor in children, but the resolution of this issue requires further studies. In conclusion, in children with IgA nephropathy, elevated baseline serum IgA may be associated with mesangial hypercellularity and segmental sclerosis, but has no effect on the presence of proteinuria or decline in renal function in the course of the disease.

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

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Product Failures in Respirators and Consumables: Analysis of Field Safety Notices of 2005–2013 Publicized by the Federal Institute for Drugs and Medical Devices in Germany

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Abstract

The current European system governed by the three EC directives 93/42/EEC (Medical Device Directive), 98/79/EC (In-Vitro Diagnostic Directive) and 90/385/EEC (Active Implantable Medical Device Directive) regulates marketing and post-market surveillance of medical devices in the European Economic Area (EEA). In cases of incidents raising the field safety corrective actions (FSCA), manufacturers have to inform the responsible Competent Authority (CA; in Germany this is the Federal Institute for Drugs and Medical Devices, BfArM) and the public by field safety notices (FSN). In this study we analyzed FSN of respirators and consumables directly required for their function, whereas devices for anesthesia and gas delivery were excluded. FSCA and FSN of 2005–2013 publicized by BfArM for the included products were analyzed with respect to the MEDDEV 2.12-1 rev. 8. In total, 60 FSCA were publicized. German and English FSN were found in 59/53 cases, respectively. FSN were clearly characterized as FSN in 44/38 cases and declaration of the type of action in 45/44 cases, respectively. Product names were provided in all cases. Lot numbers or other information for product characterization were available in 7/7 and 43/40 cases, respectively. Detailed information regarding FSCA and product malfunction was found in all cases. Information on product related risks with previous use of affected devices was provided in 42/38 cases. In 53/53 cases manufacturers provided information to mitigate product related risks. Requests to pass FSN to persons needing awareness in the organization

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were found in 27/24 cases. Contact data were provided in 53/48 cases, respectively. Confirmation that a CA was informed was found in 28/26 cases and in 19/15 cases a customer confirmation was included. The identified risks were: total loss of function (19/16), short circuit (1/1) and burn (3/3), and inhalation of foreign particles (1/1) which might cause severe risk to patients and users. The most frequent FSCA were product modifications and customer information. The data suggest that there is an annually increasing number of FSCA on devices included in our study. Most FSN fulfill the criteria of MEDDEV 2.12-1 rev. 8. However, there are differences between German and English FSN, e.g., regarding the distribution to persons needing awareness, missing statement that a CA was informed, and missing customer confirmation. Due to the importance of FSN for reduction of product related risks in FSCA, the type and content of FSN should be further improved.

Keywords
Directive 93/42/EEC • MEDDEV 2.12-1 rev. 8 • Medical devices • Respirators

1 Introduction

The European Directive 93/42/EEC, which has been finally amended in 2007, regulates conformity assessment, marketing and post-marketing surveillance of medical devices in Europe (Council Directive 93/42/EEC of 14 June 1993 concerning medical devices; Directive 2007/47/EC of the European Parliament and of the Council of 5 September 2007). Its substance has been implemented in Germany by means of the German Law on Medical Devices (MPG, Medizinproduktegesetz) in 1994, latest amended in 2012 (Gesetz über Medizinprodukte (Medizinproduktegesetz – MPG) 1994; Gesetz über Medizinprodukte (Medizinproduktegesetz – MPG) 2002). It is flanked by the Ordinance on the Medical Devices Vigilance System (MPSV, Medizinprodukte-Sicherheitsplanverordnung) from June 24th 2002, which has been subject of revision, too (Verordnung über die Erfassung, Bewertung und Abwehr von Risiken bei Medizinprodukten 2002).

Manufacturers shall be obliged to systematically review the experience gained from devices on the market, to implement corrective actions, where necessary and to report incidents and recalls to the responsible competent authority (CA). In Germany, according to the Ordinance on the Medical Devices Vigilance System also professional operators and users have to report incidents that they observe when using the products to the CA (Verordnung über die Erfassung, Bewertung und Abwehr von Risiken bei Medizinprodukten 2002; Guidelines on a Medical Devices Vigilance System 2013; Medical Devices Post Market Surveillance 2006). The same obligation applies to pharmacies and other retail traders if they notice incidents related to over-the-counter products (OTC) sold by them to lay people. Except a few *in vitro* diagnostics (IVD) listed in Annex II of Directive 98/79/EC, the Federal Institute for Drugs and Medical Devices (BfArM, Bundesinstitut für Arzneimittel und Medizinprodukte) in Germany is responsible for the registration and examination of issues related to all medical devices and most IVD (Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998; Verordnung über die Erfassung, Bewertung und Abwehr von Risiken bei Medizinprodukten 2002; Siekmeier et al. 2008; Siekmeier et al. 2009).

The task of the CA in evaluating the reports or other relevant information is to characterize the risk (concerning the likeliness of occurrence of harm and the severity of harm) in case of product failure and to assess it for acceptability. In case of unacceptable risks, the necessary corrective action is to be determined. If manufacturers have already taken measures in their own responsibility, the CA has to take a decision on whether or not these are adequate. Field safety corrective actions (FSCA) performed by the manufacturers must be properly communicated to customers and users. This is typically done by contemporary sending of field safety notices (FSN), which have to be sent to the BfArM for information and for publication on the homepage of this CA. Then, BfArM publicizes brief information regarding the affected product and the corresponding FSN on its homepage (BfArM, Homepage 2014).

In principle, CE-marked medical devices and IVD enjoy free movement in the entire European Economic Area (EEA). In consequence, there is a need for an exchange of information between CAs, in particular when an FSCA is to be taken. According the Directive European CAs inform each other and the European Commission by means of a national Competent Authority report in cases that lead to FSCA. After being informed, all CAs can monitor the FSCA in their area of responsibility (if this is deemed to be necessary) and also consider if similar products of other manufacturers may also be affected by the observed problem.

Although Directive 93/42/EEC concerning medical devices and German law on medical devices have been implemented 20 years ago and IVD were included into European and national regulation only about 5 years later, there are up to now limited data regarding the experience on market surveillance in Germany reflecting specific medical devices except IVD (Halbauer et al. 2009; Siekmeier and Lütz 2007a, b; Siekmeier et al. 2008). For IVD, large differences with respect to causes of product failure and consecutive risks for patients and users, and corrective measures performed by the manufacturers have been reported (e.g., IVD for lay use *vs*. IVD for professional use, analyzers *vs*. tests, reagents, control materials, calibrators, and microbiological growth media, IVD for use in clinical chemistry, hematology, coagulation diagnostics, or infection testing) (Siekmeier and Lütz 2007a, b; Siekmeier et al. 2008; Siekmeier and Wetzel 2013a, b). However, medical devices also show large differences with respect to their design and use. Thus, it is likely that there are also relevant differences with respect to types and causes of product failure, risks for patients due to product failure, and the type of the performed measures for risk mitigation. In consequence, product specific analysis should be made for the identification of product specific risks, which may serve for further risk reduction. In cases of product failure related to products already in the market, FSN play a central role for risk reduction. The requirements for FSN are described in MEDDEV 2.12-1 rev. 8 (Guidelines on a Medical Devices Vigilance System 2013; Hannig and Siekmeier 2015). However, up to now there are only few studies investigating the quality of FSN sent by the manufacturers in case of FSCA, which focus on IVD (Hannig and Siekmeier 2015; Siekmeier et al. 2010). Therefore, in the present study we analyzed FSCA and FSN of respirators and their consumables with respect to the criteria of MEDDEV 2.12-1 rev. 8.

2 Methods

In the present study, all notifications on medical devices received by the BfArM between 1.1.2005 and 31.12.2013 were included as there had been no relevant number of publications of FSCA on the BfArM homepage before that time. Detailed analysis was made for respirators (separately for clinical or intermediate care and home care respirators, based on product description or most likely use) and consumables needed for their operation (e.g., valves and tubes but not devices in direct contact with patients, e.g., tracheal cannulae and tubes). Other medical devices also used in respiratory care (e.g., anesthetic apparatuses, humidifiers, and equipment

Table 1 Required content of the field safety notice (FSN) according the guideline MEDDEV 2.12-1 rev. 8

1.	A clear title, with "Urgent field safety notice" followed by the commercial name of the affected product, an FSCA-identifier (e.g., date) and the type of action.
2.	Specific details to enable the affected product to be easily identified, e.g., type of device, model name and number, batch/lot or serial numbers of affected devices, and order number.
3.	A factual statement explaining the reasons for FSCA, including description of the device deficiency or malfunction, clarification of the potential hazard associated with the previous use of the device and the associated risk to the patient, user or other person and any possible risks to patients associated with previous use of affected devices.
4.	Advice on actions to be taken by the user. Include as appropriate:
	identifying and quarantining the device,
	method of recovery, disposal or modification of device,
	recommended review of patients previous results or patient follow up, e.g., implants, IVD,
	time lines.
5.	A request to pass the field safety notice to all those who need to be aware of it within the organisation and to maintain awareness over an appropriate defined period.
6.	A request for the details of any affected devices that have been transferred to other organizations, to be given to the manufacturer, and for a copy of the field safety notice to be passed on to the organization to which the device has been transferred.
7.	A request that the recipient of the field safety notice alerts other organizations to which incorrect test results from the use of the devices have been sent; e.g., failure of diagnostic tests.
8.	Confirmation that the relevant National Competent Authorities have been advised of the FSCA.
9.	Any comments and descriptions that attempt to:
	serve to play down the level of risk in an inappropriate manner
	advertise products or services should be omitted.
10.	Contact point for customers how and when to reach the designated person. An acknowledgment form for the receiver might also be included (especially useful for manufacturer's control purposes).

Guidelines on a Medical Devices Vigilance System (2013)

for gas supply) were also excluded. For the included FSCA, FSN were analyzed with respect to the criteria of MEDDEV 2.12-1 rev. 8 (Table 1) and analyzes were carried out separately for FSN in German and English language, and for different product groups.

3 Results

3.1 Number of Reports and FSCA

A total of 61,904 and 50,053 reports regarding medical devices was received by the BfArM between January 1, 2000 – December 31, 2013 and January 1, 2005 – December 31, 2013, respectively, which demonstrates a strong increase in the number of notifications (2005 *vs.* 2000: +75.1 %, 2013 *vs.* 2000: +326.7 %, and 2013 *vs.* 2005: +143.6 %) (Table 2). The analysis of notifications regarding the product groups of medical devices in total (both active and non-active), active devices, non-active devices, and IVD demonstrated that the vast majority of notifications were related to medical devices (57,356, 28,715, 28,641, and 4548 notifications between January 1, 2000 and December 31, 2013, and 45,938, 23,394, 22,544, and 4115 notifications between January 1, 2005 and December 31, 2013, respectively). Between January 1, 2005 and December 31, 2013, a total of 6465 FSCA were publicized by the BfArM, which also demonstrates a strong increase (2013 *vs.* 2005: +123.0 %). Again, most notifications were related to medical devices (active and non-active) and only a minor number to IVD (4912 *vs.* 1553 notifications, respectively). Based on the inclusion criteria of our study, only FSCA related to respirators and their direct consumables were analyzed. In detail, there were 60 FSCA, from which 53 were related to respirators (35 for clinical or intermediate care and 18 for home care) and 7 to consumables (for clinical or intermediate care and for home care).

Table 2 Number of notifications to the BfArM 2000–2013 and number of FSCA publicized by the BfArM 2005–2013

Year	Active medical devices except IVD	Non-active medical devices except IVD	All medical devices except IVD	IVD	All
	Notifications	Notifications	Notifications (FSCA)	Notifications (FSCA)	Notifications (FSCA)
2000	926	987	1913 (---)	21 (---)	1934 (---)
2001	906	1080	1986 (---)	33 (---)	2019 (---)
2002	995	1213	2208 (---)	58 (---)	2266 (---)
2003	1116	1298	2414 (---)	121 (---)	2535 (---)
2004	1378	1519	2897 (---)	200 (---)	3097 (---)
2005	1515	1665	3180 (348)	207 (135)	3387 (483)
2006	1769	1858	3627 (391)	235 (116)	3862 (507)
2007	2179	1884	4063 (388)	583 (150)	4646 (538)
2008	2290	2087	4377 (533)	506 (143)	4883 (676)
2009	2361	2141	4502 (615)	392 (149)	4894 (764)
2010	2792	2506	5298 (544)	482 (180)	5780 (724)
2011	3269	2395	5664 (666)	474 (194)	6138 (860)
2012	3400	4238	7638 (646)	573 (190)	8211 (836)
2013	3819	3770	7589 (781)	663 (296)	8252 (1077)
All	28,715 from these 23,394 since begin 2005	28,641 from these 22,544 since begin 2005	57,356 from these 45,938 since begin 2005 (4912 FSCA since begin 2005)	4548 from these 4115 since begin 2005 (1553 FSCA since begin 2005)	61,904 from these 50,053 since begin 2005 (6465 since begin 2005)

Siekmeier and Lütz (2007b), Siekmeier et al. (2008), Siekmeier et al. (2009), Siekmeier and Wetzel (2013b), BfArM homepage (2014)

3.2 Fulfillment of MEDDEV Criteria

German and English FSN were found in 34/34 cases (18/13; results for home care respirators in parentheses), respectively (Table 3). FSN were clearly characterized as FSN in 26/24 (12/9) cases and a declaration of the type of action was found in 28/30 (13/9) cases, respectively. Product names, detailed information regarding FSCA and description of product malfunction were found in all German and English FSN of both subgroups. Lot numbers or other information for product characterization were available in 1/1 (1/1) and 28/29 (9/7) cases, respectively. The information on product related risks with previous use of affected devices was provided in 26/26 (12/9) cases. In 31/34 (17/13) cases manufacturers provided information to mitigate product related risks. Comments or descriptions downplaying the risk, e.g., "Quality and safety are our highest priority", "Please be assured that maintaining a high level of safety and quality is our highest priority", "Quality and safety have always been and remain our highest priority", were observed in 8/11 (3/2) cases. However, there were no cases of direct advertising, e.g., demonstration of new products and articles. Requests to pass FSN to persons needing awareness in the organization were found in 20/18 (6/5) cases. Contact data were provided in 31/30 (16/12) cases. The confirmation that a CA was informed was found in 16/16 (9/7) cases and in 7/7 (8/4) cases a customer confirmation form was included.

The analysis for fulfillment of MEDDEV criteria was also made for the group of consumables with 7 FSCA. However, as the number of FSN was small (7 German and 6 English) we did not further differentiate between consumables for clinical or intermediate care respirators and those for home care respirators (5/4 and 2/2 cases, respectively). German and English FSN were clearly characterized as FSN in 6/5 cases, respectively (Table 3). A declaration of the type of action was stated in 4/5 cases, respectively. Product names, detailed information regarding

Table 3 Formal compliance of the FSN with the criteria of Guideline MEDDEV 2.12-1 rev. 8

	Clinical care respirators	Home care respirators	All respirators	All consumables	Total
	German/ English	German/ English	German/ English	German/ English	German/ English
Number of FSCA	35	18	53	7	60
Number of FSN	34/34	18/13	52/47	7/6	59/53
FSN is clearly to identify as FSN for users	26/24	12/9	38/33	6/5	44/38
Declaration of the product name in the FSN	34/34	18/13	52/47	7/6	59/53
Declaration of the type of action	28/30	13/9	41/39	4/5	45/44
Declaration of the Lot-No.	1/1	1/1	2/2	5/5	7/7
Other attributes for product identification	28/29	9/7	37/36	6/4	43/40
Information regarding the reason of the FSCA	34/34	18/13	52/47	7/6	59/53
Description of the device malfunction in the FSN	34/34	18/13	52/47	7/6	59/53
Clarification of potential hazard associated to use	26/26	12/9	38/35	4/3	42/38
Directions for mitigation of product related risk	31/34	17/13	48/47	5/6	53/53
Request to pass the FSN to other persons who need to be aware	20/18	6/5	26/23	1/1	27/24
Comments to downplay the situation	8/11	3/2	11/13	1/0	12/13
Declaration of a contact person or phone number	31/30	16/12	47/42	6/6	53/48
Confirmation that the relevant national CA has been informed	16/16	9/7	25/23	3/3	28/26
Acknowledgement form for the receiver included in the FSN	7/7	8/4	15/11	4/4	19/15

Guidelines on a Medical Devices Vigilance System (2013)

FSCA and description of product malfunction were found in all German and English FSN. Lot numbers or other information for product characterization were available in 5/5 and 6/4 cases, respectively. The information on product related risks with previous use of affected devices were provided in 4/3 cases. In 5/6 cases manufacturers provided information to mitigate product related risks. Comments or descriptions downplaying the risk, e.g., "We all see the patient in the center of our work" were observed in 1/0 case, respectively; however there were again no cases of direct advertising. Requests to pass FSN to persons who need to be aware in the organization were found in 1/1 case and contact data were provided in 6/6 cases. The confirmation that a CA was informed was found in 3/3 cases and in 4/4 cases a customer confirmation form was included.

We further analyzed the FSN for information regarding types and causes of product malfunction, risks for patients or users, and the type of measures recommended by the manufacturers. In case of respirators, corresponding analyzes were made separately for the subgroups of clinical care and intermediate care respirators and home care respirators (Table 4). In clinical care and intermediate care respirators, the information on product malfunction was given in 34/34 of German and English FSN and most frequent types of product malfunction were software issues (11/10 cases, e.g., stop of ventilation, display failure, or incorrect alarm). Other types of failure were faulty electrical components (8/8 cases, e.g., fault in conductor board, installation of a defective wire, switch or central processing unit (CPU), or decreased battery life time). Further cases were incomplete instructions for use

Table 4 Qualitative analysis of FSN according to the criteria of Guideline MEDDEV 2.12-1 rev. 8

	Clinical care respirators	Home care respirators	All respirators	All consumables	Total
	German/ English	German/ English	German/ English	German/ English	German/ English
Number of FSCA	35	18	53	7	60
Number of FSN	34/34	18/13	52/47	7/6	59/53
Cause of product failure	34/34	18/13	52/47	7/6	59/53
Manufacturing error	1/1	3/1	4/2	3/2	7/4
Electrical failure	8/8	7/7	15/15	1/1	16/16
Alarming issue	2/2	1/1	3/3	---	3/3
Software issue:	11/10	6/4	17/14	---	17/14
Error of communication	---	2/1	2/1	---	2/1
Error of control system	1/0	1/0	2/0	---	2/0
Alarm issue	3/3	1/1	4/4	---	4/4
Battery issue	---	1/1	1/1	---	1/1
Failure of ventilation	4/4	---	4/4	---	4/4
Display error	3/3	1/1	4/4	---	4/4
Incomplete instruction for use	2/2	---	2/2	---	2/2
No clear identification	10/11	1/0	11/11	3/3	14/14
Risk for patients/users	26/26	12/9	38/35	4/3	42/38
No or minor risk	6/7	4/3	10/10	1/0	11/10
Not clarified risk	5/5	1/1	6/6	1/1	7/7
Stop of ventilation	14/13	5/3	19/16	---	19/16
Electrical shock	1/1	---	1/1	---	1/1
Burn	---	2/2	2/2	1/1	3/3
Inhalation of foreign particle	---	---	---	1/1	1/1
Instructions for user	31/34	17/13	48/47	5/6	53/53
Stop of use	---	4/3	4/3	3/4	7/7
Return to manufacturer	1/1	1/0	2/1	---	2/1
Additional safety instructions	15/16	2/2	17/18	2/2	19/20
Product modification	15/17	10/8	25/25	---	25/25

Guidelines on a Medical Devices Vigilance System (2013)

(2/2 cases), alarms triggered without software failure (2/2 cases), and manufacturing error (1/1 case, failure in valve assembly). The remaining failures (10/11) were difficult to discriminate. In the same subgroup, the information on product related risks with previous use of affected devices was provided in 26/26 of German/ English FSN. Described risks were ventilation errors (14/13 cases, e.g., due to breakdown or malfunction of the respirator, missing or delayed alarms, or monitor failure), or electrical shock to patients or users (1/1 due to a defective power pack). In 5/5 FSN, customers were informed about a risk for patients, but a precise indication of the risk with further use was not provided in the FSN. In 6 German and 7 English FSN the manufacturer informed customers that no risk was identified and normal and safe handling of the device is possible. The information to mitigate product related risks was given in 31/34 German and English FSN. In detail, these were provision of additional safety instructions (15/16 cases) and modification of the product (15/17 cases, e.g., installation of a software-update, or exchange of defective components either by service engineers or users), whereas stop of use (0/0) and return to the manufacturer (1/1) obviously played minor roles.

In home care respirators information on product malfunction was given in 18/13 of German and English FSN, respectively. Typical types of product malfunction were also software issues

(6/4 cases; e.g. error in communication, erroneous alarming or display errors), issues with electrical components (7/7 cases; e.g., failure in CPU boards, defective capacitor or line connectors, depletion of battery) and errors in production (3/1 cases; e.g., wrong attachment of a bogie wheel or deviation during manufacturing). The remaining case (1/0) was difficult to classify; however, the manufacturer performed a reduction of the recommended replacement intervals of the blower assembly to avoid loss of function.

Information on product related risks with previous use of affected devices was provided in 12/9 of German/English FSN and described risks were also erroneous ventilation of patients and stop of therapy (5/3 cases, e.g. due to break down, severe malfunction of the respirator, missing or delayed alarms or monitor failure) and fire hazard including burn of patients and users (2/2 cases, e.g. due to a thermal damage of the device or a higher probability of spark formation). No risk with the further use of the product was described in 4/3 cases, and in one case (1/1) affected customers were only informed about a possible, but no more specified risk for patients. Finally, information to mitigate product related risks was found in 17/13 German and English FSN. Most frequently these were modification of the product (10/8 cases, e.g. exchange of defective components, installation of a software-update or sticking of a warning label on the device). Other measures were stop of use, product destruction or quarantine (4/3 cases) or additional instructions for use (2/2 cases, e.g. revision of the user manual or restriction of the application) and request for sending the affected product back to the manufacturer (1/0). In summary, our analysis for types and causes of product malfunction, risks for patients or users and types of measures recommended by the manufacturers revealed no obvious differences between respirators for clinical and intermediate care compared to those for home care.

The corresponding analysis in the group of consumables did not differentiate between the distinct types of respirators, as the number of cases was much smaller. However, types and causes of product malfunction, risks for patients or users and types of measures recommended by the manufacturers showed some differences when compared to the respirator groups. In detail, in German and English FSN (7/6 cases) reported causes of product malfunction were manufacturing errors (3/2 cases; e.g., remains of glue or saw dust or misalignment of components during manufacturing), and electrical failure (1/1 case, failure in the first charging process of the rechargeable battery). In the remaining 3/3 cases, a definite characterization was not possible. The information on product related risks with previous use of affected devices was provided in 4/3 of German/English FSN and risks were ventilation failure in patients (1/1, particle inhalation) and risk of thermal damage and smoke generation (1/1 case). In the remaining FSN, there was only the information on a remote risk (1/0) or the risk was not exactly explained (1/1). Finally, information to mitigate product related risks was found in 5/6 German and English FSN. Most frequently, this information was to stop the use, reshipment or use of a substitute product (3/4 cases), and corrective measures like additional instructions for use (2/2 cases, e.g., modification of the user manual or an addendum to the instruction for use).

Comparison of German and English FSN in few cases demonstrated differences in the number of complying MEDDEV criteria, differences of the FSN for the affected products (product or lot numbers) and differences of provided contact data of the manufacturers. In contrast, comparison of German and English FSN for potential differences of the measures to be taken by the users (e.g., due to differences in customer education) revealed no relevant deviations.

4 Discussion

MEDDEV 2.12-1 rev. 8 regulates the requirement of FSN publicized by the manufacturers in cases of FSCA due to product failure (Guidelines on a Medical Devices Vigilance System 2013). However, the number of publications investigating the quality of FSN is sparse

(Hannig and Siekmeier 2015; Siekmeier et al. 2010). The present study analyzes the quality of FSN publicized for FSCA related to respirators and their direct consumables with respect to the criteria of MEDDEV 2.12-1 rev. 8. The observation period of 2005–2013 was chosen, since there is an increasing number of European CAs publicizing information on FSCA on their homepages from 2005. However, CAs usually provide only information regarding FSCA affecting the country for which the CA takes responsibility and there are large differences regarding the publication policy between distinct CAs. Thus, the number of publicized FSCA and affected products differs strongly between distinct European CAs. The highest number of FSCA is publicized on the homepage of the German BfArM. In principle, this may reflect both the size of the German market for medical devices and IVD, which is the largest in Europe and one of the largest worldwide, and the publication policy of this CA. Therefore, we analyzed only data publicized on the homepage of BfArM.

The number of notifications to the BfArM related to medical devices and IVD since the year 2000 showed a strong increase (2005 *vs*. 2000: +75.1 %, 2013 *vs*. 2000: +326.7 %, and 2013 *vs*. 2005: +143.6 %). There was also a strong increase of the corresponding FSCA (2013 *vs*. 2005: +123.0 %). The data obtained for the German market are in some accordance with the results of Heneghan et al. (2011) for the number of FSCA in the UK, showing an increase from 62 in 2006 to 757 in 2010; i.e., by 1220 %. However, the reported strong increment is influenced by two factors, the low number of FSCA at the beginning in 2006 and the number of Medical-Device Alerts additionally publicized by the Medicines and Healthcare Products Regulatory Agency, which showed a much smaller increase but were not included in the total number (from 73 in 2006 to 100 in 2010, +37.0 %). The ongoing increase of notifications may reflect both the increasing acceptance of the European system (firstly established in 1994 for medical devices and 1998 for IVD (Council Directive 93/42/EEC of 14 June 1993 concerning medical devices; Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998) and an underreporting in the past.

Within the observation period of our study a total of 50,053 notifications were received by the BfArM and a number of 6465 FSCA was publicized for medical devices and IVD on the homepage of this CA (BfArM Homepage 2014). The comparison of the relative numbers of notifications and FSCA for medical devices and IVD indicates a relevant difference between the major group of medical devices (91.8 % of notifications, 76.0 % of FSCA) and the minor group of IVD (8.2 % of notifications but 24.0 % of FSCA). Likely, inclusion of IVD in the responsibility of the Paul-Ehrlich-Institute (IVD listed in Annex II of Directive 98/79/EC serving for infection testing and immune hematological diagnostics) would not abolish this difference as the number of notifications to this German CA is much smaller than that to BfArM (Halbauer et al. 2009; Hannig and Siekmeier 2015). However, the Paul-Ehrlich-Institute provides no information regarding FSCA related to IVD. The obvious difference between medical devices and IVD points to differences between these types of products with respect to their clinical use (e.g., patient treatment *vs*. diagnostics), product type (active or non-active medical device *vs*. IVD), and potential risk (risk of direct harm for users and patients mainly in medical devices *vs*. risk for indirect harm for patients mainly in IVD). Due to the strong heterogeneity of products, there are also large differences within the groups of IVD (e.g., lay use *vs*. professional use, tests and reagents *vs*. analyzers and clinical indications (e.g., clinical chemistry, hematology, or microbiology)) (Siekmeier and Lütz 2007a, b; Siekmeier et al. 2008; Siekmeier and Wetzel 2013a, b), and medical devices (e.g., active *vs*. non-active devices, implantable *vs*. non-implantable devices, and devices risk classes) demonstrating the need for a product specific analysis.

In the present study we analyzed 60 FSCA related to respirators and their direct consumables and also looked for product specific differences between different types of respirators

(clinical and intermediate care *vs*. home care) and their direct consumables. The included products resemble only 1.2 % of all FSCA related to medical devices, except IVD publicized by the BfArM in 2005–2013, but this very specific group of devices bears a high potential risk for patients in case of product failure. German and English FSN were found in 59 and 53 out of 60 FSCA, respectively. Most likely, the differences between the numbers of FSCA and FSN and the numbers of German and English FSN are caused by a number of missing FSN in cases where no FSN has been sent to the BfArM (e.g., information of few customers by phone only). The analysis for fulfillment of formal criteria of MEDDEV 2.12-1 rev. 8 revealed a very homogenous pattern with only minor differences between the different subgroups of devices included in this study. Most FSN were clearly characterized as FSN (44/38), specified a type of action (45/44, e.g., recall, corrective measure, or safety information) and included the names of the affected products (59/53). Lot numbers (7/7) and other attributes for product identification (43/40) were also provided by most FSN, but the number of FSN complying with the MEDDEV criteria was slightly smaller even though distinct product identification is essential in cases of FSCA. Obviously, provision of lot numbers was more frequent in FSN for consumables, whereas in FSN for respirators identification of affected products was mainly based on product name, serial-, item-, or list number, a difference which is most likely due to product specific characteristics. However, there was also a minor number of FSN providing different types of these criteria for product identification. Sufficient information regarding the reason for FSCA and description of product malfunction was provided in all FSN analyzed in the present study. The proportion of FSN with description of product malfunction was higher than in our prior study analyzing IVD for infection testing (144 out of 157 German and 136 out of 154 English FSN in a total of 169 FSCA), indicating a better consideration of the MEDDEV criteria in these high risk medical devices (Hannig and Siekmeier 2015). A similar behavior was observed for the confirmation that the relevant national CA was informed (respirators and consumables: 28 out of the 59 German (47.5 %) and 26 out of the 53 English (49.1 %) FSN, IVD for infection testing: 39 out of the 157 German (24.8 %) and 37 out of the 154 English (24.0 %) FSN) also reflecting the higher risk in the group of medical devices. In contrast, there was an unexpectedly lower proportion of FSN containing the request to pass the FSN to other persons who need to be aware (respirators and consumables: 27 out of the 59 German (45.8 %) and 24 out of the 53 English (45.3 %) FSN, IVD for infection testing: 108 out of the 157 German (68.8 %) and 87 out of the 154 English (56.5 %) FSN) (Hannig and Siekmeier 2015). Such a spread of information is important because different users and split responsibilities may exist within organizations and the corresponding information should be included especially in FSN for high risk medical devices. A critical non-compliance to the requirements of MEDDEV 2.12-1 rev. 8 was found regarding the clarification of the potential hazard associated to previous use of the affected product (42/38 out of the 59/53 German and English FSN, i.e., only 71.2 % and 71.7 % of FSN, respectively). However, these data are in accordance with the results of our previous study in IVD for infection testing (116/116 out of the 157/154 German and English FSN, i.e., 73.9 % and 75.3 % of FSN, respectively) (Hannig and Siekmeier 2015). Such noticeable deviation from the requirements of the MEDDEV 2.12-1 rev. 8 should be avoided as clear descriptions in these points serve as the basis for understanding the FSCA and required measures to be performed by the users for risk reduction. Information to mitigate product related risks was found in 53 out of the 59 German and 53 out of the 53 English FSN for respirators and consumables (89.8 % and 100.0 %, respectively) and the obtained proportions are very similar to those reported for IVD for infection testing (156 out of the 157 German and 152 out of the 154 English FSN, 99.4 % and 98.7 %, respectively). Although these numbers are high, one should consider that this information is essential in FSN in cases of

product failure. However, a strong difference was observed in comparison to IVD. In the present study there were no recommendations for retesting or control of obtained results, whereas such recommendations were found in 69 out of the 157 German and 75 out of the 154 English FSN for IVD for infection testing (Hannig and Siekmeier 2015). This difference can be explained by different types of included products in both studies. In the present study medical devices served for patient treatment and therefore control of results or retesting played no role. In contrast, IVD serve exclusively for diagnostics and recommendation of control of results or retesting serves for correction of obtained erroneous results due to product failure. Furthermore, it is likely that product failures in medical devices for use in respirators (e.g., obvious lack of function or impairment of ventilation) are more easily detectable than plausible but incorrect results in IVD tests. In the present study, contact data were provided in 53 out of the 59 German and 48 out of the 53 English FSN (89.8 % and 90.6 %, respectively) and the observed proportions were similar to those reported before for IVD (127 out of the 157 German and 131 out of the 154 English FSN, 80.9 % and 85.1 %, respectively). However, provision of contact data may be critical as users may have queries regarding the FSCA in cases of product failure. Comments, which mitigate the situation, were found in a minor number of FSN (12 out of the 59 German and 13 out of the 53 English FSN, 20.3 % and 24.5 %, respectively) and the proportion was similar as reported for IVD for infection testing before (21.6 % and 20.1 %, respectively) (Hannig and Siekmeier 2015). Even though proportions are low, in our view this is critical because FSN are issued in cases of product failure and product related risk and these comments may foil the intention of the FSN. Customer confirmation forms were found in only 19 out of the 59 German and 15 out of the 53 English FSN (32.2 % and 28.3 %, respectively) and the observed proportions were much smaller than in IVD for infection testing (132 out of the 157 German and 111 out of the 154 English FSN, 84.1 % and 72.1 %, respectively). A possible explanation might be that customer information forms were not sent to the BfArM by the manufacturers. However, even though a miss of this form is not relevant for risk reduction they should be included in the FSN as they are useful for manufacturer's control purposes.

Most cases of product failures were due to design or manufacturing deficiencies, e.g., fault in construction and software issues and product manufacturing. In principle, this confirms prior results reported by Davis et al. (2011) who analyzed FSN relating to medical device recalls in 2005–2009 publicized on the European Commission (EC) National Competent Authority Report (NCAR) database. Those authors observed that the issues related to medical devices in connection to cardiovascular recalls are almost exclusively design or manufacturing issues, while other therapeutic areas have more diverse reasons for a recall. It also confirms the results reported by the Battelle Memorial Institute which reviewed FDA high-priority Class I recalls from January 2005 to May 2010 and reported that approximately 50 % of the recall causes of devices were attributed to design deficiencies (flaw inherent in the design of the device, either created initially or through approved design changes), 29 % to manufacturing deficiencies (failure to maintain sterility, failure to follow Good Manufacturing Practices, or manufacturing quality control (QC) deficiencies), and 6 % to labeling deficiencies. It should be considered that this study analyzed Class I recalls (high risk) according to FDA classification representing only a subset of all recalls which may differ from Class II (moderate risk) and Class III (low risk) recalls (Battelle Memorial Institute 2010; Davis et al. 2011; Heneghan et al. 2011), whereas our study did not discriminate FSCA with respect to the underlying risk. Moreover, authors of the Battelle Memorial Institute did not differentiate between the distinct types of medical devices including IVD and it is likely that there are relevant differences. For example, analyzing IVD for diagnostics of infectious diseases (mostly tests and reagents, few analyzers based on cultural means) in a prior study we observed a very

heterogeneous spectrum of product failures described in the FSN released by the manufacturers. In detail, in these IVD manufacturing issues and raw material failures were also an important source of malfunction (27 out of the 147 cases), but many other examples of product deficiencies, e.g., microbiological contaminations of the product, packaging issues or failure in the package inserts were also found (Hannig and Siekmeier 2015).

A more detailed analysis of respirators and consumables concerning design or manufacturing deficiencies identified a very high risk of electrical failures (clinical respirators (8/8; German/English FSN), respirators for home use (7/7), and consumables (1/1)), most likely due to the electrical engineering of this product group. Errors in manufacturing seem only to be a minor problem in respirators (respirators for home use (3/1) and clinical respirators (1/1). In contrast, the product group of consumables is mainly characterized by manufacturing failures (3/2). However, it should be mentioned that the identification of the design or manufacturing deficiencies was rather difficult and a specific characterization was not possible in (14/14) cases.

Due to the specific character of the product, all software issues were found in respirators (respirators for clinical use (11/10) and home use (6/4)). Most frequent consequences of software issue in clinical respirators were ventilation failures (4/4), display errors (3/3), and alarm-linked issues (3/3). The control system was affected by a software issue in one case of clinical respirators (1/0). In home care respirators, communication issues provoked by software issues were most frequent (2/1), whereas other consequences of software issues were heterogeneous (error of control system (1/0), alarm-linked issue (1/1), battery issue (1/1) and display error (1/1)).

In principle, failures of medical devices and IVD can cause two types of harm for patients, users, or third persons. These are direct harm, e.g., due to burn, electrical hazard, bleeding and infection (mostly medical devices), and indirect harm due to erroneous or delayed results of analyzes (typically IVD (Siekmeier and Lütz 2007a, b; Siekmeier et al. 2008, 2009)). Respirators and their consumables only bear the risk of direct harm. For example, respirators, but usually not their consumables, can cause direct harm to patients and users by electrical failure and burn. On the other hand, both respirators and consumables may cause direct harm to patients, but not users, due to ventilation failure, e.g., failure of the respirator, missing alarms, and inhalation of particles. Therefore, there is a need for product specific analyzes considering differences between the distinct medical devices. Analysis of 59/53 German and English FSN out of the 60 FSCA in the present study demonstrated cessation of lung ventilation as the predominant risk due to respirator failure (19/16 German and English FSN, respectively), whereas other risks, e.g., electrical shock, burn, and inhalation of foreign particles played minor roles, and the information that there was no or only a remote risk was given in 11/10 FSN. The comparison with the corresponding results of our previous study of IVD (4 out of the 157 German and 4 out of the 157 English FSN) shows that the proportion of described risks for direct harm in FSN for respirators and their consumables was largely higher and the type of risk in IVD (risk of poisoning, contamination, stick injury, or infection) was largely different (Hannig and Siekmeier 2015). Most likely, the difference is caused by a high number of active (electrical) medical devices in the present study compared to IVD (mostly tests and reagents) in that prior study. In 7/7 FSN of the present study, information regarding the risk for the patients was missing which should be avoided because FSN serve for mitigation of risk due to product failure.

According to the requirements of MEDDEV 2.12-1 rev. 8, FSN should include directions for mitigation of product related risks, which should consider the types of product and product failure and the risk for the patient. In the present study, FSN in all cases specified the product failure, but

the risk for the patient was only described in 42/38 German and English FSN, respectively. Typically, manufacturers provided directions for mitigation of product related risks (53/53). These included mostly additional safety instructions (19/20) and modification of the product (25/25), whereas the stop of use (7/7) and return to the manufacturer (2/1) obviously played minor roles. These results strongly contrast our observations in IVD for infection testing, where recall of the products (including discarding the product) played a major role (102 of the 157 German and 102 of the 154 English cases) (Hannig and Siekmeier 2015).

Comparison of German and English FSN not only demonstrates differences in the number of complying MEDDEV criteria but also differences in the affected products, provided contact data of the manufacturers, and the names of the informed CA. These differences are most likely caused by distribution of divergent products or lots, different national subsidiaries of the manufacturers and different responsible CA within different countries and assumed to be not critical. However, there were also cases of differences of requests for passing the FSN to other persons and organizations (e.g., due to different types of hospital organization within different countries), which may be critical and therefore should be subject of thorough evaluation.

In summary, the data show a strong increase of notifications to the BfArM and in consequence also in the number of FSCA, which was due to increases in all groups of medical devices (active medical devices, non-active medical devices, and IVD). The high numbers of notifications and FSCA publicized by the BfArM demonstrate that the European surveillance system is an established tool for ensuring the safety of medical devices and IVD. However, there is a need for some further optimization. For example, as the distribution of FSN in cases of FSCA is an important means to reduce product related risks of medical devices already in the market, the type and content of publicized FSN should comply with the requirements of the Guideline MEDDEV 2.12-1 rev. 8.

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

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Comparative Expression of Apoptotic Markers in Lung Adenocarcinoma and Squamous Cell Carcinoma

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Abstract

Lung cancer is still an oncology challenge. A 5-year survival reaches less than 20 % of patients. Apoptosis disturbances are a key step in cancer development. The evaluation of apoptosis markers has a great potential in lung cancer. The goal of our study was a comparative evaluation of apoptosis regulators: p53, Bcl-2, Bax, COX-2, and survivin in lung adenocarcinoma (AC) and squamous cell carcinoma (SCC). We also evaluated the relationship between apoptosis markers and clinicopathological parameters. Fifty six patients with non-small cell lung cancer (NSCLC) were included into the study (20 women and 36 men). AC was diagnosed in 30 and SCC in 26 cases. The evaluation of markers was performed using an immunohistochemical method on paraffin embedded tissue specimens. We used monoclonal antibodies for p53, bcl-2, and COX2-proteins (clone DO7, bcl-2/100/D5, and 4H12, respectively), Bax (B-9 clone) and survivin (clone 12C4). The results of immunostaining were viewed by light microscopy. We revealed significantly more frequent expression of Bax and survivin in lung AC than SCC ($p < 0.01$ and $p < 0.019$). Bcl-2 immunoreactivity was seen more often in AC without lymph node metastases than with metastases ($p = 0.046$). There was no correlation between the apoptosis markers and gender or the presence of vessel emboli. A greater variability in markers expression was seen in lung AC than SCC. There were significant differences in the Bax and survivin expression in the two major pathological types of NSCLC. We did not revealed any correlation between the markers and TNM characteristics, accept for Bcl-2 presence along with the lymph node involvement in the AC group.

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Keywords
Bax • Bcl-2 • COX-2 • Immunohistochemistry • Lung cancer • p53 • Survivin

1 Introduction

Apoptosis is a very important factor for tissue homeostasis. Programed cell death allows eliminating cells with genetic errors occurred during cell cycle or cells which fulfilled its function and their further existence is not required for tissue integrity. Alterations in apoptosis are key in the development of cancer since they allow surviving of cells with modified genotypes (Shivapurkar et al. 2003). The accumulation of genetic errors triggers tumor formation, changes the function of microenvironment to achieve growth advantage by tumor tissue and finally leads to the creation of metastases (Cheng-Xiong et al. 2009). Apoptosis depends on a large number of proteins whose proper function determines the effectiveness of this multistage process. p53 protein is encoded by the *p53* gene located in 17 chromosome. This protein takes part in cell cycle regulation and plays especially important function in apoptosis initiation. It activates DNA repair after injury or induces programed cell death as a result of critical DNA damage; being called as a guardian of genome integrity. Survivin is a member of the family of apoptosis inhibitor proteins (IAP). The main function of survivin is inhibition of caspase activation and thus stopping the late phase of apoptosis. Survivin plays an active role in mitosis and its level is regulated during cell cycle (Dohi et al. 2005). There is a functional connection between p53 and survivin since p53 regulates survivin gene transcription (Mirza et al. 2002). Bcl-2 and Bax proteins are members of the Bcl-2 family which regulates the intrinsic apoptotic pathway. Bcl-2 blocks the late phase of apoptosis. Bax is important for the mitochondrial membrane permeability and in this way regulates the proapoptotic proteins movement from mitochondria into cytoplasm (Cory et al. 2003). COX-2 is an inducible multifunctional protein involved mainly in inflammatory processes. It also promotes tumor invasion, being proangiogenic, and increases resistance to apoptosis, particularly during anticancer treatment (Greenhough et al. 2009).

Lung cancer is a leading cancer in the world. In 2012, 1.80 million people suffered from lung cancer and 1.59 million people died of it (GLOBCAN 2012). Despite progress in oncology, the prognosis in lung cancer remains poor, with a 5-year survival rate less than 20 %. It is the most common cause of death among men and runs in second place as the cause of death from cancer among women. For many years, two major histological types of non-small cell lung cancer (NSCLC), adenocarcinoma (AC) and squamous cell carcinoma (SCC), were treated in the same way, which explains the deficiency of comparative research and investigation designed separately for the two subtypes. Recently, treatment of advanced lung cancer involved targeted therapy that is directed at a specific histological subtype. This approach follows from molecular diversity, which is still poorly understood with respect to AC and SCC. There are some data that AC and SCC, differ significantly in the pathogenesis and molecular profile and thus probably require a different therapeutic approach (Daraselia et al. 2012). SCC develops usually as a tumor located in the proximal part of bronchial tree and is strongly smoking-dependent. AC, on the other hand, usually arises from the peripheral part of the lung and is more often diagnosed in never smoking patients. The pathogenic steps leading to SCC are fairly well understood, including the appearance of the preceding lesions such as dysplasia or carcinoma *in situ*, but the premalignant lesions of AC are not well characterized. Some molecular changes are more frequent in AC, including mutation in the

genes: *EML-4ALK*, *EGFR*, *KRAS*, but *FGFR1* and *EGFR* amplifications are more frequent in SCC (Wistuba 2012). These differences are as yet inexplicable, so that there is room for further research on disorders of programed death and other fundamental life processes of cancer cells.

Taking into account distinctly different pathogenesis and molecular physiology of NSCLC subtypes and a key role of apoptosis disturbances in lung carcinogenesis, the goal of the present study was to compare the immunohistochemical expression of apoptosis regulators: p53, survivin, Bcl-2, Bax, and COX-2 in AC and SCC.

2 Methods

The study was approved by the institutional Ethics Committee. Fifty six lung cancer patients hospitalized in the South Silesian Center of Pulmonology in the city of Wroclaw, Poland, were enrolled into study. Lung cancer tissue was acquired by curative or diagnostic surgery and diagnostic bronchoscopy. AC was diagnosed in 30/56 (53.6 %) and SCC in 26/56 (46.4 %) cases. The clinical stage was based on the TNM system guidelines (Mirsadraee et al. 2012). The patients' characteristics are presented in Table 1.

Table 1 Patients characteristics

	AC *(n = 30)*	SCC *(n = 26)*
Female/Male	12/18	8/18
TNM		
I	13/30 (43.3 %)	9/26 (34.6 %)
II	5/30 (16.7 %)	8/26 (30.8 %)
IIIA	7/30 (23.3 %)	8/26 (30.8 %)
IIIB + IV	5/30 (16.7 %)	1/26 (3.8 %)
N		
N0	12/30 (40.0 %)	11/26 (42.3 %)
N + (N1 + N2 + N3)	18/30 (60.0 %)	15/26 (57.7 %)
Vessel emboli[a]		
Present	10/26 (38.5 %)	14/25 (56.0 %)
Absent	16/26 (61.5 %)	11/25 (44.0 %)

AC adenocarcinoma, *SCC* squamous cell carcinoma
[a]Evaluation of vessel embolism was made in 51 cases (26 AC and 25 SCC)

The expression of markers of apoptosis was investigated by immunohistochemistry in formalin fixed and paraffin embedded tissue sections. We used monoclonal antibodies against the p53, Bcl-2, and COX-2 proteins (DO7 clone, bcl-2/100/D5, and 4H12, respectively; Novocastra Laboratories, Newcastle, UK), Bax (B-9 clone; Santa Cruz Biotechnology, Santa Cruz, CA), and survivin (clone 12C4; DakoCytomation, Glostrup, Denmark). The immunohistochemistry of p53, Bcl-2, and Bax was performed using an avidin-biotin complex (ABC) technique (Vectastain Universal ABC kit; Vector Laboratories, Burlingame, CA). Survivin and COX-2 were visualized by EnVision technique (System HRP (DAB), DakoCytomation, Glostrup, Denmark). The procedures were performed according to manufacturers' instructions. Negative control was always done by excluding the primary antibody.

The results were evaluated by two independent observers. The p53 immunostaining was evaluated as positive if nuclear accumulation occurred in at least 10 % of cells. The expression of the other tested proteins, except survivin, was defined as positive if more than 20 % of cells showed the presence of a chromogen reaction in the cytoplasm. Survivin was positively assessed if more than 20 % of cells presented cytoplasmic or nuclear immunoreactivity. In addition, the intensity of staining was evaluated as follows: + (low), + + (medium), and + + + (high).

The expression of markers in AC and SCC, and mutual relations among the studied proteins studied were compared using the chi square test with Yates's correction as needed. Differences were regarded as statistically significant at $p < 0.05$. The analysis was performed using CSS Statistica for Windows ver. 5.

3 Results and Discussion

p53 was revealed in tumor cell nuclei; there were no cytoplasmic reaction. The percentage of positive cells varied from 20 to 100 % in individual AC and SCC cases, but most of them presented more than 50 % of stained nuclei. Likewise, survivin was detected in more than 50 % of

Table 2 Comparison of markers in AC and SCC taking into account the percentage of cases with stained cancer tissue

Marker	AC	SCC	P-value
p53	14/30 (46.7 %)	18/26 (69.2 %)	>0.05
Bax	25/30 (83.3 %)	14/26 (53.8 %)	0.006
Bcl-2	5/30 (6.7 %)	9/26 (34.6 %)	>0.05
Survivin	22/30 (73.3 %)	10/26 (38.5 %)	0.019
COX-2	17/30 (56.7 %)	15/26 (57.7 %)	>0.05

tumor cells in both groups. Only in three cases (1 AC and 2 SCC) weak nuclear immunoreactivity was seen along with the cytoplasmic one. Bcl-2 protein immunoreactivity was seen in the cytoplasm and the percentage of Bcl-2 positive cells did not exceed 60 % in both AC and SCC. The COX-2 and Bax presence was noticed in the cytoplasm of cancer cells, but the expressions were variable, from 20 to 100 % of cells in both cancer types. Comparison of markers' expression according to the pathological type of lung cancer is shown in Table 2.

There was a considerable variation in the percentage of cases expressing the markers of apoptosis in the AC group. Most cases in this group revealed the presence of Bax and surviving; the other markers were present in smaller percentage. Moreover, the expression of Bax and survivin were observed more often in AC than SCC (Chi^2 with Yates's correction 7.53; $p = 0.006$ and 5.57; $p = 0.019$, respectively). There was a clearly trend toward a higher frequency of the Bcl-2 presence in SCC compared with AC, although the expression of this protein was not prevalent in either group. The p53 protein occurred more often in SCC than AC, although the difference failed to be significant statistically. There was no major variation in the frequency of occurrence of the apoptosis markers in the SCC group, except for p53 which appeared in a slightly higher percentage of cases than the other markers.

As a next step we examined the relationship between the proteins expressed and clinical features of both types of lung cancer. In AC, a higher proportion of tumors expressing Bcl-2 was revealed in patients without metastases to lymph nodes (4 out of 12; 30 %) compared with those who had the involvement of lymph nodes (1 out of 18; 0.6 %); this difference was significant ($Chi^2 = 4.0$, $p = 0.046$). An inverse relationship was detected in the latter group for survivin (7 out of 12; 58 % for N0 cases and 14 out of 18; 78 % for N+) and for COX-2 (6 out of 12; 50 % for N0 cases and 14 out of 18; 78 % for N+), but this trend was not statistically confirmed. On the other hand, in SCC there were no significant differences in the expression of proteins tested according to the involvement of lymph nodes. In both subgroups of NSCLC, there were no associations of the proteins with tumor size (T status), advanced clinical stage (combined stages I- II *vs*. III-IV were analyzed due to a small number of cases), blood vessel emboli, patients' gender or age. Nor were there any interrelationships present between the studied molecular markers in individual cases of AC or SCC.

Immunohistochemistry is an important tool for the analysis of tumor markers (Szutowicz and Dziadziuszko 2010). There are many data on the expression of apoptosis markers in NSCLC, but few studies examine these markers separately for AC and SCC (Vischioni et al. 2004; Mori et al. 2004). Differences in histological subtypes constitute now the basis for new molecular therapeutic approaches. Thus, unraveling the relationship between the expression of apoptotic markers and clinical features manifested by NSCLC histological subtypes would enable to better diversify patients suffering from the two subtypes of lung cancer into appropriate treatment plans.

Vischoni et al. (2004), similarly to our present results, detected more frequent survivin expression in the AC than SCC subtype of lung cancer. However, this result has not been confirmed by other authors (Han et al. 2009). The results of studies on the expression of the antiapoptotic Bax protein are also ambiguous. Mori et al. (2004) found a higher proportion of AC, compared with SCC, expressing the Bax protein, while Yaren et al. (2006) did not report such a relationship. Kim et al. (2011) demonstrated that COX-2 is more frequent in AC than in SCC. In our present study, there were no differences in COX-2 expression between AC and SCC. Concerning the

expression of the antiapoptotic Bcl-2 protein, it is debatable if it depends on the histological subtype of lung cancer. Lai et al. (2002), in line with the present results, showed a higher percentage of Bcl-2 immunoreactivity in SCC than in AC. This difference has not been observed by Ohmura et al. (2000). Differences in the expression of molecular markers revealed in the present study, although they may underlie molecular distinctiveness of the two types of lung cancer are too small to provide a basis for differentiating AC from SCC.

The association of apoptosis markers with clinical features of NSCLC has been the subject of numerous studies. However, there is lack of detailed analysis in relation to histological cancer subtypes. In a previous study we showed that the Bcl-2 protein is frequently expressed in SCC patients who have lymph node metastases (Porebska et al. 2006), whereas in the present study the protein was frequently found in AC patients without the presence of metastases. The detection of Bcl-2 in AC patients without metastases may indicate that this protein characterizes a slightly less aggressive cancer.

The association of COX-2 immunoreactivity with the clinical and pathological features of lung cancer remains unclear. Achiwa et al. (1999) found no association of COX-2 expression with the presence of lymph node metastases in AC patients. In contrast, Hida et al. (1998) found more extensive expression of COX-2 in the lymph node metastases of AC patients than in the primary tumor focus. In the present study we examined only the primary focus of lung cancer and found a higher COX-2 expression in tumors that spread to lymph nodes. The COX-2 protein seems thus not only to participate in the early stages of lung cancer as suggested by Hosomi et al. (2000), but also in progression of cancer, which is consistent with the opinion of other investigators (Milas et al. 2003). The biological role of survivin, involving the inhibition of the late phase of apoptosis, may be important for the aggressive course of cancer. A trend toward a larger number of cases characterized by the presence of survivin in AC with the lymph node involvement suggests the possible role of this protein in the metastatic phenotype.

The evaluation of apoptotic markers may have important therapeutic implications. p53 protein revealed by immunohistochemistry in NSCC has been associated with a poor response to cisplatin chemotherapy (Harada et al. 2003). It has also been shown that cytotoxic drugs can induce the expression of COX-2 mediated by the wild type p53 (Duarte et al. 2009). Contrary to expectations, COX-2 inhibitors do not prolong survival (Groen et al. 2011). However, addition of celecoxib to carboplatin and gemcitabine has prolonged survival of patients with a high expression of COX-2 in cancer tissue (Edelman et al. 2008). Molina et al. (2010) have shown that patients with epidermal growth factor receptor (EGFR) mutation in cancer tissue and the lack of survivin immunoreactivity survive longer. Taken together, immunohistochemical analysis of apoptotic markers may be associated with significant benefits in the selection of therapy, but more information is required concerning different histological subtypes of lung cancer.

4 Conclusions

Our comparative analysis of the expression of apoptosis markers in lung cancer revealed their greater diversification in AC than in SCC. Survivin and Bax were observed in a significantly higher percentage of AC than SCC patients. Among the AC cases, Bcl-2 immunoreactivity was observed more often in tumors without lymph node metastases than with the lymph node involvement. We did not reveal any other association between the proteins studied and clinical features of lung cancer. Further studies are required to substantiate the predictive and prognostic value of apoptosis markers in the AC and ACC subtypes of lung cancer.

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Competing Interest The authors declare that they have no competing interests in relation to this article.

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