Advances in Experimental Medicine and Biology 952
Neuroscience and Respiration

Mieczyslaw Pokorski *Editor*

Advancements in Clinical Research

Springer

Advances in Experimental Medicine and Biology

Neuroscience and Respiration

Volume 952

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ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-48032-9 ISBN 978-3-319-48033-6 (eBook)
DOI 10.1007/978-3-319-48033-6

Library of Congress Control Number: 2016957574

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 1–8
DOI 10.1007/5584_2016_67

Published online: 30 August 2016

Oxygen Sensing Mechanisms: A Physiological Penumbra

Mieczyslaw Pokorski, Kotaro Takeda, and Yasumasa Okada

Abstract

This review tackles the unresolved issue of the existence of oxygen sensor in the body. The sensor that would respond to changes in tissue oxygen content, possibly along the hypoxia-normoxia-hyperoxia spectrum, rather than to a given level of oxygen, and would translate the response into lung ventilation changes, the major adaptive process. Studies on oxygen sensing, for decades, concentrated around the hypoxic ventilatory response generated mostly by carotid body chemoreceptor cells. Despite gaining a substantial insight into the cellular transduction pathways in carotid chemoreceptors, the exact molecular mechanisms of the chemoreflex have never been conclusively verified. The article briefly sums up the older studies and presents novel theories on oxygen, notably, hypoxia sensing. These theories have to do with the role of transient receptor potential cation TRPA1 channels and brain astrocytes in hypoxia sensing. Although both play a substantial role in shaping the ventilatory response to hypoxia, neither can yet be considered the ultimate sensor of hypoxia. The enigma of oxygen sensing in tissue still remains to be resolved.

Keywords

Astrocytes • Chemoreflex • Hypoxia • Oxygen content • Oxygen sensor • Respiration • TRPA1 channels

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1 Oxygen Sensing

Oxygen is required for life on the Earth as it is a prerequisite for cellular respiration, notably at the mitochondrial level, in all aerobic organisms. In higher concentrations than those present in the ambient air, oxygen is however harmful and toxic for organisms due to the enhancement of oxidative stress through the formation of reactive oxygen and nitrogen species, fostering aging or even death at high concentrations that damage lung structure and function, and ultimately decreasing delivery of oxygen to the arterial blood through the respiratory tract. This dichotomous action of oxygen requires that the living organisms be able to keep the physiological level of oxygen in balance and adapt to its changes. The adaptive processes suggest the possibility of forming an oxygen sensor during the evolution of life; a sensor that would pick up the level of oxygen in real time and transfer the message down to the effector systems. The presence of O_2 sensor is still elusive despite decades of research on the subject.

The hitherto research has focused mostly on hypoxia, a life-threatening condition, also underlying neurodegenerative diseases, and the aging process. The attention centered on the hypoxic reflex, generating rapid lung hyperventilation, relatively easy to study and producing spectacular responses. The time sanctioned location of this reflex is the arterial chemoreceptors (Izumizaki et al. 2004). These receptors are located in the carotid body, a minute paired sensory organ whose discharge increases in proportion to reduction of oxygen and, in turn, is relayed to the brainstem respiratory network to induce an increase in lung ventilation, being proportional to the chemoreceptor discharge (Pokorski 1999). The molecular mechanisms of carotid chemoreceptor activation have been worked out relatively well (Gonzalez et al. 1994). They consist of inhibition of outwardly directed K^+ channels, voltage-dependent (Buniel et al. 2008) or belonging to the TASK channel family (Buckler 2013), in the plasma membrane of chemoreceptor cells and subsequent accumulation of K^+ ions leading to cell depolarization, activation of Ca^{2+} channels, followed by influx of Ca^{2+}, accompanied by mobilization of Ca^{2+} from intracellular stores, exocytosis of putative neurotransmitters, such as dopamine, and finally excitation of the sinus nerve afferent discharge. In particular, inhibition of background TASK-like channels is considered essential for triggering the stimulus transduction cascade in chemoreceptor cells (Ortiz et al. 2012; Ortega-Sáenz et al. 2010). The inhibition of K^+ channels has to do with CO formation by hemeoxygenase in chemoreceptors (Peng et al. 2010; Kumar 2007; Williams et al. 2004).

Hypoxia depletes ATP in chemoreceptor cells, which is conducive to depolarization, as K^+ channels are modulated by cytosolic ATP and Ca^{2+} ions. However, there is a multitude of K^+ channels that respond to hypoxia, with an appreciable difference depending on the species and on the kind of cell (Peers et al. 2010). There is evidence that K^+ channels also are sensitive to regulation by factors originating beyond the cytosol. Jiang and Haddad (1994) have found in cell-free neuronal membrane patches that large-conductance K^+ channels are inhibited by oxygen deprivation, with 50 % channel inhibition at 10 mmHg and no effect at PO_2 over 20 mmHg. The level of PO_2 at which the opening probability of K^+ channels distinctly decreases is contentious and a source of uncertainty regarding the chemosensing mechanisms. A major criticism persists that inhibition of K^+ channels is realized when the level of O_2 drops to about 70–80 mmHg (Gonzalez et al. 1994; Ganfornina and López-Barneo 1991), which is much above the level producing maximal hypoxic activation of carotid sensory discharge. Therefore, closure of K^+ channels outruns the hypoxic excitation of chemoreceptor cells, the two being out of phase concerning the maximum chemoreceptor discharge and ventilatory effects (Lahiri et al. 2006; Pokorski 1999). The cascade of events above outlined has a reason and support, albeit fragmentary rather than all-encompassing, of various studies. These mechanisms have never been fully verified. Further, some fragments like,

for instance, the action of dopamine and a number of other neurotransmitters present in chemoreceptor cells, are controversial and shadowy.

Other hypoxia responsive systems have been unraveled along the tedious research way, such as vagally-innervated aortic bodies and airway neuroepithelial bodies (Cutz et al. 2013). There also are hypoxia-responding catecholaminergic neurons in the brain (Gonzalez et al. 1994). These additional systems seem however secondary and of less influence, as they are incapable to take over the generation of hypoxic lung hyperventilation when the carotid body action is switched off. Hyperoxia, on the other hand, decreases membrane depolarization of and neurotransmitter release from chemoreceptor cells, and consequently also chemoreceptor sensory discharge. Sensing hyperoxia seems as essential as sensing hypoxia due to oxygen toxicity mostly related to the formation of reactive oxygen species. The mechanisms of hyperoxia sensing are even more enigmatic than in case of hypoxia. The search for hypoxia/hyperoxia sensor, i.e., a sensor responding smoothly to changes in O_2 content, continues. To this end, a possibility has recently arisen of the involvement of transient receptor potential cation channel, subfamily A, member 1, also known as TRPA1 channels, as the core O_2 sensing element.

2 TRPA1 Channels and Chemosensing

These membrane cation channels are basically engaged in airway smooth muscle contractility and inflammation and are activated by noxious stimuli and cold. Takahashi et al. (2011) have recently demonstrated that TRPA1 are capable of sensing O_2, intriguingly along the hypoxia-normoxia-hyperoxia continuum rather that at either extreme. The O_2 sensing would however be underlain be disparate mechanisms. In normoxia, with sufficient physiological, evolution-set level of O_2, TRPA1 are rendered inactive by O_2-dependent activity of prolyl hydroxylases (PHD). In hypoxia, with insufficient oxygen, and consequently less protein hydroxylation by PHD, TRPA1 would be relieved from the PHD-mediated inhibition. In hyperoxia, in turn, TRPA1 would also be relieved from the PHD-mediated inhibition, but this time due to cysteine-mediated oxidation. TRPA1's cysteine sulfhydryl groups are highly sensitive to oxidation, the event that activates the channel.

These mechanisms have been elegantly proven in the sensory vagal system, raising a specter of a potentially uniform theory for O_2 sensing across other tissues. The theory would undoubtedly reconcile a great deal of hitherto discrepant, often opposing, results and views concerning the enigma of O_2 sensing. It also could shed light on the mechanism of some stimulatory effects on ventilation of extended oxygenation (Marczak and Pokorski 2004). Nonetheless, a full verification of the essential role of TRPA1 in O_2 sensing would require a confirmation of the presence of TRPA1 channel receptors in the carotid body and/or their functional role in the generation of the powerful hypoxic reflex taking place in chemoreceptor cells, a time proven tenet of physiology. At this juncture, TRPA1 issue has become complicated. Investigations to identify TRPA1 in the carotid body have failed; the results are equivocal if not outright null (Gallego-Martin et al. 2015). In an effort to tackle the functional role of TRPA1 we have performed a major study in the conscious unrestrained mouse in which we pharmacologically blocked TRPA1 channels while investigating the ventilatory response to O_2 changes in hypoxic, normoxic, and hyperoxic conditions as well as to hypercapnia (Pokorski et al. 2014). The main hypothesis was that if TRPA1 channels were a prerequisite for O_2 responses, then O_2 sensitivity would be abrogated when the channels are rendered inactive. We examined the acute two-minute-long chemosensory ventilatory responses before and after intraperitoneal injection of the specific TRPA1 antagonist HC-030031 in two doses of 50 and 200 mg/kg at approximately 1 h interval. The antagonist is a long-lived, slowly degradable agent in the body. Thus, cumulative dose of it amounted to 250 mg/kg; a dose being close to the

highest ever reported antagonistic dose of HC-030031 used in a study of nocifensive behaviors (McNamara et al. 2007). Ventilation and its responses to mild 13 % and severe 7 % hypoxia, pure O_2, and 5 % CO_2 balanced with O_2 were recorded in a whole-body plethysmograph. The results failed at several points to meet our expectation to lend support for the universality of TRPA1 channels in O_2 sensing. The ventilatory augmentation in response to the severe hypoxia was diminished by about half after the TRPA1 antagonist pretreatment in the higher dose compared with the control condition without the antagonist, but it remained clearly distinct (Fig. 1). The response to hyperoxia fluctuated around the baseline both before and after the antagonist pretreatment. No real stimulation of ventilation by oxygen was noticeable, nor was there any appreciable effect of TRPA1 antagonism. The magnitude of a vigorously hyperventilatory response to hypercapnia was, likewise, unaffected by the antagonist (Fig. 2). Intriguingly, however, ventilatory response to the mild 13 % hypoxia was nearly abolished by TRPA1 antagonist pretreatment in the higher dose (Fig. 3). Taken together, the findings hardly support the concept of TRPA1 channels being a unifying sensor of O_2 changes, and if so, only in the part regarding the hypoxia sensing, as posited by Takahashi et al. (2011), and only in a limited, mild range of hypoxia.

Nonetheless, the TRPA1 antagonism study has spurred novel ideas on the hypoxia sensing. The plausibility arises that hypoxia sensing consists of two components; each of different mediation depending on the level of hypoxia. Mild hypoxia would be sensed and adaptively offset by specialized chemoreceptive cells, such as airway neuroepithelial bodies, laryngeal or aortic chemoreceptors, and other vagally innervated sensors (Brouns et al. 2012; Piskuric et al. 2011). These sensors are thought of as

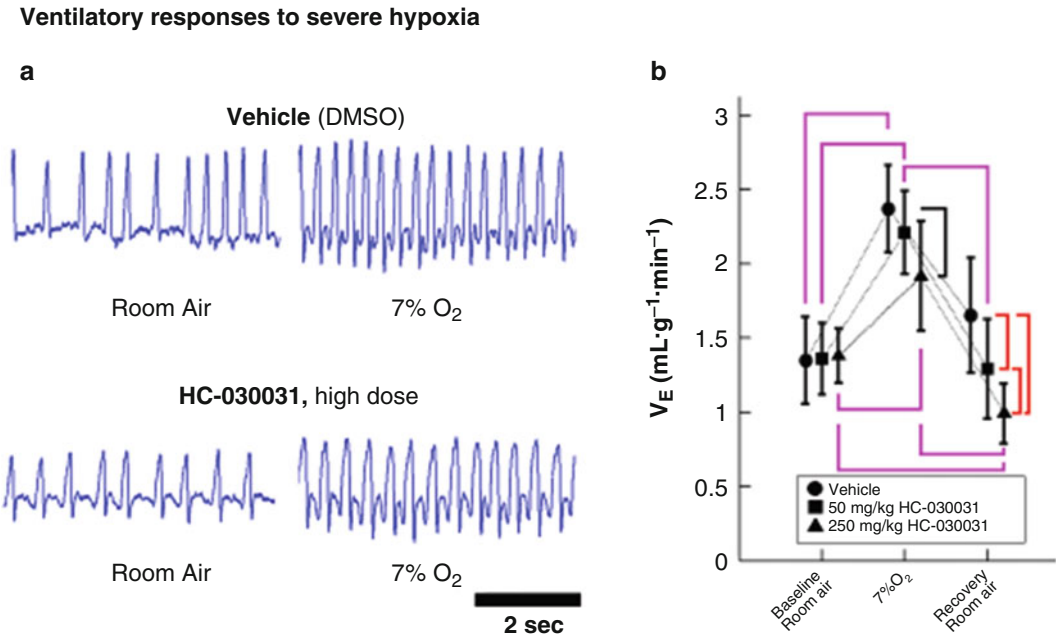

Fig. 1 Ventilatory responses to severe hypoxia (7 % O_2 in N_2) in the conscious mouse before, i.e., at the background of the vehicle DMSO, and after pharmacological blockade of TRPA1 channels with the inhibitor HC-030031. (**a**) an example of original recordings, (**b**) minute ventilation at baseline, peak hypoxic response, and recovery in the three pharmacological conditions: vehicle, and two doses of the HC-030031 inhibitor 50 and 250 mg/kg; values are means (SD), brackets of the same color connecting pairs of symbols indicate significant differences at $p < 0.05$ (two-factor within-subject ANOVA with the Bonferroni correction). (*Panel b is reproduced with permission from* Pokorski et al. 2014.)

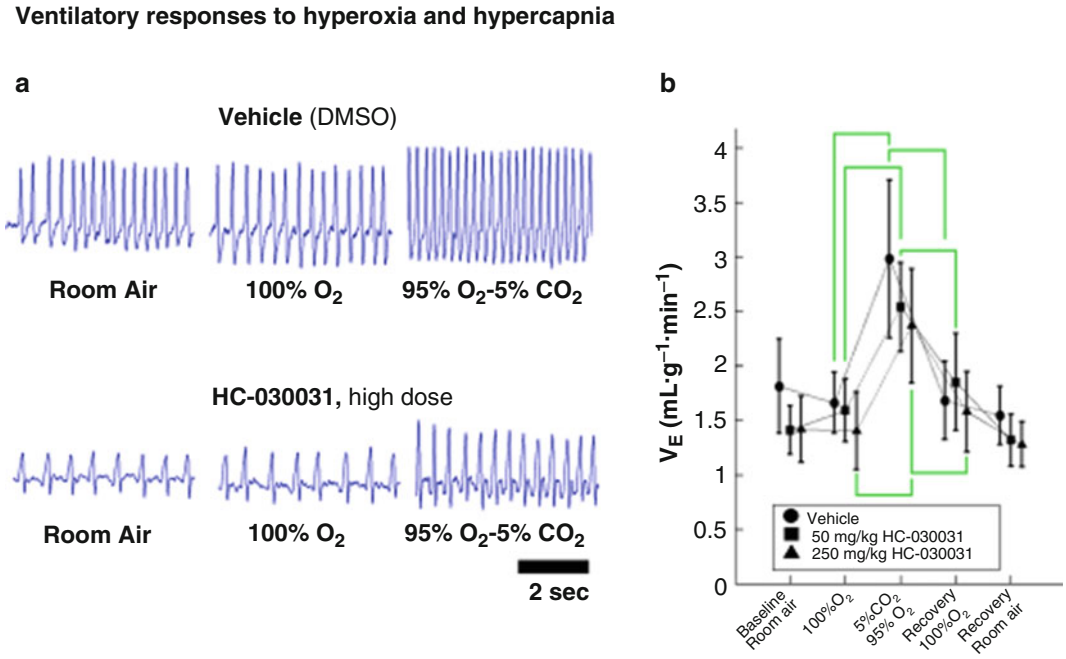

Fig. 2 Ventilatory responses to hyperoxia (100 % O_2) and hypercapnia (5 % CO_2 in O_2) in the conscious mouse before, i.e., at the background of the vehicle DMSO, and after pharmacological blockade of TRPA1 channels with the inhibitor HC-030031. (**a**) an example of original recordings, (**b**) minute ventilation at baseline, peak hyperoxic and hypercapnic responses, and recovery in the three pharmacological conditions: vehicle, and two doses of the HC-030031 inhibitor 50 and 250 mg/kg; values are means (SD), brackets of the same color connecting pairs of symbols indicate significant differences at $p < 0.05$ (two-factor within-subject ANOVA with the Bonferroni correction). (*Panel b is reproduced with permission from* Pokorski et al. 2014.)

secondary chemoreceptors, responsive to hypoxia but incapable of generating a full-fledged ventilatory response to severe hypoxia or upholding hypoxic ventilation in case the carotid bodies are rendered inactive or removed (Honda 1985). When life-threatening hypoxia is encountered, carotid chemoreceptors come to the rescue by generating a powerful defensive chemoreflex. These mechanisms would overlap throughout hypoxic exposure, with the carotid body increasing involvement along the progressively increasing lack of oxygen, which explains the suppressant effect of TRPA1 antagonism present also in severe hypoxia. The existence of such double mechanisms could also explain many a discordant, variable, or enigmatic result in investigations on the hypoxic ventilatory response, but do not set the TRPA1 channels at the core of the universal O_2 sensing mechanism.

3 Astroglia and Chemosensing

Recently, attention has been diverted from the peripheral arterial chemoreceptor cells toward the brain astroglia as the potentially outstanding player in chemosensing. Astrocytes, the macroglia cells, are ubiquitous in the brainstem respiratory regions where they form extensive functionally specialized network (Kasymov et al. 2013). Aside from the interneuronal localization of astrocytes, these cells have cytoplasmic processes that terminate on blood microvessels. Astrocytes, like carotid chemoreceptor cells, express ion channels, notably K^+ channels, and a spate of neurotransmitters and receptors (Bélanger and Magistretti 2009). They perform a number of active functions, known to be engaged also in the chemosensing process in the carotid body, such as influx and propagation

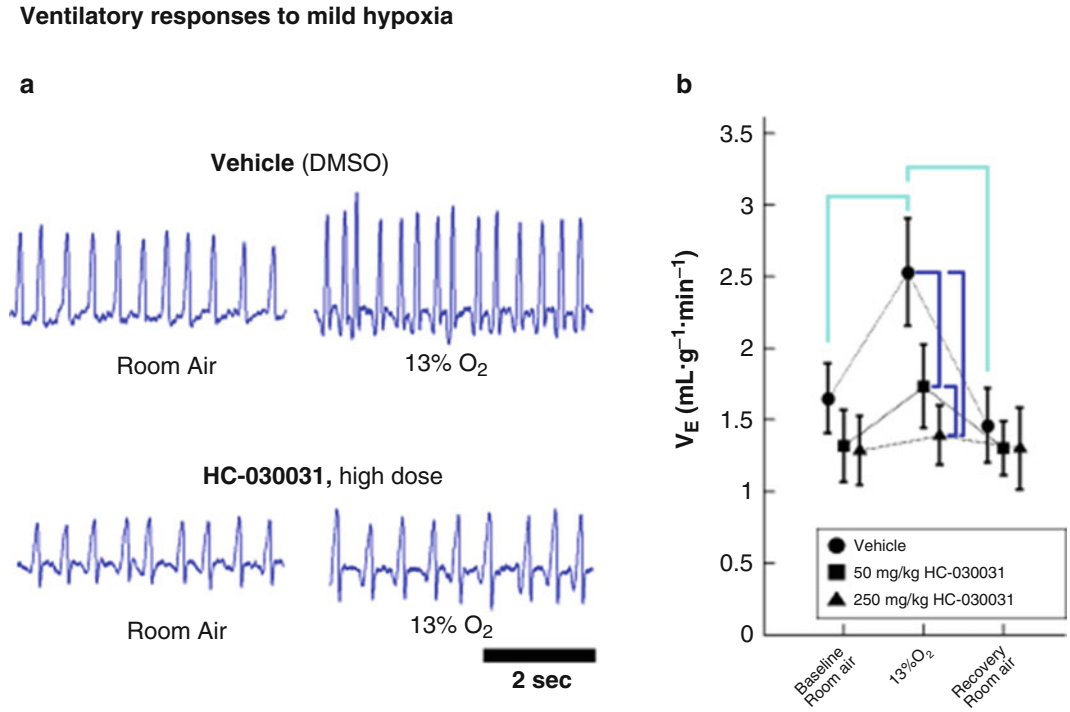

Fig. 3 Ventilatory responses to mild hypoxia (13 % O_2 in N_2) in the conscious mouse before, i.e., at the background of the vehicle DMSO, and after pharmacological blockade of TRPA1 channels with the inhibitor HC-030031. (**a**) an example of original recordings, (**b**) minute ventilation at baseline, peak hypoxic response, and recovery in the three pharmacological conditions: vehicle, and two doses of the HC-030031 inhibitor 50 and 250 mg/kg; values are means (SD), brackets of the same color connecting pairs of symbols a indicate significant differences at $p < 0.05$ (two-factor within-subject ANOVA with the Bonferroni correction). (*Panel b is reproduced with permission from* Pokorski et al. 2014.)

of intracellular Ca^{2+} on stimulation or Ca^{2+}-dependent neurotransmitter release. They are also vulnerable to redox changes (Angelova and Abramov 2016). These similarities between astrocytes and carotid chemoreceptor cells make astrocytes well-suited to sense and dynamically modulate the brainstem neuronal activity in response to O_2 changes. Further, astrocytes residing in the central chemosensitive areas of the ventrolateral medullary surface respond to a drop in pH with intracellular Ca^{2+} increase and release of ATP (Kasymov et al. 2013). The astroglia excitatory response, radiating to the brainstem respiratory network, may thus also participate in shaping the central hyperventilatory response to CO_2.

Hypoxia, particularly if prolonged or severe, causes brain depression which manifests, among others, in depressed lung ventilation. That is a delayed, depressant phase of the ventilatory response to hypoxia. Astrocytes, with their multifarious neuroprotective and homeostatic functions, could be engaged in the maintenance of cerebral function and ventilation in the hypoxic condition. We have investigated this hypothesis in a recent study in which the EEG activity of the forebrain and ventilation in a body box were simultaneously recorded in conscious mice (Fukushi et al. 2016). Astrocytic function was modulated pharmacologically by intraperitoneal administration of arundic acid, a specific inhibitory moderator of intensity of astrocytic activity. Two doses of arundic acid of 100 and 200 mg/kg (cumulative dose of 300 mg/kg) were employed at one hour intervals. A rather severe hypoxia of 6 % O_2 balanced with N_2 was induced, and ventilation and EEG responses were compared before and after pretreatment

with each dose of arundic acid (Fig. 4). Arundic acid had no appreciable effects on EEG or ventilation in the normoxic condition. At the time of the development of hypoxic ventilatory depression in hypoxia, EEG got depressed as well. Arundic acid pretreatment caused further suppression of the hypoxic ventilation, accompanied by a dose-dependent suppression of the EEG gamma frequency band. These functional changes were accompanied by a strongly decreased expression of *c-Fos* in the dorsomedial hypothalamic nucleus, respiratory-regulating nucleus, after arundic acid pretreatment. The arundic acid-induced EEG suppression is suggestive of dimmed consciousness and hypothalamic activity, with resultant deepening of ventilatory depression in hypoxia. The corollary is that astrocytes counteract the ventilatory depression. The findings reveal that astrocytes promote cerebral function, inclusive of consciousness and ventilation, in hypoxia. This function of astrocytes leaves no doubt about their involvement in the control of ventilation. Astrocytes have been recently suggested to be directly involved in the hypoxic ventilatory response (Angelova et al. 2015).

In synopsis, we believe we have shown that astroglia is an essential component of central nervous system sensitivity to hypoxia and is an active modulator of adaptive ventilatory responses. The issue of whether astrocytes could be the elusive hypoxia sensor remains conjectural. There is too little hard information on the innate mechanisms of astrocytic function to form an ultimate opinion on the role of astrocytes in the ventilatory chemoreflex. For the time being, connection between astrocytes and ventilation represents a holistic approach to respiratory regulation and poses an intriguing and interesting avenue of research. What science is all about is the meticulous building up on previous research, which gives rise to new questions

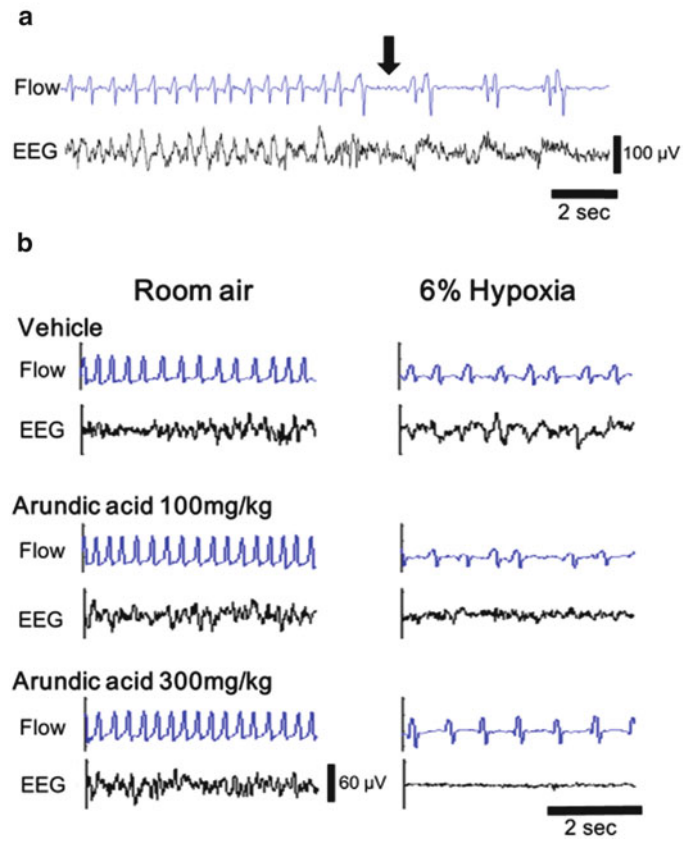

Fig. 4 Respiratory flow (inspiration upward) and EEG raw recordings in a conscious mouse. (**a**) Arrow exemplifies the onset of ventilatory fall-off during the stimulatory ventilatory response to hypoxia; (**b**) Respiratory flow and EEG signals during room air breathing (*left* column) and during the depressant phase of response to 6 % O_2 in N_2 (*right* column) at baseline (vehicle) and after pretreatment with increasing doses of arundic acid. Arundic acid caused dose-dependent suppression of both respiratory flow and EEG (*Reproduced with permission from* Fukushi et al. 2016.)

and ideas. That undoubtedly also concerns the jigsaw puzzle of O_2 sensing.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 9–15
DOI 10.1007/5584_2016_64

Published online: 26 August 2016

Nocturnal Blood Pressure Variability in Patients with Obstructive Sleep Apnea Syndrome

H. Martynowicz, I. Porębska, R. Poręba, G. Mazur, and A. Brzecka

Abstract

Obstructive sleep apnea (OSA) is a common respiratory disorder associated with hypertension and cardiovascular complications. Blood pressure variability may be a sign of risk of cardiovascular events. The aim of this study was to investigate the hypothesis that severe OSA syndrome is associated with increased blood pressure variability. Based on respiratory polygraphy, 58 patients were categorized into two groups: severe OSA with apnea/hypopnea index (AHI) greater than 29 episodes per hour (mean 52.2 ± 19.0/h) and mild-to-moderate OSA with AHI between 5 and 30 episodes per hour (mean 20.2 ± 7.8/h). A 24-h noninvasive blood pressure monitoring was performed. The standard deviation of mean blood pressure was used as the indicator of blood pressure variability. In patients with severe, compared with mild-to-moderate OSA, a higher mean nocturnal systolic blood pressure (133.2 ± 17.4 mmHg *vs.* 117.7 ± 31.2 mmHg, $p < 0.05$) and diastolic blood pressure (80.9 ± 13.1 mmHg *vs.* 73.8 ± 9.2, $p < 0.01$), nocturnal systolic blood pressure variability (12.1 ± 6.0 *vs.* 7.6 ± 4.3, $p < 0.01$) and diastolic blood pressure variability (10.5 ± 6.1 *vs.* 7.3 ± 4.0 $p < 0.05$), nocturnal mean blood pressure variability (9.1 ± 4.9 mmHg *vs.* 6.8 ± 3.5 mmHg) were detected. The findings of the study point to increased nocturnal systolic and diastolic arterial blood pressure and blood pressure variability as risk factors of cardiovascular complications in patients with severe OSA.

Keywords

Apnea/hypopnea index • Arterial blood pressure • Arterial oxygen saturation • Hypertension • Obstructive sleep apnea • Cardiovascular risk factor • Sleep disordered breathing

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1 Introduction

Obstructive sleep apnea (OSA) is a common sleep disorder characterized by a collapse of upper airways in the setting of continued respiratory effort, leading to cessation of airflow, large swings in intrathoracic pressure, and arterial oxygen desaturation, often terminated by an arousal.

OSA has been independently associated with cardiovascular diseases, such as hypertension (Peppard et al. 2000), stroke (Munoz et al. 2006), myocardial ischemia (Peled et al. 1999), and arrhythmias (Mehra et al. 2006), which all increases risk for sudden cardiac death. OSA and hypertension, in turn, have common risk factors such as age, obesity, and sedentary lifestyle. OSA syndrome is found in about 50 % of patients with hypertension (Pedrosa et al. 2011) and in 70 % of patients with resistant hypertension (Florczak et al. 2013). There are some data indicating that OSA is associated with hypertension independent of age and obesity (Pankow et al. 1997). The pathogenesis of systemic arterial hypertension in the course of OSA is complex and not fully explained. The most important factor seems hypoxia observed during or immediately after apneic or hypopneic incidents underlying OSA. Sympathetic excitation, expressed by increased catecholamine level and chemoreceptor alterations, caused by intermittent hypoxia has been suggested as a hypertension promoting factor (Fung et al. 2014; Freet et al. 2013). Other pathogenic factors leading to hypertension in OSA patients include systemic inflammation and endothelial dysfunction (Chen et al. 2015).

Hypertension is the most common risk factor for cardiovascular disease and the single most important risk factor for stroke (Roger et al. 2012). Traditionally, cardiovascular risk in hypertension has been attributed to the mean blood pressure load (Taylor et al. 2015). However, inherent variability of an individual's blood pressure may also be contributory (Rothwell 2010). Systemic arterial blood pressure undergoes marked variations during day and night (Mancia 2012). Blood pressure variability (BPV) is influenced by multiple factors, including neural dysregulation due to age, diabetes, or neuropathies, vascular, humoral, and central nervous system disorders, and mental and environmental stress (Kai et al. 2014). Association between BPV and organ damage (Matsui et al. 2011), cardiovascular events (Johansson et al. 2012), stroke (Shimbo et al. 2012), and mortality (Muntner et al. 2011) have been described. However, some other studies have failed to substantiate such associations or found the BPV of lesser importance than the actual level of blood pressure (Schutte et al. 2012).

Taking into account that OSA is accompanied by autonomic cardiovascular dysregulation which may be related to the frequency of obstructive apneic and hypopneic episodes and to increased sympathetic excitation, we hypothesized that BPV could be associated with the severity of OSA. In the present study we addresses this issue by examining BPV in the patients with mild-to-moderate and severe OSA during a 24-h period.

2 Methods

The study was approved by the Bioethics Committee of the Medical University in Wroclaw, Poland and was conducted according to the principles set in the Declaration of Helsinki for Human Research. Fifty eight patients, with newly diagnosed OSA syndrome, of the mean age of 54.3 ± 10.3 years and the mean body mass index (BMI) of 37.8 ± 6.7 kg/m^2 were enrolled into the study. Thirty one (53 %) of the patients were on antihypertensive treatment. Based on the severity of OSA, patients were categorized into two groups: severe OSA with AHI ≥30/h (35 men, 5 women) and mild-to-moderate OSA with AHI between 5 and 30/h (4 men, 14 women). All patients underwent in-hospital, nocturnal polygraphic examination at the Department of Pulmonology and Lung Cancer of Medical University in Wroclaw. The following parameters were recorded during sleep: thoracic and abdominal respiratory

movements, oro-nasal airflow, and arterial oxygen saturation (SaO_2) with finger pulsoximetry. Abnormal respiratory events: apneas, hypopneas, and episodes of desaturations were evaluated according to the standard criteria of the American Academy of Sleep Medicine Task Force (Berry et al. 2012). The following parameters were calculated: apnea/hypopnea index (AHI) – mean number of apneic and hypopneic episodes per hour of sleep, oxygen desaturation index (ODI) – mean number of arterial oxygen desaturations per hour of sleep, and the mean of the minimal values of SaO_2 at the end of apneic and hypopneic episodes.

During the day following the polygraphic examination, arterial blood (BP) pressure was monitored noninvasively, using the oscillometric method, along with pulse rate. Readings were obtained every 30 min during diurnal (6:00 a.m. to 10:00 p.m.) and every 60 min during nocturnal (10:00 p.m. to 6:00 a.m.). The BP data were calculated as means of total systolic (TSBP) and total diastolic blood pressure (TDBP) collected over the 24-h period, and separately as diurnal mean BP (DMBP), diurnal systolic (DSBP), diurnal diastolic BP (DDBP) and nocturnal mean BP (NMBP), nocturnal systolic (NSBP), nocturnal diastolic BP (NDBP). Based on the mean standard deviations (SD) of the data above listed, BP variability was calculated: total systolic BP variability (TSBPV), total diastolic BP variability (TDBPV), diurnal systolic BP variability (DSBPV), diurnal diastolic BP variability (DDBPV), nocturnal systolic BP variability (NSBPV), nocturnal diastolic BP variability (NDBPV), total mean BP variability (TMBPV), diurnal mean BP variability (DMBPV), and nocturnal mean BP variability (NMBPV). The thresholds for increases in blood pressure were taken as those set by the European Society of Hypertension/European Society of Cardiology 2013 criteria (Kjeldsen et al. 2013). Nocturnal BP changes were classified as follows: deep fall if a drop was greater than 10 % of the diurnal baseline, mild fall if a drop was between 0 and 10 % off the diurnal baseline level, and a rise if BP went over the diurnal baseline.

Data were presented as means ± SD. Distribution of variables was tested with the Shapiro-Wilk test. Inter-group data with normal distribution were statistically compared with a *t*-test, and those with skewed distribution were compared with the Mann-Whitney *U* test. Relationship between variables was estimated with the Spearman correlation coefficient. Significance of differences was considered at $p < 0.05$. Statistical analysis was performed using Statistica 6.0 software (StatSoft, Tulsa, OK).

3 Results

There were no significant differences in the anthropometric characteristics of patients with severe and milder OSA. The main OSA characteristics such as AHI, ODI, and SaO_2 dips during breathless episodes were clearly intensified in the group of patients with severe OSA compared with those in milder OSA. However, due to a large scatter of individual data, the man values did not differ statistically between the two groups (Table 1).

The mean values of TSBP, TDBP, DSBP, and DDBP were similar in both groups of OSA patients. However, NSBP and NDBP were significantly higher in the severe OSA than those in the milder OSA patients (Table 2). The mean nocturnal falls in systolic and diastolic BP were between 0 and 10 % off the diurnal baseline levels in the severe OSA. These BP falls were greater than 10 % in the milder OSA.

Blood pressure variability, assessed from the magnitude of the standard deviation of the mean value, was significantly greater for the mean, systolic, and diastolic blood pressure during nighttime in the severe OSA than those in the milder OSA patients (Table 3). Positive linear correlations were found between AHI, on the one side, and NSBPV ($r = 0.57$, $p < 0.05$), TDBPV ($r = 0.58$, $p < 0.05$), and DDBPV ($r = 0.70$, $p < 0.05$), on the other side.

Table 1 Anthropometric and polygraphic features of obstructive sleep apnea (OSA) patients

	Severe OSA (n = 40)	Mild-to-moderate OSA (n = 18)	
Age (yr)	53.9 ± 10.1	57.2 ± 12.3	ns
Weight (kg)	112.1 ± 17.8	110.0 ± 26.5	ns
BMI (kg/m^2)	38.3 ± 6.2	35.6 ± 5.7	ns
AHI (per h)	52.2 ± 19.2	20.2 ± 7.8	ns
ODI (per hour)	52.7 ± 18.1	22.3 ± 9.3	ns
SaO_2 at the end of sleep apneas/hypopneas (%)	83.7 ± 4.9	88.9 ± 3.2	ns

Data are means ± SD. *BMI* body mass index, *AHI* apnea/hypopnea index, *ODI* oxygen desaturation index, *ns* nonsignificant

Table 2 Diurnal, nocturnal, and day blood pressure changes in severe and milder forms of obstructive sleep apnea (OSA)

	Severe OSA	Mild-to-moderate OSA	p
TSBP	137.0 ± 14.0	133.1 ± 1.9	ns
TDBP	86. 8 ± 11.5	83. 3 ± 10.3	ns
DSBP	136.6 ± 13.8	135.3 ± 13.7	ns
DDBP	87.8 ± 10.5	85. 3 ± 11.6	ns
NSBP	133.3 ± 17.4	117. 7 ± 31.2	<0.01
NDBP	80.9 ± 13.2	73. 8 ± 9.2	<0.05

All data are presented as mmHg and are means ± SD. *TSBP* total (over the 24-h period) systolic blood pressure, *TDBP* total diastolic blood pressure, *DSBP* diurnal systolic blood pressure, *DDBP* diurnal diastolic blood pressure, *NSBP* nocturnal systolic blood pressure, *NDBP* nocturnal diastolic blood pressure, *ns* nonsignificant

4 Discussion

The major finding of the present study is that nocturnal blood pressure variability was significantly greater in patients suffering from severe OSA, with the AHI over 29 episodes per hour of sleep, than that in milder forms of OSA. The potentially confounding factors such as age, weight, or BMI were similar in both groups of patients and thus may be excluded as the underlying reason of blood pressure variability, acting via increased sympathetic drive (Charkoudian and Rabbits 2009). There are a number of methods to assess blood pressure variability, such as based on the coefficient of variation, weighted standard deviation (mean of diurnal and nocturnal standard deviation values of blood pressure measurements weighted for the number of hours covered by these two periods during ambulatory monitoring), average real variability, or the difference between maximum and minimum blood pressure levels. In the present study we assessed blood pressure variability on the basis of the standard deviation of the mean values of the amplitude of blood pressure measured. This method is regarded as a good index of apnea-related blood pressure elevations (Planès et al. 2002). Our finding of increased nocturnal blood pressure variability in severe OSA is, generally, in line with that of Steinhorst et al. (2014), although those authors investigated clearly hypertensive OSA patients. Planès et al. (2002) have also shown that systemic hypertension is associated with increased short-term blood pressure variability during sleep in OSA patients.

The present study also unraveled some distortions in the day profile of arterial blood pressure in severe OSA consisting of the lack of a physiological decrease in blood pressure at night. Four categories of nocturnal blood pressure changes are considered: extreme dippers (a fall in blood pressure of more than 20 % compared with diurnal level), dippers (a fall greater than 10 % but less than 20 %), non-dippers (a fall less than 10 %), and reverse dippers, i.e., risers (blood pressure increases at night). Non-dipping pattern in the 24-h blood pressure monitoring has been largely described in patients with OSA syndrome (Loredo et al. 2001; Suzuki et al. 1996); the finding attributable to autonomic dysfunction. In addition, we also found that nocturnal systolic and diastolic blood pressure were higher in patients with severe OSA (AHI ≥30 episodes per hour) compared with those present in milder forms of OSA. Changes in the circadian rhythm of blood

Table 3 Blood pressure variability (BPV) assessed from the magnitude of standard deviation of the mean value in patients with severe and mild-to-moderate OSA syndrome

	Severe OSA	Mild-to-moderate OSA	p
TSBPV	14.8 ± 3.7	16.1 ± 5.5	ns
TDBPV	12.2 ± 3.3	12.3 ± 3.8	ns
TMBPV	11.8 ± 2.8	12.8 ± 4.0	ns
DSBPV	13.9 ± 4.2	15.5 ± 5.7	ns
DDBPV	11.0 ± 3.7	11.2 ± 4.0	ns
DMBPV	10.9 ± 3.1	3.1 ± 4.5	ns
NSBPV	12.1 ± 6.0	7.6 ± 4.3	<0.01
NDBPV	10.5 ± 6.1	7.3 ± 4.0	<0.05
NMBPV	9.1 ± 4.9	6.8 ± 3.5	<0.05

All data are presented as mmHg and are means ± SD. *TSBPV* total (over the 24-h period) systolic blood pressure variability, *TDBPV* total diastolic blood pressure variability, *DSBPV* diurnal systolic blood pressure variability, *DDBPV* diurnal diastolic blood pressure variability, *NSBPV* nocturnal systolic blood pressure variability, *NDBPV* nocturnal diastolic blood pressure variability, *TMBPV* total mean blood pressure variability, *DMBPV* diurnal mean blood pressure variability, *NMBPV* nocturnal mean blood pressure variability, *ns* nonsignificant

pressure have been described by Noda et al. (1993) who show that the severity of OSA has an impact on nocturnal blood pressure elevation. Lavie et al. (1993) have also shown that blood pressure during sleep significantly correlates with the apnea/hypopnea index.

The observed changes in blood pressure variability, impaired 24-h blood pressure profile and a greater nocturnal blood pressure, in patients with severe OSA give rise to cardiovascular complications. The lack of nocturnal dipping in blood pressure has been related to more pronounced target organ damage (Verdecchia et al. 1993) and increased risk of cardiovascular events (Parati and Valentini 2006). Moreover, findings of the International Database of Ambulatory Blood Pressure in Relation to Cardiovascular Outcome (IDACO) have revealed that the night-to-day blood pressure ratio predicts both cardiovascular and non-cardiovascular mortality (Boggia et al. 2007). Sympathetic neural activity is increased in both OSA, even during the awake state, and hypertension. Sympathetic activation in patients with hypertension is associated with increases in cardiovascular risk and in end-organ damage (Mancia et al. 1999). The present study has some practical applications. The measurement of blood pressure performed once daily, or even several times a day, is clearly insufficient to determine the presence of nocturnal hypertension, the effectiveness of antihypertensive treatment, and the cardiovascular risk in OSA patients. In addition, assessment of blood pressure variability, based on the 24-h monitoring, enables to determine cardiovascular risk independently of the absolute values of blood pressure. The findings of the study indicate that in patients with severe OSA there are two important risk factors of cardiovascular complications occurring during sleep, i.e., increased nocturnal systolic and diastolic blood pressure as well as increased nocturnal blood pressure variability.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 17–29
DOI 10.1007/5584_2016_63

Published online: 30 August 2016

Frequent Attenders with Chronic Respiratory Diseases in Primary Care Settings

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Abstract

Governments struggle to fund health services and there is a growing interest in the cost, clinical characteristics, and interventions for high utilizers of care, such as persistent frequent attenders to primary care. The purpose of this study was to determine the components shaping the phenomenon of frequent attendance in patients with chronic respiratory diseases in primary care settings. We examined 200 adult patients with chronic diseases (median age 65, range 18–90) recruited from 126 general practitioners. We conclude that, in patients with chronic respiratory diseases, frequent attendance can be expected among those with a low level of satisfaction with their quality of health, a low level of QoL in the physical domain as much as QoL in the social relationships domain, making multiple visits to a doctor (more than 4 visits), taking more than five drugs, being treated for more than three chronic diseases, waiting at the doctor's office for no more than 30 min, receiving a greater number of primary care services, and requiring the assistance of a district nurse. Such patients may need social support interventions and monitoring of their clinical status.

Keywords

Chronic respiratory disease • General practice • Healthcare • Health expenditure • Health services • Needs assessment • Patient care • Quality of life

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1 Introduction

In the current global financial climate, where most governments struggle to fund health services, there is a growing interest in the cost, clinical characteristics, and interventions associated with high utilizers of care, such as persistent frequent attenders to primary care (Morriss et al. 2012). Patients who visit their general practitioner exceptionally frequently (frequent attenders; FA) are responsible for 33.4–39.0 % of all face-to-face consultations with their GPs, while persistent frequent attenders are responsible for about 8.0–15.5 % of such consultations (Pymont and Butterworth 2015; Smits et al. 2009). This group may be problematic for the whole healthcare system, as it contributes to a drain on limited healthcare resources, increases provider workload, and contributes to physician frustration (Savageau et al. 2006). Medically unexplained symptoms in FA can cause additional diagnostic difficulties for GPs, which in turn is associated with increased healthcare costs, as somatizing patients require additional medical consultations (Reid et al. 2002).

A review of the literature does not provide an unequivocal definition of FA. Savageau et al. (2006) argue that patients who make 5–12 (or more) visits *per* year pose a significant issue for primary healthcare. Different cut-off points for the definition of frequent attendance are described, e.g., the top 3 %, the top 10 %, or the top 25 % of all outpatient visits in primary care over a year or longer time period (Pymont and Butterworth 2015; Rifel et al. 2013; Luciano et al. 2010; Savageau et al. 2006). Other researchers define FA as those adult patients whose attendance rates were above the 90th centile for their age group (15–30, 31–45, 46–60, $\geq$ 61 years) and sex (Smits et al. 2008a; b). On the other hand, Hauswaldt et al. (2013) define FA in a 'traditional' sense, meaning that there are 24 or more contacts in at least one calendar year, and a 'new' sense based on intercontact intervals, i.e., the time in days between each two consecutive face-to-face meetings of a patient with the doctor. Persistent FA are these patients who have been FA for a three-year period (Smits et al. 2009) or who have visited the health center at least eight times a year for at least three out of four follow-up years (Koskela et al. 2010).

Despite the diversity in definitions of FA, researchers have identified many common characteristics for this specific group of patients. A number of studies have shown that FA consume more healthcare resources and are diagnosed with more somatic diseases, as well as more social problems, psychiatric problems, and medically unexplained physical symptoms. They also have a high degree of unmet health needs and difficulties in dealing with everyday life (Wiklund-Gustin 2013; Luciano et al. 2010; Reid et al. 2002). They receive more prescriptions for psychotropic medication (Smits et al. 2009). More than 50 % of FA suffer from psychological distress; more than 50 % have a physical disease. Social factors (low social support, unemployment, and divorce) are associated with frequent attendance in more than half of FA. Multiple problems (physical, psychological, and social) were found in one-third of FA (Vedsted and Christensen 2005). Frequent use of health services also appears to be associated with a low sense of coherence and a low internal locus of control (Wiklund-Gustin 2013). The number of life events in three years, panic disorder, illness behavior, and lack of mastery are also associated with the persistence of FA (Smits et al. 2014). Compared to non-frequent attenders, FA are statistically significantly more likely to report a lower quality of life, to be unmarried, have no educational qualifications, to experience greater health anxiety, and to suffer from morbid obesity, pain, and long-term pathological and ill-defined physical conditions. They are also more likely to have more depression, including dysthymia, anxiety, and somatoform disorders (Patel et al. 2015).

The available literature on patients with chronic respiratory diseases provides a lot of evidence for poor quality of their lives (Antwi et al. 2013; Kurpas et al. 2013; Ross et al. 2013), for negative influence of diseases (mainly asthma and COPD) on free-time activities, such as

hobbies and sports, as well as on sexual, family, and professional aspects of life (Kupryś-Lipińska and Kuna 2014; Polverino et al. 2008), for the connection between illness perception and health-related quality of life (Weldam et al. 2014), and for an increase in the number of unscheduled visits to family physicians in patients with worse overall functional status and lower quality of life (Ross et al. 2013).

The creation of a primary care model for FA with chronic respiratory diseases, accompanied by developing interventions such as customized social report cards and elements of the Chronic Care Model, can help those involved in the management of the healthcare and welfare sectors select appropriate (cheap and safe) interventions for this specific group of patients (Koskela et al. 2010; Savageau et al. 2006). As a result, community resources may prove less expensive and more satisfying solutions for patients' individual needs than medical services do.

The conclusions from the present study may be useful in developing health policy based on advanced primary care medical homes, because those practices are supported by payment, health information technology, and data which allow the quality of healthcare to be adjusted to patients' needs in terms of cost. One of the most up-to-date models of the management of healthcare and welfare institutions is Comprehensive Primary Care Plus (CPC+), which extends to such issues as planning care for chronically ill patients and risk-stratified care management (Sessums et al. 2016).

The literature lacks reports on frequent attenders with chronic respiratory diseases in primary care settings. Given this, the purpose of the present study was to determine the components shaping the phenomenon of frequent attendance in patients with chronic respiratory diseases and to determine the impact of these factors on the number of visits to the family doctor.

2 Methods

The research was performed in line with the Declaration of Helsinki and was approved by the Bioethical Commission of the Medical University in Wrocław (approval no. KB-422/2014). The main inclusion criteria were age (at least 18 years old) and diagnosis of at least one chronic respiratory disease.

The study group consisted of 200 adult patients with chronic diseases who answered a question about the frequency of their visits to a GP: 107 respondents had visited GP eight or more times, and 93 had fewer than eight visits. The median age was 65 (min–max: 18–90 years). The sociodemographic data of the patients are presented in Table 1. Participants were recruited from the patients of 126 GPs between January 2014 and October 2015. Only face-to-face consultations with GPs were included.

QoL was assessed using the Polish version of the WHO Quality of Life Instrument Short Form (WHOQOL-BREF), which measures QoL in

Table 1 Sociodemographic data of patients ($n = 200$)*

		n	%
Gender	Women	107	53.5
	Men	93	46.5
Age (yr)	24 and below	5	2.5
	25–44	23	11.6
	45–64	64	32.3
	65–84	97	49.0
	85 and above	9	4.5
Place of residence	Rural area	67	33.8
	Urban population:		
	< 5000	27	13.6
	5000–10,000	13	6.6
	10,000–50,000	47	23.7
	50,000–100,000	16	8.1
	100,000–200,000	17	8.6
	> 200,000	11	5.6
Education	Primary	38	19.3
	Vocational	59	29.9
	Secondary	54	27.4
	Post-secondary	31	15.7
	Higher	15	7.6
Marital status	Single	26	13.1
	Married	112	56.6
	Separated	4	2.0
	Divorced	5	2.5
	Widowed	51	25.8

*The figures in column *n* do not sum up to 200 due to gaps in the questionnaires completed by the patients

four domains: physical, psychological, social relationships, and environmental. Answers to all questions, including two questions on satisfaction with QoL and health, are given on a five-point Likert-type scale. The reliability of the Polish version of the WHOQOL-BREF questionnaire, measured using Cronbach's α coefficient, proved acceptable for the parts that evaluate each domain (with coefficients ranging from 0.81 to 0.69) and for the questionnaire as a whole (with a coefficient of 0.90).

The authors also used Juczyński's Health Behavior Inventory, which consists of 24 statements measuring four categories of prohealth behavior: healthy eating habits, preventive behavior, positive mental attitudes, and health practices. Respondents select the frequency with which they perform the healthy behavior or healthy activity: 1: almost never; 2: rarely; 3: from time to time; 4: often; 5: almost always. The total of the results from all four scales gives the score for the general health behavior (range 24–120); the higher the score, the healthier the behavior. The inventory's internal consistency, measured using Cronbach's α, equals 0.85.

The patients' adaptation to life with disease was assessed using the Acceptance of Illness Scale, which contains eight statements on the negative consequences of the health state, each statement being rated on a five-point Likert-type scale: 1 denotes poor adaptation to a disease and 5 its full acceptance. The score for illness acceptance is the total of all points and can range from 8 to 40. Low scores (0–29) indicate a lack of acceptance and adaptation to the disease and intense feeling of mental discomfort. High scores (35–40) indicate acceptance of the illness, manifesting as a lack of negative emotions associated with the disease. Cronbach's α was 0.85 for the Polish version and 0.82 for the original version.

The assessment of met and unmet needs was done with the Modified Short Camberwell Needs Assessment. This questionnaire focuses on 22 problem areas for patients with chronic somatic diseases (but without severe mental disorders); 0 denotes a need that was unmet, while 1 denotes a satisfied need. Next, 24 questions present 22 needs and enquire whether they are met (1) or unmet (0). In this way, the number of needs satisfied (M) out of the total number of needs (N) can be established and the Camberwell index is calculated as M/N. The internal consistency of the assessment was $\alpha = 0.96$.

The somatic index was calculated for each patient. Somatic symptoms reported by patients were assigned values from 1 (occurring once a year) to 7 (constant symptoms). The index was calculated by adding the values assigned and dividing by 49 (the highest possible score for somatic symptom frequency).

The service index was calculated by summing the services received and dividing by the number of types of services received during visits to doctors over the last 12 months.

2.1 Statistical Analysis

We took into account 52 variables, including the frequency of patients' visits to a GP. The subject of the analysis was the relationship between this variable and other variables.

The Shapiro-Wilk test showed only three of the 52 variables to be normally distributed (QoL in psychological domain, healthy eating habits, and BMI). Medians and variability ranges (extremes) were calculated for measurable (quantitative) variables; for qualitative variables, the frequency (percentage) was determined. The analysis of qualitative variables was based on contingency tables and the chi-squared test, or on Fisher's exact test for count data. The relationship between the number of visits and other variables was analyzed by Cramér's V coefficient and Spearman's rho rank correlation coefficient. The V coefficient was calculated using categorized variables, and the r correlation coefficient using the source variables (alternatively, nonnumerical variables are coded with numbers, e.g., 'no' is replaced by '0' and 'yes' by '1'). A significant linear relationship (r significantly different from '0') was confirmed by the significant strength of the relationship (the

V coefficient being significantly different from '0'). Where there was no linear relationship, the strength of the relationship was insignificant. Therefore, 27 variables significantly correlating with the number of visits were selected for hierarchical cluster analysis, the aim of which was to divide the initial group of variables into clusters of variables that correlate similarly with the number of visits. In the cluster analysis, a feature by which the objects will be classified needs to be chosen. In our study, this feature was the degree of correlation with the number of visits as expressed by the correlation coefficient *r*. The absolute value of the difference between correlation coefficients was taken as a measure of a distance between variables. Thus, the distance is close to '0' between variables with similar correlation coefficients and higher between variables with different correlation coefficients. Division into classes was performed using Ward's method. After several repeated calculations for the various numbers of classes, the conclusion was drawn that division into seven classes is possible and useful.

Finally, we calculated the odds ratios for certain events between groups of patients visiting a GP more and less often. Patient groups with above-median and below-median variable values were created. A 95 % confidence interval was set for each odds ratio. For the odds ratio analysis, we selected variables that correlated significantly with the number of visits to the GP's office, as well as other quantitative variables that did not correlate with the number of visits to the GP. Events were defined using variables. In the definition of an event we used medians for numerical variables, and for the categorized variables we combined categories into two groups. This choice is justified by the fact that the lack of correlations between quantitative variables does not imply the lack of relationship between two categorical variables created from those two initial variables (in our case by the comparison with the median). In all cases in which no correlation was observed between variables, there was also no relationship between the two categorical variables.

The R 3.1.3 (for Mac OS X 10.11.5) statistical software was used for all analysis. The critical level of significance was set at 0.05 for all tests.

3 Results

3.1 Significant Correlations

The number of visits to GP practices correlated positively with the following variables: healthcare services index ($r_s = 0.66$, $p < 0.001$), number of medications ($r_s = 0.42$, $p < 0.001$), number of chronic diseases ($r_s = 0.40$, $p < 0.001$), district nurse interventions ($r_s = 0.37$, $p < 0.001$), number of visits to a doctor due to chronic diseases ($r_s = 0.32$, $p < 0.001$), number of phone consultations ($r_s = 0.29$, $p < 0.001$), number of hospitalizations over three years ($r_s = 0.27$, $p < 0.001$), somatic index ($r_s = 0.27$, $p < 0.001$), age ($r_s = 0.25$, $p = 0.001$), level of preventive behaviors ($r_s = 0.23$, $p = 0.002$), average duration of chronic disease ($r_s = 0.21$, $p = 0.004$), home visits ($r_s = 0.21$, $p = 0.003$), and the level of health practices ($r_s = 0.18$, $p = 0.018$).

The number of visits to GP practices correlated negatively with the following variables: satisfaction with quality of health state ($r_s = -0.34$, $p < 0.001$), level of QoL in physical domain ($r_s = -0.31$, $p < 0.001$), satisfaction with QoL ($r_s = -0.30$, $p < 0.001$), level of QoL in psychological domain ($r_s = -0.29$, $p < 0.001$), level of illness acceptance ($r_s = -0.27$, $p < 0.001$), waiting time (in minutes) for the patient at the doctor's office ($r_s = -0.24$, $p = 0.001$), Camberwell index ($r_s = -0.23$, $p = 0.001$), level of education ($r_s = -0.21$, $p = 0.003$), level of QoL in social relationships ($r_s = -0.18$, $p = 0.010$), feeling that the waiting room is comfortable ($r_s = -0.16$, $p = 0.028$), expected waiting time (in days) for a visit to the doctor ($r_s = -0.15$, $p = 0.042$), waiting time (in days) for a visit to the doctor ($r_s = -0.15$, $p = 0.047$), level of QoL in environmental

domain ($r_s = -0.14$, p = 0.042), and the problems with obtaining a referral for additional tests (blood count, X-ray, USG, etc.) ($r_s = -0.14$, p = 0.049).

3.2 Results of Hierarchical Cluster Analysis

The results of the hierarchical cluster analysis are presented in Table 2 and Fig. 1.

Table 2 Results of statistical analysis: frequent attender (FA) group *vs.* non-frequent attender (non-FA) group

		NVP < 8		NVP ≥ 8		OR	pF	rho	pS
Variables		n	%	n	%	CI1	CI2		
Home visits	x = 0	73	78.5	75	70.1	1.55	0.198	0.21	0.003
	x > 0	20	21.5	32	29.9	0.78	3.15		
Number of phone consultations	x = 0	66	71.0	63	58.9	1.70	0.078	0.29	<0.001
	x > 0	27	29.0	44	41.1	0.91	3.23		
District nurse interventions	x = 0	78	83.9	71	66.4	2.62	0.006	0.37	<0.001
	x > 0	15	16.1	36	33.6	1.27	5.62		
Waiting time (days) for a visit to the doctor	x ≤ 1	45	54.2	54	63.5	1.47	0.272	−0.15	0.047
	x > 1	38	45.8	31	36.5	0.76	2.86		
Expected waiting time (days) for a visit to the doctor	x ≤ 3	52	57.8	68	67.3	1.50	0.181	−0.15	0.042
	x > 3	38	42.2	33	32.7	0.80	2.83		
Wait time (minutes) in the doctor's office	x ≤ 30	52	57.8	79	74.5	2.13	0.015	−0.24	0.001
	x > 30	38	42.2	27	25.5	1.12	4.10		
The waiting room comfort	yes	80	86.0	101	94.4	2.72	0.054	−0.16	0.028
	no	13	14.0	6	5.6	0.92	9.14		
Problems with obtaining a referral to additional blood and other tests	no	80	86.0	101	94.4	0.37	0.054	−0.14	0.049
	yes	13	14.0	6	5.6	0.11	1.09		
Education									
At least vocational		37	41.1	60	56.1	0.55	0.045	−0.21	0.003
Higher than vocational		53	58.9	47	43.9	0.30	1.002		
Level of illness acceptance	x ≤ 27	39	44.8	59	59.0	0.57	0.058	−0.27	<0.001
	x > 27	48	55.2	41	41.0	0.30	1.05		
Satisfaction with QoL	x ≤ 4	86	92.5	101	96.2	0.49	0.354	−0.30	<0.001
	x > 4	7	7.5	4	3.8	0.10	2		
Satisfaction with quality of health	x ≤ 3	55	60.4	84	80.0	0.38	0.003	−0.34	<0.001
	x > 3	36	39.6	21	20.0	0.19	0.76		
Level of QoL:									
Physical domain	x ≤ 13.14	34	36.6	54	51.4	0.55	0.045	−0.31	<0.001
	x > 13.14	59	63.4	51	48.6	0.30	0.100		
Psychological domain	x ≤ 13.33	38	40.9	58	54.7	0.57	0.065	−0.29	<0.001
	x > 13.33	55	59.1	48	45.3	0.31	1.04		
Social relationships domain	x ≤ 14.67	52	55.9	76	71.7	0.50	0.026	−0.18	0.010
	x > 14.67	41	44.1	30	28.3	0.27	0.94		
Environmental domain	x ≤ 13.50	45	48.4	56	52.8	0.84	0.571	−0.14	0.042
	x > 13.50	48	51.6	50	47.2	0.46	1.52		
Level of preventive behaviors	x ≤ 3.83	43	48.3	35	35.7	1.68	0.102	0.23	0.002
	x > 3.83	46	51.7	63	64.3	0.90	3.15		
Level of health practices	x ≤ 3.58	45	55.6	39	44.8	1.53	0.217	0.18	0.018
	x > 3.58	36	44.4	48	55.2	0.80	2.96		
Age	x ≤ 65	53	58.2	49	45.8	1.65	0.089	0.25	0.001
	x > 65	38	41.8	58	54.2	0.90	3.02		
Number of hospitalization in 3 years	x ≤ 1	70	75.3	67	62.6	1.81	0.067	0.27	<0.001
	x > 1	23	24.7	40	37.4	0.94	3.53		
Average duration of chronic disease	x ≤ 8	54	58.1	49	45.8	1.63	0.091	0.21	0.004
	x > 8	39	41.9	58	54.2	0.90	2.99		

(continued)

Table 2 (continued)

Variables		NVP < 8		NVP ≥ 8		OR	pF	rho	pS
		n	%	n	%	CI1	CI2		
Number of visits to a doctor due to chronic diseases	x ≤ 4.33	58	62.4	41	38.3	2.65	0.001	0.32	<0.001
	x > 4.33	35	37.6	66	61.7	1.45	4.93		
Camberwell index	x ≤ 0.78	42	46.2	61	57.5	0.63	0.118	−0.23	0.001
	x > 0.78	49	53.8	45	42.5	0.35	1.15		
Somatic index	x ≤ 0.39	54	58.1	48	44.9	1.70	0.067	0.27	<0.001
	x > 0.39	39	41.9	59	55.1	0.94	3.10		
Healthcare services index	x ≤ 4.67	71	76.3	28	26.9	8.64	<0.001	0.66	<0.001
	x > 4.67	22	23.7	76	73.1	4.39	17.62		
Number of medications	x ≤ 5	67	72.0	52	48.6	2.71	0.001	0.42	<0.001
	x > 5	26	28.0	55	51.4	1.45	5.15		
Number of chronic diseases	x ≤ 3	62	66.7	47	43.9	2.54	0.002	0.40	<0.001
	x > 3	31	33.3	60	56.1	1.38	4.74		

NVP Number of visits to GP practice, *OR* odds ratio, *CI1*, *CI2* 95 % confidence interval for the odds ratio, *pF* p-value of Fisher's exact test, *rho* Spearman's rank correlation coefficient between rough variables, *pS* p-value of test for correlation between paired samples using Spearman's rho, *QoL* quality of life

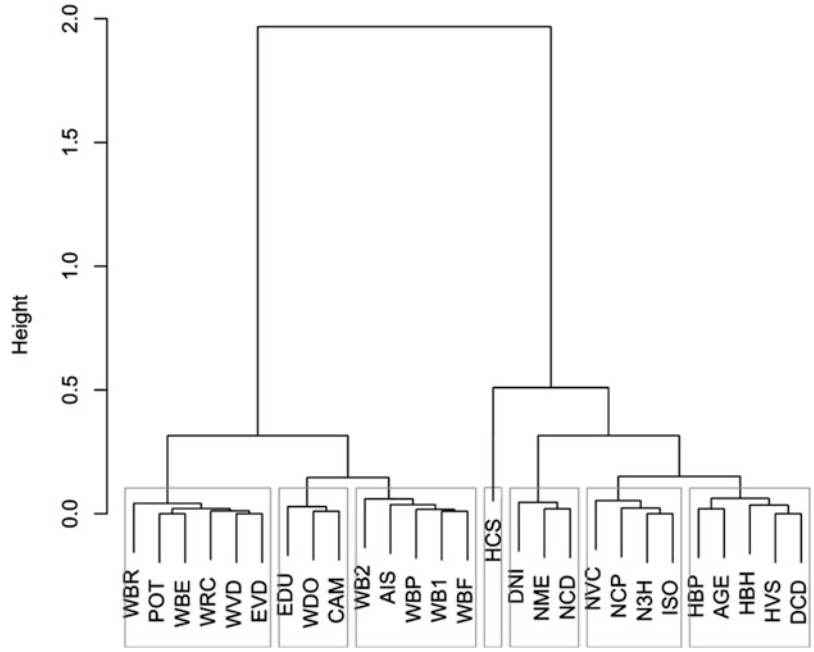

Fig 1 Cluster analysis results for the number of visits to GP's practice.
AGE Age, *AIS* Level of illness acceptance, *CAM* Camberwell index, *DCD* Average duration of chronic disease, *DNI* District nurse interventions, *EDU* Education, *EVD* Expected waiting time (days) for a visit to the doctor, *HBH* Level of health practices, *HBP* Level of preventive behaviors, *HCS* Healthcare services index, *HVS* Home visits, *ISO* Somatic index, *N3H* Number of hospitalizations in three years, *NCD* Number of chronic diseases, *NCP* Number of phone consultations, *NME* Number of medications, *NVC* Number of visits to a doctor due to chronic disease, *POT* Problems obtaining a referral to additional tests (blood count, X-rays, etc.), *WB1* Satisfaction with QoL, *WB2* Satisfaction with quality of health state, *WBE* Level of QoL in environmental domain, *WBF* Level of QoL in physical domain, *WBP* Level of QoL in psychological domain, *WBR* Level of QoL in social relationships, *WDO* Waiting time (minutes) in the doctor's office, *WRC* Waiting room comfort, *WVD* Waiting time (days) for the visit to a doctor

3.3 Odds Ratios for Selected Variables

Odds ratios for selected variables are presented in Table 2. The odds ratio for a healthcare services index above 4.67 in the FA group was almost nine times higher than in the non-FA group (OR 8.64, 95 % CI 4.39-17.62). The proportions of patients with an index higher than 4.67 in both groups were 73.1 % and 23.7 %, respectively ($p < 0.001$). The odds ratio for taking more than five medicines was almost three times higher in the FA group than in the non-FA group (OR 2.71, 95 % CI 1.45-5.15). The proportions of patients taking more than five drugs in these groups were 51.4 % and 28.0 %, respectively ($p < 0.001$). The odds ratio for the number of visits to the GP due to chronic diseases, higher than four, was almost three times higher in the FA group than in the non-FA group (OR 2.65, 95%CI 1.45-4.93). The proportions of patients who had more than four visits due to chronic disease were 61.7 % and 37.6 %, respectively ($p = 0.001$). The odds ratio for district nurse visits was almost three times higher in the FA group than in the non-FA group (OR 2.62, 95%CI 1.27–5.62). The proportions of patients visited by a district nurse in the two groups were 33.6 % and 16.1 %, respectively ($p = 0.006$). The odds ratio for having more than three chronic diseases was almost two and a half times higher in the FA group than in the non-FA group (OR 2.54, 95 % CI 1.38-4.74). The proportions of patients with more than three diseases in both groups were 56.1 % and 33.3 %, respectively ($p < 0.001$). The odds ratio for waiting no more than 30 min at the GP's office was almost twice as high in the FA group as in the non-FA group (OR 2.13, 95 % CI 1.12-4.10). The proportions of patients who waited no more than 30 min were 74.5 % and 57.8 %, respectively ($p = 0.015$). The odds ratio for the level of satisfaction with health state being above three was almost three times lower in the FA group than in the non-FA group (OR 0.38, 95 % CI 0.19-0.76). The proportions of patients with a level of satisfaction above three were 20.0 % and 39.6 %, respectively ($p = 0.003$). The odds ratio for quality of life in the physical domain, greater than 13.14, was almost two times smaller in the FA group than in the non-FA group (OR 0.55, 95 % CI 0.30-0.99). The proportions of patients with quality of life in the physical domain above 13.14 were 48.6 % and 63.4 %, respectively ($p = 0.045$). The odds ratio for quality of life in the social relationships domain, greater than 14.67, was almost two times smaller in the FA group than in the non-FA group (OR 0.50, 95 % CI 0.27-0.94). The proportions of patients with quality of life in the social relationships domain above 14.67 in the two groups were 28.3 % and 41.4 %, respectively ($p = 0.026$).

4 Discussion

Frequent attenders (FA) to general practices receive great attention in primary care research, because they demand large amounts of time, manpower, technical equipment, and financial resources (Hauswaldt et al. 2013). They also substantially increase the cost, not only of primary, but also specialist care (Smits et al. 2013). Our findings support the above statements. We demonstrate that an increase in the number of visits to a GP is strongly positively correlated with the healthcare services index. What is more, the probability of the health service index being above the median was almost nine times higher in FA than in non-FA. The former had fewer problems obtaining a diagnostic examination request (blood test, X–rays, and others) than the latter. In addition, FA were almost twice as likely to wait for a shorter time (30 min at the most) in the GP's office.

From an economic standpoint, the subgroup of FA with medically unexplained symptoms (MUPS) is especially problematic. It has been shown that such patients receive a greater number of referrals to secondary care and are more likely to undergo particular tests. This group accounts for the level of service and expenditure that are comparable with other FA, but the use of

cost of medical investigation in this group is significantly greater (Reid et al. 2002). A three-year observation of a Dutch population clearly indicates that the median and mean costs of both primary and specialist care are substantially higher in all FA groups than in non-FA groups. As compared to non-FA, the adjusted mean expenditures were €1723 and €5293 higher in FA for one-year and three-year observations, respectively (Smits et al. 2013).

The present study shows that FA consume more additional GP services as a greater number of visits to the GP correlated positively with the numbers of the following variables: home visits, phone consultations, and district nurse interventions. Further, the odds ratio for the visit of a district nurse was nearly three times higher in the FA group than in the non-FA group. Hauswaldt et al. (2013) also have shown that FA more likely need home visits and emergency attention than non-FA do. Still, an increasing number of medical services should not be looked at purely from the economic point of potential costs and expenses. In a study of elderly Koreans, Choi and Joung (2016) have demonstrated that, among long-term care service users, the hazard ratio for death of institutional service users is significantly higher than for in-home service users. These authors have also emphasized that the main goal of long-term care insurance for the elderly should be health promotion, life stabilization, and the prevention of institutional care. It is possible to attain this goal through the provision of a higher number of the above-mentioned services, while simultaneously taking into account the patients' opinions on the quality of healthcare services (Marcinowicz et al. 2015).

In the present study, an increase in the number of visits was accompanied by a higher level of preventive behaviors and a higher level of health practices in the FA group, which shows that the phenomenon of frequent attendance can contribute to prohealth behaviors. In a longer perspective, such behaviors may prove cost-effective for the healthcare system. Smits et al. (2014) have also shown that persistent FA score higher on illness behavior, as measured by the Illness Attitude Scales.

Additionally, we demonstrate that variables such as age and the level of education correlate significantly with the number of visits to a GP in patients with chronic respiratory diseases. The number of visits increased significantly with age, though advanced age was not a variable differentiating the FA and non-FA groups. Savageau et al. (2006) have shown that patients aged 45–64, and those 65 and older, are more likely to visit frequently. In another study, proportion of FA was 28 % higher among those aged 55–59, and 54 % higher among those aged 60–65, relative to the 50–54 years of age group (Jørgensen et al. 2016).

Women are more likely to be FA than men (Pymont and Butterworth 2015; Smits et al. 2013; Koskela et al. 2010). This may generally be associated with postmenopausal hormone therapy (Jørgensen et al. 2016), although in case of women with chronic respiratory diseases, the reasons may be different. Di Marco et al. (2006) have drawn attention to the fact that women with chronic respiratory diseases endure symptoms worse than men who have the same pulmonary function, and more often suffer from depression, anxiety, and dyspnea. Panic disorder, negative life events, illness behavior, and the lack of mastery are independently associated with the persistence of frequent attendance (Smits et al. 2014). Underrecognized and untreated depression and anxiety have deleterious effects on physical functioning and social interaction, increasing fatigue and healthcare utilization in patients with COPD (Yohannes and Alexopoulos 2014). The results of the present study correspond with those mentioned above, as we confirm a negative relationship between the psychological domain of QoL and the number of visits to a GP. Another study of COPD patients has revealed that for men alone, higher age is associated with a higher probability of GP visits, but not with emergency room visits or hospitalizations (de Miguel Díez et al. 2015). A longitudinal study of Slovenian primary care shows that male gender is significantly associated with frequent attendance after 12 months, but not 24 months, although age and

living alone have no significant effects on frequent attendance after 12 and after 24 months follow-up (Rifel et al. 2013).

The present study does not demonstrate any significant influence of gender or marital status on the FA phenomenon, but we do demonstrate that low QoL scores for social relationships and environmental domains were related to a significant increase in the number of visits to the GP. What is more, the odds for QoL in the social relationships domain being above the median were twice as small for the FA group as for the non-FA group. A Danish study has shown that married persons are less likely to be FA than unmarried ones. Living alone also correlates with an increased number of unscheduled visits to the primary care physician in patients older than 65 years with asthma (Ross et al. 2013). Occupational status is a strong determinant of frequent attendance, with employed persons being less likely to be FA than the unemployed ones (Jørgensen et al. 2016). Another study addressing the influence of social factors suggests that male frequent attendance is associated with living alone, being out of work, or being on disability pension, whereas female frequent attendance seems unaffected by social factors when the analysis is adjusted for physical and psychological health (Vedsted and Olesen 2005). The above-mentioned study of Rifel et al. (2013) has shown that being a housewife, a student, or unemployed is a protective factor for frequent attendance at 12 months, but not at 24 months.

The relationship between patients' educational level and the number of visits to the GP is of note. Jørgensen et al. (2016) have mentioned that a high education level (>4 years higher education *vs*. no vocational training) is inversely associated with frequent attendance. Rifel et al. (2013) have demonstrated that people with more than elementary school education are less likely to become frequent attenders. These results correspond with the present findings, showing that the number of visits to a GP significantly decreases with an increase in the level of the patient's education.

Persistent frequent attendance in primary care is associated with poor quality of life and high clinical complexity, characterized by diverse and often persistent physical and mental multimorbidity (Patel et al. 2015). How patients perceive the symptoms of their chronic diseases is crucial to the FA phenomenon (Wiklund-Gustin 2013). Weldam et al. (2014) have indicated that the health-related quality of life in COPD patients is associated with illness perception. A study of Antwi et al. (2013) has shown that the prevalence of self-reported COPD is associated with poor health- related quality of life and is highest among women, those aged 65 and older, current smokers, and with low levels of education and income.

Patients with chronic respiratory diseases are not satisfied with their quality of life and health state. In a study of Kurpas et al. (2013) in 315 adults with chronic respiratory diseases, the majority (66.1 %) of respondents were dissatisfied (39.0 %) or very dissatisfied (27.1 %) with their health state. Most respondents (81.1 %) were also dissatisfied with the quality of their lives (47.7 % were very dissatisfied and 33.7 % were dissatisfied). An important component of quality of life in patients suffering from chronic illnesses is sexual activity. A reduction in sexuality is reported in patients with COPD, cystic fibrosis, and respiratory failure on noninvasive mechanical ventilation (Polverino et al. 2008). The factors associated with a worse self-rated health in patients with COPD are the lowest educational level, having three or more chronic diseases, mental disorders, and no leisure time physical activities. In relation to the health resources use, having three or more chronic diseases is associated with a higher probability of general practitioner visits (de Miguel Díez et al. 2015). In the present study we confirm that a low level of satisfaction with QoL (lower than 4) was significantly related to a higher number of visits to a GP. Moreover, FA were almost three times less likely to have a level of satisfaction with health above the median (higher than 3) than non-FA were.

One issue that needs particular attention in discussing the FA phenomenon is the connection between the number of visits to a GP and the patients' functioning in physical domain

regarding the number and duration of chronic diseases. This is an important problem, because patients with chronic respiratory diseases often suffer from comorbidities, such as hypertension, gastroesophageal reflux, arthritis, Alzheimer's disease, allergic rhinitis, denutrition, obesity, anemia, cancer, cardiovascular diseases, osteoporosis, and diabetes (Brinchault et al. 2015; Ross et al. 2013). Pymont and Butterworth (2015) have suggested that the likelihood of frequent attendance is increased by diabetes, asthma, thyroid problems, arthritis, and heart conditions. The total number of medical complaints, both somatic and psychological, is higher among FA than among non-FA (Smits et al. 2013). FA are more likely to suffer from pneumonia, stroke, dementia, or severe substance abuse (Hauswaldt et al. 2013). Savageau et al. (2006) have also shown that the conditions associated with higher visit frequency include hypertension, diabetes, and depression.

It has been shown that lower physical and mental quality of life scores on the SF-12 questionnaire predict future frequent attendance (Rifel et al. 2013). This is confirmed by the present results which show that the general number of visits to GP practice increases with the number of visits to a doctor due to chronic diseases, the number of chronic disease, the average duration of a chronic disease, and the somatic index.

Smits et al. (2009) have concluded that, compared to one-year frequent attenders, persistent frequent attenders have even more consultations with their GP, and suffer not only from more somatic diseases but especially from more social and psychiatric problems, and from medically unexplained physical symptoms. They are also prescribed more psychotropic and analgesic medications. In the present study we confirm that the probability of taking more than five drugs was almost three times higher in the FA group. However, as stated by Hirsikangas et al. (2016), FA adhere well to health regimens and exceptionally well to medication. The variables that predict the best adherence of FA to health regimens are carrying out self-care, receiving medical care, and feeling responsible for self-care.

Several limitations of this study should be mentioned. Considering a small size of the study sample and a short observation period (12 months for the number of visits, three years for the hospitalization rate), as well as the lack of a uniform definition of FA in the literature, we defined FA as patients whose number of visits during a year was equal to, or higher than, the median (8 visits). However, we adopted the approach concerning the number of visits to define frequent attendance used by other authors (Hirsikangas et al. 2016; Koskela et al. 2010).

5 Conclusions

In patients with chronic respiratory diseases, frequent attendance should be expected among those with low levels of satisfaction with quality of health state, low level of QoL in the physical domain as much as QoL in the social relationship domain, those with the number of visits to a doctor due to chronic diseases higher than four, taking more than five drugs, receiving treatment for more than three chronic diseases, waiting at the doctor's office for no more than 30 min, receiving a greater number of primary care services, and requiring the interventions of a district nurse. These patients may need social support interventions and monitoring of their clinical status.

Conflicts of Interest The authors have no financial or other relations that might lead to a conflict of interest.

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 31–34
DOI 10.1007/5584_2016_71

Published online: 30 August 2016

Damaging Effects of Cannabis Use on the Lungs

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Abstract

Cannabis is the most widely smoked illicit substance in the world. It can be smoked alone in its plant form, marijuana, but it can also be mixed with tobacco. The specific effects of smoking cannabis are difficult to assess accurately and to distinguish from the effects of tobacco; however its use may produce severe consequences. Cannabis smoke affects the lungs similarly to tobacco smoke, causing symptoms such as increased cough, sputum, and hyperinflation. It can also cause serious lung diseases with increasing years of use. Cannabis can weaken the immune system, leading to pneumonia. Smoking cannabis has been further linked with symptoms of chronic bronchitis. Heavy use of cannabis on its own can cause airway obstruction. Based on immuno-histopathological and epidemiological evidence, smoking cannabis poses a potential risk for developing lung cancer. At present, however, the association between smoking cannabis and the development of lung cancer is not decisive.

Keywords

Cannabis • Cannabinoids • Lung diseases • Marijuana • Respiratory health • Respiratory risk

1 Cannabis

Cannabis, known as marijuana, is the most popular illicitly used substance worldwide. The substance contains hundreds of compounds, including several scores of psychoactive ones, the most prevalent of which is tetrahydrocannabinol (THC). Apart from its recreational use, it can also be a medicinal drug. The use of cannabis, especially among young people, has increased dramatically in recent times. Dealing with cannabinoids is a topic that is actively discussed in politics and in the public (Kreuter et al. 2016; Lutchmansingh et al. 2014). Despite legislative

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reform, cannabis is still the most commonly used drug by smokers. The use of cannabinoids may lead to harmful side effects. Moreover, composition of commercial cannabis has barely changed over recent years (Imtiaz et al. 2016). Although cannabis smoke contains dangerous and carcinogenic substances similar to tobacco smoke, the knowledge on the health effects and impact on the lungs cannabis smoking is an area of limited understanding. Data published before widespread legalization cannabis smoking are contradictory and inconclusive (Imtiaz et al. 2016). Therefore, it is imperative to develop sound scientific information relating to the impact of smoking cannabis on lung health.

The purpose of this review is to explore the effects of inhaling marijuana smoke on the respiratory tract system through recent studies. To determine these effects, a search was conducted in the PubMed database for appropriate publications dated from 1997 to 2016 and the results were analyzed.

2 Cannabis and Tobacco

High doses of cannabinoids may cause lung diseases, particularly with a long-term use. Side effects from the use of cannabis are often not sufficiently separated by the simultaneous effects of tobacco (Kreuter et al. 2016). Smoking ordinary marijuana can cause several respiratory symptoms such as coughing, wheezing, and sputum. These symptoms are similar to those experienced by tobacco smokers. Moreover, endobronchial biopsies of habitual marijuana and tobacco smokers have shown that both drugs cause significant histopathological changes to bronchial mucosa. Although marijuana smokers show minimal changes to lung function compared with tobacco smokers, they can develop bullous lung disease and spontaneous pneumothorax (Lutchmansingh et al. 2014).

The effects of marijuana and tobacco are additive. The use of cannabinoids in combination with tobacco can lead to the development of chronic bronchitis and pulmonary emphysema. Cannabinoids also induce allergic diseases, such as asthma. The use of cannabis weakens the immune system, resulting in inflammatory lung diseases such as pneumonia (Kreuter et al. 2016).

Marijuana smoke contains polycyclic aromatic hydrocarbons and carcinogens at higher concentrations than those in in tobacco smoke (Underner et al. 2014). In the worst-case scenario, lung cancer arises as a synergistic effect of chronic addiction to both cannabis and tobacco (Imtiaz et al. 2016; Kreuter et al. 2016; Biehl and Burnham 2015). However, examination of the relationship between smoking cannabis and lung cancer gives little evidence of increased risk of cancer among occasional or long-term cannabis smokers, barring the harmful effects of heavy consumption (Zhang et al. 2015). Simultaneous smoking tobacco and cannabis is rarely accompanied by lung cancer with tuberculosis (Cadelis and Ehret 2015). In fact, the occurrence of lung cancer together with active tuberculosis is rather rarely reported.

3 Impact of Cannabis on Respiratory Health

A recent increase in the use of cannabis has been recorded all over the world, although marijuana is as yet an illegal drug in many countries. Marijuana is used *via* smoke inhalation and there are concerns about its possible adverse effects on the lungs; such effects have been actually documented. The evidence clearly suggests that habitual or regular marijuana smoking is a detriment for respiratory health. Table 1 summarizes

Table 1 Lung damage caused by smoking cannabis

Cough
Sputum
Wheezing
Bullous lung disease
Spontaneous pneumothorax
Chronic bronchitis
Pulmonary emphysema
Allergic asthma
Pneumonia
Tuberculosis
Lung cancer

the possible harmful effects on the lungs of smoking cannabis.

Smoking cannabis can particularly lead to chronic obstructive pulmonary disease (COPD) and lung cancer, both of which are major causes of morbidity and death worldwide. Unlike smoking tobacco, risks of cannabis use have not been extensively studied, resulting in a serious gap in the knowledge of the actual effects on the respiratory system of cannabis. A limited number of studies have shown that chronic cannabis use is consistently associated with an increased prevalence of symptoms related to chronic bronchitis (Tashkin 2014; Aldington et al. 2007). Studies have demonstrated endoscopic and microscopic evidence of inflammatory damage to the central airways in habitual smokers of cannabis alone, without tobacco. The damage is accompanied by a loss of ciliated epithelium and a replacement thereof with mucus secreting goblet cells (Roth et al. 1998; Fligiel et al. 1997).

In contrast, effects of chronic cannabis use on lung function are less clear. Some studies have shown little differences in cannabis smokers compared to nonsmoking controls, while other show mild obstruction and insignificant trend toward reduced forced expired volume in 1 s (FEV1) only in the heaviest marijuana smokers (Pletcher et al. 2012; Aldington et al. 2007). Some of these differences could be due to variations in the populations studied, the age of participants, and the amount and duration of cannabis use (Tashkin 2014; Hancox et al. 2010). Since COPD has generally not been studied in people over the age of 40 in relation to cannabis use, and because COPD prevalence increases with age, there is a need for the assessment of the potential effects of the former or current cannabis use in elderly participants of future studies.

It is disputed whether or not smoking cannabis is conducive to lung cancer. While there are detectable carcinogens in cannabis smoke and histological and immunopathological evidence of potentially pre-cancerous changes to the bronchial epithelium of smokers of cannabis, without tobacco, the evidence from epidemiological studies is contentious (Barsky et al. 1998; Fligiel et al. 1997).

It also is uncertain whether or not using cannabis carries an increased risk of developing pneumonia. THC exerts an immunosuppressive effect. That combined with cannabis-induced alterations in alveolar macrophage function and the replacement of bronchial epithelium by hyperplastic mucous secreting cells lowers epithelial defense against infections and predisposes to pneumonia as well as tuberculosis (Shay et al. 2003; Fligiel et al. 1997). In addition, it has been reported that cannabis is often contaminated by fungal and bacterial pathogens (Tashkin 2014). While some case reports and a few past epidemiological studies suggest a possible link between cannabis and pneumonia in immunocompromised subjects, additional well-designed controlled studies are needed to firmly establish a causal relationship between cannabis and pneumonia.

Lastly, numerous cases of pneumothorax, pneumomediastinum, and bullous pulmonary diseases in heavy smokers of cannabis have been described (Aldington et al. 2007). However, the prevalence of these lung diseases have not been studied in the general population, so that a cause-effect relationship cannot be discerned from those isolated case reports.

4 Conclusions

A connection between smoking cannabis and deterioration of lung function, particularly in elderly subjects who carry a higher risk for developing COPD, which could be further enhanced by cannabis use, should be investigated. Additional studies should explore and clarify the potential risks of lung cancer and infectious contagions from cannabis use. Since cannabis use, both recreational and medicinal, is expected to further grow, there is an urgent need for medical savvy on how to mitigate the adverse effects on the lungs of regular cannabis use.

Conflict of Interest The authors declare no competing financial or otherwise interests in relation to this article.

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 35–39
DOI 10.1007/5584_2016_70

Published online: 30 August 2016

Neurogenic Pulmonary Edema in Aneurysmal Subarachnoid Hemorrhage

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Abstract

Neurogenic pulmonary edema (NPE) is observed in cerebral injuries and has an impact on treatment results, being a predictor of fatal prognosis. In this study we retrospectively reviewed medical records of 250 consecutive patients with aneurysmal subarachnoid hemorrhage (SAH) for the frequency and treatment results of NPE. The following factors were taken under consideration: clinical status, aneurysm location, presence of NPE, intracranial pressure (ICP), and mortality. All patients had plain- and angio-computer tomography performed. NPE developed most frequently in case of the aneurysm located in the anterior communicating artery. The patients with grades I-III of SAH, according to the World Federation of Neurosurgeons staging, were immediately operated on, while those with poor grades IV and V had only an ICP sensor's implantation procedure performed. A hundred and eighty five patients (74.4 %) were admitted with grades I to III and 32 patients (12.8 %) were with grade IV and V each. NPE was not observed in SAH patients with grade I to III, but it developed in nine patients with grade IV and 11 patients with grade V. Of the 20 patients with NPE, 19 died. Of the 44 poor grade patients (grades IV-V) without NPE, 20 died. All poor grade patients had elevated ICP in a range of 24-56 mmHg. The patients with NPE had a greater ICP than those without NPE. Gender and age had no influence on the occurrence of NPE. We conclude that the development of neurogenic pulmonary edema in SAH patients with poor grades is a fatal prognostic as it about doubles the death rate to almost hundred percent.

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Keywords
Cerebral injury • Intracranial aneurysm • Intracranial pressure • Mortality • Pulmonary edema • Subarachnoid hemorrhage

1 Introduction

Neurogenic pulmonary edema (NPE) is a life threatening complication of central nervous system injuries (Mrozek et al. 2015; Davison et al. 2012; Maramattom et al. 2006). It develops mainly in cerebral injury, but may also constitute a sequela of spinal cord compression (Sedy et al. 2015). The most important role in NPE development plays increased intracranial pressure (Mrozek et al. 2015; Vespa and Bleck 2004). Intracranial hypertension activates the sympathetic nervous system, which affects the cardio-pulmonary system *via* release of catecholamines, leading eventually to NPE (Chen et al. 2014; Inamasu et al. 2012; Friedman et al. 2003). NPE can develop within minutes to hours after injury, but it can also present delayed forms that develop 12–24 h after cerebral injury (Piazza et al. 2011; Kahn et al. 2006). Aneurysmal subarachnoid hemorrhage (SAH) is one of the most common cause of NPE. The development of NPE is usually associated with poor grade patients, and the incidence of NPE in aneurysmal SAH ranges from 2 to 30 % (Mrozek et al. 2015; Veeravagu et al. 2014; Sato et al. 2012; Piazza et al. 2011; Wartenberg et al. 2006). In the present study we analyzed the occurrence of NPE in patients with aneurysmal subarachnoid hemorrhage and the influence of NPE on the results of treatment.

2 Methods

The study was accepted by the Ethics Board for Human Research of the Kazimierz Pulaski University of Technology and Humanities in Radom, Poland. We retrospectively analyzed the files of 250 patients admitted to neurosurgical wards with a diagnosis of aneurysmal SAH. Only were the patients admitted directly after SAH, with CT and angio-CT performed on admission, included into the study. The patients with large intracerebral hematomas, requiring prompt evacuation, were excluded from the analysis. The clinical condition on admission was assessed according to the modified WFNS grading scale (Sano et al. 2015; Rosen and Macdonald 2004). The patients with grades I-III were surgically treated within the first 3 days after admission. Poor grade patients underwent only the implantation of an intracranial pressure measurement device. All patients had chest X-ray performed on admission, which was repeated when pulmonary disturbances appeared. NPE was diagnosed on the basis of clinical criteria such as the auscultation of crackles and the presence of pink tracheal exudate, and radiographic criteria such as a sharp delineation of pulmonary markings, blurring or haziness of the perivascular outlines, and the loss of demarcation of hilar shadows. The patients with a suspicion of choking or with a history of a previous serious pulmonary disease were excluded from the analysis.

There were 140 men and 110 women, 25–69 years old, included into the analysis. One hundred and eighty six (74 %) of them were in I-III grade in WFNS scale, 32 (13 %) were in grade IV, and another 32 (13 %) in grade V. All 186 patients admitted in grade I to III were treated surgically by one of the authors of this article (ZK) within the first three days. Sixty four poor grade patients had only the implantation of an intracranial pressure (ICP) measurement device for continuous ICP monitoring. Poor grade patients who had improved were also surgically treated between the 8th and 20th day after aneurysmal SAH. The patients were treated microsurgically, no endovascular treatment was employed (Sandström et al. 2013).

Categorical data are presented as the number of patients. Numerical data of the intracranial

pressure are presented as means ± SD and their differences are compared with a *t*-test. A p-value of less than 0.05 defined statistical significance.

3 Results

None of the 186 patients admitted in grade I-III developed NPE. All these patients were operated on within 72 h; 90 % of them within the first 24 h after admission. Seven patients (3.8 %) died after surgery due to a cerebral vasospasm. For comparison, 39 (60.9 %) of the 64 poor grade patients died. NPE was diagnosed in 20 (31.3 %) of the poor grade patients, which makes 8.0 % of all 250 patients analyzed in this study. Clinical and radiological symptoms of NPE appeared in up to 12 h after admission. Of the 20 patients with NPE 19 died, all within the first seven days after aneurysmal SAH. The NPE patients had higher ICP values (mean 45.0 ± 7.2 mmHg) than the patients without this pulmonary complication (mean 26.0 ± 4.2 mmHg); the difference between the two groups was significant ($p < 0.001$). In general, the higher the ICP the more cases of NPE were noted (Table 1). Of the 44 poor grade patients who did not develop NPE, 17 (38.6 %) died within the first week. All the remaining 27 patients in this group, who survived the first week, were operated on 10 to 21 days after aneurysmal SAH and three of them died after surgery.

The most frequent location of an aneurysm was the middle cerebral artery (36.0 %), followed by the internal carotid artery (31.5 %), and the anterior communicating artery (29.0)%. Eight patients had a different aneurysm location, four in the anterior cerebral artery and another four at the tip of the basilar artery. NPE developed in 13 (59.1 %) of the 22 poor grade patients with the anterior communicating artery aneurysms, in two (50.0 %) with the basilar artery aneurysms, and in five (12.8 %) of the 39 patients with another location of aneurysms. Thus, NPE developed most frequently in SAH resulting from a rupture of the anterior communicating artery aneurysm. Table 2 delineates the location of aneurysms in relation to clinical grading. We failed to substantiate the presence of an association between the development of NPE and gender or age of patients.

4 Discussion

Neurogenic pulmonary edema is the most common extracerebral complication of subarachnoid hemorrhage and its development is a predictor of bad treatment outcome (Mrozek et al. 2015; Davison et al. 2012; Maramattom et al. 2006). NPE usually develops during the first 12–24 h after aneurysmal SAH, mainly in poor grade patients (Veeravagu et al. 2014; Piazza et al. 2011; Wartenberg et al. 2006). The present findings are in line with the reports outlined above, as we did not notice NPE in any of better-grade patients. Of the poor-grade patients, grade IV and V, NPE developed in 31.3 % of patients within several hours after brain hemorrhagic injury. NPE developed in patients with significantly increased ICP, above 30 mmHg, irrespective of patient gender or age. Aneurysmal SAH produces a massive sympathetic nervous system activation which enhances catecholamine concentration in extracerebral tissues. Except for the effects of catecholamines on the endothelium, which provoke cerebral vasospasm and

Table 1 Intracranial pressure (ICP) and the development of neurogenic pulmonary edema (NPE) in poor-grade patients

ICP (mmHg)	<21	21–30	31–40	41–50	>50	
Number of poor grade patient						Total
Grade IV	0	19	4 (2)	3 (2)	6 (5)	32 (9)
Grade V	0	15	6 (3)	4 (3)	7 (5)	32 (11)
Total	0	34	10 (5)	7 (5)	13 (10)	64 (20)

The number of patients with NPE in parenthesis

Table 2 Location of aneurysms in relation to clinical grading

Patient's grading according to modified WFNS scale	I–III	IV	V	Total
Aneurysm location				
ACoA	51	10 (6)	12 (7)	73 (13)
MCA	66	11 (1)	13 (2)	90 (3)
ICA	64	10 (1)	5 (1)	79 (2)
ACA	4	0	0	4
BA	1	1 (1)	2 (1)	4 (2)
Total	186	32 (9)	32	250 (20)

The number of patients with neurogenic pulmonary edema (NPE) in parenthesis
ACoA anterior communicating artery, *MCA* middle cerebral artery, *ICA* internal carotid artery, *ACA* anterior cerebral artery, *BA* basilar artery

consequently a secondary ischemic cerebral injury, NPE increases the formation of toxic cytokines, resulting in pulmonary edema or myocardial myocytolysis (Chen et al. 2014; Cinotti et al. 2014; Fontes et al. 2003). Lung dysfunction can also be a result of disturbed hypothalamo-pituitary adrenal axis (Davison et al. 2012; Inamasu et al. 2012; Friedman et al. 2003). In the present study, NPE appeared mainly in patients with the anterior communicating artery aneurysms. The lesion location nearby the hypothalamus and pituitary gland, and a direct irritation of these structures by hemorrhage can play an important role in the NPE development (Kahn et al. 2006). It seems, however, that increased intracranial pressure plays the most essential role in the occurrence of NPE (Mrozek et al. 2015; Sato et al. 2012; Piazza et al. 2011; Ochiai et al. 2001). Pulmonary disturbance, leading notably to decreased oxygen delivery to tissues, causes hypoxic brain injury that amplifies intracranial pressure increase. The vicious cycle created leads to a worsening of symptoms of brainstem insufficiency and is conducive to fatal treatment results. The present findings show that NPE develops in the first 12 h after brain hemorrhagic injury and almost all patients with NPE die in the first week after the injury.

5 Conclusions

Neurogenic pulmonary edema developed in 8.0 % of patients with aneurysmal SAH. In patients in clinical grades 1–3, pulmonary edema was unobserved. Pulmonary edema appeared explicitly in poor grade patients, grade IV and V, with the prevalence of 31.3 % (20 out of 64 patients). Patients with neurogenic pulmonary edema had a significantly worse result of treatment outcome, only 1 in 20 survived, which makes up a 5 % survival rate, while from the 44 poor grade patients who did not develop pulmonary edema mortality rate amounted to 45.5 % (20 patients died). The main predictors of neurogenic pulmonary edema after aneurysmal SAH are poor grade of the patient, enhanced intracranial pressure, and a location of the aneurysm in the anterior communicating artery.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 41–49
DOI 10.1007/5584_2016_66

Published online: 30 August 2016

Clinical Implications of Hepatocyte Growth Factor, Interleukin-20, and Interleukin-22 in Serum and Bronchoalveolar Fluid of Patients with Non-Small Cell Lung Cancer

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Abstract

Hepatocyte growth factor (HGF) is involved in tumorigenesis, interleukin-20 (IL-20) is an inhibitor of angiogenesis, and interleukin-22 (IL-22) stimulates tumor growth. The aim of this study was to determine the level of HGF, IL-20, and IL-22 in both serum and bronchoalveolar lavage fluid (BALF) of non-small cell lung cancer (NSCLC) patients before onset of chemotherapy, the nature of the interrelationships between these markers, and their prognostic significance regarding post-chemotherapy survival time. We studied 46 NSCLC patients and 15 healthy subjects as a control group. We found significantly higher serum levels of HGF and IL-22 in the NSCLC patients than those in controls [pg/ml: HGF – 1911 (693–6510) *vs*. 1333 (838–3667), p = 0.0004; IL-22 – 10.66 (1.44–70.34) *vs*. 4.69 (0.35–12.29), p = 0.0007]. In contrast, concentrations of HGF and IL-22 in BALF were lower in NSCLC patients than those in controls [pg/ml: HGF – 72 (6–561) *vs*. 488 (14–2003), p = 0.0002; IL-22 – 2.28 (0.70–6.52) *vs*. 3.72 (2.76–5.64), p = 0.002]. In the NSCLC patients, there was a negative correlation between the serum level of IL-20 and time to tumor progression (r = −0.405, p = 0.04) and between the serum

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level of HGF and survival time ($r = -0.41$, $p = 0.005$). In addition, a higher serum level of HGF and a higher BALF level of IL-22 in patients were linked with a shorter overall survival. We conclude that HGF, IL-20, and IL-22 in the serum and BALF of NSCLC patients before chemotherapy may be a prognostic of cancer progression.

Keywords
Bronchoalveolar lavage fluid • Hepatocyte growth factor • Interleukin-20 • Interelukin-22 • Non-Small Cell Lung Cancer

1 Introduction

Lung cancer is a fatal malignant tumor. There are no tumor markers that are sufficiently useful for predicting the effect of treatment and survival time. Hepatocyte growth factor (HGF) is a key cytokine in pulmonary alveolar homeostasis, which is mainly produced by lung fibroblasts, macrophages, and pneumocytes. An elevated level of HGF has been found in the serum and bronchoalveolar lavage fluid (BALF) of patients with interstitial lung diseases and lung cancer (Wislez et al. 2003). HGF is involved in the activation of signaling pathways leading to increased invasion and motility, proliferation, and stimulation of angiogenesis. It has been shown that serum HGF is an independent prognostic marker for non-small cell lung cancer (NSCLC) (Ujiie et al. 2012). Recently, Heynen et al. (2014) have demonstrated that HGF confers resistance to a number of kinase inhibitors in a variety of cancer cell lines. However, clinical significance of this cytokine has not yet been determined.

Interleukin-20 (IL-20) and interleukin-22 (IL-22) also are known to have modulatory, albeit seemingly opposing, effects on cancer cells. Lim and Savan (2014) have demonstrated that IL-20 is an inhibitor of angiogenesis but IL-22 stimulates tumor growth. IL-20R2 and IL-20R1 protein expression is elevated in tumor tissue of NSCLC patients (Hsu et al. 2012). Since the role of these markers in cancerogenesis is not fully clear, in the present study we seek to determine the level of HGF, IL-20, and IL-22 in both serum and BALF of patients with NSCLC before onset of chemotherapy, the nature of the associations between these markers, and their prognostic significance regarding post-chemotherapy survival time.

2 Methods

The present study was performed in conformity with the Declaration of Helsinki for Human Experimentation of the World Medical Association and the study protocol was approved by a local Ethics Committee. Written informed consent was obtained from all participants.

2.1 NSCLC Patients and Control Subjects

The study involved 46 male patients (mean age of 63 ± 3 years) with the histological diagnosis of NSCLC. Adenocarcinoma was diagnosed in 10 (22 %) patients, squamous cell carcinoma in 25 (54 %), and large cell carcinoma in 11 (24 %) patients. The diagnosis of NSCL was confirmed by standard procedures such as computed tomography and fiberoptic bronchoscopy with histopathological investigation of specimens. The bronchoscope was inserted into the bronchial segment nearest the tumor. Clinical staging of NSCLC was performed using the TNM system of the International Association for the Study of Lung Cancer (IASLC) (Rami-Porta et al. 2014). There were 20 patients at stage IIIB, 26 patients at stage IV of NSCLC. All tumors were rendered inoperable and the patients were scheduled to

receive four cycles of cisplatin and gemcitabine chemotherapy in the 21-day cycle routine; cisplatin – 30 mg/m^2 on Day 1 and gemcitabine – 1000 mg/m^2 on Day 1 and Day 8 of the cycle. The effects of chemotherapy were evaluated by the Response Evaluation Criteria in Solid Tumors (RECIST) criteria (Therasse et al. 2002)

The study material consisted of samples of bronchoalveolar lavage fluid (BALF) taken during diagnostic bronchoscopy, with a second 50-milliliter aliquot of recovered fluid considered as suitable for analysis, and serum samples obtained from the whole blood taken by venipuncture before onset of chemotherapy. Blood samples were taken to measure the level of HGF, IL-20 and IL-22.

HGF, IL-20, and IL-22 were measured by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instruction (R&D Systems, MN). The samples were assessed in duplicate and the average of the two measurement was taken for further analysis. The range of minimal detectable levels of HGF, IL-20, and IL-22 was 0–40 pg/ml, 1.6–16.6 pg/ml, and 0.7–5.8 pg/ml, respectively. For the analysis, serum samples were instantly centrifuged at 3500 rpm and BALF samples at 1500 rpm for 15 min at 4 °C, and all were stored at −80 °C until further use.

In addition, BALF samples were assessed for the total and differential cell counts. The total cell count was investigated in Nageotte's chamber and the results were expressed as cells x 10^5/ml. The differential cell profile was investigated under light microscopy by counting at least 400 cells (magnification 1 k).

Serum and BALF samples were also obtained and investigated in like manner in the control group consisting of 15 healthy volunteers (12 men/3 women; mean age of 60 ± 4 years).

2.2 Statistical Analysis

Data are presented as medians with interquartile (IQR, 25th–75th percentile) ranges and minimum-maximum values. The Shapiro-Wilk test was used to assess data distribution. Data with normal distribution were compared with a *t*-test and with skewed distribution with the Mann-Whitney U and Wilcoxon tests. Associations between cancer markers were evaluated with the Spearman rank test. The overall survival was assessed with the Kaplan-Meier method. The log-rank test was used to determine the significance of differences in survival rates. A p-value < 0.05 was considered to indicate statistical significance. We used Statistica 12 software (StatSoft Inc., Tulsa, OK) for all data analyses.

3 Results

There were no appreciable differences in regard to age and gender between the patients and healthy subjects. The serum levels of HGF and IL-22 were higher in the NSCLC patients than those in healthy controls [*HGF:* 1911 (693–6510) *vs.* 1333 (838–3667) pg/ml, p = 0.0004; *IL-22:* 10.66 (1.44–70.34) *vs.* 4.69 (0.35–12.29) pg/ml, p = 0.0007]) (Fig. 1a and c). The serum IL-20 did not appreciably differ between the NSCLC and healthy groups, [*IL-20:* 40.35 (29.86–63.81) *vs.* 37.73 (32.49–50.80) pg/ml, respectively] (Fig. 1b).

In contrast, BALF levels of HGF and IL-22 were lower in the NSCLC group than those in healthy control subjects (Fig. 2a and c) [*HGF:* 72 (6–561) *vs.* 488 (14–2003) pg/ml, p = 0.0002; *IL-22:* 2.28 (0.70–6.50) *vs.* 3.72 (2.76–5.64) pg/ml, p = 0.002]. The BALF IL-20 tended to be higher in the NSCLC patients than that in controls, but the difference failed to achieve statistical significance [*IL-20:* 64.26 (1.88–202) *vs.* 41.15 (1.88–133) pg/ml] (Fig. 2b).

There were no significant differences in the levels of HGF, IL-20, or IL-22 between stages IIIB and IV of tumor [*serum HGF IIIB vs. serum HGF IV:* 1866 (693–5285) *vs.* 2146 (1036–6510) pg/ml, p = 0.612; *BALF HGF IIIB vs. BALF HGF IV:* 69 (6–245) *vs.* 83 (6–561) pg/ml, p = 0.655; *serum IL-20 IIIB vs. serum IL-20 IV:* 36.42 (29.86–63.81) *vs.* 40.35 (32.49–61.21) pg/ml, p = 0.114; *BALF IL-20 IIIB vs. BALF IL-20 IV:* 64.26 (4.19–202.86) *vs.* 64.26 (2.88–179.76) pg/ml, p = 0.395; *serum*

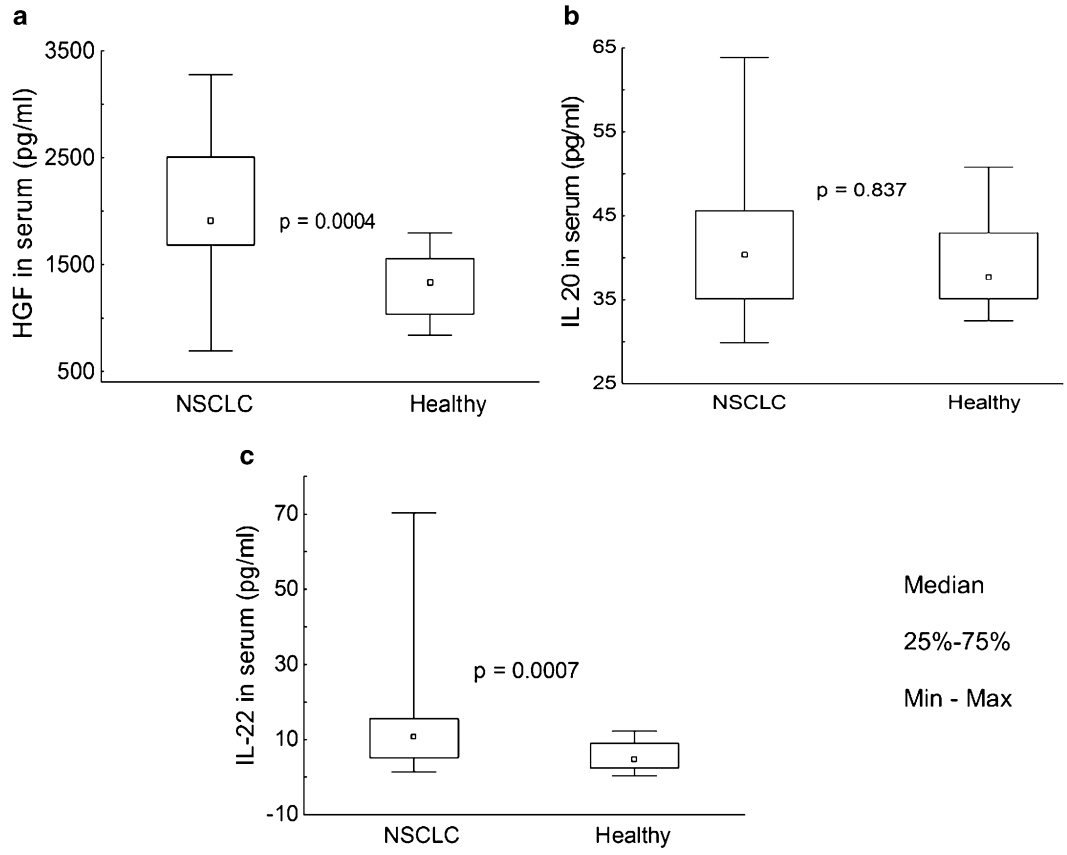

Fig. 1 (**a**) Hepatocyte growth factor (HGF), (**b**) interleukin-20 (IL-20), and (**c**) interleukin-22 (IL-22) concentrations in serum of NSCLC patients and healthy control subjects

IL-22 IIIB vs. serum IL-22 IV: 6.86 (1.44–33.99) *vs.* 11.74 (1.98–70.43) pg/ml, p = 0.052; *BALF IL-22 IIIB vs. BALF IL-22 IV:* 2.28 (0.84–4.64) *vs.* 2.76 (0.71–6.52) pg/ml, p = 0.961].

There was any apparent association between pre-chemotherapy levels of HGF, IL-20, or IL-22 and the effect of chemotherapy. Chemotherapy resulted in a partial response (PR) in 17 (38 %), stabilization (SD) in 13 (28 %), and a progressive disease (PD) in 16 (34 %) patients [*serum HGF:* PR *vs.* SD *vs.* PD – 1833 (1283–6257) *vs.* 1827 (693–6510) *vs.* 2146 (1644–4619) pg/ml, p = 0.319; *BALF HGF:* PR *vs.* SD *vs.* PD – 54 (14–329) *vs.* 72 (6–561) *vs.* 83 (6–415) pg/ml, p = 0.227; *serum IL-20:* PR *vs.* SD *vs.* PD – 35.79 (29.86–63.81) *vs.* 40.35 (35.11–61.21) *vs.* 40.34 (32.49–50.8) pg/ml, p = 0.281; *BALF IL-20:* PR *vs.* SD *vs.* PD – 87.36 (2.88–202.01) *vs.* 41.15 (18.05–179.76) *vs.* 41.16 (4.19–179.76) pg/ml, p = 0.099; *serum IL-22:* PR *vs.* SD *vs.* PD – 6.32 (1.44–36.15) *vs.* 10.66 (4.69–70.34) *vs.* 12.28 (1.98–33.45) pg/ml, p = 0.454; *BALF IL-22:* PR *vs.* SD *vs.* PD – 2.28 (0.76–6.52) *vs.* 1.8 (0.78–5.08) *vs.* 2.76 (0.70–4.68) pg/ml, p = 0.764].

We found negative correlations between the serum level of IL-20 and time to progression of NSCLC patients (Fig. 3) ($r = -0.405$, $p = 0.04$) and between the serum level of HGF and survival time ($r = -0.41$, $p = 0.005$).

There were no associations among the levels of HGF, IL-20, IL-22 and macrophages, lymphocytes, neutrophils, or eosinophils in BALF. Total cell count in BALF did not differ significantly between the NSCLC patients and controls (734 ± 311 *vs.* 640 ± 278 x 10^5/ml, p = 0.453, respectively). There were, however,

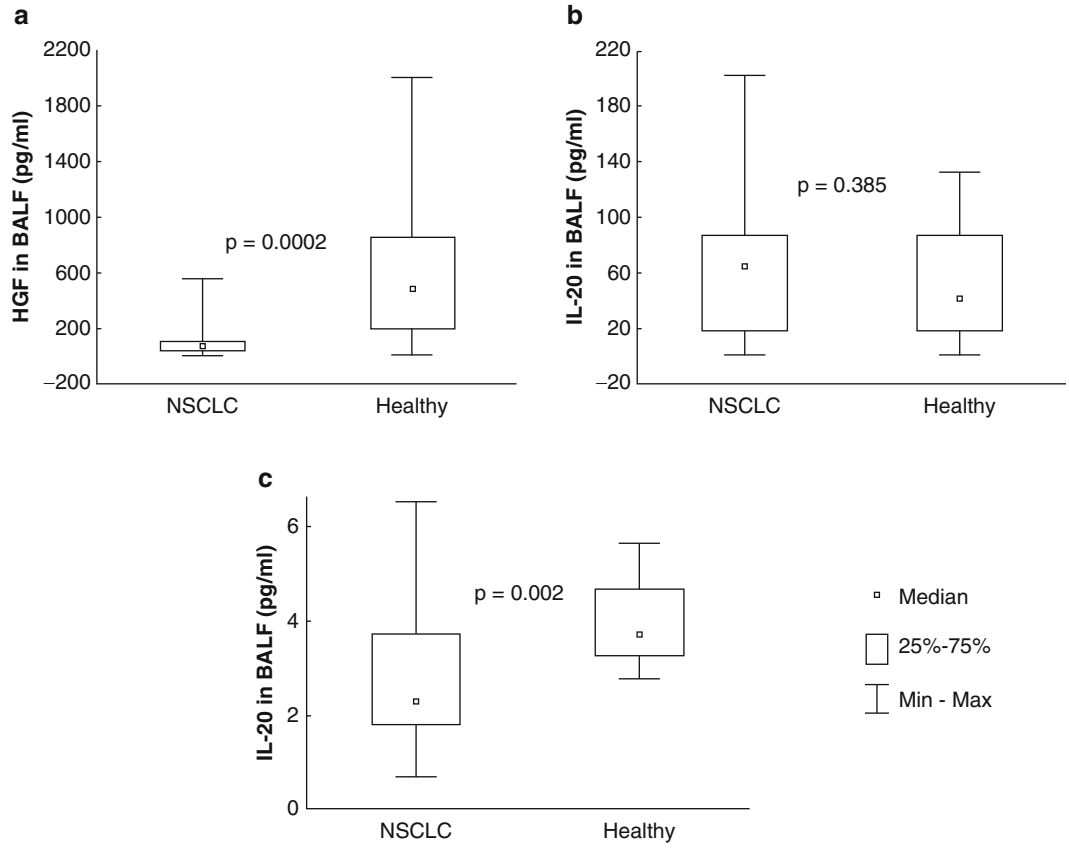

Fig. 2 (a) Hepatocyte growth factor (HGF), (b) interleukin-20 (IL-20, and (c) interleukin-22 (IL-22) concentrations in serum of NSCLC patients and healthy control subjects

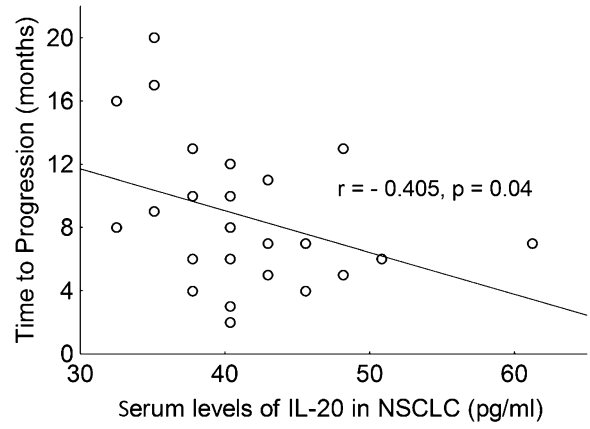

Fig. 3 Higher level of serum interleukin-20 (IL-20) is associated with faster advancement of non-small cell lung cancer (NSCLC)

differences in differential counts. Macrophage count was higher in patients than in controls (81.0 ± 16.0 *vs.* 75.4 ± 17.0 %, $p = 0.041$) and lymphocyte count was lower in patients than in controls (12.5 ± 6.0 *vs.* 18.6 ± 5.0 %, $p = 0.005$, respectively). Neutrophil and eosinophil counts were similar in both groups.

The mean overall survival time of patients was 14.4 ± 12.0 months. The median values of serum HGF and BALF IL-22 in the NSCLC

group were taken as the cut-off level distinguishing between high and low concentration of these markers. The patients with the serum HGF above the cut-off level of 1911 pg/ml had a significantly shorter survival time than those below that level; 13.0 *vs.* 9.6 months, respectively (Fig. 4a). Likewise, patients with the BALF IL-22 above the cut-off level of 2.28 pg/ml had a shorter survival than those below that level; 7.0 *vs.* 15.0 months, respectively (Fig. 4b).

4 Discussion

In the present study we found that patients with NSCLC had higher serum levels of HGF than those present in healthy subjects. These results are in line with those of Ujiie et al. (2012), who have demonstrated that elevated HGF at the time of diagnosis is an unfavourable prognostic for survival in NSCLC, as we found that patients with a higher serum level of HGF had a shorter survival than those with lower HGF (9.6 *vs.* 13 months, respectively). Thus, we confirmed the notion that HGF may be involved with cancer growth and metastasis, possibly through enhancing motility of cancer cells and stimulating angiogenesis (Mittal et al. 2014). These observations are, however, at variance with those of Pan et al. (2015) who have failed to show that HGF may be a prognostic marker in patients with lung cancer. A possible explanation for the discrepancy may lie in different stages of NSCLC in both studies. The patients of the present study were in advanced stages of NSCLC, where tumor escapes from immune control and proliferates in an unrestricted manner (Mittal

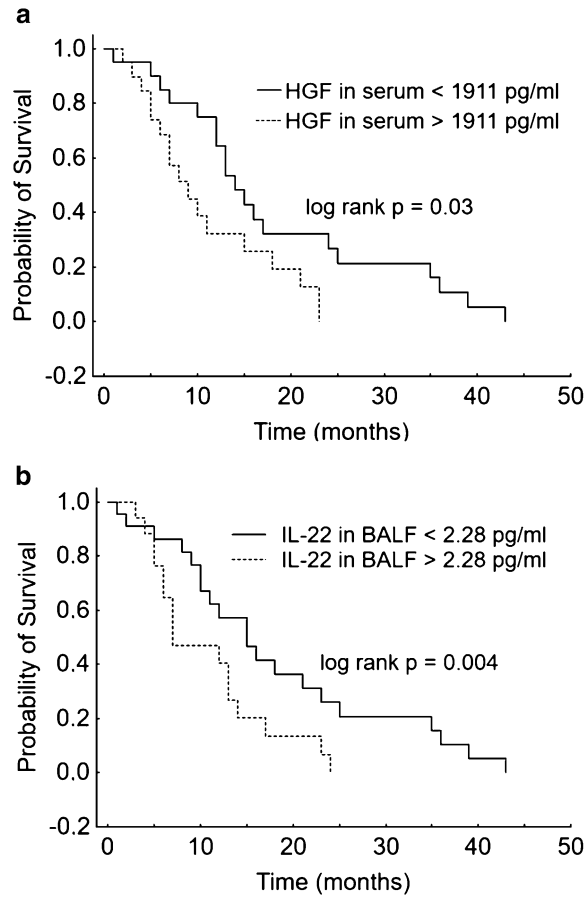

Fig. 4 Kaplan-Meier survival curves: (**a**) patients with serum HGF <1911 pg/ml (*solid line*) and those with HGF > 1911 pg/ml (*dashed line*); (**b**) patients with BALF IL-22 < 2.28 pg/ml (*solid line*) and those with BALF IL-22 > 2.28 pg/ml. Patients with serum HGF > 1911 pg/ml and BALF IL-22 > 2.28 pg/ml had significantly shorter survival times

et al. 2014). HGF initiates angiogenesis, stimulates proliferation, differentiation, and motility of cells (Wislez et al. 2003). The synthesis of HGF is regulated by prostaglandins, cytokines, and acute phase proteins (e.g., tumor necrosis factor-α, interleukin-1, or interleukin-6) (Heynen et al. 2014).

There is also increasing evidence that tumors are able to create an immunosuppressive microenvironment and recruit specific immune cells that favor tumor growth and progression, particularly in advanced stages of cancer (Joyce and Fearon 2015). In early stages of cancer, the immune system is more apt to control tumor growth (Koebel et al. 2007), which may explain the lack of association between the level of HGF and overall or disease-free survival of patients with stage I-II of NSCLC observed in a study by Pan et al. (2015). This issue remains, however, contentious. Other studies have shown an association between the serum level of HGF and cancer progression in case of breast, gastric, and also small cell lung cancer (Ujiie et al. 2012). Recently, Gibot et al. (2016) have revealed that HGF collaborates with VEGF-C in the formation of lymphatic vasculature. Thus, HGF appears an attractive therapeutic target in oncology (Garajová et al. 2015).

Wislez et al. (2003) have shown that patients with adenocarcinoma NSCLC have elevated HGF in BALF. The present findings are different in this regard. We noted a lower level of HGF in BALF in NSCLC than those in healthy subjects, which may be explainable by the lack of HGF expression in airway mononuclear cells and in pulmonary interstitium. Moreover, squamous cell carcinoma NSCLC, predominantly present in our patients, may release a smaller amount of HGF to the alveolar space than adenocarcinoma does. That reasoning is supported by the observations of strong expression of HGF in the cytoplasm of adenocarcinoma cells (Wislez et al. 2003) and the production of HGF by stromal fibroblasts in this type of NSCLC (Masuya et al. 2004).

Fibroblasts are a major component of the extracellular matrix which forms the tumor milieu containing a variety of proteins, such as growth factors, cytokines, or tumor promoters, all of which may be associated with enhanced invasion of NSCLC and metastasis (Heuzé-Vourc'h et al. 2005). One of the key cytokines expressed in the tissue microenvironment, which stimulates proliferation of cancer cells, is IL-22 (Park et al. 2011). Our present findings of a higher level of IL-22 in the serum of patients with NSCLC compared with healthy subjects lend support to the possible cancerogenic role of IL-22. Other authors have also demonstrated elevated serum IL-22 in NSCLC patients, which is associated with poor prognosis (Kobold et al. 2013; Zhang et al. 2008). IL-22 enhances inducible nitric oxide synthase (iNOS) expression and activity induced by interferon-γ; contributing to the conversion of nitrites associated with tumorigenic inflammation (Ziesché et al. 2007). An oxidative inflammatory microenvironment containing IL-22 is predisposed to mitogenic signaling, increasing the risk of tumor formation (Dąbrowska et al. 2011). Further, IL-22 activates nuclear factor (NF-kB) which promotes the expression of genes involved in cell cycle, cell proliferation, and prevention of apoptosis (Cho et al. 2012).

In the present study we failed to find an association between the probability of survival and serum levels of IL-22 in NSCLC patients, which is in line with other reports (Kobold et al. 2013). However, we found that IL-22 in BALF of patients was negatively associated with the probability of survival, although the level of IL-22 in BALF was lower in patients compared with that in healthy controls. A possible explanation for a decreased level of IL-22 in BALF of NSCLC patients could lie in the distribution of IL-22 receptors (IL-22R1). IL-22 has a restricted tissue specificity as its unique receptor IL-22R1 is exclusively expressed in epithelial and tissue cells, but not immune cells (Lim and Savan 2014). Kobold et al. (2013) have revealed that IL-22R1 is particularly expressed in lung cancer tissue. In the present study we assessed the unbound IL-22. The BALF concentration of IL-22 in NSCLC patients could plausibly be reduced due to its binding to the receptor. Further, chemoresistant lung cancer cells express

higher levels of IL-22R1 receptor and are more proliferative in response to IL-22 than non-resistant cells. Our results are in line with these findings as patients with an elevated level of IL-22 in BALF had a shorter survival than those with lower levels of IL-22.

IL-20 and its receptors are frequently dysregulated in NSCLC. The receptors may be a candidate anti-angiogenic therapeutic target in NSCLC due plausibly to IL-20-mediated downregulating of vascular endothelial growth factor (VEGF) (Hsu et al. 2012; Baird et al. 2011). Yet the exact role of IL-20 remains unclear in view of reports to the contrary demonstrating that it promotes angiogenesis and activates cell signaling in the lymphangiogenic processes (Dudakov et al. 2012; Lee et al. 2013). The present finding are in line with the latter as the serum level of IL-20 was negatively associated with time to cancer progression. Others have recently reported that IL-20 exerts proangiogenic effects through IL-20R1 receptor and simultaneous anti-angiogenic effects through IL-22R1 receptor (Lim and Savan 2014); the finding that seems to have further confound the issue of IL-20 in cancer.

In conclusion, tissue microenvironment of advanced NSCLC contains cytokines that hold opposing pro- and anti-tumor effects. A better understanding of these complex mechanisms may contribute to the development of new targeted therapies for lung cancer and better foreseebility of treatment effects and survival time.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 51–58
DOI 10.1007/5584_2016_69

Published online: 13 September 2016

Expression of Ceramide Galactosyltransferase (UGT8) in Primary and Metastatic Lung Tissues of Non-Small-Cell Lung Cancer

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Abstract

Ceramide galactosyltransferase (UGT8) is an enzyme that regulates the synthesis of sphingolipids of the myelin sheath in nervous systems. The protein raises an increasing research interest as a potential marker of cancer progression in various organs. In the present study we seek to determine whether UGT8 could play a role of a therapeutic marker in non-small cell lung carcinoma (NSCLC). We addressed the issue by examining the intensity of UGT8 expression in tissue specimens of primary and corresponding metastatic lung tumors in 19 NSCLC patients undergoing surgery. The methodology was one of immunohistochemical tissue staining using light microscopy. The findings were that the majority of both lung primary and metastatic tumor tissues were positive in UGT8 signals. The cytoplasmic expression of UGT8 was found in 68.4 % of cases of primary tumors and 82.2 % of metastases, with a positive correlation between the UGT8 expression in both tumor tissues. The normal tissue adjacent to tumors showed no positive UGT8 staining. However, we failed to find any appreciable difference in UGT8 expression depending on the clinical stage of NSCLC or

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lymph node involvement. Nor was there any association between UGT8 expression in tumor tissues and patients' survival time. We conclude that it is unlikely that therapeutic targeting of UGT8 could inhibit cell proliferation and invasion of NSCLC. UGT8, although enhanced in NSCLC tissues, does not meet the criteria of a lung tumor marker. Thus, UGT8 cannot be considered as having diagnostic or therapeutic utility in NSCLC. The pathophysiological meaning of enhanced expression of UGT8 in lung cancer remains to be explored in further studies.

Keywords
UGT8 • Immunohistochemistry • Lung metastases • Lung tissue • Non-small cell lung cancer • Primary tumor • Prognostic marker

1 Introduction

Lung cancer is the most common malignancy and is the first cause of cancer deaths among men and the second among women (Jemal et al. 2011). The prognosis is poor and it mainly depends on the histologic type and clinical stage of disease (Kosacka and Jankowska 2007). The basic diagnostic method used today is immunohistochemistry enabling to determine the expression of tumor markers. In case of lung cancer, determination of expression of specific proteins also gives clues on the possible use of targeted immunotherapy, enables the monitoring of disease progression, and allows predicting the effects of therapy (Jassem et al. 2010). The ceramide galactosyltransferase protein (UGT8) has been originally reported as a myelin basic protein in the oligodendrocyte lineage in the central nervous system and in Schwann cells of the peripheral nervous system (Bosio et al. 1996; Dyer and Benjamins 1989). The protein is responsible for the synthesis of the glycosphingolipid-galactosyloceramid (GalCer). In the physiological condition, GalCer takes part in muscle cell differentiation by regulating calcium levels, and it affects the rebuilding of the cellular cytoskeleton (Schulte and Stoffel 1993). The elevated level of GalCer is observed in certain pathological conditions, notably in many types of cancer lesions. Initially, increased expression of GalCer has been reported in astrocytomas and oligodendrogliomas. Then, GalCer has also been demonstrated in mammary gland cancers and in metastatic lesions of prostate cancer (Dzięgiel et al. 2010; Ruckhaberle et al. 2009; Oudes et al. 2005; Sung et al. 1995). The expression of GalCer synthase is increased about 11-fold in ovarian epithelial carcinoma cells compared with normal ovarian stromal tissue (Liu et al. 2010).

The level of UGT8 expression positively correlates with increased risk of metastasis of primary mammary gland to the lungs (Dzięgiel et al. 2010). Nonetheless, UGT8 expression in primary and metastatic tumors of non-small cell lung cancer (NSCLC) has not yet been studied. In the absence of a satisfactory treatment outcome of patients with NSCLC, we deemed it warranted to investigate the possibility that UGT8 expression could play a potential role of a therapeutic marker in NSCLC. In the present study, we addressed the issue by examining the intensity of UGT8 expression in the specimens of primary and metastatic tumor tissues in NSCLC patients undergoing surgery, and the possible association of UGT8 with the clinicopathological features.

2 Methods

The study was approved by the Bioethics Committee of Wroclaw Medical University in Wroclaw, Poland, and was conducted in accord with the principles set by the declaration of Helsinki for Human Research. This was a retrospective study that covered 19 patients (6 women

and 13 men) of the mean age of 64 ± 9, range 44–78 years. There were 5 patients below 60 and 14 above 60 years of age. All patients were treated in the Lower Silesian Center of Lung Diseases in Poland in the years 2001–2013. Lung tissue specimens were collected during surgical lobectomies of primary NSCLC and then during reoperations due to metastatic pulmonary lesions, performed after 1–93 months after the first tumor removing surgery. Clinicopathological data of patients, obtained from the hospital archives, are depicted in Table 1.

Specimens were investigated by immunohistochemistry. Reactions were carried out in 4 μm thick paraffin tissue sections. For deparaffinization, rehydration, and exposure of antigenic determinants, glass slides were boiled in an alkaline buffer (pH = 9), a target retrieval solution, for 20 min at 97 °C, using a Pre-Treatment Link Station (DakoCytomation; Glostrup, Denmark). Then, after using the Flex EnVision Peroxidase-Blocking Reagent for 5 min (DakoCytomation; Glostrup, Denmark), immunohistochemical reactions were carried out with primary polyclonal rabbit anti-UGT8 antibodies (20 min, room temperature, dilution 1:700; Prestige Atlas Antibodies; Stockholm, Sweden) and secondary horseradish peroxidase-conjugated antibodies (20 min; EnVision FLEX/HRP, DakoCytomation). The slides were developed with diaminobenzidine (10 min). Contrasting staining was performed using EnVision FLEX hematoxylin (5 min). Immunohistochemical reactions were examined under light microscopy (Olympus BX-41; Tokyo, Japan). The cytoplasmic expression of the UGT8 protein was evaluated using a semi-quantitative scale designed by Remmele and Stegner (1987); details of which are set out in Table 2.

Table 1 Clinico-pathological features of non-small cell lung cancer

Histopathological type	n
Squamous cell carcinoma	6
Adenocarcinoma	12
Macrocellular carcinoma	1
TNM	
T1	5
T2	6
T3	1
Tx	7
N0	9
N1	2
N2	1
Nx	7
M0	4
Mx	15

An analysis of data distribution was performed with the Shapiro-Wilk test and differences between groups were tested with the Mann-Whitney *U* test. Spearman's correlation coefficient was used to assess the relationship between the UGT8 expressions in primary tumor *vs.* metastases. The Kaplan-Meier survival curves were used to define the probability of patient surviving depending on the low or high level of UGT8 expression. The differences in results were considered statistically significant at $p < 0.05$. Statistical analysis was performed using the commercial statistical package Graph Pad Prism ver. 5.0.

3 Results

We examined tissues of 19 primary tumors and 19 corresponding metastatic lung cancer foci. Cytoplasmic expression of UGT8, exemplified in Fig. 1, was found in 68.4 % of cases of primary tumors and 82.2 % of metastases. Thus, the majority of lung tumor tissues were positive in UGT8 signals as opposed to adjacent normal tissues. The normal tissue showed virtually no positive UGT8 staining, irrespective of whether the tumor staining was distinctly strong or mild as demonstrated in Fig. 1. Scoring of the staining intensity corresponded to moderate or strongly positive immunoreaction. The mean UGT8 expression in primary tumors amounted to 2.7 ± 3.3, with the median of 2.0 (95 % CI 1.1–4.4). For comparison, the mean score of UGT8 expression in metastatic tumors was 3.5 ± 3.3, and the median was 3.0 (95 % CI 1.9–5.1) (Fig. 2). Increasing expression of the UGT8 protein in primary tumors was associated with its being increased also in the corresponding

Table 2 Semi-quantitative scale based on the intensity immunostaining score (IRS) designed by Remmele and Stegner (1987)

Intensity of staining	Percentage of positive cells	IRS score (0–12)	IRS classification
0 = no color reaction	no positive cells	0–1 = negative	0 = negative
1 = mild reaction	<10 % positive cells	2–3 = mild	1 = positive, weak expression
2 = moderate reaction	10–50 % positive cells	4–8 = moderate	2 = positive, mild expression
3 = intense reaction	51–80 % positive cells	9–12 = strongly positive	3 = positive, strong expression
	> 80 % positive cells		

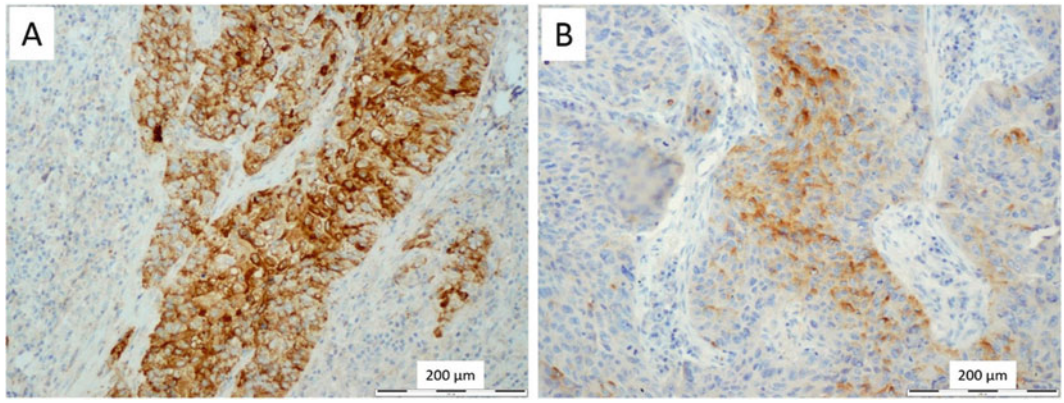

Fig. 1 Non-small cell lung cancer: (**a**) the primary site, (**b**) lung metastasis. Examples of strong and mild cytoplasmic UGT8 expressions; magnification x200

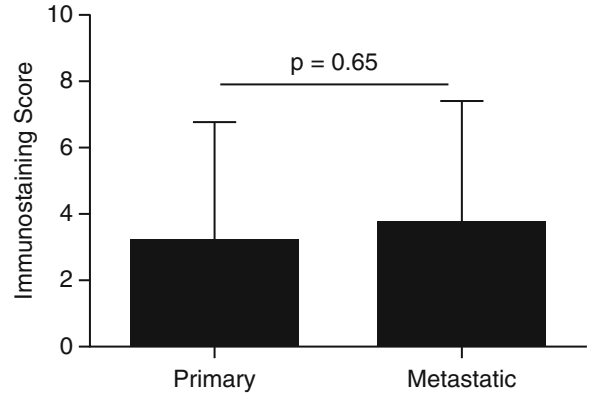

Fig. 2 UGT8 expression in primary and secondary tumors of non-small cell lung cancer (NSCLC). No difference between the two kinds of tissue was evident

lung metastases, although the correlation was rather weak ($p < 0.05$; $r = 0.48$) (Fig. 3).

We failed to find any appreciable difference in the expression of UGT8 between primary tumors and metastases in the subgroups of patients with and without the involvement of lymph nodes, i.e., in clinical stage below and above IIB, in both male and female patients. There was no significant association between the patients' age and expression of UGT8 concerning both primary and metastatic tumors. Moreover, survival time of patients had no relation to the level of UGT8 expression. It remained similar in the patients with low and high expression of UGT8 in both primary and metastatic tumors (Figs. 4 and 5).

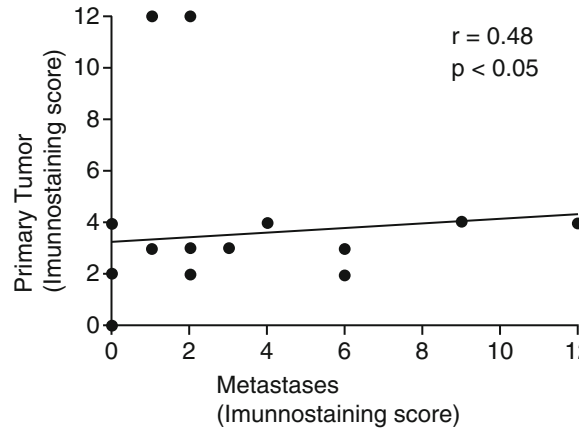

Fig. 3 Association of cytoplasmic UGT8 expression in non-small cell lung cancer (NSCLC); primary *vs*. metastatic lung tumor tissues

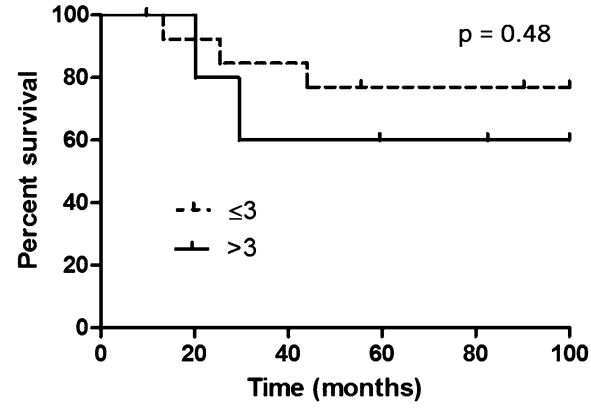

Fig. 4 The Kaplan-Meier survival curves of patients with primary tumor of non-small cell lung cancer (NSCLC) having low ≤ 3 and high > 3 immunostaining score of UGT8 expression. No significant difference between the two subgroups of patients was found

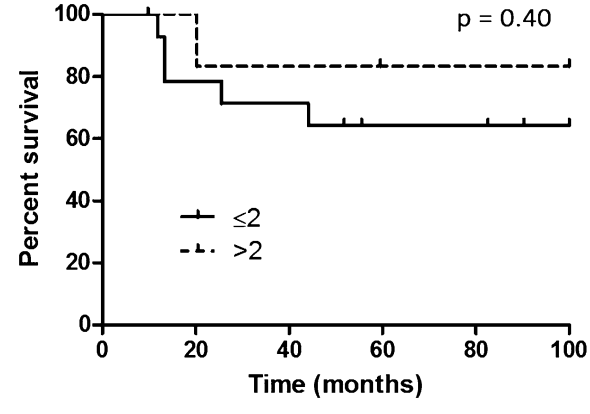

Fig. 5 The Kaplan-Meier survival curves of patients with lung metastases accompanying primary non-small cell lung cancer (NSCLC) having low ≤ 2 and high > 2 immunostaining score of UGT8 cytoplasmic expression. No significant difference between the two subgroups of patients was found

4 Discussion

In the present study we investigated the hypothesis that the cellular ceramide galactosyltransferase, UGT8, known to be engaged in tumorigenesis in various tissues, could potentially be a marker of progression in non-small cell lung cancer (NSCLC). To this end, we examined the immunohistochemical intensity of UGT8 expression in NSCLC and its metastases to the lungs, and the possible association of UGT8 with the clinical features of cancer. The study demonstrates the distinct presence of UGT8 of moderate intensity on the semi-quantitative scale of immunostaining of Remmele and Stegner (1987) in both primary NSCLC and its lung metastases. We also found that a high level of UGT8 in primary tumor was matched by a high level of UGT8 in metastases. We thus may say that overall there was a distinct increase in UGT8 expression in lung cancer tissues relative to adjacent normal lung tissue where the UGT8 expression was negligible or null. These results are, generally, in line with the results of other authors who have shown that the expression of UGT8, at both transcriptional and protein levels, is positively associated with the metastatic potential of pancreatic ductal adenocarcinoma cell lines but adjacent normal duct cells show no UGT8 expression (Li et al. 2013).

Other than the presence of UGT8 expression in cancerous lung tissue, the findings of the present study were largely negative and disappointing in terms of the possible therapeutic or marker-like role in NSCLC of UGT8. The expression of UGT8 did not appreciably differ between the primary and metastatic tumors, it had no relation to the cancer stage, assessed by the presence or lack of lymph node involvement, nor was it affected by patients' age or gender. Further, survival time of patients had no apparent relation to the magnitude of UGT8 expression. Therefore, it seems unlikely that therapeutic targeting of UGT8 could inhibit cell proliferation and invasion of NSCLC.

Ruckhaberle et al. (2009) have been the first who directed attention to the possible role of kinases involved in phospholipid activity and cancer progress. These authors have identified a group of enzymes such as sphingosine kinase-1 (SPHK1), ganglioside GD3 synthase, and the ceramide galactosyltransferase (UGT8) in the microarray studies of various subtypes of breast cancer cells. They have found that UGT8 is associated with a higher proliferation and fewer apoptotic cells in estrogen-negative breast cancer type, which is associated with a worse prognosis. Dzięgiel et al. (2010) have shown that higher expression of the UGT8 in breast cancer is associated with increased risk of metastases to the lungs. Other observations on the role of UGT8 in tumorigenesis are in line with the above outlined research. A higher degree of malignancy has been noted in breast cancer associated with a higher expression of UGT8 in dogs (Nowak et al. 2013). Likewise, Owczarek et al. (2013) in the experimental murine model of metastatic cancer cells of the breast (MDA-MB-231), with the induction of apoptosis with doxorubicin, have confirmed the association of UGT8 activity with a higher rate of cancer cell proliferation and fewer apoptotic cells; the conditions that promote survival of tumor cells and are conducive to metastases in distant organs. The polymorphism of the UGT8 gene apparently does not affect the effectiveness of chemotherapy in lung cancer, but it may increase the neutropenia of chemotherapy (Nakamura et al. 2011). In connection to those reports, Zheng et al. (2002) have reported the activity of the glucuronyl enzymes (UGT1A6) in detoxification of airway and lung tissues from carcinogenic metabolites of benzopyrene, present in tobacco smoke. The UGT1A6 gene polymorphism, expressed in leukocytes of patients with lung cancer, is associated with a higher probability of malignancy. Kua et al. (2012) have suggested that the

investigation of the polymorphisms of UGT1A6 gene may be used to detect people with increased risk for lung cancer. Other studies demonstrate that the frequency of the low activity alleles UGTA7*2 and UGTA7*3 of the UGTA7 gene is significantly higher in Taiwanese lung cancer patients than in healthy subjects. The reduced enzyme activity may hamper detoxification of carcinogens and by doing so may foster cancer progression (Lee et al. 2011).

Given the largely negative results of the present study concerning the potential role of UGT8 expression as a prognostic of NSCLC cancer development and survival we backed away from further dwelling on the issue by studying the expression of the related enzyme galactosylceramide (GalCer), whose synthesis is dependent on the action of UGT8. GalCer has been reported to have proliferative and metastatic potential due to its antiapoptotic activity as well as the ability to induce chemotherapeutic resistance in breast cancer cell lines (Nowak et al. 2013; Owczarek et al. 2013; Dzięgiel et al. 2010; Ruckhaberle et al. 2009). The role of GalCer in lung tumorigenesis remains at present unknown.

The limitation of the present study was that the immunochemistry we employed was restricted to the use of a single antibody against UGT8 and we investigated the metabolism of only one enzyme involved in glycolipid metabolism. Nonetheless, we conclude that UGT8, although enhanced in NSCLC tissues, does not meet the criteria posed for tumor markers, and therefore its potential diagnostic or therapeutic use in NSCLC cannot be considered. The pathophysiological meaning of enhanced expression of UGT8 in lung cancer remains conjectural and it should be explored in another study designs.

Acknowledgements This work was performed as a separate ramification of the project "Intratumoral freezing and dye injection during surgical resection of lung tumors: a new targeted delivery technique", funded by the statutory budget of Wroclaw Medical University in Poland.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 59–64
DOI 10.1007/5584_2016_72

Published online: 13 September 2016

Antibiotic Treatment of Hospitalized Patients with Pneumonia Complicated by *Clostridium Difficile* Infection

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Abstract

Clostridium difficile infection (CDI) is one of the most common gastrointestinal complication after antimicrobial treatment. It is estimated that CDI after pneumonia treatment is connected with a higher mortality than other causes of hospitalization. The aim of the study was to assess the relationship between the kind of antibiotic used for pneumonia treatment and mortality from post-pneumonia CDI. We addressed the issue by examining retrospectively the records of 217 patients who met the diagnostic criteria of CDI. Ninety four of those patients (43.3 %) came down with CDI infection after pneumonia treatment. Fifty of the 94 patients went through severe or severe and complicated CDI. The distribution of antecedent antibiotic treatment of pneumonia in these 50 patients was as follows: ceftriaxone in 14 (28 %) cases, amoxicillin with clavulanate in 9 (18 %), ciprofloxacin in 8 (16.0 %), clarithromycin in 7 (14 %), and cefuroxime and imipenem in 6 (12 %) each. The findings revealed a borderline enhancement in the proportion of deaths due to CDI in the ceftriaxone group compared with the ciprofloxacin, cefuroxime, and imipenem groups. The corollary is that ceftriaxone should be shunned in pneumonia treatment. The study demonstrates an association between the use of a specific antibiotic for pneumonia treatment and post-pneumonia mortality in patients who developed CDI.

Keywords

Antibiotics • Antimicrobial treatment • Diarrhea • *Clostridium difficile* infection • Gastrointestinal complications • Pneumonia

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1 Introduction

Clostridium difficile is the main agent causing the healthcare-associated infections, often being fatal (Magill et al. 2014), prolonging hospitalization, and incurring additional healthcare costs (Gabriel and Beriot-Mathiot 2014; Zimlichman et al. 2013). The incidence and virulence of *Clostridium difficile* infections (CDI) rapidly rise worldwide (Dubberke et al. 2011). The main risk factor for *C. difficile* infection is antibiotic use. The risk is dose-related and it further increases with prolonged antibiotic use or with combination therapy (Winslow et al. 2014). Other risk factors include: elderly age, recent hospitalizations, comorbidities, and a use of drugs such as proton pomp inhibitors or immunosuppressants. Alterations in gut microflora caused by antimicrobial treatment cause *C. difficile* spores germination, toxins production, and intestinal expansion, as a consequence leading to inflammation, colon epithelial lesions, and colitis (Britton and Young 2014). Biological features of the *C. difficile* spores make them resistant antimicrobial treatment, heat, disinfectants, and thus to hung on in the environment (Lund and Peck 2015). *C. difficile* may be transmitted from the community, other patients (Brown et al. 2015), health care workers, hospital ward surfaces, or contaminated food (Paredes-Sabja et al. 2014; Hoover and Rodriguez-Palacios 2013). The symptoms vary from asymptomatic shedding, mild diarrhea to severe and complicated colitis, and death. A diagnostic pathway is based on multistep algorithms using enzyme immunoassays to detect toxins, polymerase chain reaction (PCR) for the toxin genes encoding a protein 'poison', or a single step PCR in a liquid stool sample (Bagdasarian et al. 2015). Treatment includes a withholding of contributing antibiotics, oral metronidazole for the mild-onset infection, and vancomycin for severe cases. In recurrent CDI, fidaxomycin and fecal microbiota transplantation can be effective (Britton and Young 2014; DuPont 2013). Many studies show that CDI is highly prevalent during the course of pneumonia antibiotic treatment and is associated with post-pneumonia mortality (Becerra et al. 2015). The research demonstrates that CDI is peculiarly related to the use of cephalosporins and clindamycin (Slimings and Riley 2014).

In the present study we attempted to get insight into the association between mortality from severe post-pneumonia CDI and the kind of antecedent antibiotic treatment. We also sought to determine and compare the proportion of patients who died due to CDI in regard to the use of most commonly recommended antibiotics for pneumonia treatment.

2 Methods

The study was approved by a local Ethics Committee and was conducted according to the guidelines set by the Declaration of Helsinki for Human Research. Medical files of all adult CDI patients hospitalized at the Internal Medicine Ward of the Medical University of Warsaw, Poland, between May 2012 and February 2015 were retrospectively reviewed. The patients were enrolled into the study who had recovered from pneumonia but subsequently suffered from CDI.

A total of 217 patients, F/M – 128/89, of the overall mean age of 80 $\pm$ 11 years, met the diagnostic criteria of CDI. There were 94 (43.3 %) patients with CDI in the group of post-pneumonia treatment. Fifty (53.2 %) out of the 94 CDI patients, which amounted to 23.0 % of all 217 pneumonia cases, ran a course of severe or severe and complicated CDI according to the guidelines of the American College of Gastroenterology (ACG) and the Society for Healthcare Epidemiology of America and the Infectious Diseases Society of America (SHEA/IDSA) (Table 1) (Surawicz et al. 2013; Cohen et al. 2010).

The diagnosis of pneumonia was based on clinical symptoms and signs (productive cough, fever, increased inflammatory indices such as leukocyte count and C-reactive protein), along with the radiological findings in chest X ray consistent with pneumonia. Other clinical indices

Table 1 Staging systems for severity of *Clostridium difficile* infection (CDI)

Staging of CDI	ACG guidelines.	SHEA/IDSA guidelines.
Stage 0 – Mild-to-moderate	Diarrhea plus any additional signs or symptoms not meeting severe or complicated criteria below outlined	WBC $\leq$15,000 cells/mm^3 and serum creatinine <1.5 times the premorbid level
Stage 1 – Severe	Serum albumin <3 g/dl plus one of the following:	WBC $\geq$15,000 cells/mm^3 and serum creatinine >1.5 times the premorbid level
	WBC $\geq$15,000 cells/mm^3	
	abdominal tenderness	
Stage 2 – Severe and complicated	Any of the following attributable to CDI:	Hypotension or shock, ileus, megacolon
	admission to intensive care unit for CDI	
	hypotension with or without use of vasopressors	
	fever $\geq$38.5 °C	
	ileus or significant abdominal distention	
	mental status changes	
	WBC $\geq$35,000 cells/mm^3 or $\leq$ 2000 cells/mm^3	
	serum lactate $\geq$2.2 mmol/l	
	end-organ failure (mechanical ventilation, renal failure)	

ACG American College of Gastroenterology guidelines, *SHEA/IDSA* Society for Healthcare Epidemiology of America/Infectious Diseases Society of America

such blood morphology, serum creatinine, urea and albumin levels, arterial blood pressure, and mental status were assessed at the time of CDI diagnosis. In addition, anamnesis included comorbidities, place of patients' residence, and the kind of antibiotics used in the past two months. The CDI was defined as a passage of three and more unformed stools in a 24-h-period after admission, coupled with a positive result of stool assay for toxins A or B of *C. difficile,* evaluated by either an enzyme-immunoassay or stool culture, and not attributable to other cases (Schiller et al. 2014). From the epidemiological standpoint, CDI included nosocomial and community acquired cases. The antibiotics for pneumonia treatment were used on the empiric basis. The following, most commonly prescribed antibiotics, were taken into consideration: ceftriaxone, amoxicillin with clavulanate, clarithromycin, cefuroxime, imipenem, and ciprofloxacin.

Continuous variables were reported as means $\pm$ SD and categorical variables as the number of patients and percentages. We estimated the influence of antimicrobial treatment on the mortality rate in CDI. The null hypothesis was that there are no significant differences between the proportions of patients who died from CDI depending on the kind of antibiotic used. These differences were assessed with two-tailed one-sample *t*-test. A p-value <0.05 defined statistical significance. The analysis was performed using R Statistical Software ver. 3.1.2 (GNU General Public License).

3 Results

There were 94 patients with post-pneumonia CDI. Fifty (53.2 %) of these patients ran a course of severe or severe and complicated CDI. The distribution of antibiotic treatment in the 50 patients with CDI was as follows: ceftriaxone in 14 (28 %) cases, amoxicillin with clavulanate in 9 (18 %), ciprofloxacin in 8 (16.0 %), clarithromycin in 7 (14 %), and cefuroxime and imipenem in 6 (12 %) each. The mortality rate was the greatest (64 %) in the patients who received ceftriaxone and the smallest in those who received cefuroxime and imipenem (17 % each) for the antecedent treatment of pneumonia (Table 2). The *t*-statistic was not significant at the 0.05 critical alpha level for the differences in the proportion of deaths among the antibiotic-groups. However, there was a borderline significance between the highest proportion of deaths in

Table 2 Number of death cases in patients with post-pneumonia severe (stage 1) and very severe and complicated (stage 2) *C. difficile* infection (CDI), according to ACG and SHEA/IDSA classification guidelines, with respect to the kind of antibiotic used for treatment of pneumonia

Antibiotic	Patients with post-pneumonia CDI; n (%)	No. of deaths n (%)	ACG severity		SHEA/IDSA severity	
			Stage 1	Stage 2	Stage 1	Stage 2
Ceftriaxone	14 (28)	9 (64)	n = 6	n = 8	n = 2	n = 12
Amoxicillin & clavulanate	9 (18)	3 (33)	n = 4	n = 5	n = 3	n = 6
Ciprofloxacin	8 (16)	2 (25)	n = 4	n = 4	n = 3	n = 5
Clarithromycin	7 (14)	2 (28)	n = 3	n = 4	n = 2	n = 5
Cefuroxime	6 (12)	1 (17)	n = 2	n = 4	n = 3	n = 3
Imipenem	6 (12)	1 (17)	n = 2	n = 4	n = 3	n = 3

ACG American College of Gastroenterology guidelines, *SHEA/IDSA* Society for Healthcare Epidemiology of America/Infectious Diseases Society of America

Table 3 Statistical probability (p) of death from post-pneumonia *Clostridium difficile* infection (CDI), calculated from the proportion of patients who died in each antibiotic-group of pneumonia treatment

	Ceftriaxone	Amoxicillin & clavulanate	Ciprofloxacin	Clarithromycin	Cefuroxime	Imipenem
Ceftriaxone		0.16	**0.09**	0.14	**0.07**	**0.07**
Amoxicillin & clavulanate	0.16		0.72	0.83	0.50	0.50
Ciprofloxacin	**0.09**	0.72		0.90	0.73	0.73
Clarithromycin	0.14	0.83	0.90		0.65	0.65
Cefuroxime	**0.07**	0.50	0.73	0.65		1.00
Imipenem	**0.07**	0.50	0.73	0.65	1.00	

The probability of death in the ceftriaxone group was borderline higher compared with the ciprofloxacin, cefuroxime, and imipenem groups

the ceftriaxone-treated, on the one side, and the smaller proportions of deaths in ciprofloxacin, cefuroxime, and imipenem-treated, on the other side ($0.05 < p < 0.10$). Statistical details of the cross-correlations between the antibiotic patient-groups are displayed in Table 3.

We failed to find any appreciable differences concerning blood morphology and clinically relevant biochemical indices as well as basic demographic, physical, and social conditions of CDI patients allocated to the antibiotic-groups (data not shown).

4 Discussion

The studies show that CDI is highly prevalent after pneumonia treatment, which is associated with a significant increase in inhospital mortality (Becerra et al. 2015). In the present study we attempted to investigate the influence of antibiotic treatment of community or nosocomial pneumonia on the development of post-pneumonia CDI, its severity, and mortality. We identified 94 (43.3 %) out of the 217 patients who developed CDI as an after-effect of the antibiotic use for pneumonia treatment. Fifty of the 94 (53.2 %) CDI patients, which amounts to 23.0 % of all pneumonia cases, ran a course of severe or severe and complicated CDI. Concerning the antecedent antibiotic treatment of pneumonia, ceftriaxone was employed most frequently and, notably, it was responsible for the highest mortality rate of 64 %. This rate was nearly two-fold greater than that for amoxicillin with clavulanic acid, the second in frequency

antibiotic used. CDI developed less frequently after pneumonia treatment with the other antibiotics assessed in this study, such as ciprofloxacin, clarithromycin, cefuroxime, and imipenem, with a lower mortality rate ranging between 17 % and 28 %. The probability of death due to CDI that developed as an after-effect of pneumonia treatment with ceftriaxone was borderline higher compared with the ciprofloxacin-, cefuroxime-, and imipenem-treated patients ($0.05 < p < 0.10$). The exact influence of the antibiotics investigated on CDI mortality cannot be precisely set due to a small number of patients in each antibiotic-group. Nonetheless we believe we have shown that ceftriaxone carries the greatest mortal potential, it should be used with caution or not used at all whenever feasible for treatment of post-pneumonia CDI.

Concerning substantial mortality in *C. difficile* infection it is crucial to identify patients at high risk of severe or very severe and complicated infection. The antibiotic exposure related to the treatment of an antecedent infection is a main risk factor for CDI. The antecedent use of antibiotics also factors in the scoring of infection severity (Hernández-García et al. 2015). There are reports concerning the exposure to specific antibiotics or antibiotics belonging to the same structural class. Cephalosporins and carbapenems are considered to be involved with a higher risk of developing CDI. Further, penicillines and quinolones are commonly delivered to patients as a treatment preceding CDI (Büchler et al. 2014). Fluoroquinolone exposure, in particular, may be associated with severe CDI and more frequent transition of a patient to the intensive care unit (Kurti et al. 2015). It is strongly suspected that the antibiotics devoid of activity against *C. difficile* disrupt the gastrointestinal flora and increase the production of toxins (Phillips et al. 2011).

This study affirms the influence of treatment choices in case of risk of nosocomial infections. *C. difficile* infection carries a high deadly potential, harms patients' immunity, increases the probability of CDI recurrence, prolongs hospitalization, and generates extra costs to healthcare systems (Ghantoji et al. 2010; Dubberke et al. 2008). It is essential to make a proper treatment decision on admission to decrease nosocomial infection risk. In patients on antibiotic therapy and thus with high risk of CDI, antibiotic drugs should be chosen with caution to decrease the possible complications. Ceftriaxone is one of the broad spectrum antibiotics, widely used either in patients with pneumonia and other respiratory tract infections. The present findings point to the possible relation of ceftriaxone treatment to a high incidence and mortality from CDI. We conclude that ceftriaxone ought not to be advocated as the first line antimicrobial drug in pneumonia infections. Treatment of respiratory infections should be cautiously tailored having in mind the real risk of developing post-antibiotic *Clostridium difficile* infection.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 65–73
DOI 10.1007/5584_2016_62

Published online: 11 September 2016

Concha Bullosa in Paleoanthropological Material

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Abstract

Concha bullosa is a variant of the sinonasal anatomy in which the middle nasal turbinate contains pneumatized cells, which leads to turbinate enlargement. The reason for concha bullosa formation is unclear, but the variant is seen in up to half the modern population and it may predispose to paranasal sinusitis. The variant has hitherto featured little in paleopathology. Therefore, in the present study we seek to determine the presence of concha bullosa, with the coexisting hypertrophy of the middle turbinate and signs of sinusitis or other pathology of the paranasal complex, in a population living in Tomersdorf-Toporow in the Upper Lausatia, a historical region in Germany and Poland, presently Zgorzelec County in the Lower Silesian voivodeship, at the turn of the nineteenth and twentieth century. The material consisted of 32 skeletons (24 males, 8 females). The gender, age, and stress indicators and the presence of pathological signs were assessed, followed by CT of the skulls. We found 2 skulls (6.3 %) with concha bullosa. In one case septal nasal deviation was present. We conclude that the incidence of concha bullosa could be lower in the past

A genetic component is suggested since differences in the presence of concha bullosa are observed between populations from different regions of the world and different climatic conditions.

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times than at present. Wider research is necessary to settle whether concha bullosa is indeed a rare respiratory paleopathology or a missed, and thus underreported observation.

Keywords
Concha bullosa • Middle turbinates • Paleoanthropology • Paranasal sinuses • Sinusitis

1 Introduction

Diseases of the ostiomeatal complex are a serious health problem due to the prevalence and potential clinical consequences. Anatomical variations in the nasal cavity such as concha bullosa, nasal septum deviation, paradoxical middle turbinate, medial or lateral deviation of the uncinate process, pneumatization of ager nasi, spheno-ethmoidal cells, and oversized bulla may predispose to sinusitis. Modern therapeutic methods based on endoscopic techniques have created more interest in the anatomy of nasal cavity and paranasal sinuses and of the incidence of abnormalities and developmental variations (Hatipoğlu et al. 2005; Subramanian et al. 2005). The assessment of computer tomography images is the gold standard in the studies on nasal anatomy (Badia et al. 2005).

The analysis of bone material from archaeological excavations provides the information on the presence and spread of many diseases and their evaluation over the centuries, including diseases of the upper and lower respiratory tracts (Gawlikowska-Sroka et al. 2013; Kwiatkowska et al. 2011). Paleopathological studies have been carried out for over 150 years. The first examples of such studies are those carried out by Johann Friedrich Esper of the eighteenth century concerning the presence of osteosarcoma in the bear's femur. Studies on the bone material by Rudolf Virchof have significantly changed the view on the origin of syphilis. On the basis of these studies, Joseph Jones diagnosed syphilis in pre-Columbian archaeological remains (see Ortner 2011). The use of modern research techniques for the analysis of paleopathological materials helps unravel significant etiological details concerning various pathologies and the influence of pathologies on human biological and cultural development.

Roberts (2007) has reported that the presence of signs of respiratory diseases in bone material indicates poor air quality in populations exposed to pollution and toxic agents emitted into the air by the mines, workshops, etc. He has also observed a higher incidence of chronic sinusitis in urban populations. This was usually associated with the lower socioeconomic status of these populations and faster spread of diseases. Paleoanthropological studies have demonstrated that urbanization and change of lifestyle by past populations, which switched from a hunter-gatherer to a settled lifestyle, were usually linked with a deterioration in living conditions and a higher incidence of stress indicators (Gawlikowska-Sroka et al. 2013; Kwiatkowska et al. 2011; Skinner and Goodman 1992).

Radiological examinations provide extensive information on the ethnic variations in the formation and pneumatization of sinonasal structures (Badia et al. 2005). The middle turbinate is part of the ethmoid bone forming the lateral wall of the nasal cavity. This turbinate limits the opening of the maxillary sinus, the largest paranasal sinus, and consists of the lamellar and bulbous portions. Pneumatization of these portions leads to the formation of concha bullosa. Specific fragments of the middle turbinate are used as a

reference in the most popular classification system for concha bullosa created by Bolger et al. (1991), which distinguishes lamellar concha bullosa (LCB), bulbous concha bullosa (BCB), and extensive concha bullosa (ECB). Superior, middle, and inferior turbinates covered with mucous membranes are responsible for the filtration, heating, and humidification of inhaled air. Dysfunction of these structures can cause symptoms such as recurrent rhinitis, nasal obstruction, olfactory disorders, eustachitis, paranasal sinusitis, snoring, pharyngitis, and laryngitis. Turbinate dysfunction is mainly caused by hypertrophic changes involving local soft tissues and variations in bone structures, e.g., excessive pneumatization of concha bullosa in the middle turbinate. The cause of pneumatization has not been fully explained. Some researchers suggest the involvement of a genetic component (Chaiyasate et al. 2007). The incidence of concha bullosa is quite variable in populations from different regions of the world and different climatic conditions. Studies carried out in the US have revealed the presence of concha bullosa in 9 % of the population, while in the Turkish population the incidence is estimated at 56 % (Mays et al. 2014). Perhaps, such high disproportions are caused by the adoption of different criteria for the diagnosis and classification, or may result from the environmental factors. A clear answer to this issue has not yet been found. The studies above outlined were conducted on contemporary populations. The presence of concha bullosa in archaeological materials has been scarcely investigated (Mays et al. 2014; Kwiatkowska et al. 2011; Mays et al. 2011; Pospísilová et al. 2001; Derums 1978). There is a relatively high number of research articles dealing with the paleopathological signs of sinusitis in bone material, but only the study by Mays et al. (2014) has concurrently analyzed the presence of concha bullosa and its relationship with the osseous signs indicative of chronic sinusitis, such as new bone formation, erosion, pits, and spicules. That study has estimated the prevalence of concha bullosa in a population from Medieval England. The scarcity of information on this anatomical variation in the past times, quite often observed today, makes it unsettled whether the incidence of concha bullosa was indeed low in historical populations or it is a missed respiratory paleopathology, due to the lack of assessment or perhaps the postmortem damage to fragile structures. The aim of the present study was, therefore, to estimate the incidence of concha bullosa in a population living in Tomersdorf-Toporow in the Upper Lausatia, a historical region in Germany and Poland, presently Zgorzelec County in the Lower Silesian voivodeship, at the turn of the nineteenth and twentieth century. A second objective was to assess the relationship between the presence of concha bullosa and inflammatory lesions in paranasal sinuses. We addressed the issue by examining the excavatory skull material.

2 Methods

The study material was obtained during archeological excavations carried out in 2014–2015 at a cemetery near the village of Prędocice (Serbian-Lusatian *Tornow*, German *Tormersdorf*) located in the western part of the Toporow forestry district (forest division Ruszów). This area belongs to the macro-region of the Silesian-Lusatian Lowland. The investigation of concha bullosa was part of a broader research project aimed at reconstructing the history of the settlement in the area and understanding the biological condition of local populations, their culture, and the relationship with the natural environment. Geophysical analyses were carried out to precisely locate burial sites within the cemetery. Magnetic prospecting carried out with a magnetometer revealed large areas of point-dipole anomalies corresponding with modern iron elements and indicating the use of the site as a cemetery (crosses, cemetery plots, and brickwork). A georadar demonstrated the presence of diffractors, possibly the margins of graves and boundaries of land use. Light detection and ranging (LIDAR) technology, and air prospection were also used to identify the site plan of the cemetery and nearby village (Figs. 1 and 2).

Test pits were established in two graves located in the eastern part of the cemetery. During the works 32 graves arranged in distinct rows were uncovered. Only one phase of the cemetery use was identified, and it was dated to the beginning of the twentieth century. The material was excellent for research purposes due to its good state of preservation, which allowed assessing of changes within the nasal cavity directly after excavation.

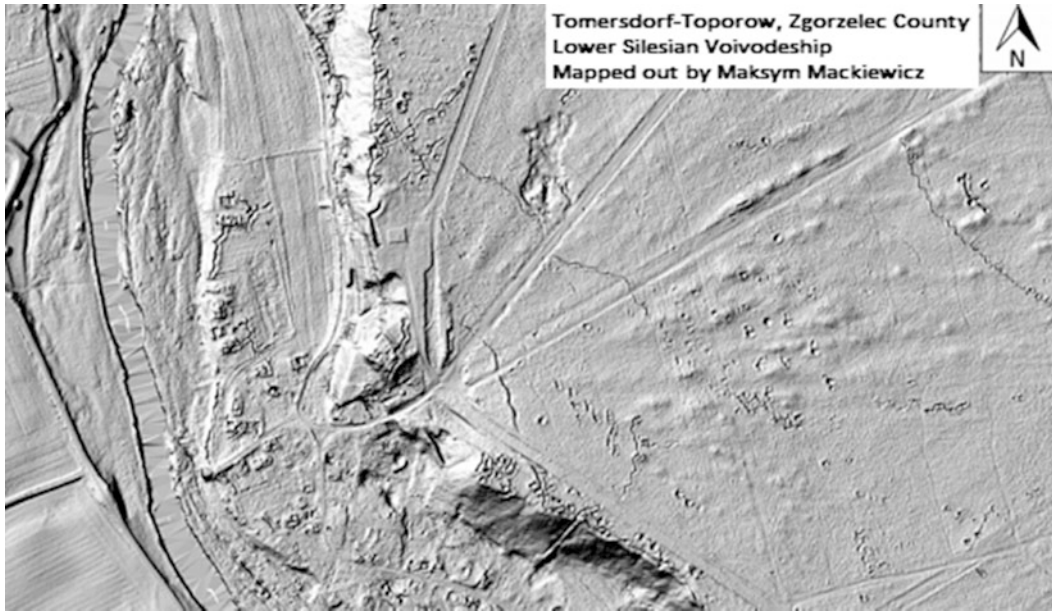

Fig. 1 Laser scanning (LIDAR) of the examined area of the microregion Tomersdorf-Toporow

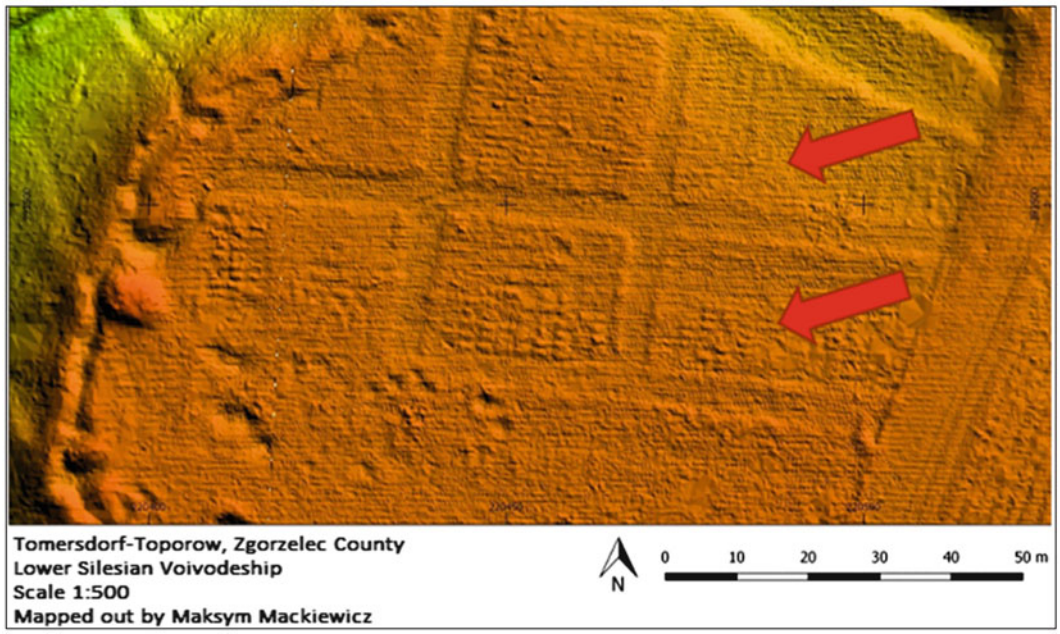

Fig. 2 Laser scaning (LIDAR) of the cemetery; localization of excavation shown by *red arrows*

Gender of buried individuals was identified based on the morphology of the skull and pelvic bones, using accepted standards of anthropology (Acsádi and Nemeskéri 1970; Loth and Henneberg 1996). The age-at-death of individuals was estimated from the degree of tooth wear and the morphology of the pubic symphysis surface (Buikstra and Ubelaker 1994). Stress indicators such as cribra orbitalia and enamel hypoplasia were also assessed. Skeletons were also examined for the presence of pathological changes. Computed tomography scanning was performed on the skulls to confirm or rule out the presence of pneumatization within the turbinates. Changes were classified using the system proposed by Bolger et al. (1991).

3 Results

Of all 32 skeletons found in graves 24 were male and 8 were female. According to age-at-death the individuals were classified as *senilis* (4), *maturus*/*senilis* (3), *maturus* (17), *adultus*/*maturus* (4), *adultus* (3), and the skeleton of a male individual found in grave no. JS-8/I/2014 was classified as *juvenis*/*adultus*.

The signs of pathological changes of various degrees were detectable in all excavated skeletons. Changes caused by degeneration and overloading (osteophytes, Schmorl's nodes, degenerative changes on the articular surface of the vertebra) were identified in 60 % of individuals. In addition, numerous signs of developmental, inflammatory, and posttraumatic changes were found. Dental caries was detected in 61 % and periodontal disease in 28 % of individuals. Antemortem tooth loss was observed in 80 % of subjects.

In two female skeletons concha bullosa was identified already through a macroscopic assessment; to be later confirmed by the examination of images acquired by computed tomography scanning. A massive bulbous concha bullosa was found in skull no. J.S.5/2015. Extensive pneumatization of the middle turbinate was associated with a significant nasal septum deviation (Figs. 3, 4, and 5), as well as macroscopically detectable degenerative changes in the temporo-mandibular joints and the wear of the condylar processes.

In this female skeleton there were signs of severe bilateral inflammation of the maxillary alveolar process. The bone material also showed numerous degenerative changes within the spine, possibly caused by bone tuberculosis or very advanced osteoporosis.

The second case of concha bullosa was found in skull no. J.S.3/2015. Here, changes were less advanced, and were classified as lamellar concha bullosa. No other changes, such as

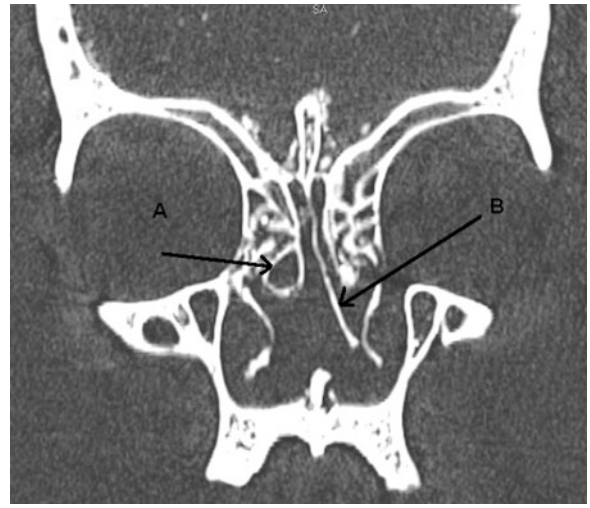

Fig. 3 CT scan, frontal projection; *arrow A* concha bullosa – bulbous type, *arrow B* deviated nasal septum (skull no. J.S.5/2015)

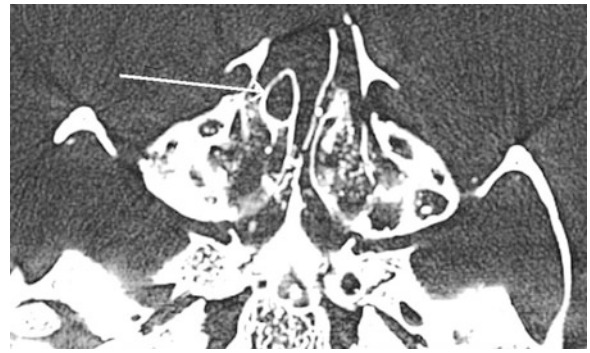

Fig. 4 CT scan, transverse projection; *arrow* points to concha bullosa – bulbous type (skull no. J.S.5/2015)

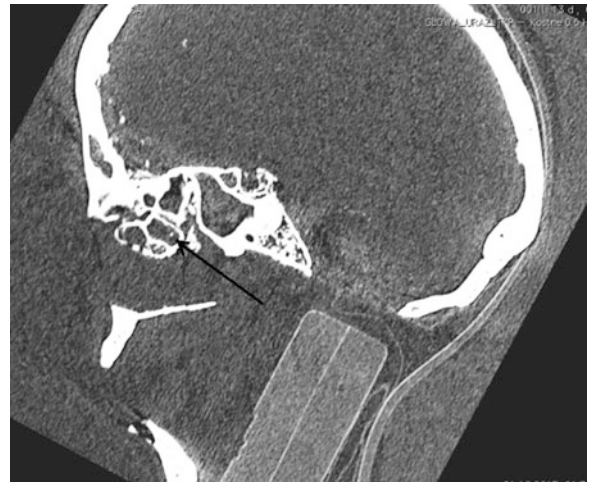

Fig. 5 CT scan, sagittal projection; *arrow* points to concha bullosa – bulbous type (skull no. J.S.5/2015)

pneumatization or nasal septum deviation, were detected (Figs. 6 and 7).

The skeleton showed evidence of the antemortem loss of many teeth, extensive calculus, and carious lesions. Degenerative changes within the spine were less advanced compared with those in the other case described above, but there was an ossified ligament within the atlanto-occipital joint.

4 Discussion

Concha bullosa, i.e., the pneumatized lamella of the middle turbinate, is not an abnormality but a developmental variation. However, in some cases it may predispose to the onset and progression of upper respiratory tract diseases (Alkire and Bhattacharyya 2010). These are usually associated with abnormal air flow and impaired patency of the openings of sinuses into the middle nasal passage, because the hypertrophy of the middle turbinate obstructs the ethmoidal infundibulum and leads to recurrent sinusitis. Several investigators have reported a higher prevalence of concha bullosa in patients with sinusitis compared to controls (Calhoun et al. 1991; Lloyd 1990; Clark et al. 1989). However, no such association has been found by others (Cho et al. 2011; Alkire and Bhattacharyya 2010; Tonai and Baba 1996). Treatment of hypertrophic turbinates consists of corrective resection of the osseous fragment and restoration of patency to the sinus structures. This type of treatment has already been advocated by Hippocrates in ancient times, who proposed the removal of 'hard growths' using a sinew looped around.

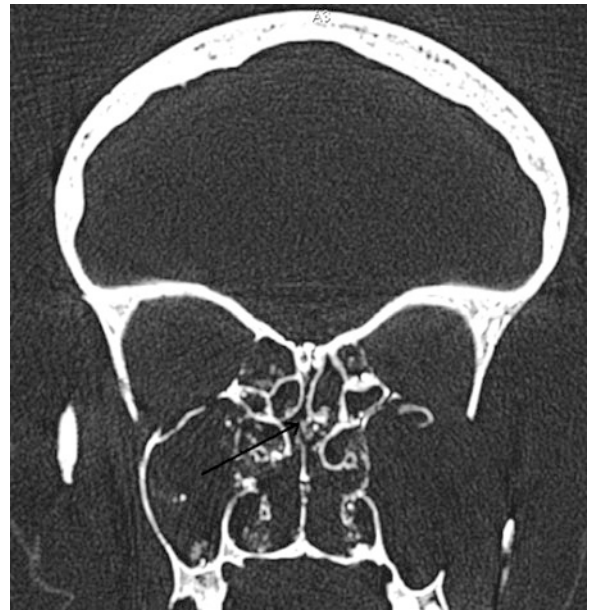

Fig. 6 CT scan, frontal projection; *arrow* points to concha bullosa – lamellar type (skull no. J.S.3/2015)

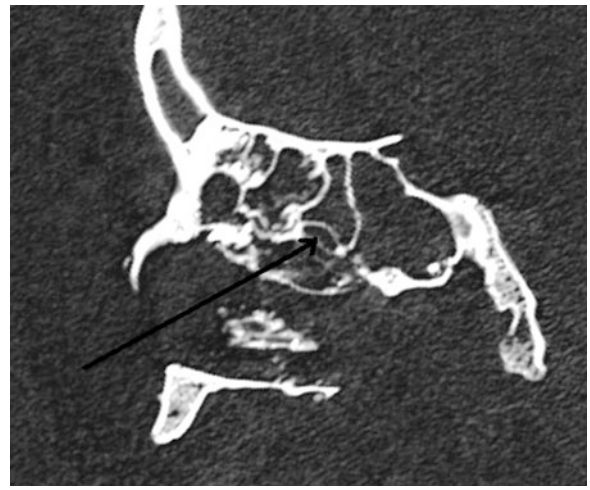

Fig. 7 CT scan, sagittal projection; *arrow* points to concha bullosa – lamellar type (skull no. J.S.3/2015)

In the available literature we found only a few papers reporting the presence of concha bullosa in paleopathological material (Mays et al. 2014; Kwiatkowska et al. 2011). Studies carried out on a large sample of bone material from the Baltic Sea region dated to the Bronze Age have revealed the presence of concha bullosa in only 0.2 % of the skulls (Derums 1978). Such a low prevalence can be associated with a low general pneumatization of skulls in this skeletal series, or with the fact that partly or completely damaged bone material was not excluded from the analysis. To validate findings, examination should be carried out only in skulls with a well preserved nasal cavity because the conchae and other bony structures of nasal cavity are fragile and highly vulnerable to fragmentation and destruction in archeological burials (Mays et al. 2014). Following this principle, Mays et al. (2014) have examined bone material coming from Wharram Percy, a medieval site in England, representing 360 adult individuals, and identified only

45 skulls that the met inclusion criteria. In the present study we examined skulls only with well-preserved structures of the nasal cavity, and found concha bullosa in 6.3 % of individuals, which is a rate much lower than that reported for the contemporary Caucasian population. Pospísilová et al. (2001) have demonstrated the presence of concha bullosa in 52 % of skulls excavated from a medieval site of Broumov Ossuary. Hatipoğlu et al. (2005) has reported a greater prevalence of left-sided concha bullosa. In the present study, concha bullosa was identified on the right side, and was bulbous or lamellar. Concha bullosa may be unilateral or bilateral. It usually contains a single large air cell, as found in our study. However, variations containing numerous, smaller air cells, have also been reported, albeit less frequently (Subramanian et al. 2005).

Studies by Mays et al. (2014) have not confirmed the presence of an association concha bullosa and features indicating chronic sinusitis, which may stem from a rather small size of concha bullosa observed by the authors. Calhoun et al. (1991) have argued, however, that the coexisting sinusitis is not determined by the presence of concha bullosa *per se*, but rather by its considerable size. Other findings seem to confirm this notion. A single case of extremely large concha bullosa, confirmed by CT, has been reported by Kwiatkowska et al. (2011), who examined the bone material from a medieval site in the city of Glogow in Poland. The extensive concha bullosa coexisted with numerous inflammatory lesions in the maxillary sinus penetrating to an eye socket. Likewise, in the present study the larger concha bullosa coexisted with periodontal inflammatory lesions, but in the second case no signs of inflammation within the skull were detected.

Investigators try to explain the potential effect of geographical region, climatic conditions, and gender on the prevalence of concha bullosa and pneumatization of sinuses. A low-degree pneumatization and aplasia of sinuses is more frequently noted in individuals from colder climatic zones (Koertvelyessy 1972), but it is usually more pronounced in populations from temperate and warm regions. Studies performed on contemporary populations from Australia have shown the presence of concha bullosa in 55 % of subjects, while Earwaker (1993) has observed turbinate pneumatization in 53 % of Swiss individuals. Thus, the effect of climate cannot be considered as the underlying reason of concha bullosa in this case. Nevertheless, regional and ethnic differences in the prevalence of concha bullosa have been reported. Badia et al. (2005) have shown that the incidence of concha bullosa is significantly higher, and it is associated with greater pneumatization of the ethmoid labirynth, in Caucasians compared to Chinese people. In contrast, incidence of a complete absence of a sinus was higher in the Chinese. Subramanian et al. (2005) have found no effect of ethnicity on the incidence of concha bullosa in patients. However, they report a significantly higher incidence of this anatomical variation in women. The present examination of bone paleomaterial also revealed the presence of concha bullosa in women. In historical populations, incidence of concha bullosa seems to have been lower. That may not result just from the damage to bone material, and thus underreporting the presence of this anatomical variation, but also from the fact that the analysis covers the entire population. The above-mentioned contemporary studies most frequently analyze groups of patients with upper respiratory tract diseases, including sinusitis, and therefore the probability of detecting concha bullosa is much higher.

5 Conclusions

The use of radiological studies for the analysis of paleoanthropological material and interdisciplinary collaboration significantly extends the ability to detect conditions of historical populations, and thus enriches the knowledge about the evolution of health condition and the underlying etiological mechanisms of diseases throughout the ages. Wider research is necessary to answer the question about the presence or absence of concha bullosa paleopathology. In the historical material hitherto examined the frequency of concha bullosa changes seems to have been lower than

that present in the contemporary Caucasian populations.

Acknowledgments Project financed by the National Center of Science, grant. DEC-2013/10/E/HS3/00368.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 75–84
DOI 10.1007/5584_2016_65

Published online: 30 August 2016

IgA Nephropathy in Children: A Multicenter Study in Poland

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Abstract

IgA nephropathy (IgAN) is the most common form of glomerulonephritis in pediatric population. The clinical presentation of the disease in children ranges from microscopic hematuria to end-stage kidney disease. The aim of the study was to retrospectively assess clinical and kidney biopsy features in children with IgAN. We assessed a cohort of 140 children, 88 boys, 52 girls with the diagnosis of IgAN in the period of 2000–2015, entered into the national Polish pediatric IgAN registry. The assessment

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included the following: proteinuria, hematuria, glomerular filtration rate (GFR), arterial blood pressure, and the renal pathological changes according to the Oxford classification and crescents formation, as modifiable and unmodifiable risk factors. The incidence of IgAN in Poland was set at 9.3 new cases *per* year. The mean age at onset of IgAN was 11.9 ± 4.3 years, and the most common presentation of the disease was the nephritic syndrome, recognized in 52 % of patients. Kidney biopsy was performed, on average, 1.3 ± 2.0 years after onset of disease. Based on the ROC analysis, a cut-off age at onset of disease for GFR <90 mL/min/1.73 m^2 (risk factor of progression) was calculated as 13.9 years. Unmodifiable lesions: segmental sclerosis, tubular atrophy/interstitial fibrosis (S1, T1-2) in the Oxford classification and crescents in kidney biopsy were significantly more common in Gr 1 (>13.9 years) compared with Gr 2 (<13.9 years), despite a significantly shorter time to kidney biopsy in the former. We conclude that IgAN in children may be an insidious disease. A regular urine analysis, especially after respiratory tract infections, seems the best way for an early detection of the disease.

Keywords
Children • Glomerulonephritis • Hematuria • IgA nephropathy • IgA protein • Kidney • Proteinuria • Respiratory infection • ROC analysis

1 Introduction

IgA nephropathy (IgAN) is the most common type of chronic glomerulonephritis worldwide (Pesce et al. 2016; Wyatt and Julian 2013; McGrogan et al. 2011; Coppo and D'Amico 2005; D'Amico 1987). The incidence of this condition in children is estimated at 0.03/100,000/year in Venezuela, 0.5–1.0/100,000 children/year in the US, and 9.9/100,000 children/year in Asia (Shibano et al. 2015; Orta-Sibu et al. 2002; Wyatt et al. 1998). Based on the findings reported, a map of genetic susceptibility to IgAN worldwide has been created (Kiryluk et al. 2012). The disease is more common in boys and typically manifests with episodic hematuria occurring in the course or after an upper respiratory tract infection (Wang et al. 2012; Schena 1990).

The pathogenesis of IgAN is not entirely clear, but recent studies favor a four-hit hypothesis (Suzuki et al. 2011). A key pathogenetic role is played by galactose deficiency in the hinge region of the IgA1 molecule (Hit 1), produced in response to infections, mostly of the upper airways (Mestecky et al. 2005). This results in the formation of an aberrant galactose-deficient IgA1 (GdIgA1). Then, GdIgA1 induces the formation of IgG or IgA1 antibodies (Hit 2). These antibodies recognize GalNAc-containing epitopes in the hinge region O-glycans of GdIgA1 (Tomana et al. 1999). The presence of GdIgA1 and anti-GdIgA1 antibodies in the serum leads to the formation of pathogenic IgA1 that contain immune complexes (Hit 3). In patients with IgAN and Henoch-Schönlein nephritis (HSN), a large size of IgG-IgA1 immune complexes does not allow their hepatic degradation *via* the asialoglycoprotein receptor (ASGPR), resulting in their entry to the renal circulation. Mesangial localization of immune complexes induces an autoimmune inflammatory

response, proliferation of mesangial cells, and expression of extracellular matrix components (Hit 4), leading to glomerular and interstitial damage and frequently to renal failure (Suzuki et al. 2011). The inflammatory process in the glomerular structure is associated with inflammation in the tubulointerstitium and infiltration by immune cells of variable intensity (Gluhovschi et al. 2009). The diagnosis can be made only on the basis of kidney biopsy which unravels IgA deposits by immunofluorescence staining (Berger and Hinglais 1968).

Previous histopathologic classifications of IgAN included various elements of biopsy findings. The Oxford classification introduced in 2009 includes four adverse prognostic components: mesangial proliferation (M), endocapillary hypercellularity (E), segmental sclerosis (S), and tubular atrophy/interstitial fibrosis (T) (Coppo et al. 2014; Cattran et al. 2009). In recent years, a distinction between modifiable (M, E, and crescents) and unmodifiable (S and T) changes has been added to the classification (Coppo and Davin 2015). In Japan, a school urine screening program has been functioning since 1974. It enables early diagnosis and treatment of glomerulopathy, resulting in a decreasing rate of end-stage renal failure secondary to glomerulonephritis (Shibano et al. 2015).

The clinical course of IgAN is milder in children (Nozawa et al. 2005; Haas 1997). However, the age at onset can be an independent predictor of poor outcome and renal failure (Radford et al. 1997). A pediatric registry of IgAN has been established in Poland in 2013. The present study was undertaken to analyze epidemiological, clinical, and histopatological data of IgAN children, retrieved from this registry.

2 Methods

The Research Review Board of Warsaw Medical University in Poland approved this study. The consent requirement was waived because of the retrospective nature of the analysis of medical records. We retrospectively examined records of 140 children, including 88 boys and 52 girls, (mean age of 11.4 $\pm$ 4.3 years) with the diagnosis of IgAN, based on the presence of IgA as the predominant immunoglobulin in the glomerular mesangium according to the Oxford classification. The patients from nine pediatric nephrology units in Poland were entered into the registry in the years 2000–2015.

In all patients, demographic data, symptoms at disease onset, including proteinuria, microscopic hematuria, hypertension, glomerular filtration rate (GFR), history of a preceding infection, family history of glomerulopathy, and kidney biopsy findings were analyzed. Proteinuria was determined using the Exton method and expressed in mg/kg/day. Nephrotic range proteinuria was defined as $\geq$50 mg/kg/day, and nephritic (non-nephrotic) range proteinuria was defined as $<$50 mg/kg/day. Nephritic syndrome was defined as microscopic hematuria combined with nephritic (non-nephrotic) range proteinuria. Microscopic hematuria was defined as the presence of $>$5 erythrocytes in the urine sediment *per* viewfield in light microscopy at 400-fold magnification, and gross hematuria was diagnosed when a change in urine color was present. Isolated microscopic/gross hematuria was recognized when it was unaccompanied by other abnormalities in urinalysis. Hypertension was diagnosed when blood pressure on three occasions was above the 95th percentile for height, age, and gender (National High Blood Pressure Education Program 2005). Glomerular filtration rate was calculated using the Schwartz formula (eGFR = 0.413 x height/serum creatinine) (Schwartz et al. 2009). A positive history of a preceding infection was defined as an infection within 3 weeks before the development of IgAN symptoms. A positive family history of glomerulopathy was based on the kidney biopsy results in a family member.

Diagnostic kidney biopsy findings were evaluated in the participating units and further confirmed by a reference center, the Department of Pathology of Warsaw Medical University in Poland. The diagnosis was made when immunofluorescence testing showed predominant IgA deposits. Kidney biopsies were also scored using the Oxford classification (Coppo et al. 2014; Cattran et al. 2009), with the presence

of a given finding scored 1, and the absence scored 0. The following components were included: M – mesangial proliferation (M1 or M0); E – endocapillary hypercellularity (E1 or E0); S – segmental sclerosis/adhesions (S1 or S0); and T – tubular atrophy/interstitial fibrosis (T0 – 0–25 %, T1 – 26–50 %, and T2 > 50 %). The MEST score was calculated as the sum of M + E + S + T, ranging from 0 to 5. The M1 and E1 changes and crescents were considered modifiable (active), and S1 and T1-T2 changes were considered unmodifiable (chronic).

Based on the severity of baseline proteinuria, which is an adverse prognostic, patients were categorized as having nephrotic range proteinuria (Group A), non-nephrotic range proteinuria (Group B), or isolated hematuria (Group C). We also analyzed patients in relation to the age at onset of disease.

2.1 Statistical Elaboration

Data were shown as means ± SD for normally distributed variables, and medians and ranges for non-normally distributed variables. Normality of distribution of variables was verified using the Lilliefors test. The Student *t*-test for independent samples was used to compare the mean values of normally distributed continuous variables between two groups, and the Mann-Whitney *U* test was used for non-normally distributed variables. Three-group comparisons were performed using univariate ANOVA and the Kruskal-Wallis test for normally and non-normally distributed variables, respectively.

Categorical variable frequencies in 2 or 3 groups were compared using the chi-squared test. The receiver operating characteristic (ROC) curve was used to determine a cut-off age optimal for dichotomization between normal and reduced GFR, i.e., for two-class categorization of GFR values ≥90 *vs.* <90 mL/min/1.73 m^2. The area under the curve (AUC) was considered statistically significant when greater than >0.5 at $p < 0.05$. A p-value <0.05 defined statistically significant differences.

Table 1 Demographic, clinical, and histopathologic characteristics of pediatric patients

	All (n = 140)
Male (n%)	88 (63 %)
Female (n%)	52 (37 %)
Age at onset (yr)	11.4 ± 4.3
Proteinuria (mg/kg/day)	
Mean	38.4 ± 99.0
Median (range)	13.0 (0–967)
GFR (mL/min/1.73 m^2)	94.2 ± 36.2
Time to biopsy (yr)	1.3 ± 2.0
MEST score	1.5 ± 1.1
M 1, n (%)	109 (78)
E 1, n (%)	30 (21)
S 1, n (%)	40 (29)
T 1–2, n (%)	24 (17)
Active lesions only	
M1/E1, n (%)	65 (46)
Chronic lesions	
S1/T1, n (%)	50 (36)

3 Results

Demographic, biochemical, and histological characteristics of the cohort studied are shown in Table 1. Based on the registry data, the incidence of IgAN in children was 9.3/100,000 *per* year, with the male to female ratio of 1.7:1, and the mean age at onset of disease of 11.4 ± 4.3 years. The mean baseline proteinuria was 38.4 ± 99.0 (median 13.0) mg/kg/day, and the mean GFR was 94.2 ± 36.2 mL/min/1.73 m^2.

The frequency of clinical manifestations of IgAN in children is shown in Table 2. The most common initial manifestation was nephritic syndrome with microhematuria, present in 50 % of patients, followed by isolated hematuria in 29 % of patients, and nephrotic range proteinuria with hematuria in 21 % of patients. Hypertension, as one of the initial manifestations of IgAN, was present in 17 % of patients, accompanying nephritic syndrome in 11 % of patients, nephrotic syndrome in 5 % of patients, and isolated microscopic/gross hematuria in 1 % of patients. GFR reduced below 90 mL/min was seen in 39 % children, including 23 % with nephritic syndrome, 10 % with nephrotic

Table 2 Clinical characteristics of pediatric patients with IgA nephropathy (IgAN)

Symptoms at onset of IgAN	n (%)	HTN	↓GFR	Gross hematuria	Previous infection	Family history of GN
Nephrotic proteinuria + hematuria	29 (21 %)	7 (5 %)	14 (10 %)	8 (6 %)	17 (12 %)	3 (2 %)
Nephritic proteinuria + hematuria	70 (50 %)	15 (11 %)	33 (23 %)	22 (16 %)	39 (28 %)	1 (1 %)
Isolated hematuria/gross hematuria	41 (29 %)	2 (1 %)	8 (6 %)	10 (7 %)	29 (21 %)	4 (3 %)
Overall	140 (100 %)	24 (17 %)	55 (39 %)	40 (29 %)	85 (61 %)	8 (6 %)

IgAN IgA nephropathy, *HTN* hypertension, *GRF* glomerular filtration rate, *GN* glomerulopathy

Table 3 Characteristics of pediatric patients in relation to the severity of proteinuria

	Group A Nephrotic proteinuria (n = 29)	Group B Non-nephrotic proteinuria (n = 70)	Group C Isolated hematuria (n = 41)	p
Age at onset (yr)	10.2 ± 4.7	12.1 ± 4.2	11.2 ± 4.3	0.09
Proteinuria – median (range) (mg/kg/day)	100 (50–967)	14 (4–50)	0	
GFR (mL/min/1.73 m^2)	90.3 ± 41.4	86.2 ± 34.2	110.9 ± 31.5	$<0.05^1$
GFR <90 mL/min/ 1.73 m^2; n (%)	14 (40)	33 (47)	8 (19)	$<0.05^2$
Infection at onset; n (%)	17 (59)	39 (56)	29 (70)	ns
Time to biopsy (yr)	0.7 ± 1.1	2.8 ± 10.8	1.6 ± 1.7	$<0.05^3$
MEST score	1.9 ± 1.2	1.6 ± 1.1	1.3 ± 0.8	$<0.05^4$
Active lesions M1/E1; n (%)	10 (34)	33 (47)	22 (54)	ns
Chronic lesions S1/T1; n (%)	11 (38)	30 (43)	9 (22)	$<0.05^5$

Significant differences: [1]A *vs.* C, B *vs.* C, [2]A *vs.* C, B vs C, [3]A *vs.* C, B *vs.* C; [4]A *vs.* C, [5]B *vs.* C

syndrome, and 6 % with isolated hematuria. GFR below 60 mL/min was noted at baseline in 16 (11 %) patients. Episodic hematuria as an initial disease manifestation was seen in 29 % of patients, most commonly with nephritic syndrome (16 %). An infection, mostly involving airways, preceded disease symptoms in 54 % of patients, and a positive family history of glomerulopathy was noted in 6 % of patients.

Kidney biopsy was performed in all children at the mean of 1.3 ± 2.0 (median 0.5) years since the initial manifestation of the disease (Table 1). The most common indication for kidney biopsy was nephritic syndrome (52 %). The most frequent changes according to the Oxford classification were M1 (mesangial proliferation) noted in 109 (78 %) of patients, while chronic S1 and T1/T2 changes were noted in 29 % and 17 % of children, respectively. The most common MEST score was 1, found in 61 (45 %) of patients.

Isolated active M1 and/or E1 changes were found in 65 children (46 %), including 10 (15 %) with nephrotic range proteinuria, 33 (51 %) with non-nephrotic range proteinuria, and 22 (34 %) with isolated hematuria. Crescents were present in 33 (23 %) children, most frequently fibrocellular in 17 (51 %) and fibrous in 12 (36 %). Unmodifiable S1 and/or T1/T2 changes were found in 50 (36 %) patients, most commonly with non-nephrotic range proteinuria or isolated hematuria. We found a positive correlation between the MEST score and baseline proteinuria ($r = 0.20$, $p < 0.05$), and a negative correlation between the MEST score and GFR ($r = -0.27$, $p < 0.05$).

Table 3 shows the correlation between MEST and GFR *vs.* proteinuria/hematuria (Group A –

nephrotic range proteinuria, Group B – non-nephrotic range proteinuria, and Group C – isolated hematuria). The age of patients in Group A tended to be lower than that in Group B ($p = 0.09$), but it did not differ significantly in relation to Group C. The mean GFR values were significantly lower, and the number of children with GFR <90 mL/min/1.73 m^2 was significantly higher in Groups A and B compared with Group C. The time to kidney biopsy was longer in Groups B and C compared with Group A. The mean MEST score was the highest in Group A (1.9 ± 1.2), with a significant difference compared with Group C. There was an insignificant trend toward more frequent MEST scores of 1 and 2 in Groups B and C compared with Group A, and toward MEST scores of 3 and 4 in Group A. No significant differences in the rates of isolated active M1/E1 changes and the presence of crescents were found among Groups A, B, and C. Unmodifiable changes were most common in Group B (43 %), significantly more common compared with Group C (21 %) ($p < 0.05$) and Group A (38 %) ($p > 0.05$). A negative correlation between the age at onset of disease and GFR was found ($r = -0.24$, $p < 0.05$).

Based on the analysis of ROC curves, we determined the cut-off level of age at ≥13.9 years, associated with GFR <90 ml/min/1.73 m^2 at onset of disease in children with IgAN [(AUC = 0.62, sensitivity 0.768 (95 % CI 0.62–0.854), specificity 0.466 (95 % CI 0.33–0.601), $p < 0.05$)] as risk factor of poor prognosis (Fig. 1).

We also defined two age-groups: above (Group 1) or below (Group 2) the cut-off age level of 13.9 years, to compare laboratory and other findings as shown in Table 4. We failed to find any significant differences in the severity of baseline proteinuria, the presence of hematuria and hypertension, and the rate of preceding infections between the two age-groups, and between the subgroups A1/A2, B1/B2, and C1/C2 defined by the greater (A1, B1, and C1) or smaller (A2, B2, and C2) severity of baseline proteinuria.

Kidney biopsy was performed after a significantly shorter time from the initial disease symptoms in Group 1 (aged >13.9 years) compared with Group 2 (aged <13.9 years) ($p < 0.05$). The time from initial disease symptoms to kidney biopsy did not differ between subgroups A1 and A2, and between subgroups C1 and C2, but kidney biopsy was performed significantly earlier in subgroup B1 (age >13.9 years with non-nephrotic range proteinuria) compared with subgroup B2 (age

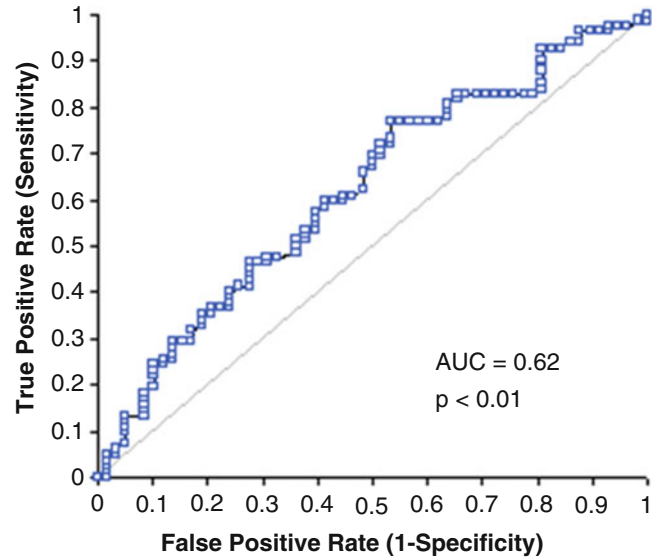

Fig. 1 Receiver operating characteristic (ROC) curve designating a cut-off level of age associated with glomerular filtration rate (GFR) < 90 ml/min/1.73 m^2 at onset of disease in children with IgA nephropathy (IgAN)

Table 4 Clinical and histopathological data of pediatric patients with IgA nephropathy (IgAN) in relation to age

	Overall (n = 140)			Nephrotic proteinuria (n = 29)			Non-nephrotic proteinuria (n = 70)			Isolated hematuria (n = 41)		
	Group 1 (n = 46)	Group 2 (n = 94)	p	Group A1 (n = 9)	Group A2 (n = 20)	p	Group B1 (n = 23)	Group B2 (n = 47)	p	Group C1 (n = 14)	Group C2 (n = 27)	p
Age at disease onset (yr)	16.1 ± 1.8	9.1 ± 3.2	<0.001	15.4 ± 1.2	7.3 ± 3.0	<0.001	16.4 ± 2.0	10.2 ± 3.1	<0.001	16.0 ± 1.8	8.8 ± 2.9	<0.001
Proteinuria (mg/kg/day)												
mean proteinuria	70.7	45.8	ns	245.0	116.4	ns	20.4	15.2	ns	0	0	ns
median (min-max)	18.0 (2.5-967)	20.0 (3.5-500)		133.0 (51–967)	73.5 (50–500)		14.5 (2.5-50)	14.1 (2.5-39)		0	0	
GFR <90 mL/min/1.73 m^2, n (%)	25 (54)	30 (32)	<0.05	7 (78)	7 (35)	<0.05	14 (60)	19 (40)	ns	4 (29)	4 (15)	ns
Gross hematuria, n (%)	12 (26)	28 (30)	<0.01	0	8 (40)	<0.01	9 (39)	13 (28)	ns	3 (21)	7 (26)	ns
Hypertension, n (%)	8 (17)	16 (17)	ns	3 (33)	4 (20)	ns	3 (13)	12 (25)	ns	2 (14)	0	<0.05
Previous infection, n (%)	23 (50)	62 (66)	ns	3 (33)	12 (60)	ns	13 (56)	32 (68)	ns	7 (50)	18 (67)	ns
Family history of glomerulonephritis, n (%)	1 (2)	7 (7)	ns	1 (11)	2 (10)	ns	0	1 (2)	ns	0	4 (15)	<0.05
Time to biopsy (yr)	0.7 ± 0.8	1.6 ± 2.3	<0.05	0.2 ± 0.2	0.9 ± 3.0	ns	0.7 ± 0.8	1.8 ± 2.7	<0.05	1.2 ± 0.9	1.8 ± 2.1	ns
Active lesions, n (%)	18 (39)	47 (50)	ns	1 (11)	9 (45)	<0.05	10 (43)	23 (50)	ns	7 (50)	15 (55)	ns
M1	13 (28)	42 (45)	=0.05	1 (11)	8 (40)	ns	6 (26)	20 (42)	ns	6 (43)	14 (52)	ns
E1	1 (2)	0 (0)	ns	0 (0)	0 (0)	ns	1 (4)	0 (0)	ns	0 (0)	0 (0)	ns
M1 + E1	4 (9)	5 (5)	ns	0 (0)	1 (5)	ns	3 (13)	3 (6)	ns	1 (7)	1 (4)	ns
Chronic lesions n (%)	30 (65)	35 (37)	<0.01	6 (67)	10 (50)	ns	18 (78)	21 (45)	<0.05	6 (43)	4 (15)	ns
S1	18 (39)	22 (23)	ns	4 (44)	6 (30)	ns	10 (43)	14 (30)	ns	4 (29)	2 (7)	ns
T1/T2	12 (26)	13 (14)	ns	2 (22)	4 (20)	ns	8 (35)	6 (13)	ns	2 (14)	2 (7)	ns
(S1 + T1/T2)	9 (19)	4 (4)	<0.05	1 (11)	3 (15)	ns	7 (30)	1 (2)	<0.05	1 (7)	0 (0)	ns

Group 1 – above and *Group 2* – below the cut-off age level of 13.9 years, *A1*, *B1*, and *C1* greater degree of proteinuria, *A2*, *B2*, and *C2* smaller degree of proteinuria, *GFR* glomerular filtration rate, *M1* mesangial proliferation, *E1* endocapillary hypercellularity, *S1* segmental sclerosis; *T1* tubular atrophy/interstitial fibrosis; *ns* nonsignificant

<13.9 years with non-nephrotic range proteinuria) ($p < 0.05$).

Isolated active M1 and E1 changes were observed in 18 (39 %) children in Group 1 and 47 (50 %) children in Group 2; the difference was insignificant. Crescents were significantly more common in Group 1 than Group 2 (35 % *vs.* 18 %, $p < 0.05$); most frequently of fibrocellular and fibrous type. The presence of chronic unmodifiable S1 and T1/T2 changes was significantly more frequent in in Group 1 compared with Group 2; 30 (65 %) *vs.* 35 (37 %) of patients, $p < 0.01$, respectively. The rate of these changes did not differ significantly between age-groups of patients with nephrotic range proteinuria or isolated hematuria, but they were significantly more frequent in older children with non-nephrotic range proteinuria ($p < 0.05$).

4 Discusion

The incidence of IgAN in children in Poland is 9.3 cases *per* year, i.e., about 0.155/100,000 children *per* year, which is lower compared with the Japanese data reporting 9.9/100,000 new cases *per* year (Shibano et al. 2015) and Chinese data where one center has reported 110 cases over 12 years, albeit without giving the information on the size of the pediatric population in the area (Wang et al. 2012). According to McGrogan et al. (2011), the incidence of IgAN in children usually ranges from 0.2/100,000/year to 2.8/100,000/year. The difference in disease frequency is associated with geographic differences in genetic susceptibility to IgA nephropathy (Kiryluk et al. 2012).

The disease is more common in boys than in girls, and the male to female ratio in our registry was similar to that reported by other authors. The mean age at onset in the present study was around 11 years of age, similar to that in Japan (Shibano et al. 2015), but lower than the 16 years of age reported in China (Wang et al. 2012). The most common disease manifestations included microscopic hematuria and non-nephrotic range of proteinuria, found in 52 % of patients. Yoshikava et al. (1999) have reported 62 % of children with microscopic hematuria and proteinuria and Wang et al. (2012) have reported similar clinical findings in 47–66 % of children. Gross hematuria has been observed in 29 % of patients in the present study, as compared to 26 % of Japanese patients, 54 % of Chinese patients, and up to 80 % in different cohorts from Europe and USA (Wang et al. 2012; Yoshikava et al. 2001; Lévy et al. 1985; Linné et al. 1982).

Infections, mostly involving the upper airways, preceded urinary manifestations in 54 % of patients in the present study, similarly to the observations of other authors (Wang et al. 2012). GFR reduction at baseline, which is an established poor prognostic marker, was found in 55 % of children in the present study, significantly more frequently among older children. By using the ROC curve analysis, we were able to determine the threshold age of 13.9 years associated with worse outcomes.

Kidney biopsy was performed in all children at the mean of 1.3 years after the initial disease symptoms; significantly earlier (at 0.7 years) in older children (above 13.9 years of age). For comparison, time to kidney biopsy was 7.7 months in children and 10.7 months in adults in a study by Wang et al. (2012). Of note, chronic unmodifiable changes in kidney biopsy were significantly more frequent among older children despite a shorter time from the initial symptoms to biopsy in that group.

Some authors argue that sclerotic lesions are more characteristic for children with the presence of abnormalities in urinalysis detected earlier than a year or perhaps even 3 years before kidney biopsy, which supports the implementation of a wide urine screening program in children (Shima et al. 2015). In the present study, time to biopsy in older children (Group 1) was less than one year and sclerotic and fibrotic lesions were significantly more frequent than in the younger children (Group 2), where the time to biopsy usually exceeded one year. The greater frequency of fibrocellular or fibrous crescents and other unmodifiable chronic kidney lesions may be attributable to a longer duration of

disease. We also noted chronic lesions in biopsy specimens from children with isolated microscopic hematuria with no apparent proteinuria, which clearly speaks against the suggestions of some nephrologists to perform renal biopsy when proteinuria is greater than 0.5 g/g creatinine (Hama et al. 2015).

Due to the association between IgAN and respiratory infections, Wang et al. (2012) have recommended urinary screening, particularly in children, which might be of major meaning for early discovery of IgAN. Shima et al. (2015) have confirmed that the policy of school urinary screening is a very effective measure for prompt diagnosis and subsequent treatment of IgA nephropathy in children, preventing the development of end-stage renal disease. We conclude that IgA nephropathy may often be an insidious disease in children. The employment of mass urinary screening tests, especially after respiratory infections, has an undeniable potential to detect asymptomatic chronic renal disease in children.

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

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Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 28: 85–86
DOI 10.1007/5584_2016

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