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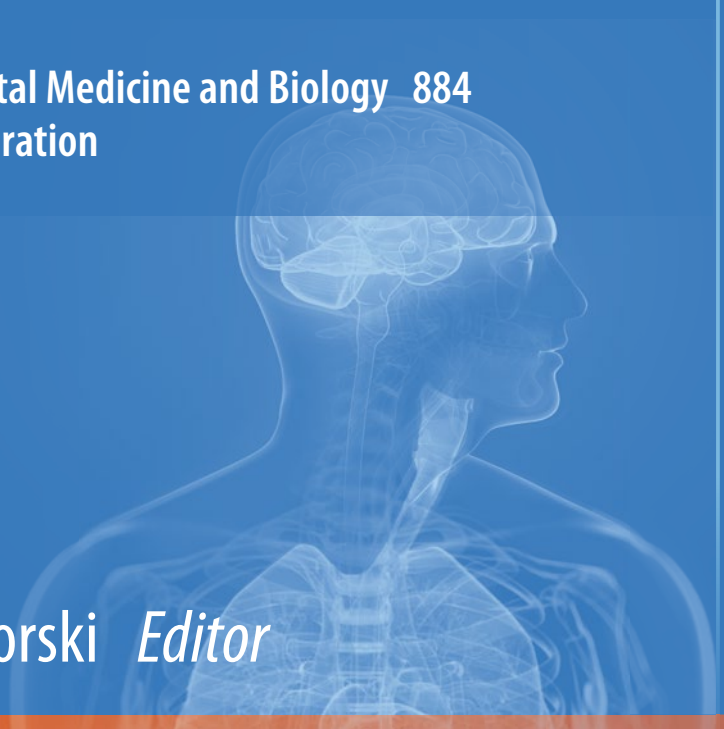
Neuroscience and Respiration



Mieczyslaw Pokorski *Editor*

Pathophysiology of Respiration

Springer

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Neuroscience and Respiration

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Pathophysiology of Respiration

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Preface

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

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

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

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

I would like to express my deep gratitude to Martijn Roelandse, Paul Roos, and Tanja Koppejan from Springer's Life Sciences Department for their genuine interest in making this scientific endeavor come through and for the expert management of this novel book series. My thanks also go to Pavitra Arulmurugan from SPi Technologies India Private Ltd. for the skillful handling of the book production details.

Opole, Poland Mieczyslaw Pokorski

Volume 18: Pathophysiology of Respiration

This book presents a range of research and clinical contributions, including observational studies, practical treatment examples, diagnostic test assessment, biomarkers, and systematic reviews. The objective is to seek to explain the processes or mechanisms whereby abnormal or undesired conditions develop and progress. The attention is given to disturbed carbohydrate metabolism in perpetuation of respiratory ailments or raising therapeutic problems. The psychosocial context of chronic respiratory conditions is tackled as well. Oxidative stress is conducive to both physical and cognitive aging, which raises the specter of using its biochemical markers in future antiaging therapeutic strategies. Critical information provided in the contributions will allow readers to make informed judgments. The book will hopefully serve to create awareness and apprehension about the developments in the field of pneumology and related areas of medical research.

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Breathing in Parkinsonism in the Rat

Monika Bialkowska, Paweł Boguszewski, and Mieczyslaw Pokorski

Abstract

Parkinsonism is underlain by dopamine (DA) deficiency in the mid-brain, a neurotransmitter innately involved with respiratory regulation. However, the state of respiration in parkinsonism is an unsettled issue. In this study we seek to determine ventilation and its responses to hypoxia in a reserpine – alpha-methyl-tyrosine model of parkinsonism in the rat. We also attempted to differentiate between the role of discrete brain and carotid body DA stores in the modulation of the hypoxic ventilatory response (HVR). To this end we used domperidone, a peripheral D2 receptor antagonist, and levodopa, a central D2 receptor agonist. The HVRs to acute 12 % and 8 % hypoxia were studied in a whole body plethysmograph in the same rats before and after the induction of parkinsonic symptoms in conscious rats. We found that resting ventilation and the HVR were distinctly reduced in parkinsonism. The reduction was particularly evident in the peak hypoxic hyperpneic augmentation. Domperidone, which enhanced ventilation in the control healthy condition, failed to reverse the reduced parkinsonic HVR. In contrast, levodopa, which did not appreciably affected ventilation in the healthy condition, caused the parkinsonic HVR to return to and above the baseline healthy level. The findings demonstrate the predominance of a lack of the central DA stimulatory element and minimize the role of carotid body DA in the ventilatory impediment of parkinsonism. In conclusion, the study

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provides the pathophysiological savvy concerning the respiratory insufficiency of parkinsonism, a sequela which carries a risk of chronically impaired blood oxygenation, which may drive the disease worsening.

Keywords
Domperidone • Dopamine • Hypoxic ventilatory response • Levodopa • Parkinsonism • Reserpine • Ventilation

1 Introduction

Parkinsonism or Parkinson's syndrome is one of the leading progressive neurodegenerative diseases. The prevalence of parkinsonism increases sharply in people older than 60 years of age, and the anticipation is its incidence will continue to rise as the human population ages worldwide. The disease is relatively easy to diagnose on the basis of clinical symptoms, such as muscle rigidity, tremor, hypokinesia, or catatonic symptoms, combined with balance problems. These classical motor disorders are accompanied by non-motor neuropsychological symptoms of impaired cognition, fatigue, depression, or pain. Parkinsonism is underlain by insufficient formation and activity of dopamine (DA) or an accelerated loss of dopaminergic neurons in the mid-brain areas of substantia nigra, striatum, and basal ganglia, which underlies motor dysfunction of the disease (Hirsch et al. 1988). This also is associated with insufficient DA availability in other neighboring brain areas, such as the amygdala (Chang and Grace 2015), and with disruption of fronto-striatal dopamine pathways, related to non-motor cognitive defects (Gratwicke et al. 2015).

Respiration, generated in the brainstem neuronal network, is an archetype of fine motor tuning, but the possibility of respiratory insufficiency, bound to decrease the delivery of oxygen into the arterial blood, has gone virtually unrecognized as a possible underlying cause of gradual worsening of parkinsonic patients with time. Chronic hypoxia, developing as a sequela of inadequate ventilation, is well known to be able to induce both motor and non-motor features that are characteristic of parkinsonism (Bouquet et al. 1999; Stuss et al. 1997).

Lack of DA, a special quality of Parkinson's disease, should have a compelling effect on ventilation since DA is a recognized regulator of ventilation. DA is a putative neurotransmitter in the carotid body. This sensory paired organ contains type I or glomus cells that sense reductions in arterial oxygen content and generate the stimulatory hypoxic ventilatory response (HVR). Carotid body chemoreceptors contain abundant DA (Steele and Hintergerge 1972; Dearnaley et al. 1968; Chiocchio et al. 1966). The prevailing consensus is that DA, acting through postsynaptic D2 receptors, is inhibitory for ventilation at the carotid body level (Ward and Bellville 1982; Welsh et al. 1978). Therefore, peripheral antagonism of D2 receptors enhances ventilation and its responses to hypoxia. The central limb of the hypoxic chemoreflex seems, however, to be different. DA and D2 receptors are present in the solitary tract nucleus (NTS), a site in the brainstem where the ventral respiratory neuronal groups reside, which integrates the input emanating from the carotid body (Kline et al. 2002; Lawrence et al. 1995; Hsiao et al. 1989; Smatresk et al. 1983). Hypoxia releases neuronal DA (Goiny et al. 1991) and central antagonism of D2 receptors dampens the HVR; all of which implicates an enhancing effect of central DA on hypoxic ventilation (Huey et al. 2000; Osanai et al. 1997; Hsiao et al. 1989).

Central DA action, functionally opposite to the carotid body-mediated effect, is strengthened by the findings showing that chronic hypoxia

enhances dopamine D2-receptor mRNA in rostral NTS (Huey and Powell 2000). Hypoxia also enhances tyrosine hydroxylase mRNA in the NTS, which is a rate limiting enzyme in DA synthesis; the effect persisting after carotid body chemodenervation (Pascual et al. 2004; Dumas et al. 1996). The corollary is that the lack of brain DA might impair ventilation in parkinsonism. However, studies on respiration in parkinsonism are scanty and the results are contentious. Even less is known about the HVR that imposes a substantial hyperventilatory strain on the respiratory system. Reports show either no change in the HVR (Seccombe et al. 2013) or some impairment, intensified in advanced parkinsonism (Seccombe et al. 2011; Onodera et al. 2000; Serebrovskaya et al. 1998).

In the present study we seek to determine the course of the HVR in parkinsonism and to differentiate between the central and carotid body chemoreceptor-mediated role of DA in ventilatory changes. We addressed the latter issue by using domperidone, a peripheral D2 receptor antagonist and levodopa, a central D2 agonist, as tools to investigate DA-modulated dynamic changes of ventilatory responses. The study was performed in a reserpine model of parkinsonism in the rat.

2 Methods

2.1 Animals and Study Protocol

The study was approved by a local Ethics Committee for Animal Research and was conducted in accord with the guiding principles for the Animal Care and Use of the Declaration of Helsinki of the Medical World Association. A total of 42, 11–12 weeks old conscious male adult Wistar rats, weighing 250–300 g were used. The animals were kept individually in steel cages at a 12 h light/dark cycle in room temperature of 21 °C, with commercial rodent chow and water *ad libitum*.

The core of the study had to do with the HVR in a reserpine model of parkinsonism. In this part of the experimental paradigm each rat served as its own control. The experimental protocol consisted of the following sequential stages. Firstly, the control response to two levels of acute hypoxia (see below) was taken in the untreated rats. This group consisted of 15 rats in all. Then, the rats were dosed with reserpine (2.5 mg/kg; Polfa, Warsaw, Poland) dissolved in a mixture solution consisting of 0.25 % citric acid, 2 % benzyl alcohol, and 10 % Tween-80. The effectiveness of reserpine action was preliminarily assessed from the occurrence of body mass loss of 5–6 % the following day. When proved effective, α-methyl-tyrosine (α-MT; dissolved in distilled water) (Sigma-Aldrich, St. Louis, MO) was injected in a dose of 250 mg/kg, 16 h after reserpine. The development of full-fledged parkinsonic symptoms was checked 1 h after α-MT using the open field test (see below). The completion of the test required about 15 min, after which the HVRs were reinvestigated. Then, domperidone (DOM; 1 mg/kg) dissolved in 300 μl of 0.9 % NaCl with the addition of 10 μl of 5 % HCl and 10 μl MeOH, or levodopa (L-DOPA) with benserazide (25 mg/kg + 6.25 mg/kg, respectively) (Sigma-Aldrich, St. Louis, MO) were injected. Benserazide was injected 20 min before L-DOPA. DOM injection was made in 8 rats and L-DOPA with benserazide in 7 different rats. Forty minutes after the injection of domperidone or L-DOPA the HVRs were reinvestigated again, which was followed by the final open field test. All injections were made intraperitoneally; the volume of injectant would not exceed 0.3 ml.

In addition, there were the following separate control investigations performed: open field tests in healthy untreated rats ($n = 15$), hypoxic ventilatory responses to DOM ($n = 7$), and to L-DOPA with benserazide ($n = 5$) in otherwise healthy untreated rats, using the same doses as in the reserpinized rats above outlined.

The open field test assesses locomotor activity and motivation to explore the surrounding environment (Stanford 2007). The test was performed in a rectangular open field and was assisted by a video camera with associated software to automate the process of distance quantification. The immobility time of rats in the center of the field

Fig. 1 Open field test in rats. The *yellow tangled lines* show the trajectory of movement of a healthy untreated rat during 15 min of the test. The *inset* in the *upper right corner* shows the rat immobilization in the reserpine model of parkinsonism

in the reserpinized rats was taken as corresponding to the development of muscle stiffness and rigidity characteristic of parkinsonism. For comparison, the distance covered by the healthy untreated rats amounted to 4346 ± 155 (SE) cm/15 min (yellow trajectory lines in Fig. 1), whereas that in reserpinized rats was barely 11 ± 2 cm/15 min, as the rats practically did not move beyond the central area of the field (Fig. 1; inset in the right upper corner).

2.2 Ventilation Measurements

Ventilation and its poikilocapnic responses to hypoxia were investigated in a whole-body flow-through plethysmograph (model PLY3223; Buxco Electronics, Wilmington, NC). Rats remained unrestricted in the recording chamber. As soon as the rat familiarized with the chamber environment (about 30–40 min) basal ventilation was recorded in room air. Acute hypoxia was achieved by a rapid flushing of air balanced with N_2. Two levels of the hypoxic stimulus were applied in a random manner: 12 % and 8 % O_2 in N_2. The gas equilibration time in the chamber took 45–60 s from a gas switch, after which time the recording started. Pressure difference between the recording and reference chambers was measured with a differential pressure transducer. The breath-by-breath signal was integrated and further processed by data analysis software (Biosystem XA for Windows SFT3410 ver. 2.9; Buxco Electronics, Wilmington, NC), yielding instantaneous minute ventilation (V_E, $ml \cdot min^{-1}$, BTPS), as the product of tidal volume (V_T, ml) and breathing frequency (f, $breaths \cdot min^{-1}$). The hypoxic tests were of 3 min duration, with a 10–15 min recovery interval of room air breathing between the tests. A bias flow at a rate of 2.5 l/min was employed between the tests to remove the CO_2 build-up from the chamber. Data were tallied off-line at 30 s time marks of the hypoxic course; each variable was expressed as an average of the 10 s bin preceding the time mark.

2.3 Statistical Elaboration

Data were expressed as means ± SE. Variables were normalized to kg of body weight. The analysis focused on three main points of interest characterizing ventilatory changes: prehypoxic

basal control (0 s point), peak hypoxic response (30 s point), and the end-test hypoxic fall-off (180 s point). These three main time marks of the HVR were compared with one-way ANOVA with repeated measures, followed by the *post hoc* Tukey test if significant changes were detected. A two-tailed paired *t*-test was used for comparison of ventilatory data at the corresponding time points. Finally, two-way ANOVA with repeated measures in time factor was applied to compare time courses of HVRs in various pharmacological conditions employed (second factor). $P < 0.05$ was used as a definer of statistical significance.

3 Results

3.1 Ventilation in Parkinsonism

The HVR had a classical biphasic stimulatory/inhibitory character in both healthy untreated and parkinsonic reserpine-treated rats. The peak V_E response developed promptly within 30 s from hypoxia commencement followed by a gradual fall-off; with ventilation remaining above the resting baseline level within the test time. Peak ventilatory augmentation at both hypoxic levels employed, as shown in all relevant figures below, was invariably significant ($0.05 > p > 0.0001$). Parkinsonic V_E was significantly dampened across the course of the hypoxic course compared with that in the healthy group (Fig. 2a, b). The dampening was present in response to both 12 % and 8 % hypoxia and was notably expressed in relation to the peak response at 30 s. The ventilatory response to the stronger 8 % hypoxic stimulus expectedly demonstrated a more conspicuous hyperpneic stimulation as well as reserpine-induced inhibition; where the mean peak V_E decreased from 1644 ± 82 ml·min^{-1}·kg^{-1} in the healthy to 1264 ± 73 ml·min^{-1}·kg^{-1} in the parkinsonic rats ($p < 0.0001$) (Fig. 2b). The parkinsonic condition also decreased the baseline resting V_E before the initiation of hypoxia. This decrease, albeit significant, was disproportionally smaller than that of peak V_E. Therefore, the dampening ventilatory effect of the parkinsonic condition was even more noticeable in the augmentation of V_E from baseline to peak amounting to 771 ± 52 and 532 ± 47 ml·min^{-1}·kg^{-1} ($p < 0.01$) in the healthy and parkinsonic rats, respectively, in response to 8 % hypoxia. The three null

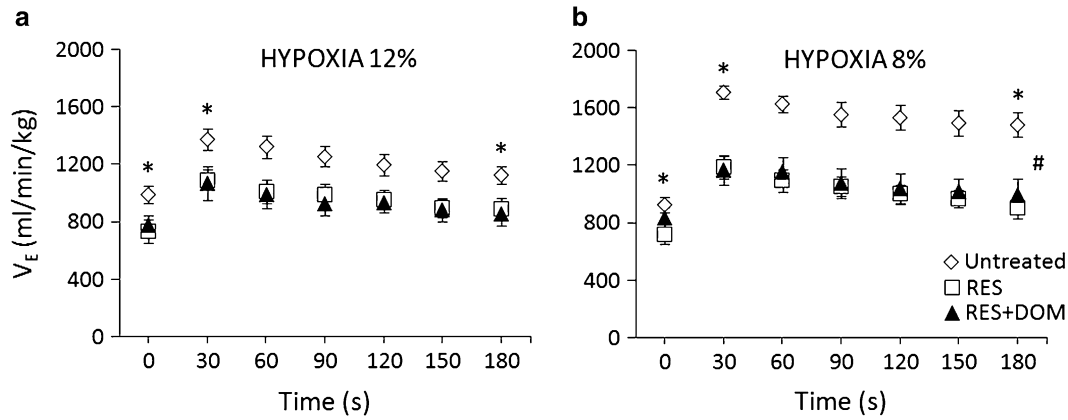

Fig. 2 Ventilatory responses to acute 12 % and 8 % hypoxia in the healthy untreated condition (*open diamond-shaped symbols*), in reserpine-induced parkinsonism (*open squares*), and in domperidone (DOM)-treated reserpinized rats (*solid triangles*). The rats were their own controls ($n = 8$). Parkinsonic condition caused an overall decrease in ventilation and its responses to hypoxia. (**a**) – *$p < 0.02$ for the decreases in ventilation in reserpine-treated and reserpine + DOM-treated *vs.* untreated rats. at 0, 30, and 180 s time points; (**b**) – *$p < 0.001$ for the difference between ventilation in reserpine-treated *vs.* untreated rats at 0 s, and *$p < 0.0001$ between reserpine-treated and reserpine + DOM-treated *vs.* untreated rats at 30 s and 180 s; #$p < 0.001$ for the effects of type of treatment, time, and the interaction of treatment with time

hypotheses tested by two-way ANOVA that time has no effect on the ventilatory outcome, reserpine treatment makes no ventilatory difference, and there is no interaction between time and treatment regarding the ventilatory outcome were disproved ($p < 0.001$) (Fig. 2b). No differences between the effects of reserpine and reserpine + DOM were detected in the V_E responses to either 12 % or 8 % hypoxia.

3.2 Peripheral and Central Dopamine Modulation of Hypoxic Ventilation in Parkinsonism

Domperidone, a peripheral antagonist of DA D2 receptors which does not cross the blood-brain barrier, given on top of reserpine failed to appreciably influence baseline ventilation and its responses to hypoxia (Fig. 2a, b). That was in contrast to a waxing effect of domperidone on the HVR in the untreated healthy rats, which was particularly expressed during the stronger 8 % hypoxic stimulation (Fig. 3b).

Conversely, L-DOPA with benserazide, the latter inhibits peripheral L-DOPA metabolism which enables it to cross the blood-brain barrier in full, a central DA D2 receptor agonist, given on top of reserpine significantly enhanced reserpine-dampened resting ventilation and its responses in both mild and strong hypoxia (Fig. 4a, b). The enhancement exceeded, although insignificantly, the baseline ventilatory level over the course of the hypoxic responses, yielding a stimulatory response overshoot. The peak ventilatory responses to the stronger 8 % hypoxic stimulus at 30 s were as follows: baseline in the untreated rats of 1409 ± 62 ml·min^{-1}·kg^{-1} decreased to 1017 ± 117 ml·min^{-1}·kg^{-1} in reserpine-induced parkinsonism, and rebounded to 1661 ± 168 ml·min^{-1}·kg^{-1} after giving L-DOPA with benserazide on top of reserpine; differences in V_E between reserpine-treated *vs.* untreated and reserpine + L-DOPA-treated rats were significant ($p < 0.01$) (Fig. 4b). There were significant treatment ($p < 0.04$) and time ($p < 0.001$) effects on the ventilatory outcome, and an interaction between the two ($p < 0.001$) regarding the HVR after reserpine *vs.* reserpine + L-DOPA for 12 % and 8 % hypoxia and the HVR in untreated *vs.* reserpine-treated rats for 8 % hypoxia (two-way ANOVA).

L-DOPA with benserazide given to otherwise untreated rats, showed just a tendency to enhance ventilation and its responses to both 12 % and 8 % hypoxia, which failed to reach significance across the response course (Fig. 5).

The inspection of the breathing pattern shows that the frequency component contributed more

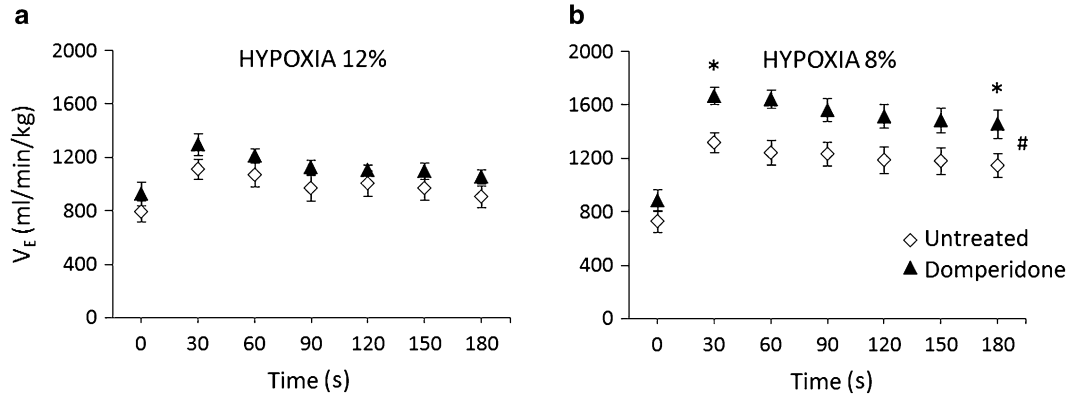

Fig. 3 Influence of domperidone (*solid triangles*) on resting and hypoxic ventilation in control untreated rats (*open diamond-shaped symbols*) (n = 7). (**a**) – 12 % hypoxia; (**b**) – 8 % hypoxia, *$p < 0.03$ for the difference between ventilation in domperidone-treated *vs.* untreated rats at 30 s and 180 s; #$p < 0.001$ for the effects of time, type of treatment, and the interaction of treatment with time

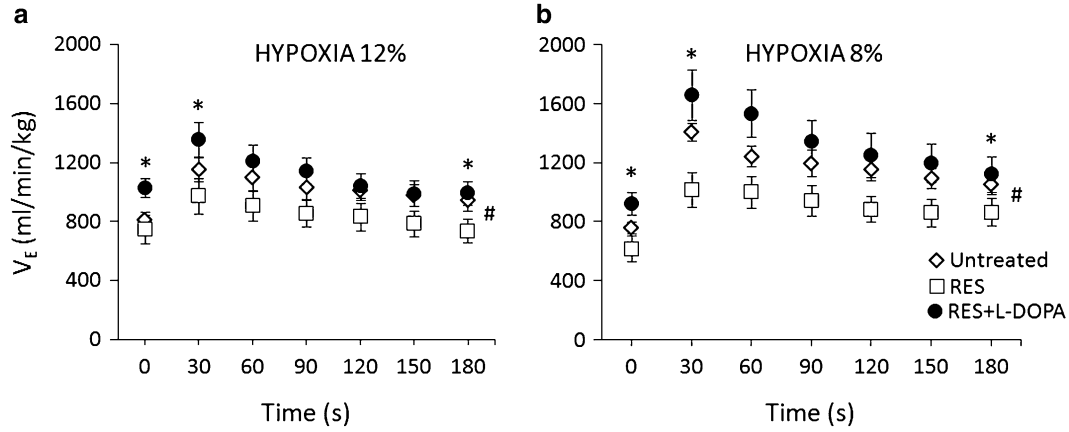

Fig. 4 Ventilatory responses to acute 12 % and 8 % hypoxia in the healthy untreated condition (*open diamond-shaped symbols*), in reserpine-induced parkinsonism (*open squares*), and in L-DOPA + benserazide-treated reserpinized rats (*solid circles*). The rats were their own controls (n = 7). Parkinsonic condition caused an overall decrease in ventilation and its responses to hypoxia and L-DOPA caused ventilation to rebound beyond the level present in healthy untreated rats. (**a**) – *$p < 0.005$ for the differences in ventilation between reserpine + L-DOPA-treated *vs.* untreated and reserpine-treated rats at zero time point and *$p < 0.002$ for differences between reserpine + L-DOPA-treated *vs.* reserpine-treated rats at 30 and 180 s; (**b**) – *$p < 0.01$ for the differences between reserpine-treated *vs.* reserpine + L-DOPA-treated rats at 0 s, reserpine-treated *vs.* untreated and reserpine + L-DOPA-treated rats at 30 s, and reserpine-treated *vs.* reserpine + L-DOPA-treated rats at 180 s; #significant effects of type of treatment ($p < 0.04$), time and the interaction of treatment with time ($p < 0.001$) for reserpine *vs.* reserpine + L-DOPA (panels a and b) and for untreated *vs.* reserpine-treated rats (panel b)

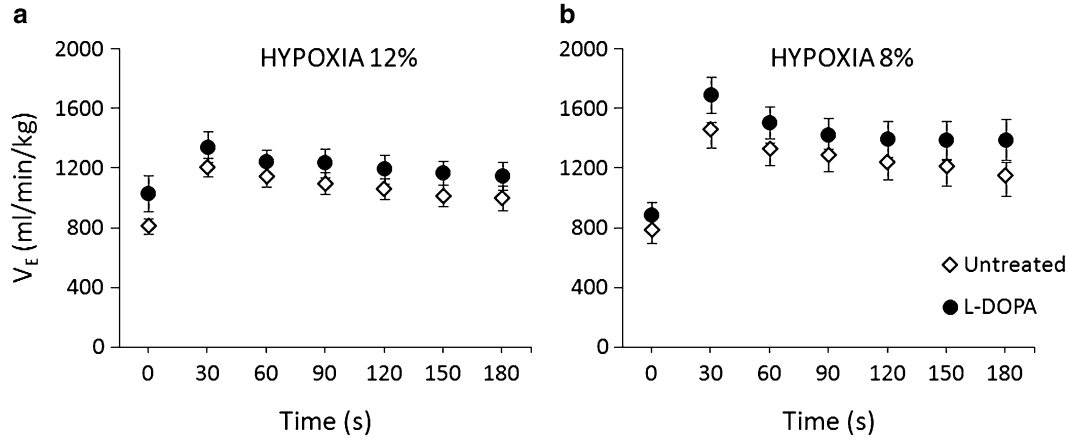

Fig. 5 Influence of L-DOPA (*solid circles*) on resting and hypoxic ventilation in healthy untreated rats (*open diamond-shaped symbols*) (n = 5). (**a**) – 12 % hypoxia; (**b**) – 8 % hypoxia

to shaping the ventilatory changes. Breathing rate decreased by about 30–40 % in reserpinized rats along the HVR. This decrease tended to be compensated by increases in tidal component, which were disproportionately smaller and thus fell short of offsetting the overall decrease in V_E (Table 1). Domperidone failed to affect the breathing patter. L-DOPA, on the other hand, increased both components, in particular breathing rate reverted, on average, about halfway toward the baseline level present in the healthy untreated condition.

Table 1 Contribution of frequency (f) and tidal (V_T) components to hypoxic ventilatory changes at baseline (0 s), peak (30 s), and nadir (180 s) of response in the reserpine (*RES*) model of parkinsonism

	f (breaths·min^{-1})			V_T (ml·kg^{-1})		
Condition	0 s	30 s	180 s	0 s	30 s	180 s
Domperidone (DOM) experiment						
Hypoxia 12 %						
Untreated	104.8 ± 5.6	123.8 ± 4.3	123.5 ± 6.4	9.6 ± 0.8	11.5 ± 0.8	9.4 ± 0.6
RES	59.5 ± 3.4*	84.1 ± 2.0*	72.7 ± 3.5*	12.1 ± 0.7*	13.1 ± 1.0*	12.2 ± 0.9*
RES + DOM	65.9 ± 3.7*	79.4 ± 4.0*	68.7 ± 4.2*	11.8 ± 0.7*	13.3 ± 1.2*	12.4 ± 0.8*
Hypoxia 8 %						
Untreated	108.6 ± 4.4	139.3 ± 7.3	141.3 ± 7.4	8.6 ± 0.4	12.7 ± 0.7	10.9 ± 0.5
RES	63.8 ± 2.7*	86.1 ± 3.9*	73.8 ± 2.7*	11.1 ± 0.7*	13.9 ± 0.6	12.3 ± 0.8
RES + DOM	71.3 ± 4.3*	85.5 ± 2.8*	74.0 ± 5.2*	11.7 ± 0.8*	13.6 ± 1.0	13.5 ± 1.0*
Levodopa (L-DOPA) experiment						
Hypoxia 12 %						
Untreated	104.8 ± 4.0	122.5 ± 7.8	115.2 ± 6.8	7.8 ± 0.5	9.7 ± 0.7	8.4 ± 0.6
RES	68.4 ± 3.9*	83.2 ± 4.8*	71.7 ± 4.3*	10.8 ± 1.0*	11.6 ± 1.2	10.2 ± 0.9*
RES + L-DOPA	88.2 ± 4.3*#	100.7 ± 5.0*#	85.0 ± 4.0*#	11.8 ± 1.0*#	13.6 ± 1.3*	12.1 ± 1.1*
Hypoxia 8 %						
Untreated	103.3 ± 6.8	129.8 ± 9.3	133.2 ± 8.3	7.5 ± 0.6	11.1 ± 0.3	8.1 ± 0.5
RES	60.0 ± 6.7*	82.4 ± 6.2*	78.3 ± 5.5*	10.1 ± 0.9*	12.4 ± 1.2	11.3 ± 1.2*
RES + L-DOPA	86.3 ± 3.2#	110.0 ± 3.9#	95.2 ± 4.4*#	10.9 ± 0.9*	15.1 ± 1.4*#	11.8 ± 1.2*

Domperidone (DOM), a peripheral dopamine antagonist, and levodopa (L-DOPA), a central dopamine agonist, were administered on top of reserpine action
Data are means ± SE. *$p < 0.05$ for the differences between reserpine alone, or reserpine with domperidone, or reserpine with L-DOPA and the healthy untreated condition; #$p < 0.05$ for the differences between reserpine with L-DOPA and reserpine alone

4 Discussion

The main finding of the present study was that ventilation and its stimulatory responses to hypoxia were substantially dampened in the reserpine-induced model of parkinsonism in the rat. Reserpine is an alkaloid that irreversible blocks the vesicular monoamine transporter (VMAT), which depletes monoamine neurotransmitters in the synapses (Betarbet et al. 2002). The depletion of dopamine concerns both central and peripheral neural systems using the dopamine pathway and is enhanced by the addition of alpha-methyl-tyrosine, a blocker of tyrosine hydroxylase, a rate-limiting enzyme in dopamine synthesis. DA depletion by reserpine is notably pronounced in the striatum and is accompanied by muscle rigidity, hypokinesia, and decreased explorative motivation; thus emulating the basic features of parkinsonic symptoms. Deficit of VMAT, whose presence has been confirmed in the striatum of parkinsonic patients *post mortem* (Fernandes et al. 2012), increases oxidative stress, neurodegeneration, and pathologic neuronal accumulation of alpha-synuclein, the protein that further inhibits tyrosine hydroxylase activity. Symptomatic sequelae of DA deficit in the reserpine model were verified in the present study in behavioral tests.

The impairment of ventilatory responsiveness found in the present study is in harmony with the sparse reports that point to degradation of breathing in parkinsonism (Onodera et al. 2000; Serebrovskaya et al. 1998). Lung function, in general, is not in the clinical focus of Parkinson's disease, even though chronic hypoxia, due to insufficient ventilation, may form and intensify both motor and non-motor features of the disease (Bouquet et al. 1999; Stuss et al. 1997). Impaired ventilation is usually linked to muscle rigidity and stiffness leading to postural changes and dysfunction of the chest respiratory muscle pump. However, DA deficiency in its own nature may impair ventilation, which particularly concerns the strenuous for respiratory system chemoreflex hyperpnea.

DA is a well-recognized regulator of lung ventilation. The prevailing consensus of opinion is that DA is stimulatory for ventilation at the central level and inhibitory peripherally at the carotid body, a paired sensory organ that generates the hyperpneic responses to hypoxia. A second objective of the present study was, therefore, to attempt to distinguish between the central and peripheral DA-related ventilatory effects in the reserpine model of parkinsonism. We addressed the issue using the following, specifically peripheral or central, dopaminergic modifiers to investigate dynamic changes of ventilatory responses: domperidone, a peripheral D2 receptor antagonist, unable to cross the blood-brain barrier, and L-DOPA, a D2 receptor agonist, crossing the barrier after antecedent inhibition of its peripheral metabolism with benserazide – a DOPA decarboxylase inhibitor. Injection of either agent was made on top of reserpine action and the HVRs were reinvestigated in the same rat.

An enhancing ventilatory effect of domperidone in the healthy condition noted in the present study, also known from other studies (Walsh et al. 1998; Bascom et al. 1991; Delpierre et al. 1987), underlies the mediation of the HVR inhibition by carotid body DA. Decreased ventilation and its responses in parkinsonic rats ran somewhat against our initial presumption that, assuming an inhibitory effect on ventilation of carotid body DA and its plausible deficiency in parkinsonism, ventilation should rather be shifted upward. Further, domperidone failed to cause a rebound of the HVR impairment, which was in opposition to domperidone-mediated increase in the HVR in control, healthy rats. The lack of improvement of impaired HVR in parkinsonism by domperidone makes ventilatory inhibition by the innate carotid body DA an unlikely possibility. Further, it seems a reasonable assumption that DA was exhausted in the carotid body in the reserpine model of parkinsonism employed, which nullified the action of domperidone. The findings discount the role of carotid body DA in the chemoreflex changes of parkinsonism. However, the content of DA in the parkinsonic carotid body has by far never been assessed and its exploration requires alternative study designs.

L-DOPA, on the other hand, which had inappreciable effect on the HVR in the healthy condition, caused a clear rebound of both resting and hypoxic ventilation in parkinsonic rats. The reversal of impaired ventilation actually tended to overshoot the baseline profile of the HVR present in healthy rats. These findings point to the predominance of the lack of the central DA stimulatory ingredient in the ventilatory impediment of parkinsonism. The ability of the respiratory system to take on the strenuous hyperpneic effort after L-DOPA administration, on par with the healthy condition, also raises doubts on the leading part of the deformed skeletal-muscle structure of the chest respiratory pump in ventilatory insufficiency and points to the extensive compensatory capability of the respiratory system.

In conclusion, we believe we have shown that lung ventilation is distinctly diminished in parkinsonism. The diminution has to do notably with the insufficient brain DA stimulatory component, although respiration is a multifaceted process engaging also peripheral neural, muscle, skeletal, and postural functions. Respiratory insufficiency of parkinsonism has often been neglected or left unrecognized. Thus, health professionals should be cognizant of the restrictions that come with impaired blood oxygenation, which may worsen the disease process.

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

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Lund-Mackay System for Computed Tomography Evaluation of Paranasal Sinuses in Patients with Granulomatosis and Polyangiitis

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Abstract

Granulomatosis with polyangiitis (GPA), a disease capable of affecting any organ, most often acts upon the upper respiratory tract. Diagnostic imaging is primarily represented by computed tomography (CT) of paranasal sinuses. The aim of this study was to define the characteristic changes in paranasal CT in patients with GPA and to evaluate diagnostic usefulness of the Lund-Mackey scoring system (L-M System). The study encompassed 43 patients with GPA of the mean age of 47.7 ± 12.8 years who were treated topically with mupirocin. We found that inflammation occurred mainly in the maxillary sinuses (72 %). The mean L-M score was 5.8 ± 6.1. The right maxillary sinus had the highest percentage (12.6 %) of score hits of 1, i.e., partial opacification and the left ostiomeatal complex had the highest percentage (7.6 %) of score of 2, i.e., complete opacification or obstruction. The following changes were the most characteristic for GPA: sinus mucosal thickening, widespread bone damage, and osteogenesis. We conclude that the long-term topical mupirocin treatment of GPA may inhibit nasal bone damage, but also may led to permanent rhinological changes of the rhinosinusitis type. The Lund-Mackey staging system is a useful diagnostic imaging option in GPA patients.

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Keywords
Computed tomography • Granulomatosis with polyangiitis • Lund-Mackay staging system • Mupirocine • Osteogenesis • Sinusitis

1 Introduction

Granulomatosis with polyangiitis (GPA) is a rare disease of unknown origin. It may damage all organs and systems, but most often it affects the upper and lower respiratory tract and kidneys. Diagnostic imaging is primarily represented by computed tomography (CT) of paranasal sinuses and, if necessary, by magnetic resonance imaging. No clear radiological characteristics for the establishment of precise diagnostic criteria have been found. However, several typical signs could be enumerated as follows:

- Unilateral changes appear very rarely;
- Patients with GPA show symptoms of chronic rhinosinusitis, especially those patients who had undergone paranasal sinus surgery. Mucous membrane thickening in the nasal cavity and paranasal sinuses is not a pathognomonic GPA sign. The thickening may stem from coexisting disease or be a result of vasculitis-induced rhinosinusitis (Lohrmann et al. 2006; Grindler et al. 2009);
- Variable bone damage may appear in various forms: from a slight thinning of the nasal septum and paranasal sinus walls, to perforation or a widespread nasal bone destruction resulting in the occurrence of one large nasal space and occasional changes affecting the orbit (Benoudiba et al. 2003; Razek and Castillo 2009). According to some sources, a classic pattern of bone destruction can be observed, starting in the nasal septum and spreading laterally to the orbit (Lloyd et al. 2002);
- Destruction of bone is relatively often (50–78 %) accompanied by paransal sinus wall sclerosis, probably of neoosteogenetic origin. Lloyd et al. (2002) perceive widespread bone damage and sclerosis, with no other causes in the medical history, e.g., paranasal sinus surgery, as pathognomonic for GPA;
- Orbit involvement and suspected orbital tumor symptoms appear relatively often (30–50 %); and
- Lack of CT changes does not exclude the occurrence of GPA.

Dedicated CT has been a useful tool in assessing patients with a sinus disease, including the sinus involvement in GPA. Various staging systems have been proposed for CT evaluation; however, no single system has yet been accepted as the gold standard for use in chronic rhinosinusitis. Many a study uses the Lund-Mackay scoring system (L-M System) to evaluate radiographic images (Moghaddasi et al. 2009). The L-M System seems an effective and easy to use assessment method in both clinical practice and research, and it can also predict the response to functional endoscopic sinus surgery. The scoring scale grades the right and left side sinuses independently. This radiological staging system enables not only an effective sinus evaluation but also facilitates an interspecialist communication about the severity of rhinosinusitis.

The purpose of the present study was to define the characteristics of GPA changes in nasal computed tomography, to evaluate the effectiveness of the Lund-Mackey system, and to analyze correlations between the achieved scores and the disease duration and activity.

2 Methods

The study protocol was approved by a local Ethics Committee. There were 43 patients with GPA of the mean age 47.7 ± 12.8 years (F/M – 31/12) enrolled into the study. The diagnosis of

GPA was established using the widely accepted criteria of the American College of Rheumatology (Leavitt et al. 1990) and the European League against Rheumatism. The study procedures for all patients were the following:

- Medical history;
- Physical examination with anterior rhinoscopy, followed by nasal endoscopy if rhinoscopy did not reveal signs of GPA;
- Determination of selected diagnostic biochemical and serological parameters to assess disease activity and extent;
- Paranasal sinus computed tomography evaluated with the L-M scoring system;
- Several sensitive and comprehensive indices considered disease-specific for GPA were applied to assess disease activity, extent, and organ damage as a consequence of granulomatous inflammation and vasculitis: Birmingham Vasculitis Activity Score (BVAS) (Luqmani et al. 1994), Disease Extent Index (DEI) (de Groot et al. 2001), and Vasculitis Damage Index (VDI) (Exley et al. 1997). These indices have been validated for use in a range of systemic vasculitides, correlate well with the physician's global assessment of disease activity, and have good inter- and intraobserver reliability.

Only was the patients' first sinus CT scan reviewed. Two multi-detector spiral CT system (LightSpeed VCT, GE Healthcare, Safe Harbor, PA) were employed. The patients were imaged in the supine position with their head entering the gantry. Coronal and axial sinus views were obtained from the scout images using the head top as a landmark and data from the head top to the sterno-clavicular joint were collected using continuous scanning images in the helical model (slice thickness: 1.25 mm, rotation-time: 0.8 s). The L-M scoring system was used in further analysis. The right and left sinuses were divided into six portions including: maxillary sinuses, anterior ethmoid sinuses, posterior ethmoid sinuses, sphenoid sinuses, frontal sinuses, and the ostiomeatal complex. Mild mucosal thickening without fluid accumulation was scored as 0, mild mucosal thickening with fluid accumulation that causes partial opacification as 1, and moderate or severe mucosal thickening with fluid accumulation causing complete opacification as 2. Furthermore, the ostiomeatal complex was scored as either 0 (not obstructed) or 2 (obstructed). The ten scores for all the sinuses and bilateral ostiomeatal complexes were summed up to give a bilateral total L-M score that could range from 0 (complete lucency of sinuses) to 24 (total opacification of sinuses). Additionally, five sinuses and one ipsilateral ostiomeatal complex from either side were also summed up to give a separate unilateral L-M score that could range from 0 to 12. Data were given as means ± SD. The dataset with a total L-M score was statistically elaborated with a two-tailed *t*-test. A p-value < 0.05 defined statistical significance.

3 Results

The sinonasal involvement was seen in 93 % of the patients. The mean ranges of BVAS, VDI, and DEI scores were 11.9 ± 7.8, 13.6 ± 6.4, and 8.3 ± 2.9, respectively, which points to moderate disease activity and extent. The patient CT results were analyzed for disease signs and their location. Inflammation usually occurred in the maxillary sinus (72 % of cases) (Table 1), its mean intensity on the L-M scale was 5.8 ± 6.1,

Table 1 Percentage of changes recorded in different paranasal sinuses in computed tomography

	n = 43	%
Maxillary sinus	31	72
Anterior ethmoid sinus	21	49
Posterior ethmoid sinus	13	30
Frontal sinus	13	30
Ostiomeatal complex	14	32
Sphenoidal sinus	9	21
Bone damage	14	32
Septum perforation	9	21
Neoosteogenesis	4	9
Orbit involvement	7	16
Nasal polyps	2	5

Table 2 Percentages of Lund-Mackay (L-M) scores stratified by increasing degree of sinus opacification (decreasing lucency) for each sinus examined on the right and left sides

	Right	Right	Right	Left	Left	Left
Degree of opacification	0	1	2	0	1	2
Frontal sinus (%)	97.0	1.4	1.6	98.0	1.4	0.6
Maxillary sinus (%)	82.9	12.6	4.5	82.9	11.5	5.6
Anterior ethmoid sinus (%)	92.0	6.4	1.6	92.8	5.8	1.4
Posterior ethmoid sinus (%)	95.0	3.8	1.2	95.7	3.4	0.9
Ostiomeatal complex (%)	93.6	1.1	5.3	90.3	2.1	7.6
Sphenoid sinus (%)	98.0	0.8	1.2	96.9	2.4	0.6

0 Lack of opacification (complete lucency), *1* Partial opacification, *2* Complete opacification (lack of lucency)

with the lowest result of 0 and the highest of 24. It was found that the mean bilateral total L-M score was 7.0 ± 1.9, with a range from 0 to 12. Furthermore, the right total L-M score was 4.3 ± 1.3 and the left total L-M score was 5.6 ± 1.5, with a range from 0 to 12 in both. Percentages of L-M scores corresponding to each sequential degree of sinus opacification for each sinus examined are presented in Table 2. The right sphenoid sinus and the left frontal sinus had the highest percentage (98 %) of L-M score hits of 0, which is a lack of opacification or complete lucency. The right (12.6 %) and left 11.5 % maxillary sinuses had the highest percentage of L-M scores of 1, which is a partial opacification. The right (5.3 %) and left (7.6 %) ostiomeatal complexes had the highest percentage of L-M scores of 2, which is a complete opacification or obstruction (Table 2). Apart from paranasal sinus opacification, CT also showed nasal septum perforation and widespread bone damage, in some cases reaching the orbit wall.

The study revealed some associations between the structural damage in the upper respiratory tract and a number of disease-related factors. Higher L-M scores were related to mupirocin treatment and to the presence of bloody nasal discharge. Bone damage was related to a prolonged time elapsing from the start of symptoms to diagnosis. Septum perforation occurred more frequently in patients with low D-dimer levels and higher BVAS scores. The associations outlined above were significant ($p < 0.05$).

4 Discussion

Diagnostic imaging does not show pathognomonic GPA pattern for early stage symptoms. Mucosal thickening is a non-specific symptom and may be related to other diseases. A characteristic GPA histopathological change in the upper respiratory tract is necrotizing vasculitis. It is a process responsible for the most characteristic paranasal sinus CT symptom – bone damage, accompanied by paranasal sinus opacification. Bone damage is initially centrally located, affecting the nasal septum and the turbinates, and later spreads symmetrically to paranasal sinuses. The final stage of chronic granulomatosis is the conjoining of nasal cavities and paranasal sinuses into one large space, accompanied by bone structure atrophy. The disease then spreads to other structures, including the lamina papyracea and the cribriform plate. In some cases, bone damage is associated with the formation of new bone structure. In CT imaging, bone sclerosis appears as an irregular double line on the sinus wall. This is related to a new layer of bone being formed within the sinus wall. The mechanisms responsible for this growth seem to be of neoosthogenic nature and are not due to periostitis. Similar changes occur after paranasal sinus surgery or in chronic sinusitis. Milford et al. (1986), before CT became commonly used in clinical practice, have described changes seen in the paranasal sinus X-ray in 20 GPA patients. The radiologic signs corresponded to mucosal thickening, opacification, fluid levels, bone damage, and sinus wall sclerosis. Paling

Table 3 Changes in paranasal computer tomography in patients with granulomatosis with polyangiitis observed in various studies

	Lloyd et al. (2002)	Lohrmann et al. (2006)	Grindler et al. (2009)	Zycinska et al. (2008)
	n = 28	*n = 28*	*n = 74*	*n = 43*
Sinus opacification	86 %	75 %	–	78 %
Mean L-M score	–	–	10	6
Bone damage	75 %	57 %	62 %	32 %
Neoosteogenesis	50 %	21 %	78 %	9 %
Orbit involvement	29 %	11 %	–	16 %

et al. (1982) have published similar X-ray data pertaining to 14 patients, who displayed signs of opacification in at least one sinus, and sinus osteosclerosis and osteogenesis. In other studies conducted in similarly small groups of GPA patients, sinus opacification, orbit involvement, bone damage, nasal septum perforation, and mucous inflammation have been reported (Simmons et al. 1987; Yang et al. 2001). Benoudiba et al. (2003), in a study conducted in nine patients, showed that the following characteristic CT changes in GPA patients: nodular mucosal thickening, local bone damage (mainly in the septum and lateral nasal wall, but without ethmoid involvement), ostiomeatal complex involvement and bone demineralization, and the orbit involvement.

Recently, larger groups of GPA patients have been investigated. The most important findings of those investigations are listed in Table 3. Lloyd et al. (2002), on the basis of CT images conducted in 28 GPA patients with no history of nasal or paranasal sinus surgery, have described the following symmetric changes: sinus mucosal thickening, widespread bone damage, and osteogenesis; the findings in comport with our present study. Twenty nine percent of the patients also displayed symptoms of paranasal sinus-related orbital pseudo tumor. In a different study, Lohrmann et al. (2006), apart from the disease signs outlined in Table 3, have found that a small percentage of patients are characterized by partial sinus opacification. In the largest to-date study which analyzed GPA paranasal sinus CT imaging, Grindler et al. (2009) have for the first time used the Lund-Mackay scoring system, basically confirming the findings of others. However, none of the studies outlined above have included data on GPA activity and inflammatory or serological parameters. Those studies were retrospective and dealt with rhinological changes causing sinusitis-like symptoms in GPA patients. The inflammatory changes in the paransal sinuses revealed in our present study were comparable with the results obtained by others. However, we observed less evidence of bone damage and osteogenesis. That might be due to the fact that the study also included patients with normal CT results. Given the lack of data on local and systemic GPA activity in the studies by others outlined in Table 3, no comparative analysis is feasible. However, the present CT and selected GPA activity results showed that the patients with higher L-M score suffered from stronger headaches and more frequently complained of bloody nasal discharge. Although such L-M score-symptom associations have initially been examined for GPA, similar associations occur in case of chronic rhinosinusitis (Nair 2009). That leads to a conclusion that rhinological manifestations and CT findings may appear similar in patients with GPA and chronic rhinosinusitis. The present study also showed an association between the bone damage, visible in CT imaging, and the extension of time from the appearance of first symptoms to diagnosis. In all patients, diagnosis was linked with the initiation of systemic glucocorticosteroid and cyclophosphamide treatment. It appears that this treatment considerably decreases nasal bone damage. On the other hand, the prolonged presence of symptoms prior to diagnosis facilitates

destruction of sinonasal anatomy. We found no associations between L-M score, symptom duration, and the time from diagnosis. However, L-M score was apparently associated with mupirocin treatment. Mupirocin is a topically applied antibiotic produced by *Pseudomonas fluorescens*. The antibiotic inhibits protein and RNA synthesis and is applied for treatment of chronic colonization by *Staphylococcus aureus*. Although *Staphylococcus aureus* carriage not always requires treatment in healthy persons, treatment is recommended in instances of immunodeficiency, e.g., during immunosuppressive therapy. The treatment is particularly important in GPA as *Staphylococcus aureus* colonization may lead to disease relapse (Zycinska et al. 2008).

In the present study, topical mupirocin treatment was associated with higher L-M scores; the corollary is that the antibiotic might increase paranasal sinus inflammation, which could lead to adverse symptomatic effects in the course of GPA. It might be that mupirocin facilitates chronic rhinosinusitis through a selection of resistant bacteria strains and impairment of physiological biofilm of paranasal mucous. That reasoning seems supported by research showing that prolonged mupirocin treatment leads to bacteria resistance to the antibiotic (Patel et al. 2009). Mupirocin also may act as a mucous irritant. Among the known antibiotic's adverse effects there are local irritation, burning, itching, sensitivity reactions, and allergies. Mupirocin could enhance the migration of inflammatory cells and thus also paranasal sinus opacification. However, clinical importance of adverse effects of mupirocin treatment seems contradicted by its lack of influence on rhinosinusitis activity, as assessed in physical examination and history in the present study. It also ought to be pointed out that mupirocin was applied in patients with more active sinusitis, which introduces a bias in the unambiguous assessment of its action. Moreover, adverse effects of mupirocin have not been uniformly confirmed. Other studies underscore benefits of an antibacterial role of mupirocin, leading to containment of chronic rhinosinusitis (Solares et al. 2006). In addition, the recommended mupirocin treatment should take approximately only 10 days, which makes serious side effects rather unlikely. In the studies pointing to adverse effects of mupirocin in the course of GPA, the antibiotic was used for months, which was related to its primary effect, i.e., lessening the possibility of disease relapse through *Staphylococcus aureus* eradication (Patel et al. 2009). The potential of mupirocin for sinusitis exacerbation should be explored in alternate study design.

In conclusion, treatment of granulomatosis with polyangiitis inhibits nasal bone damage, but may lead to chronic rhinosinusitis-like changes. The long-term topical mupirocin application might also exert an enhancing effect on paransal sinus inflammation. The disease is characterized by the following notable symmetric changes: sinus mucosal thickening, widespread bone damage, and osteogenesis. The Lund-Mackey scoring system appears a useful tool in the assessment of GPA diagnostic imaging.

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

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Oxidative Stress and Nitric Oxide in Sedentary Older Adults with Intellectual and Developmental Disabilities

E. Carmeli, A. Bachar, O. Rom, and D. Aizenbud

Abstract

Individuals with moderate-to-profound intellectual and developmental disabilities (IDD) are characterized by significant cognitive deficits, abnormal muscle tone, poor posture and balance, and inactive lifestyle. Increased oxidative stress (OS) has been implicated in a variety of chronic diseases, inflammatory conditions, aging, and even following intense physical exercise. Nitric oxide (NO) is a highly reactive mediator that has been shown to play different roles in a variety of different biological process and in aging. The aim of the study was to investigate the serum levels of global OS and NO metabolites (NOx) in sedentary and non-sedentary older adults with IDD. Global OS was measured by CR 3000 instrument, FORM system, and NOx were measured by determination of serum nitrite levels. OS and NOx levels were significantly higher in sedentary IDD comparing non-sedentary controls. The increased of OS and NOx levels suggest their possible involvement in the phenomenon of 'accelerated aging' in IDD. Our findings can provide another aspect indicating both OS and NOx as possible biochemical markers and their potential application in minimizing their negative influence through future therapeutic strategies.

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Keywords
Aging • Cognition • Intellectual disabilities • Sedentary lifestyle • Oxidative stress • Nitric oxide

1 Introduction

In recent years, the life expectancy of individuals with intellectual and developmental disabilities (IDD) has increased considerably. However, their life expectancy still remains lower than that of the general population (Coppus 2013). Individuals with IDD often experience health issues associated with aging at earlier ages and at higher rates than the general population (Dykens 2013; Heller and Sorensen 2013). The 'premature' or 'accelerated aging' process of individuals with IDD can be reflected by an increased risk of chronic diseases such as cardiovascular diseases, hypertension, type II diabetes, dementia, depression, and health changes in musculoskeletal system (Carmeli et al. 2003; Heller and Sorensen 2013). Thus, older adults with IDD are prone to develop early chronic morbidities and a sedentary lifestyle, which result in frequent hospitalization, obesity, frailty, falls, and mortality (Carmeli et al. 2003).

The treatment of patients with chronic neurological conditions including IDD has become increasingly complex for various reasons. One of the main reasons is the presence of dual neurological diagnoses (i.e., epilepsy, Down syndrome, Alzheimer's disease, and schizophrenia) or the presences of the above mentioned comorbidities (Carmeli et al. 2003; Flanagan et al. 2007). A sedentary lifestyle, common among older adults with IDD, may increase the risk for some of these comorbidities such as obesity, osteoporosis, sarcopenia, and cardiovascular diseases (Heller and Sorensen 2013).

The majority of aged persons with IDD lives at residential care centers and benefit from a social, physical, psychological, and occupational support as well as recreation activities (Merrick et al. 2006). However, health promotion and morbidity prevention among aged individuals with IDD has received little attention from policy makers in public health and welfare authorities. Indeed, health promotion interventions for adults with IDD, which focus on physical activity and exercise, have been shown to have positive effects such as improved fitness, lower blood pressure, and weight loss (Heller and Sorensen 2013). In our recent publication we have found, quote: "*low participation in physical activity for persons with IDD*" (Carmeli et al. 2012).

Oxidative stress (OS) is thought to play a central role in neuro-pathologies, metabolic diseases, aging, strenuous physical activity, poor nutrition, and can also be a result of exposure to toxins (e.g., drugs, cigarette smoking, alcohol, etc.) or pathogens (Garcez et al. 2005; Valko et al. 2007). High levels of free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) are present in conditions of oxidative stress (Floyd et al. 2000). Nitric oxide (NO) is an inflammatory mediator and an important free radical. NO plays a crucial role in the homeostatic regulation of the cardiovascular, neuronal, and immune systems (Mangge et al. 2014). NO also serves as a second messenger in various physiological processes including neurotransmission in the CNS, maintenance of vasodilator tone, and arterial pressure (Czapski et al. 2007).

Despite these important physiological functions, oxidized metabolites of nitrogen (NOx) are well-known toxic agents, being one of the constituents of air pollution and cigarette smoke (Grisham et al. 1999; Krause 2007). In biological systems, NO decomposes to nitrite and nitrate. NO is produced by mammalian cells and is constitutively synthesized by endothelial NO synthase (eNOS) and by inducible neuronal NO synthetase (iNOS). Excessive iNOS levels can be found in the hypothalamus and in aging (Floyd and Hensley 2000; Ferrini et al. 2001). Increased iNOS levels are also evident in various chronic inflammatory diseases

Table 1 Characteristics of participants

	Sedentary group (12)		Active group (10)	
	Mean ± SD	Range	Mean ± SD	Range
Sex (F/M)	8/4	–	7/3	–
Age (yr)	57 ± 5	51–63	55 ± 4	50–60

such as arthritis, obesity, hepatitis, inflammatory bowel diseases, and septic and hemorrhagic shock. Therefore, NO relates to ROS and RNS that are involved in oxidative stress and brain damage (Floyd et al. 2000).

The aim of this study was to investigate the serum levels of OS and NOx in active and sedentary older adults with IDD. We hypothesized that higher levels of OS and NOx are present among sedentary older adults with IDD. Higher levels of OS and NOx may play a role in the on-going pathological processes among older adults with IDD, leading to deterioration of their function and accelerated aging.

2 Methods

2.1 Participants

This cross-sectional study received prior approval by the Institutional Ethic Committee of Residential Care Centers under the administrative control of the Israeli Ministry of Welfare. A written consent received from guardians. The sample consisted of permanent residents who lived in residential care centers. Inclusion criteria included: (1) moderate-to-profound IDD diagnosed within 1–3 years after birth by IQ-scores defined by the Wechsler Abbreviated Scale of Intelligence (Harcourt Assessment Inc., San Antonio) (Hays et al. 2002); (2) sedentary behavior in most of the day hours (i.e., bedbound or wheelchair); (3) aged 50 or older. Exclusion criteria included: (1) diagnosis of a specified moderate mental retardation such as fragile X and Down syndrome; (2) clinical history of neurological diseases (e.g., Parkinson's disease, stroke, Alzheimer's disease, neuropathy, or brain surgery); (3) presence of peripheral neurological symptoms; (4) medical history such as pulmonary disease, type II diabetes mellitus, chronic or acute heart failure, adrenal or kidney diseases, and smoking. None of the participants received narcotic medications or glucocorticoids during the time of the experiment.

From a population sample of 253 permanent residents with IDD, 23 met the inclusion criteria. Twelve potential participants were randomly selected and included in the study as the experimental group (sedentary group). Ten IDD individuals that were independent in activities of daily living and were characterized with a mild active lifestyle served as the control group (active group). The characteristics of the participants are shown in Table 1.

2.2 NOx Assay

NOx were measured in the serum obtained after night sleep. The assay followed the exact protocol as previously described (Carmeli et al. 2009). Sera from all participants were provided by a nurse practitioner. A total of 6 mL of venous blood samples were collected after an overnight fasting and were drawn into 'vacutainer' 10 mL tubes for metabolite assays, which did not contain any additives. Briefly, 40 mL of each sample were transferred to 96-well plates and mixed with 40 μL of the reduction solution (NADPH 1.25 ng/μL; FAD 10.4 ng/μL; KH_2PO_4 0.125 M) containing 0.5 U of NO_2 reductase for 2 h at 37 °C. After this time, 80 μL of Griess reagent (one part 0.1 % N-1-naphthyl-ethylene-diamine dihydrochloride in water and one part 1 % sulfanilamide in 3 % concentrated H_3PO_4) were added to each well. The mixture was incubated for 5 min at room temperature and read at 540 nm in a microplate reader. Concentrations were determined by a standard curve of sodium nitrite. The detection limit of the assay was 1 mM of nitrite.

2.3 Global Oxidative Stress Analysis

This assay followed the same protocol as previously describe (Carmeli et al. 2008). The analyses were carried out using CR 3000 instrument (FORM system, Catellani Group, Callegari S.p.A, Parma, Italy). All measurements were performed within 0.3–1 h post blood drawing. The CR 3000 instrument includes software and photometers (505 nm) with one or three reading cells for the determination of primary tests on whole capillary blood. The CR 3000 kits include ready filled cuvettes (4.8 pH buffered chromogen) that are bar coded (so that the reader automatically recognizes the test about to be performed and the *k*-factor), and capillaries for blood collection. The prepared reagents are able to promote the iron-catalyzed Haber-Weiss reaction. After an overnight fast (8–10 h), and between 08:00 and 09:00 a.m., a total of one drop of capillary blood samples, approximately 10 μl, were drawn from the subject's finger, and inserted into the prepared cuvette. The cuvette was then gently rocked several times until the sample was completely diluted. The sample was then placed in the square cuvette which contained the solid chromogen mixture, after closing the cuvette with its cap-on, it was shaken for 30 s until the reactive has dissolved. Then placed in the table centrifuge for 1 min (at room temperature), then placed in the reading cell.

Oxidants profile is measured by a colorimetric test based on the ability of transition metals, such as iron, to catalyze the breakdown of hydroperoxides into derivative radicals according to Fentons and Haber-Weiss' reactions shown below:

$$\text{R-OOH} + \text{Fe}^{2+} \xrightarrow{\text{A}} \text{R-O}^{\cdot} + \text{OH}^{-} + \text{Fe}^{3+}$$

$$\text{R-OOH} + \text{Fe}^{3+} \xrightarrow{\text{B}} \text{R-OO}^{\cdot} + \text{H}^{+} + \text{Fe}^{2+}$$

$$\text{RO}^{\cdot} + \text{ROO}^{\cdot} + 2\text{CrNH}_2 \xrightarrow{\text{C}} \text{RO}^{-} + \text{ROO}^{-} + \left[\text{Cr} - \text{NH}_2^{+\cdot}\right]$$

Reference values are

160–200 = 1.22–1.58 mmol/l H_2O_2 – Excellent

201–250 = 1.59–1.97 mmol/l H_2O_2 – Very good-Good

251–310 = 1.98–2.17 mmol/l H_2O_2 – Good – Fair

311–500 = 2.18–2.36 mmol/l H_2O_2 – Bad

2.4 Statistical Analysis

A group comparison was assessed using a two-tailed Mann-Whitney U test (for abnormally distributed values) and a *t*-test (for normal distributed values). Data were expressed as means ± SD. Analyses were performed using the SPSS statistical package (ver. 12 for Windows). Differences were considered significant if $p < 0.05$.

Due to a small sample size, the effect of size was used to quantify differences in OS and NOx measures between the participants. The size-effect for each measure was computed as standardized mean differences in the score change between the two groups, namely, $(d_1 - d_2)/\delta_p$, where d_1 and d_2 denote the scores of the sedentary and active groups, respectively, and δ_p is the pooled standard deviation of the score difference. For the sake of completeness, despite the small sample size, we also conducted a two sample *t*-test and Mann-Whitney *U* test for the mean score differences (between the final groups).

3 Results

3.1 Level of Global Oxidative Stress

The CR3000 results for global oxidative stress (OS) in IDD participants were significantly higher in the sedentary group when compared with the active control group (Fig. 1).

3.2 Level of Oxidized Metabolites of Nitrogen

Oxidized metabolites of nitrogen (NOx) were significantly higher in sedentary group when

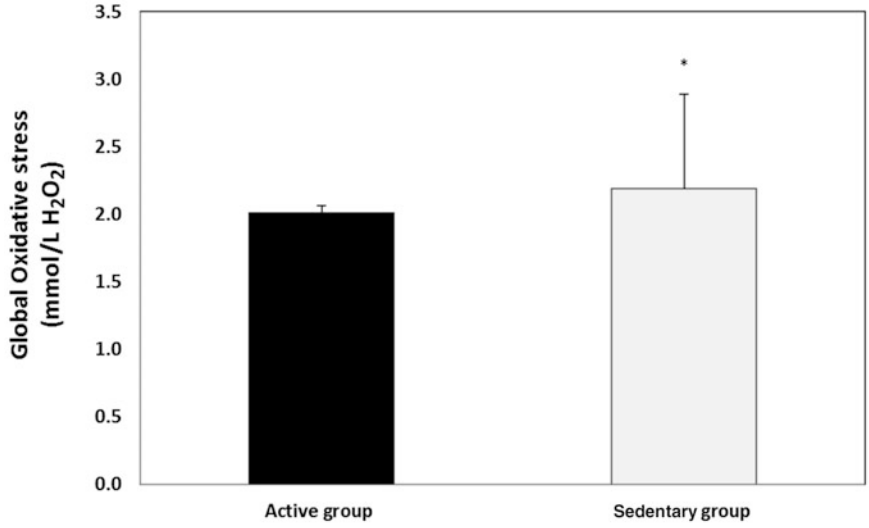

Fig. 1 Global oxidative stress in active *vs.* sedentary individuals with intellectual and developmental disabilities (IDD); *p $<$ 0.05 *vs.* the active group

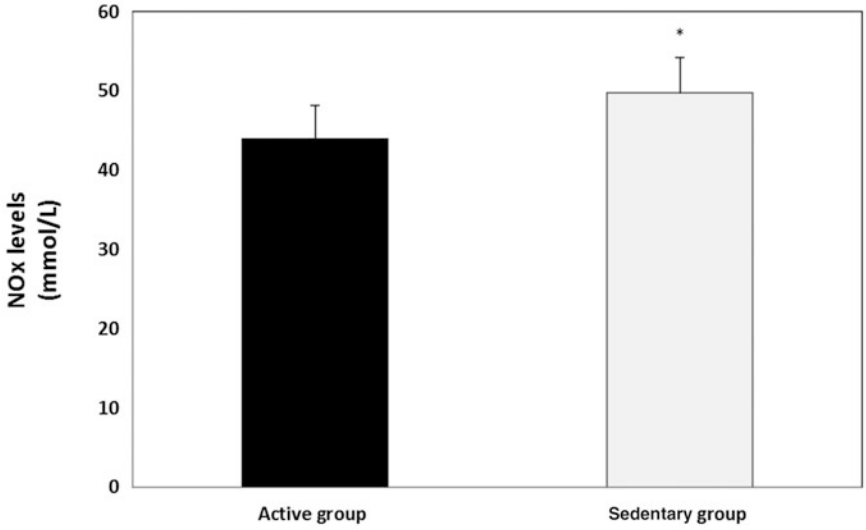

Fig. 2 NO_x in active *vs.* sedentary individuals with intellectual and developmental disabilities (IDD); *p $<$ 0.05 *vs.* the active group

compared with active control group (Fig. 2). The mean NOx level of females and males in both groups were not statistically different. In the sedentary group, the level of OS correlated significantly with NOx level of the same participants at the same point of time ($r = 0.78$, $p < 0.05$). Insignificant correlation was found between NOx and OS in the active control group ($r = 0.45$).

4 Discussion

Early aging, chronic morbidities, and a sedentary lifestyle are more common among older adults with IDD. Higher levels of OS and NOx may promote various pathological processes and accelerated aging in older adults with IDD. The aim of the present study was to assess the OS and

NOx levels in sera of active and sedentary older adults with IDD.

Despite research progress in the field of early-onset aging among IDD persons, our knowledge and understanding of what mechanisms are involved is still ambiguous. One of the main factors that attribute to accelerated aging, particularly among IDD persons, is a passive lifestyle or lack of physical activity. The consequences of physical activity on one hand, and physical inactivity on the other hand are widely known. Metabolic and physiologic adaptation to explain inactivity is generally supported by a model of metabolic homeostasis based on 'use it or lose it' assumption (Baskin et al. 2015). Following prolong inactive periods, certain biological pathways are induced such as apoptosis and inflammation, and often they are irreversible, especially in the aged (Russ and Lanza 2011).

Among the most prominent signal transduction inducers of apoptosis there are a few molecules accountable to OS. Because an analysis of a single molecule does not reflect the complexity of passive lifestyle, a multivariate approach is required to better illustrate the complex dynamic networks of physical inactivity. In the present study, high serum levels of circulatory NOx and systemic OS were revealed in sedentary older adults with IDD. Oxidative stress is thought to have a pivotal role not only in a number of chronic diseases, but also in aging and in physical inactivity (Lawler and Hindle 2011). In the present study, a high serum global OS concentration was demonstrated in healthy, yet sedentary IDD people. Therefore, we suggest that there is a direct mechanistic link among the systemic OS and inactivity status.

The role of NO in many diseases is controversial, yet most researchers agree that measuring NOx levels in the serum is related to NO production (Andrukhov et al. 2013). There are both direct effects of NO, mediated by the NO molecule itself, and indirect effects of NO, mediated by reactive nitrogen species produced by the interaction of NO with superoxide radicals. High serum concentrations of NOx induce toxicity by tyrosine residues and by inducing lipid peroxidation which might lead to vascular atherosclerosis in the brain, heart, and kidneys (Eiserich et al. 1998).

A high level of NOx was observed in muscles of young sedentary subjects (Saltin 2007). The levels of OS and NOx are progressively increasing with aging. Aging and inactivity have synergetic and harmful effects leading to biological and physiological damage. As reported by Nyberg et al. (2012), lifelong physical activity opposes this harmful effect. Thus, accumulation of NOx and free radicals with aging can be attenuated by maintaining a physically active lifestyle. Higher levels of OS and NOx in sedentary individuals with IDD in comparison with active individuals with IDD are explainable by the 'cytokine hypothesis of inactivity' (Dammann and Leviton 1998), or 'diseasome of physical inactivity' (Pedersen 2009). According to these hypotheses, disuse of skeletal muscles releases cytokines that induce inflammation, causing muscle atrophy and fat accumulation.

The main limitation of the present study relates to the controversy regarding the specificity of OS serum values. Moreover, if OS values are increased, it has been recommended that a more specific assay such as HPLC should be performed, which can be measured by fluorometry or spectrophotometry with higher sensitivity (Mehanna et al. 2011). Yet, the statistical deviation percentages in the present results confirmed that measuring OS with CR3000 is accurate enough. Because the source of NOx is not only endothelial cells (eNOS), but also macrophages (iNOS) and neuronal cells (nNOS), the serum level of NOx may reflect the status of eNOS and, to a small extent, that of iNOS.

5 Conclusions

Serum oxidative stress and NOx levels in sedentary older individuals with intellectual and developmental disabilities are elevated in comparison to active controls. These results indicate that inactive lifestyle may be comparable to a pathological condition of a disease. Future programs of health promotion and morbidity prevention in patients with intellectual and developmental disabilities should focus on healthy behaviors

such as physical activity, but also on nutritional regimes and anti-inflammatory drugs or supplements for either improving the anti-oxidant defense mechanism or decreasing the accumulation of free radicals.

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

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Foreign Body in the Airway a Female Patient with Myasthenia Gravis

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Abstract

Aspiration of a foreign body occurs rarer in adults than it does in children. Advanced age and swallowing disorders, often caused by neuromuscular diseases, predispose for aspiration. Symptoms due to aspiration are mainly cough and wheezing. However, clinical and radiological symptoms may mistakenly suggest lung cancer. Making a proper diagnosis could be difficult and time consuming. In this study we report a case of a 73-year old woman who has been diagnosed and treated myasthenia gravis for 10 years. The patient manifested chronic cough for over a year, weight loss, lung lesions on chest X-ray and computed tomography images in the form of atelectasis and inflammatory infiltrations. The results of cytological tests of bronchoalveolar lavage fluid were 'atypical cells' which suggested a lung cancer. Flexible bronchoscopy set the diagnosis as a foreign body in right upper bronchus, which turned out to be a piece of a plant obstructing the bronchus. The patient came down with pneumonia. Laboratory examinations revealed leucocytosis and a high level of C-reactive protein. A complete removal of foreign body took place during rigid bronchoscopy. Concomitantly, but unrelated to the finding of a foreign body in the bronchus, the patient was diagnosed with digestive tract perforation on the basis of X-ray images, which remained otherwise asymptomatic. Explorative laparotomy revealed a perforated colonic diverticulum, which was successfully treated surgically.

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Keywords
Aspiration • Bronchoscopy • Bronchus • Foreign body • Myasthenia gravis

1 Introduction

Foreign body aspiration may be caused by deglutition disorders, which may, in turn, be caused by disorders of the neuromuscular junction. An increased risk of aspiration is present in the elderly. The clinical presentation of a foreign body in the lower respiratory tract may be suggestive of a pulmonary tumor. We present a case of a 73-year-old female with myasthaenia gravis and over a year's history of a foreign body (a plant fragment) in the right upper lobe bronchus. Rigid bronchoscopy was required to remove the foreign body in this patient.

2 Case Presentation

This case presentation was accepted by an institutional Ethics Committee. In August of 2012 a 73-year-old female, a non-smoker, was admitted to the Department of Respiratory Medicine at the Center for Pulmonary Diseases in Olsztyn, Poland, for persistent dry cough of about 6 months' duration and unintended weight loss of about 16 kg. Since 2002, the patient had been receiving treatment for myasthenia gravis (pyridostigmine 60 mg t.i.d.). In 2003, she underwent a complete thyroidectomy followed by radioactive iodine treatment for follicular thyroid carcinoma. She has been on long-term treatment with L-thyroxin (0.175 g/daily). In 2009, she underwent implantation of a DDDR pacemaker for sick sinus syndrome. She had also been taking ramipril 5 mg q.d. for hypertension and had a history of paroxysmal atrial fibrillation. In February 2012, she underwent diagnostic evaluation at another respiratory medicine inpatient facility. A chest radiogram obtained at that time revealed atelectasis of the posterior segment of the right upper lobe and the histopathological examination of the tissue obtained during flexible bronchoscopy demonstrated 'atypical cells'.

Physical examination on admission revealed bilateral ptosis, the presence of dentures, a post-surgery scar on the neck, and a soft, non-tender mass measuring 10 × 12 cm was palpated in the left epigastrium.

Blood tests demonstrated elevated C-reactive protein (CRP) (281.8 mg/l; normal range: <5 mg/l), elevated white blood cell count (28.3×10^3/μl; normal range: 4.1–10.9 × 10^3/μl) with a predominance of neutrophils, elevated platelet count (476×10^3/μl; normal range: 140–440 × 10^3/μl), elevated alanine transaminase (ALT) (75 IU/l; normal range: <41 IU/l), and elevated aspartate aminotransferase (AST) (136 IU/l; normal range: <40 IU/l).

A chest radiogram (Fig. 1) revealed a massive area of opacification involving the upper and middle zones of the right lung and free intraabdominal air under the diaphragmatic dome. A computed tomography (CT) scan of the chest (Fig. 2) revealed an area of consolidation with an air bronchogram in the upper lobe of the right lung, occlusion of the right upper lobe bronchus, enlargement of a right lower paratracheal lymph node, and it confirmed the presence of free intraabdominal gas. No tumor was visualized in the right pulmonary hilum in the CT scan. However, a tumor could be ruled out inside the atelectatic and inflammatory changes in the upper lobe. Thus, the findings were equivocal and the endoscopic assessment was required.

Flexible bronchoscopy revealed a cauliflower-like lesion at the origin of the right upper lobe bronchus. The lesion contained necrotic masses, occluded approximately 90 % of the bronchial lumen and a purulent discharge was oozing from underneath the lesion (Fig. 3). The bronchoscopy also revealed a macroscopically changed (infiltrated) upper-lobe carina. The

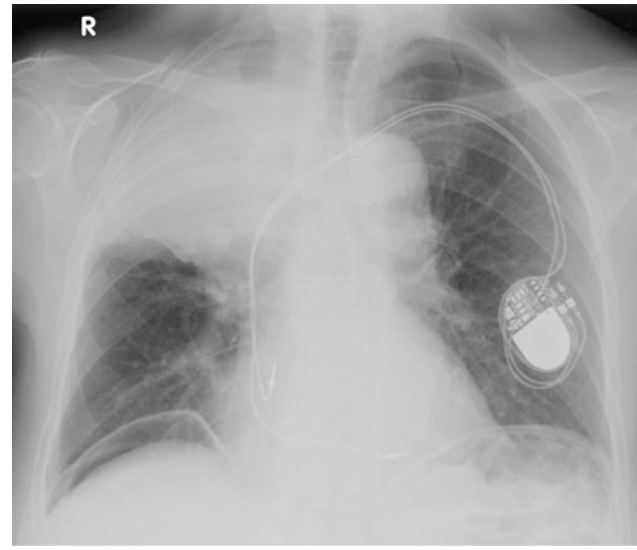

Fig. 1 A massive area of opacification involving the upper and middle zones of the right lung, enlargement of the entire cardiac silhouette, an elongated and sclerotic aorta. Free intraabdominal air visible under the diaphragm

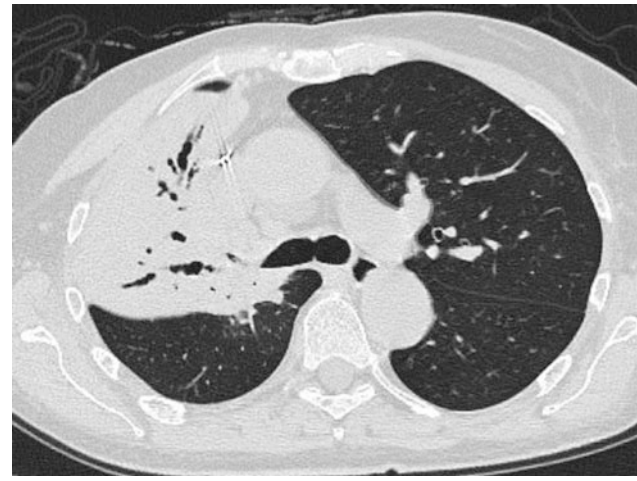

Fig. 2 An area of consolidation with an air bronchogram in the upper lobe of the right lung. The right upper lobe bronchus gradually narrows towards the described lesions

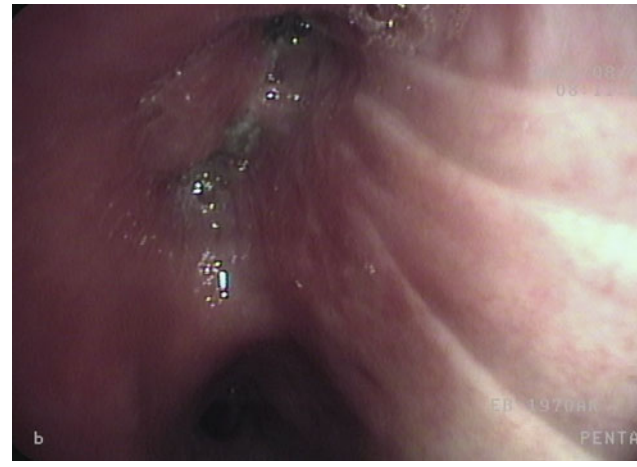

Fig. 3 Flexible bronchoscopy reveals a nearly 90 % narrowing of the right upper lobe bronchus by a cauliflower-like lesion containing necrotic masses

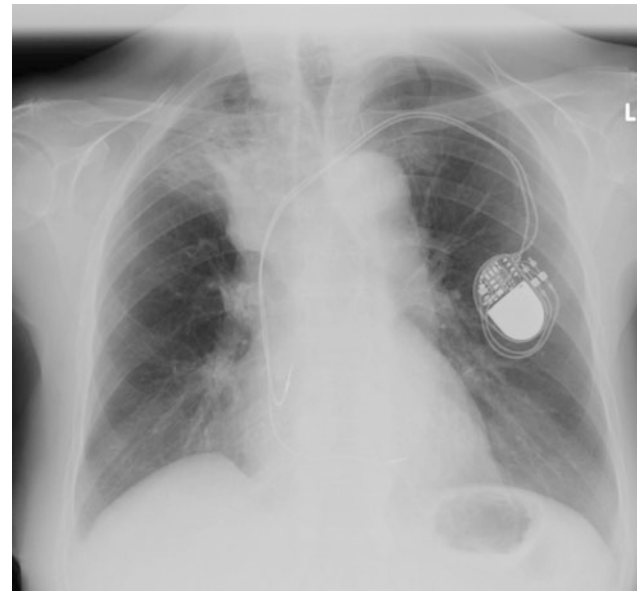

Fig. 4 Compared with the radiogram of Fig. 1, there was a partial regression of the lesions in the upper zone of the right lung and no air under the diaphragmatic domes

histopathological examination of the tissue samples collected during bronchoscopy revealed 'chronic bronchitis'. The bronchoalveolar lavage fluid cultures revealed *Citrobacter braakii* and the patient received targeted antimicrobial treatment.

Given the radiographic findings suggestive of free intraabdominal air under the diaphragmatic dome, while the patient denied any abdominal symptoms and had no history of surgery, a CT scan of the abdomen was requested. Other than the free intraabdominal gas, the scan revealed no pathologies. Following a surgical consultation a decision to perform exploratory laparotomy was made. Surgery revealed a perforated diverticulum of the large intestine. After surgery to the intestine for treatment of the perforation, the patient was re-admitted to the Department of Respiratory Medicine in April of 2013 to continue the treatment of persisting cough accompanied by a slight expectoration.

Repeated laboratory tests revealed that CRP persisted to be elevated at 111.1 mg/l. A chest radiogram (Fig. 4) and a CT scan (Fig. 5) showed a partial regression of the parenchymal changes in the right upper lung lobe seen in the previous imaging studies obtained in August of 2012. However, the CT scan revealed a complete obstruction of the right upper lobe bronchus. The patient underwent another flexible bronchoscopy, which revealed a foreign body in the right upper lobe bronchus and inflamed hypertrophic mucosa at the ostium of the bronchus (Fig. 6). An attempt was undertaken to remove the foreign body. However, not all its fragments could be removed (Fig. 7). Biopsies were collected form the mucosa lining the right upper lobe bronchus. Histopathological examinations of biopsy specimens revealed exacerbated chronic bronchitis, and necrotic and inflammatory masses. The foreign body consisted of plant fragments.

One week later in April of 2014 the patient underwent rigid bronchoscopy with jet ventilation. The foreign body in the bronchus, macroscopically, resembling a bone, completely obstructed the origins of the apical, posterior, and anterior segmental bronchi. It spontaneously defragmented, but all the fragments were successfully removed. The post-bronchoscopy course was uneventful. The patient remained under the care of a respiratory clinic. She reported no further respiratory complaints. The

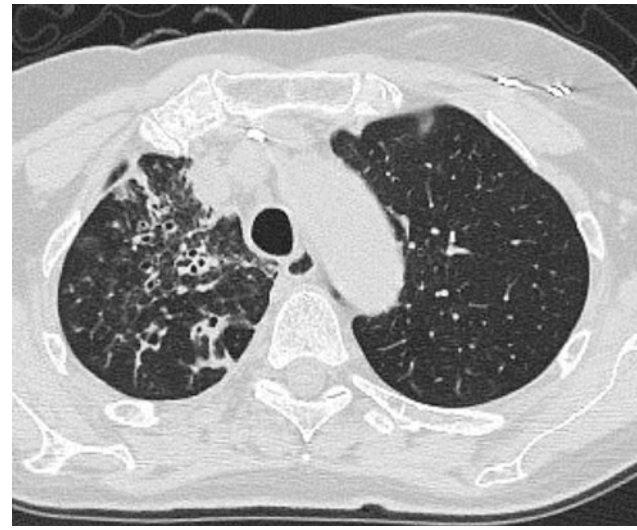

Fig. 5 Compared with the CT scan of Fig. 2, the previously revealed area of consolidation in the upper lobe of the right lung was decreased and lay along the outlines of the mediastinum and the oblique fissure. A complete obstruction of the right upper lobe bronchus was visible

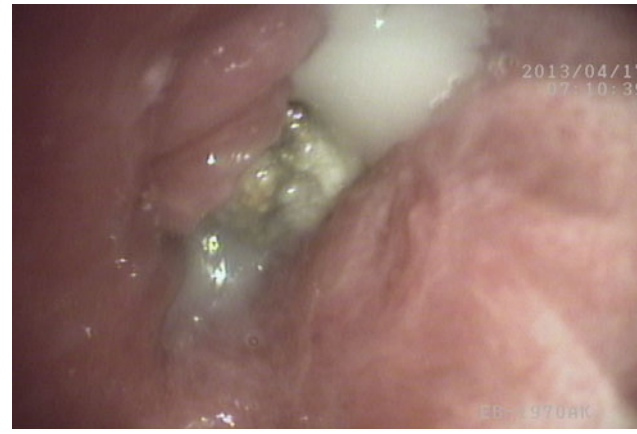

Fig. 6 Flexible bronchoscopy revealed that the upper lobe bronchus was completely obstructed by a foreign body, with overlying growth of hypertrophic mucosa. An attempt to remove the foreign body was undertaken

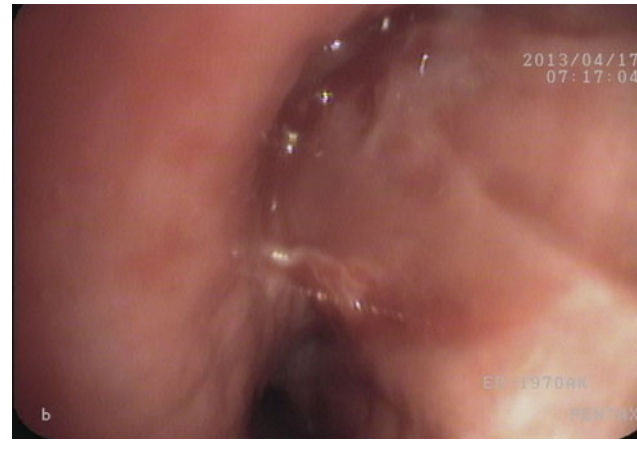

Fig. 7 The right upper lobe bronchus after removal of the foreign body fragments shown in Fig. 6. However, not all the fragments could be removed flexible bronchoscopy

radiographic lesions in the right lung have completely regressed.

3 Discussion

Foreign body aspiration is a common occurrence in children. It is far less frequent in adults and mainly occurs in elderly patients with deglutition disorders (Sharma et al. 2006), which are due mostly to neurologic, rheumatologic, or iatrogenic causes (e.g., post-radio or chemotherapy) (Sentürk and Sen 2011). The neurologic causes of deglutition disorders include myasthenia gravis (Klair et al. 2014; Sura et al. 2012).

Myasthenia gravis is an autoimmune disease caused by the formation of antibodies to neuromuscular junction proteins. The diagnosis of myasthenia is based on the history (worsening of symptoms as the day progresses is characteristic), electrodiagnostic studies, detection of elevated anti-acetylcholine receptor antibody titer, and a positive anti-cholinesterase test, i.e., a transient resolution of myasthenic symptoms. While the reports of foreign body aspiration in patients with myasthenia can be found in the literature, they all concern children with juvenile myasthenia gravis (Murray and McAllister 2001; Winter and Koopman 1990).

Foreign body aspiration may manifest as a sudden dyspnea and in some cases results in acute respiratory failure. In adults, clinical manifestations often do not develop directly after choking. Therefore, patients often cannot recall any choking episode (Sentürk and Sen 2011; Ngoo et al. 2008). In some cases, the delay in establishing the correct diagnosis and initiating the appropriate treatment after aspiration can be long. Cough and wheezing are the most commonly reported long-standing manifestations which are suggestive of an airway foreign body (Ngoo et al. 2008; Sharma et al. 2006). In some patients, hemoptysis is also present (Mishra et al. 2014; Sentürk and Sen 2011).

The patient reported herein did complain of chronic cough of several months' duration, loss of appetite and an unintentional weight loss of about 16 kg. Similar symptoms are also often present in lung cancer patients. There are reports of patients in whom the clinical presentation of a foreign body in the airways is suggestive of a lung tumor (Ngoo et al. 2008; Quereshi and Soorae 2001; Chen et al. 1997).

Radiographic abnormalities of the lungs that may develop as a result of foreign body aspiration include parenchymal consolidation, atelectasis, lung emphysema, and signs of hyperinflation (Herth 2012; Lima et al. 2008; Debeljak et al. 1999). In some patients, radiograms may be normal. Certain foreign bodies (e.g. metal foreign bodies) may be visible on a chest radiogram (Chen et al. 1997). In such cases identification of the cause of the patient's complaints and radiographic changes is easy (Philip et al. 2013). In the elderly, the predominant type of aspirated foreign bodies is food particles (Herth 2012). These most commonly include: bone fragments, grains (peas, beans, corn, or rice grains), and fragments of other plants or nuts (Debeljak et al. 1999; Chen et al. 1997). The type of aspirated food is determined by dietary habits in various parts of the world. Airway foreign bodies most frequently lodge in the right bronchial tree, with the right lower lobe bronchus being the commonest site (Rodrigues et al. 2012; Sentürk and Sen 2011; Chen et al. 1997).

The right upper lobe bronchus, where the foreign body in the presently described patient had lodged, is involved in 9–11 % of the cases, depending on the author (Debeljak et al. 1999; Chen et al. 1997). Bronchial obstruction caused by a foreign body may cause recurrent inflammation of pulmonary tissue with clinical manifestations such as cough with expectoration of mucopurulent sputum and laboratory abnormalities such as leucocytosis and elevated levels of C-reactive protein. All of these were present in our patient. Complications may also include pleural effusion, pleural empyema, and

lung abscess (Ideh et al. 2014; Luk and Chan 2014; Madsen and Madsen 2014).

Flexible bronchoscopy is the procedure of choice for the diagnosis and, in most cases, for the removal of airway foreign bodies (Rodrigues et al. 2012; Sentürk and Sen 2011; Boyd et al. 2009; Vithalani and Maniyar 2005; Swanson and Edell 2001). In cases where inflammation of bronchial mucosa is revealed during bronchoscopy, particularly if the foreign body has been present in the bronchus for a long time, the differential diagnosis should include lung cancer (Debeljak et al. 1999) and tuberculosis (Ideh et al. 2014). Histopathological examination of the collected material may be equivocal (Ngoo et al. 2008). That also was the case in our patient, as the histopathological assessment of the samples collected during flexible bronchoscopy revealed 'atypical' cells. It was justified, therefore, to include both primary lung tumor and metastatic malignancies in the differential diagnosis, particularly that the patient had had a history of treatment for thyroid carcinoma. In some patients, removal of a foreign body requires rigid bronchoscopy (Debeljak et al. 1999; Sharma et al. 2006), which was the case with our patient, and sometimes even thoracotomy (Cobanoglu and Yalcinkaya 2009; Ngoo et al. 2008).

In summary, it seems that the medical history is a crucial factor in clinical suspicion of foreign body aspiration. Such suspicion should lead to further radiographic imaging and detailed diagnostic work-up taking into account possible co-morbidities, notably including chest tumors. Although flexible bronchoscopy is increasingly used for foreign body removal from the airways, rigid bronchoscopy offers advantages in difficult cases by providing better visualization and a wider use of ancillary instrumentation if required. Delayed diagnosis increases the intensity of symptoms and leads to the formation of granulation tissue, raising the pro-inflammatory and infectious specter in the lung.

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

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Relevance of Immune-Sympathetic Nervous System Interplay for the Development of Hypertension

Pawel J. Winklewski, Marek Radkowski, and Urszula Demkow

Abstract

Historically, the sympathetic nervous system (SNS) has been mostly associated with the 'fight or flight' response and the regulation of cardiovascular function. However, evidence over the past 30 years suggests that SNS may also influence the function of immune cells. In this review we describe the basic research being done in the area of SNS regulation of immune function. Further, we show that the SNS-immune interplay during circadian rhythm may modulate the robustness of the inflammatory response, critical for survival during periods of increased activity. Finally, new concepts of a close relationship between these systems in the pathogenesis of hypertension are discussed.

Keywords

Circadian rhythm • Hypertension • Immune system • Inflammation • Sympathetic nervous system

1 Introduction

Primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymphoid organs are abundantly innervated by autonomic, mostly sympathetic efferent fibers. Norepinephrine (NE), a sympathetic nervous system (SNS) neurotransmitter, is released into the lymphoid tissue and modulates the function of immune cells. Bellinger et al. (2008) have mentioned the following criteria of neuroimmune transmission: (1) the presence of noradrenergic fibers in lymphoid organs, (2) the release of NE from noradrenergic terminals in

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these organs, and (3) the expression of adrenoceptors on lymphoid cells capable to respond functionally to the stimulation. Therefore, sympathetic innervation of lymphoid organs meets the criteria for neurotransmission, with immune cells as target cells.

2 Physiological Immune-Sympathetic Nervous System Interplay

2.1 Bone Marrow

Activation of β-adrenoceptors stimulates the proliferation and differentiation of hematopoietic/mesenchymal stem cells. Isoproterenol, a β-adrenergic agonist, added to murine bone marrow cell culture increases cellular proliferation and granulopoiesis in a dose-dependent manner (Felten et al. 1996), while the addition of a β-antagonist to cultured human bone marrow cells diminishes cellular proliferation and decreases the number of granulocyte-macrophage colony forming units (GM-CFU) (Dresch et al. 1981). Activation of α-adrenoceptors suppresses myelopoiesis and augments lymphopoiesis. Prazosin, an α_1-adrenergic antagonist, increases GM-CFU formation and accelerates myelopoiesis while it reduces the differentiation of precursor cells into thymocytes and splenic T and B cells *in vitro* (Maestroni and Conti 1994; Maestroni 1995).

Both α- and β-adrenoceptor antagonists provide protection against radiation-induced cell death. However, the protective effects of these antagonists are time-dependent. β-adrenoceptor antagonists are protective when administered prior to irradiation, while α-adrenoceptor antagonists are more effective after radiation exposure (Byron 1972; Byron and Fox 1969; Lipski 1980). It seems that the expression of α- and β-adrenoceptors on immune cells may change over the time; β-adrenoceptor expression predominates in the early phases of bone marrow stem cell activation, and α-adrenoceptor expression increases at later stages (Dresch et al. 1981). In animals exposed to stress, corticosteroids enhance, while catecholamines suppress, T cell accumulation in the bone marrow, suggesting that these two groups of stress mediators may act, at least in part, in opposition to each other (Sudo et al. 1997).

2.2 Thymus

The regulation of thymic function by the SNS was extensively explored in the 1970s and 1980s. These early studies highlighted β-adrenoceptor dependent enhancement of thymocyte differentiation and suppression of thymocyte proliferation in the presence of intact NE innervation (Singh and Owen 1975; Singh and Owen 1976; Singh 1985). An *in vivo* exposure to isoproterenol reduces thymic weight and thymocyte number in mice (Durant 1986). Similarly, catecholamines or isoproterenol decrease concanavalin A- or lipopolysaccharide-induced proliferation of murine thymocytes (Cook-Mills et al. 1995). However, thymocyte proliferation increases after treatment with epinephrine, isoproterenol, and NE. Morgan et al. (1984) reported that stimulation of β_2-adrenoceptors can elevate the level of intracellular cAMP, while stimulation of α-adrenoceptor by NE increases intracellular Ca^{2+}. Elevated cAMP production induces cell apoptosis (McConkey et al. 1990). Thymus weight and tissue cell count decrease and the number of peripheral proliferating T cells are reduced following chemical sympathectomy, whereas apoptosis increases (Kendall and al-Shawaf 1991). Sympathetic modulation of thymocyte development and maturation has been reviewed in detail elsewhere (Bellinger et al. 2008).

2.3 Lymphocytes

Human and murine T naïve, Th1, and $CD8^+$ lymphocytes express β_2-adrenoceptors (Fuchs et al. 1988; Radojcic et al. 1991; Swanson et al. 2001). In contrast, Th2 clones do not express β_2-adrenoceptors (Sanders et al. 1997; Kohm et al. 2002). *In vitro*, adrenergic agonists can

modulate all phases of the immune response (induction, proliferation, and effector function). Stimulation of β-adrenoceptors inhibits mitogen- and anti-CD3 antibody-induced T cell proliferation and diminishes naïve T cell differentiation into Th1 cells (Swanson et al. 2001; Sanders et al. 1997; Ramer-Quinn et al. 1997). However, under Th1-promoting culture conditions, β-adrenoceptor stimulation drives Th1 effector cell development from naïve T cells. These effector Th1 cells produce higher amounts of IFN-γ after re-stimulation (Swanson et al. 2001). Under Th2-promoting culture conditions, naïve T cells exposed to NE transform into Th2 cells, but adrenergic agents have no effect on IL-4, IL-5, or IL-10 production (Swanson et al. 2001; Sanders et al. 1997; Ramer-Quinn et al. 1997). Taken together, these *in vitro* data suggest that NE stimulation of β_2-adrenoceptor plays a role in the development of Th1 polarized cell-mediated immunity (Felten et al. 1996).

B lymphocytes express β_2-adrenoceptors, and their stimulation elevates the intracellular concentration of cAMP, similarly to that seen in T cells (Fuchs et al. 1988; Bishopric et al. 1980). The enhancement or inhibition of B cell proliferation through β_2-adrenoceptors depends on the type of mitogen used. Receptor stimulation or elevation of intracellular cAMP inhibits lipopolysaccharide-induced B cell proliferation (Diamantstein and Ulmer 1975; Vischer 1976) and the production of immunoglobulins (Ig) (Cohen and Rothstein 1989; Whisler et al. 1992). In contrast, activation of β_2-adrenoceptors and increased cAMP enhance ionomycin-induced B cell proliferation (Cohen and Rothstein 1989; Whisler et al. 1992). NE administration and β_2-adrenoceptor stimulation enhance IgM production in murine spleen cells cultured with a Th1 cell-dependent antigen. This effect is blocked by the addition of a β-adrenoceptor antagonist (Sanders and Munson 1984a, b).

2.4 Macrophages

α_2 and β_2-adrenoceptors are expressed on normal macrophages. Functional effects of stimulation of these adrenoceptors depend on the receptor subtype. β_2-adrenoceptor stimulation suppresses macrophage activity, whereas α_2-adrenoceptor stimulation enhances it (Bellinger et al. 2008).

2.5 Circadian Rhythm

Circadian rhythms generated by the central biological clock of the SNS regulate the majority of physiological and behavioral conditions, including locomotor activity and sleep-awake cycles, as well as autonomic, endocrine, and immune functions. In rodents, the highest SNS input to the spleen is provided during the day. Granzyme-B, perforin, INF-γ, and INF-α are expressed in natural killer (NK) cells in a rhythmic manner, with the highest levels during the dark period (a period of activity for rodents) (Arjona and Sarkar 2005). *In vitro* studies suggest that NE and other β-agonists suppress the mRNA expression of granzyme-B, perforin, and INF-γ (Dokur et al. 2004). Further, splenic denervation increases TNF-α expression under various experimental conditions (Molina 2001; Kees et al. 2003; Meltzer et al. 2004). The rhythmic pattern of NK cell function may have evolved for the benefit of having them readily available during periods of activity, when injuries and infections are more likely to occur. Circadian disruption or desynchronization between the central and peripheral systems could alter rhythms of sympathetic NE input to the spleen, thereby compromising the cytotoxic activity of NK cells. It is possible that compromised NK cell function resulting from altered release of the mediators outlined above could play a role in metastatic tumor growth or other diseases (Logan et al. 2011).

Circadian migration of leukocytes into tissues is also regulated by signals from the SNS; the peak of this recruitment occurs in rodents at night. Circadian hematopoietic cell recruitment is synchronized by the molecular clock *via* sympathetic nerves, modulated through β-adrenoceptor oscillations, the expression of endothelial cell adhesion molecules, and the chemokine release by endothelial cells (Scheiermann et al. 2012). Moreover, diurnal

mechanisms stabilizing neutrophil numbers may interfere with the course of inflammatory cardiovascular diseases (Coller 2005). Circadian rhythms modulate the robustness of the inflammatory response, critical for survival during periods of increased activity (Scheiermann et al. 2012). In humans, hypersensitivity to inflammatory stimuli associated with increased SNS tone may have a detrimental effect, potentially linked to a higher incidence of acute vascular events in the morning (Boudreau et al. 2012; Reavey et al. 2013).

3 Sympathetic-Immune-CNS Interaction Concept of Hypertension

It is widely accepted that the pathophysiology of hypertension includes autonomic nervous system dysfunction, as well as immune system involvement (Guzik et al. 2007; Zubcevic et al. 2011; de Kloet et al. 2013; Santisteban et al. 2013). However, the close relationship between these systems in the pathogenesis of hypertension and in particular the role of the brain in this interaction is still evolving. Two aspects will be discussed in depth: the effect of inflammatory cytokines on the paraventricular nucleus and subfornical organ (cardiovascular control centers in the brain), and the potential role of the bone marrow in hypertension.

3.1 Effect of Inflammatory Cytokines on the Paraventricular Nucleus and Subfornical Organ

Inflammatory cytokines such as TNF-α and IL-1β activate cyclooxygenase-2 (Cox-2) in perivascular macrophages of the blood-brain barrier. Cox-2 catalyzes the production of prostaglandin E2 which enters the brain and stimulates paraventricular nucleus neurons (PVN) which, in turn, regulate adrenocorticotropic hormone release and increase sympathetic drive (Felder et al. 2009; Felder 2010). Further, direct microinjection of TNF-α and IL-1β to PVN increases blood pressure and sympathetic outflow and enhances the cardiac sympathetic afferent reflex (Shi et al. 2011). Intracerebroventricular administration of IL-10 decreases TNF-α, IL-1α, prostaglandin E2, and Cox-2 levels in the PVN and attenuates sympathoexcitation (Yu et al. 2007). Chronic angiotensin II-induced hypertension is associated with increased expression of inflammatory cytokines and microglial activation in PVN (Shi et al. 2011; Colombari et al. 2010). Minocycline, an antibiotic that can cross the blood-brain barrier, inhibits microglial activation, attenuates angiotensin II-induced high blood pressure, decreases the amount of PVN inflammatory cytokines, and mitigates cardiac hypertrophy (Shi et al. 2011). Most likely, the subfornical organ, a forebrain circumventricular organ that lacks a blood-brain barrier, plays a major role and is a predominant site in the brain at which circulating proinflammatory cytokines act to elicit cardiovascular and sympathetic responses. Intracarotid injection of TNF-α or IL-1β significantly increases mean blood pressure, heart rate, and renal sympathetic nerve activity in rats with an intact sunfornical organ, while these excitatory responses are significantly attenuated in sunfornical organ-lesioned rats (Wei et al. 2013).

3.2 Potential Role of Bone Marrow in Hypertension

Although the bone marrow is known as a primary lymphoid organ, according to some authors it has a potential to serve as a secondary immune organ and may be involved in systemic T cell-mediated immunity. It has been demonstrated that the bone marrow may harbor memory T cells and, importantly, may be a site for the initiation of T cell activation (Feuerer et al. 2003). A critical role of T cells in hypertension has been discussed elsewhere (Guzik et al. 2007; Zubcevic et al. 2011; de Kloet et al. 2013; Santisteban et al. 2013). The bone marrow is also the primary source of endothelial progenitor cells (Asahara et al. 1999) which play a particularly important role in

endothelial repair in arterial injury following inflammatory or hypertensive stimuli (Rabelink et al. 2004; Hristov et al. 2007; Zhu et al. 2013). Acute inflammatory stimuli trigger endothelial progenitor cell mobilization, while chronic inflammation can have the opposite effect, decreasing the number of circulating endothelial progenitor cells (Andreou et al. 2006). As in other vascular beds, NE acts as a vasoconstrictor in the bone marrow and plays an important role in controlling blood flow (Feitelson et al. 2002). It has been proposed that hypertension, which presents with increased circulating NE, is responsible for extensive vasoconstriction in bone marrow, creating a hypoxic environment. Consequently, hypertension could negatively modulate stem and endothelial progenitor cells and enhance local inflammatory responses, i.e., T cell activation and cytokine production (Santisteban et al. 2013). The last hypothesis, however, has to be verified experimentally.

4 Conclusions

Current evidence suggests that the SNS plays an important role in both activating and limiting immune and inflammatory responses. This, in turn, encourage thinking that manipulation of the SNS may be useful in regulation of immune reactivity, including the inflammatory reactions in hypertension and other cardiovascular diseases. However, therapeutic manipulation will become possible only when our knowledge about the mechanisms responsible for the fine-tuning of the immune system by the SNS is significantly increased. Another important issue for all cardiovascular diseases, including hypertension, is how the disease itself influences sympathetic activity. Finally, activation of the autonomic system in the brain and periphery is quite frequently in opposition to each other, which makes therapeutic interventions even more complex and difficult.

Undoubtedly, it is necessary to develop new technologies for manipulating the SNS in time and space, in conjunction with activation of other neuroendocrine hormone systems. Some of such technologies are already being investigated in clinical trials. Of particular interest would be to investigate the full impact of new modalities used to treat resistant hypertension, such as renal innervation, carotid glomectomy or baroreflex activation therapy, on immune system. The assessment of the immune system should become a standard analysis when searching for new methods of the SNS modulation.

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

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The Influence of Insulin Therapy on the Course of Acute Exacerbation of Bronchial Asthma

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Abstract

Large doses of systemic corticosteroids are the basis of treatment of acute exacerbation of bronchial asthma. The hyperglycemic activity of systemic corticosteroids often leads to the loss of control of diabetes diagnosed earlier or to its first diagnosis during treatment of the exacerbation of asthma. We conducted a prospective, randomized study in a group of 24 adult patients treated for asthma exacerbation, with the blood glucose level at admission above 8.4 mmol/l. The patients were randomly divided into a group treated with intravenous insulin infusion by an electric syringe pump in doses controlling glycemia at 4.5–7.2 mmol/l (Group A) and a group of patients treated with insulin administered subcutaneously in three doses controlling glycemia at 7.2–10.0 mmol/l (Group B). A control group (Group C) consisted of patients without any disturbances in carbohydrate metabolism, treated for exacerbation of asthma. Asthma exacerbation was treated in all groups in a uniform way. We found that the average hospitalization time was 8.2 ± 2.4 days in Group A, 10.2 ± 5.2 days in Group B, and 5.8 ± 1.9 days in Group C; the last being significantly shorter than those in Groups A and B. We conclude that hyperglycemia is a significant factor increasing the risk of extending hospitalization time due to asthma exacerbation, regardless of the way of insulin therapy.

Keywords

Bronchial asthma • Diabetes mellitus • Exacerbation • Insulin therapy

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1 Introduction

Systemic corticosteroids are the strongest anti-inflammatory drugs. Apart from short-acting β_2-agonists they are the basic group of drugs applied in exacerbation of bronchial asthma. Serious

shortness of breath, stress and respiratory failure stimulate the secretion of adrenaline, cortisol, growth hormone and glucagon, hormones that operate opposite to insulin. This is the reason of frequent loss of control of early diagnosed diabetes or its diagnosis during treatment of exacerbation of asthma. Insulin affects the tissue metabolism by controlling glycemia, decreasing the concentration of lactates and non-esterified fatty acids in the serum and inhibiting gluconeogenesis. Additionally, insulin exerts numerous non-metabolic actions such as inhibiting the synthesis of free oxygen radicals, nuclear factor κB, plasminogen activator inhibitor type 1, and the regulation of gene transcription of pro-inflammatory cytokines. Maintaining the proper concentration of insulin also affects the operation of immune system, which particularly concerns the activity of macrophages and neutrophils (Jackson et al. 1999; Dandona et al. 2001). A study by van den Berghe et al. (2001) conducted in a group of 1548 critically ill patients has shown that maintaining glycemia at 4.4–6.1 mmol/l increases the survival rate two-fold compared with patients in whom glycemia was controlled at 10.0–12.1 mmol/l. Most of the patients in that study were after cardio-surgical operations, some had respiratory system diseases, but bronchial asthma was not singled out as a separate disease entity studied.

The aim of the present study was to assess the effect of hyperglycemia on the course of asthma exacerbation. We hypothesized that intensive intravenous insulin therapy in hyperglycemic patients hospitalized due to asthma exacerbation to control the blood glucose at a low level of 4.5–7.2 mmol/l would result in a quicker recovery in terms of hospitalization duration compared with patients in whom hyperglycemia was treated with less effective subcutaneous insulin resulting in the blood glucose level of 7.2–10.0 mmol/l.

2 Methods

2.1 Patients, Clinical Procedures, and Treatment

The study was conducted at the Department of Internal Diseases, Geriatrics and Allergology of Medical University of Wroclaw, Poland, and was accepted by a local Ethics Committee. Out of a total number of 88 patients admitted to the hospital due to acute asthma exacerbation over a 2-year period, 24 patients met the inclusion criteria which were the following:

- history: intensification of shortness of breath and cough which continued despite treatment with β_2-agonists and oral corticosteroids;
- physical examination: wheezing, frequency of breath above 30/min, pulse rate above 100 beats/min;
- additional tests: hypoxemia (partial pressure of oxygen – $PcO_2 < 70$ mmHg with no oxygen therapy) in arterialized capillary blood, peak expiratory flow (PEF) < 50 % of predicted or forced expiratory volume in one second – $FEV_1 < 50$ % of predicted.
- glycemia > 8.4 mmol/l, irrespective of the presence or no of an earlier diagnosis of diabetes.

Before hospitalization, all patients were chronically treated for asthma with short-acting and long-acting β_2-agonists (SABA and LABA). Two patients in Group A and three patients in Group B had not been using inhaled corticosteroids (ICS); all patients in Group C used ICS. Diabetes type 2 was diagnosed in 4 patients from Group A, two of them were treated with insulin, one with a sulfonylurea derivative, and one with an α-glucosidase inhibitor. In Group B, diabetes type 2 was diagnosed in five patients, two of them were treated with insulin, one with a sulfonylurea derivative, and two patients remained on a diet.

The exacerbation of asthma had the same treatment pattern in all patients: methylprednisone (MP) 2 mg/kg, i.v. q.i.d; salbutamol in nebulization 2.5 mg every 4 h, ipratropium bromide 250 μg every 4 h, oxygen supplementation 3 l/min, potassium supplementation to maintain its serum level at 4.0–5.5 mmol/l. Thiazides were withheld from the treatment regimen. The patients with respiratory tract infection received ceftriaxone 1–2 g i.v. daily, further treatment was conducted according to the result of antibiograms.

The patients were randomly divided into the following two groups of insulin therapy:

- Group A – 11 patients – short-acting insulin (Actrapid HM; Novo Nordisk, Bagsvaerd, Denmark) in solution (50 units in 50 ml 0.9 % NaCl) was given intravenously by an electric syringe pump at the dose required to control glycemia at 4.5–7.2 mmol/l. In patients weighing 50–100 kg, insulin infusion began at 1.0 unit/h during the first hour when glycemia was <10.0 mmol/l or 2.0 units/h when glycemia was >10 mmol/l. In patients over 100 kg of weight, insulin infusion was 1.5 and 3.0 units/h, respectively. When glycemia reached 4.5 mmol/l or less, the supply of insulin was stopped and it was resumed when glycemia reverted to over 6.1 mmol/l.
- Group B – 13 patients – short-acting insulin (Actrapid HM) was given subcutaneously before meals in 3 daily doses (morning 30 %, noon 40 %, and evening 30 %), allowed maintaining glycemia at 7.2–10.0 mmol/l. In patients weighing 50–100 kg, the initial daily dose of insulin was established in the following way: glycemia <10.0 mmol/l – daily dose 0.25 unit/kg, glycemia >10.0 mmol/l – daily dose of 0.5 unit/kg. In patients over 100 kg of weight, doses of insulin were 0.4 and 0.7 unit/kg, respectively. Insulin was given three times a day.
- Group C – 64 patients with no hyperglycemia were included in this control group.

The mean age of the patients in Group C (48.3 ± 14.4 years) was significantly lower than that in Group A ($p = 0.01$). Apart from significantly lower blood glucose level in Group C ($p < 0.001$), clinical condition of patients across the three groups was comparable (Table 1).

The following tests were conducted on all patients on the first day of study: complete blood count, chemistry tests, concentration of glycated hemoglobin (HbA1c), C-reactive protein, general urine test, ECG, chest X-ray, and pulmonary function tests. If an infection of the respiratory tract was suspected, sputum was taken to grow culture.

The blood glucose level was measured every hour over the initial 4 h of treatment. Further measurements were conducted eight times a day: before breakfast (8:00 a.m.), 11:00 a.m., before lunch (1:00 p.m.), 3:00 p.m., before dinner (6:00 p.m.), 8:00 p.m., before night meal (10:00 p.m.), at night (3:00 a.m.). Blood glucose was measured with an Accu-Check glucometer (Roche Diagnostics, Basel, Switzerland) the result was a mean of two repeat measurements. The diet of patients was established at 25 kcal/kg/day, 30 % of which was fats, 40 % carbohydrates, and 30 % protein.

Every morning, each patient was subjected to pulmonary function tests. PEF was measured before nebulizations at 08:00 a.m. and 8:00 p.m., and gasometry of arterialized blood was performed.

If after the first day of treatment there was no need to supply additional doses of MP or nebulization, PEF or FEV_1 were >59 %, and PcO_2 > 70 mmHg, the daily dose of MP was halved and nebulized budesonide was added in a dose of 1 mg μg b.i.d (Pulmicort, AstraZeneca; London, UK). Nebulized salbutamol and ipratropium bromide continued at initial doses. Thereafter, it was established at daily intervals whether the patient meets the above outlined criteria of MP dose reduction and inclusion of nebulized budesonide. After MP reduction and addition of budesonide, MP dose was further halved when PEF or FEV_1 increase was greater than 10 % compared with the previous day or reached 80 % of predicted value. The i.v. dose of MP was continually reduced in like manner down to 40 mg/day, after which it was replaced with oral prednisone – 20 mg/day. Then, the patient was discharged the day following that when FEF or FEV_1 remained stable or decreased no more than 10 %. In both groups, in patients with prediagnosed diabetes insulin treatment continued uninterrupted. In patients without the diagnosis of diabetes before inclusion into the study the treatment with insulin was withheld when the required daily dose fell below 12 units

Table 1 General characteristics of patients at hospital admission

	Group A (n = 11)	Group B (n = 13)	Group C (n = 64)
Women/men	6/5	7/6	38/26
Age (year)	61.4 ± 11.0	53.7 ± 12.5	48.3 ± 14.4**
BMI (kg/m^2)	30.6 ± 4.2	31.1 ± 7.2	29.6 ± 6.9
Atopy (n)	6	6	36
Asthma duration (year)	17.7 ± 8.9	17.1 ± 9.2	13.7 ± 7.5
Daily dose of prednisolone (mg)	6.25 ± 7.72	7.69 ± 9.04	5.88 ± 6.11
Daily dose of budesonide (mg)	0.68 ± 0.57	0.65 ± 0.51	0.62 ± 0.44
Patients with diabetes (n)	4	5	0
Diabetes duration (year)	5.5 ± 3.4	2.8 ± 1.3	0
FEV_1 (%pred.)	45.6 ± 12.9 (26–50)	42.5 ± 14.9 (16–48)	41.8 ± 7.2 (24–49)
PcO_2 (mmHg)	62.2 ± 5.8 (55–69)	61.2 ± 6.2 (51–70)	63.1 ± 6.0 (52–70)
Glycemia (mmol/l)	10.2 ± 1.8 (8.6–13.4)	10.9 ± 3.0 (8.5–18.1)	5.6 ± 0.9 (4.9–8.2)***
HbA1c (%)	6.1 ± 0.6 (5.3–7.0)	6.9 ± 2.1 (3.5–9.1)	–

Data are means ± SD (range). *BMI* body mass index, *FEV_1* forced expired volume in one second, *PcO_2* partial pressure of oxygen in arterialized capillary blood, *HbA_1c* concentration of glycated hemoglobin. **p < 0.01 *vs*. Group A; ***p < 0.001 *vs*. Group A and B

and three successive measurements of blood glucose were below 5.8 mmol/l.

2.2 Statistical Analysis

The main outcome variable analyzed in the study was the duration of hospitalization of patients with acute asthma exacerbation and hyperglycemia treated with intravenous insulin (Group A) or subcutaneous insulin (Group B) as compared with patients having no disturbance in the blood glucose level (Group C). A single-factor analysis was made using the Kaplan-Meier curves where a chi-squared test or Gehan-Wilcoxon test was applied for comparisons. A multi-factor analysis (combined influence of the kind of treatment, sex, age, and FEV_1) was made using the Cox proportional hazard model where the significance of individual factors was studied with the Wald test. Data were presented as means ± SD or medians. $P < 0.05$ defined the statistical significance of differences.

3 Results

The course of treatment is shown in Table 2. The patients' clinical condition in both groups improved during treatment compared with the initial condition, which was manifested by increases in FEV_1 and PcO_2. The average daily doses of MP and insulin did not differ in Group A and B. However, the daily dose of MP in Group C was significantly lower than that in Group A or B ($p < 0.05$). Two patients in Group A and 4 patients in Group B required an antibiotic due to respiratory infection. The goal to keep the blood glucose level under the set outpoint was achieved in Group B, where it was, on average, below 11.1 mmol/l, but not in Group A, where it was higher by 0.6 mmol/l than the upper limit of 7.2 mmol/l. Additionally, there were three incidents of hypoglycemia in Group A.

Differences in hospitalization duration among the three groups were studied with the Kaplan-Meier curves. The mean hospitalization durations in Group A, B, and C were 8.2 ± 2.4, 10.2 ± 5.2, and 5.8 ± 1.9 days, respectively ($p < 0.001$; chi-squared test). There was a significant difference between Group A and C as well as Group B and C ($p < 0.001$ for both; the Gehan-Wilcoxon test). No difference was found for hospitalization duration between women and men for all groups combined. The Cox model was used to study the influence of sex, age, coexistence of hyperglycemia (Group A and B), and the level of FEV_1 on hospitalization duration. Only did age ($p = 0.001$) and hyperglycemia in the patients treated with subcutaneous

Table 2 The course of treatment in both groups

	Group A	Group B	Group C
FEV_1 (%pred.)	60.6 ± 19.5	58.5 ± 25.9	62.2 ± 23.2
Morning PEF (%pred.)	47.9 ± 12.2	45.2 ± 11.7	52.7 ± 16.1
Evening PEF (%pred.)	47.3 ± 12.6	48.0 ± 13.5	56.9 ± 12.4
PcO_2 (mmHg)	68.0 ± 7.4	66.2 ± 6.2	68.9 ± 5.6
Daily dose of prednisone (mg)	89.3 ± 60.3	80.6 ± 53.4	55.2 ± 36.1*
Blood glucose level (mg/dl)	140.6 ± 40.7	160.5 ± 55.0	–
Daily dose of insulin (unit)	37.9 ± 17.7	37.2 ± 23.6	–
Duration of hospitalization (days)	8.2 ± 2.4	10.2 ± 5.2	5.8 ± 1.9***
No. of hypoglycemic events	3	0	

Data are means ± SD. *FEV*$_1$ forced expired volume in one second, *PEF* peak expired flow, *PcO*$_2$ partial pressure of oxygen in arterialized capillary blood. *p < 0.05 vs. Group A or B; ***p < 0.001 *vs.* Group A or B

Table 3 Analysis of the Cox model

n = 88	Beta	Standard error	Hazard ratio	Wald test	p
Sex	−0.129	0.222	0.878	0.339	0.560
Age	−0.031	0.009	0.969	10.998	0.001[a]
FEV1	0.015	0.012	1.015	1.509	0.219
Insulin i.v.	−0.539	0.341	0.583	2.488	0.114
Insulin s.c.	−0.944	0.362	0.388	6.817	0.009[a]

i.v. intravenous, *s.c.* subcutaneous
[a]statistically significant

insulin (Group B) appear of significant relevance (p = 0.01; the Wald test) (Table 3).

The hazard ratio (HR) for the appearance of hyperglycemia in patients treated with subcutaneous insulin (Group B) was 0.38, meaning that the chance of discharging a patient from group B from the hospital is 2.5 times smaller than that for a patient from Group C. The HR for the appearance of hyperglycemia in patients treated with intravenous insulin was 0.58, meaning that the chance of discharging a patient from Group A from the hospital was 1.7 times smaller than that for a patient from Group C. The results indicate that controlling the level of glucose in the blood within the assumed range of 4.5–7.2 mmol/l in patients receiving large doses of systemic corticosteroids during acute exacerbation of asthma is difficult and causes the risk of hypoglycemia, particularly in older patients. The mean dose of systemic corticosteroids used to treat the exacerbation of asthma was significantly lower in the group of patients with no disturbance of the blood glucose level compared with those having hyperglycemia (Table 2).

The FEV1 increased at discharge from the hospital to 83.1 ± 11.7, 76.8 ± 15.6, and 79.6 ± 17.3 % pred., in Group A, B, and C, respectively; the increases being of the same magnitude in all three groups. The patients were followed for up to 12 weeks after discharge. The beneficial effect of hospital treatment were sustained regarding both lung function and blood glucose level; the latter amounted to 5.7 ± 1.2 and 6.3 ± 1.2 mmol/l in Group A and B, respectively.

4 Discussion

The major finding of this study was that hyperglycemia accompanying systemic corticosteroid treatment and/or newly diagnosed diabetes hampers recovery from acute exacerbation of asthma requiring hospitalization, which causes an extended hospital stay compared with patients who have no disturbance of carbohydrate metabolism. In addition, the way and intensity of insulin treatment in hyperglycemic patients failed to

have an appreciable effect on the course of exacerbation and duration of hospitalization.

In the past, the adverse influence of hyperglycemia on patients in intensive care units was neglected. In life threatening situations in unconscious patients or patients after operations with parenteral nutrition achieving normoglycemia is difficult and there is a risk of hypoglycemia. Controlling blood glucose level with insulin is greatly beneficial in such patients and results in significant reductions in mortality, fewer, hospital-acquired infections, myocardial and cerebral infarct size, and less frequent blood transfusions (Golden et al. 1999; Malmberg et al. 1999; Capes et al. 2000; Stranders et al. 2004). Initial data on a favorable outcome of glycemia control with intravenous insulin have come from the studies on patients after myocardial infarction and cardio-surgical operations (Malmberg 1997; Dantona et al. 2003). Van den Berghe et al. (2001) have shown in a group of 765 intensive care unit patients that keeping the blood glucose level at around 4.5–6.1 mmol/l results in a two-fold decrease in mortality compared with the glucose level of 10.0–11.1 mmol/l. Another study has demonstrated a significant reduction in hospital mortality in patients whose blood glucose level was kept below 8.1 mmol/l, and an increase in mortality when glucose level is hard to control due to up and down shifts, which requires larger doses of insulin (Finney et al. 2003). The authors have also noted that the actual level of blood glucose is greatly influenced by numerous drugs and the patient condition.

Studies regarding hyperglycemia in the exacerbation of asthma are scarce. However, hyperglycemia in this condition is a serious issue, as about half of such patients manifest disturbed carbohydrate metabolism. The predisposing factors include age and chronic use of systemic corticosteroids. The present study included patients with acute exacerbation of asthma and symptoms of severe respiratory impairment, albeit not requiring mechanical ventilation, and with diagnosed diabetes or temporary glucose intolerance. The strategy of treatment of asthma exacerbation, applied in the study, complied with the international guidelines (GINA 2015). The incidents of hypoglycemia observed in the group treated with intravenous insulin were mild in form and required administration of glucose *per os* and temporary suspension of insulin supply. That is consistent with the observations made by other authors demonstrating a significant advantage of intravenous supply of insulin in acute medical conditions. The more frequent episodes of hypoglycemia occurring with the subcutaneous supply of insulin are apparently not life threatening to patients (Clement et al. 2004). A shorter hospitalization duration in patients with no disturbance in carbohydrate metabolism (Group C) could also be influenced by a significantly lower age of patients in this group compared with the other two groups. Maintaining the blood glucose level in a range of 4.5–7.2 mmol/l in the group of patients treated with intravenous insulin was difficult, especially at the beginning phase of the study when the doses of systemic corticosteroids were high.

We conclude that the control of glycemia in patients with asthma exacerbation is a serious challenge. Frequently, application of large doses of systemic corticosteroids results in the loss of control of diabetes. This control may be restored with intravenous insulin, which acts faster and requires smaller doses than subcutaneous insulin therapy (Kitabchi et al. 2001; Metchick et al. 2002). Nonetheless, a better control of hyperglycemia does not necessarily translate into more effective therapy of asthma exacerbation, which would end up with a shorter duration of hospitalization.

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

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Association of Allergic Rhinitis in Female University Students with Socio-economic Factors and Markers of Estrogens Levels

I. Wronka, K. Kliś, and K. Jarzębak

Abstract

The aim of this study is to investigate the association of allergic rhinitis in female university students with socio-economic factors and sex-hormone markers, including age at menarche, menstrual disorders, and selected anthropometrics indexes. The research was conducted among 640 female university students, aged 19–25 years. The measurements of body height, body mass, waist and hip circumference were taken. Each person completed a questionnaire. The occurrence of allergy was determined on the basis of answers to the questions whether the allergy and its allergens were defined on the basis of medical work-up. We found that a significantly larger number of cases of allergic rhinitis were recorded in the university students coming from families of high socio-economic level than those from lower level. Allergic rhinitis also was more frequent in the students who spent their childhood in cities than in those who lived in the countryside. The prevalence of allergic rhinitis was inversely correlated to the number of siblings. There were no differences in the prevalence of allergic rhinitis in relation to the birth order. The estrogen level seemed unassociated with rhinitis. However, there were slightly more allergic among females with an earlier age of menarche.

Keywords

Age at menarche • Allergic rhinitis • Birth order • Socio-economic status • Obesity

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1 Introduction

Many countries, especially highly industrialized ones, have recently seen an abrupt increase in the prevalence of asthma and allergic diseases. According to latest reports, in some populations nearly 50 % of individuals show symptoms of atopic sensitization. The prevalence of allergic rhinitis among European adults ranges from 17 % in Italy to 29 % in Belgium, with an overall value of 23 % (Bauchau and Durham 2004). The cause of this phenomenon is not fully explained; yet it is commonly associated with changes in terms of exposure to pathogens, mainly bacteria and parasites (Okada et al. 2010).

One of the most popular hypotheses, posited by Strachan (1989), indicates excessive hygiene as the main risk factor contributing to the occurrence of allergic diseases. Many a study has reported a connection between the socio-economic status and the prevalence of allergies. Allergic diseases tend to occur more and more frequently in children from high-status families and families living in cities, a finding explained by reduced exposure to bacteria and parasites, as well as spending more time in a 'sterile' environment compared to children of low-status families and those living in rural areas (Okada et al. 2010; Pawlińska-Chmara et al. 2008). Differences in the prevalence of allergies are also noticeable across countries. The so-called 'westernization' of lifestyle is conducive to the development of allergies, with more frequent occurrence observable in societies with high mean living standards (Gold 2006).

However, the hygiene hypothesis is not the only one which attempts to explain the growing number of persons with allergies. Atopy is listed as one of the negative effects of early onset of puberty (Macsali et al. 2012; Al-Sahab et al. 2011). In the twentieth century, the development of medicine and the overall improvement of living conditions in many countries translated into inter-generation changes in the biological condition of individuals and populations. An example of secular trends is the acceleration of age at menarche. Also, significant differences in biological development rate dependent on lifestyle and living conditions are reported within a single generation. Age at puberty is considerably earlier in wealthier countries than in states with poorer living standards; within the same country, children brought up in families of high socio-economic status reach puberty earlier than their counterparts from low-status families.

It is possible that providing a child with optimum conditions for biological development, including less exposure to bacteria, viruses and parasites, reduces the risk of diseases in childhood, and allows for rapid biological growth and earlier sexual maturation. On the other hand, there are negative consequences of early puberty, including the lability of the immune system.

The relation between the onset of puberty and the prevalence of asthma and allergies is particularly noticeable in females. It is supposed that high oestrogen level increases the risk of asthma and allergies. This theory is confirmed by studies in which the prevalence of allergies is higher in females than in males, as well as by results of the tests on the relationship between hormone levels and the occurrence of allergy or the varying intensification of allergic symptoms across the menstrual cycle in women.

An early age at menarche may be the marker of hormone levels. In females who reach puberty at early age, estrogen level is much higher both during adolescence and adulthood than in those who reach puberty at normal or later age. The amount of adipose tissue could be another factor connecting allergic diseases with early adolescence. Obesity is referred to as one of risk factors contributing to the incidence of asthma and allergies. Early menarche is also known to cause a greater deposition of adipose tissue and more frequent occurrence of overweight and obesity.

The present study was aimed at determining factors correlated with the prevalence of allergic rhinitis in university students. Socio-economic factors, as well as factors which could be indicative of estrogen level, including age at menarche, menstrual cycle regularity, waist-to-hip ratio (WHR), and body mass index (BMI) were taken into consideration.

2 Methods

The study protocol was approved by a local Ethics Committee. Data were collected following the ethical principles as stated in the Declaration of Helsinki. It was a cross-sectional study conducted among 670 female students of Krakow universities and colleges. The mean age of participants was 20.1 ± 1.4 years, with a range of 19–25 years of age. In a group of women without allergy the mean age was 20.1 ± 1.5 years and the allergic women were 20.1 ± 1.3 years of age.

Each student responded to a questionnaire. The questions concerned the incidence of allergic conditions, socio-economic status, the age at the onset of adolescence, and the menstrual cycle pattern.

The incidence of atopy was determined using the response to the question 'Have you been diagnosed with an allergy on the basis of medical tests, and if yes, which allergens are you allergic to?'. Allergic rhinitis was defined by a positive response to the question: 'Do you get attacks of 'hay fever' (i.e., sneezing, running or blocked nose, sometimes with itchy eyes or nose)?'.

Socio-economic status was established by means of variables used as standard in auxological studies: place of residence before the university or college period, mother's level of education, father's level of education, and the number of siblings. Place of residence before going to university/college was considered in three categories: village, town (up to 100,000 inhabitants), and city (more than 100,000 inhabitants). Mother and father's education levels were provided as three categories: vocational, secondary, and higher. On the basis of all the above factors, a complex socio-economic status indicator was calculated. Students were divided into three groups: low, average, and high status.

Age at menarche was established by means of the retrospective method. The questions related to the menstrual cycle patterns concerned both regularity and length of menstrual cycles.

Every subjects' height, body mass, waist and hip measurements were taken, on the basis of which BMI, WHR, and waist-to-height ratio (WHtR) were calculated.

3 Results

Twenty three percent of female subjects reported that they suffer from allergic rhinitis or both respiratory and food allergy. The most commonly named aeroallergens included pollens, house dust mite, and pet dander.

Table 1 contains socio-economic characteristics of female subjects, including the prevalence of allergic rhinitis. In groups of high socio-economic status more students reported allergic rhinitis than in groups of average and low status. The results of a chi-squared test revealed that the differences were statistically significant. Considering individual socio-economic factors, there were no differences in the prevalence of allergic rhinitis depending on mother and father's education level. A significantly higher prevalence of allergic rhinitis was noticeable in students who prior to going to university lived in an urban area, in particular in large cities, than in those who lived in rural areas. As for the 'sibling count' factor, there was a significant increase in the prevalence of allergic rhinitis along with a growing number of children in the family. The students were further divided into four groups: single children in the family, children with younger sibling(s), children with older sibling(s), and children with both younger and older sibling(s). Single children suffered from allergic rhinitis more frequently than children with sibling(s). No differences in the prevalence of allergic rhinitis were reported between the other three groups.

Another group of factors under consideration was related to estrogen levels. The first factor was the age at which the first menstruation occurred. The mean age at menarche for allergic students did not differ from that in students without allergies. The subjects were divided into

Table 1 Prevalence of allergy among students in relation to socio-economic status

Factor	Category	Allergy incidence		p-value
		no	yes	
		n (%)	n (%)	
Mother's education	Vocational	74 (72.6)	28 (27.5)	NS
	Secondary	178 (73.9)	63 (26.6)	
	University	224 (68.5)	103 (31.5)	
Father's education	Vocational	174 (72.2)	67 (27.8)	NS
	Secondary	152 (73.1)	56 (26.9)	
	University	150 (67.9)	71 (32.1)	
Number of siblings	0	72 (62.6)	43 (37.4)	<0.01
	1	214 (69.5)	94 (30.5)	
	2	129 (74.6)	44 (25.4)	
	3 and more	61 (82.4)	13 (17.6)	
Siblings	Lack	72 (62.6)	43 (37.4)	NS
	Younger	162 (71.7)	64 (28.3)	
	Older	157 (72.7)	59 (27.3)	
	Younger and older	85 (74.6)	29 (25.4)	
Place of living	Village	172 (80.8)	41 (19.3)	<0.001
	Cities below 100,000 inhabitants	174 (73.4)	63 (26.6)	
	Cities above 100,000 inhabitants	130 (59.1)	90 (40.9)	
Socio-economic status	Low	127 (79.9)	32 (20.1)	<0.001
	Average	237 (71.4)	95 (28.6)	
	High	112 (62.2)	68 (37.8)	

three groups: early onset of puberty (age at menarche lower than the 25th centile), average onset of puberty (age at menarche ranging from the 25th to 75th centile), and late onset of puberty (age at menarche higher than the 75th centile). In the group of girls with early onset of adolescence slightly more subjects reported allergic rhinitis than in groups with the average or late onset of puberty, although the difference was not statistically significant. Also other factors under analysis which may be indicative of estrogen levels, such as WHR and menstrual cycle regularity, were not significantly correlated with the prevalence of allergic rhinitis (Table 2).

The final stage of the analysis involved verifying the relationship between indicators of abnormal amount of adipose tissue and the prevalence of allergic rhinitis. No statistically significant differences in the incidence of allergic rhinitis between underweight, normal-weight, overweight, and obese students were reported; the same was also true of students with and without abdominal obesity (Table 2). Moreover, no significant differences in the mean values of BMI, waist circumference, and WHtR between students with and without allergies were found (Table 3).

4 Discussion

All variables analyzed in the present work were referred to in the scientific literature as factors correlated with risk of allergic conditions. Most studies have demonstrated that the development of allergic conditions is strictly connected with living conditions. Likewise, the present study indicates that the prevalence of allergies is to a larger extent caused by socio-economic factors than high level of estrogens. The present findings indicate that three factors showed significant in terms of their effect on the prevalence of allergies: overall socio-economic status, sibling count, and the place of residence, i.e., a degree of urbanization. Other researchers, too, have shown a significant relationship between social and

Table 2 Prevalence of allergy among female students in relation to age at menarche and obesity

Factor	Category	Allergy incidence		p-value
		no	yes	
		n (%)	n (%)	
Age at menarche	Early	62 (66.7)	31 (33.3)	NS
	Average	366 (71.8)	144 (28.2)	
	Late	48 (71.6)	19 (28.4)	
Menstrual cycles	regular	344 (71.2)	139 (28.8)	NS
	irregular	132 (70.6)	55 (29.4)	
Body mass index (kg/m^2)	Underweight	65 (65.7)	34 (34.3)	NS
	Correct	361 (72.1)	140 (27.9)	
	Overweight and obese	50 (71.4)	20 (28.6)	
Waist circumference (cm)	≤80	394 (72.0)	153 (28.0)	NS
	>80	50 (66.7)	25 (33.3)	

Table 3 Anthropometric indices in relation to the incidence of allergy

Anthropometric indices		Allergy incidence		p-value
		no	yes	
BMI (kg/m^2)	$\bar{x}$	21.3	21.5	NS
	s	2.95	3.36	
	min-max	16.6–36.8	16.7–35.6	
Waist circumference (cm)	$\bar{x}$	71.3	70.9	NS
	s	8.1	8.6	
	min-max	56–106	58–106	
WHR	$\bar{x}$	0.75	0.74	NS
	s	0.06	0.07	
	min-max	0.62–1.01	0.64–1.10	
WHtR	$\bar{x}$	0.43	0.42	NS
	s	0.05	0.05	
	min-max	0.32–0.66	0.34–0.60	

BMI body mass index, *WHR* waist-hip ratio, *WHtR* waist-height ratio

economic factors, and the incidence of allergies (Okada et al. 2010). In European and North American countries it has been observed that the percentage of people with asthma and allergic conditions grows with an increase in the gross domestic product (Bach 2002). This correlation is also visible in a single country. Allergic conditions are more common in wealthier than in poorer regions. As mentioned in the introduction, the prevalence of allergies is also connected with the socio-economic status of the family in which the child is brought up (Patterson et al. 1996). Almost all studies demonstrate that allergic conditions are related to high socio-economic status. The only exception is asthma, as studies indicate that it occurs more frequently in persons living in worse conditions. The reasons for this phenomenon are not entirely explained. It is often emphasised that the causes may be linked to differences in exposure to infections in childhood or in environmental pollution (Eggleston 2009; Bach 2002). The present study confirmed the above hypothesis. Similar to other authors' findings, we report that allergies were more often in single children in the family than in those having sibling(s). The reason for this could be that children with sibling(s) receive more exposure to infections. Of special significance is the exposure to infections during the first 6 months of life. Therefore, what is important is not only the fact of having brothers or sisters, but also the sequence in which the children are born. Older siblings lead to a greater risk of allergies than younger ones (Cardwell et al. 2008;

Strachan 1989). Latest studies provide another explanation of the relationship between the number of children in the family and the prevalence of allergies. It is believed that the prevalence of allergies depending on birth sequence is a reflection of *in utero* effects. It has been shown that exposure to a high cord blood immunoglobulin E level in the prenatal period is positively correlated with allergies later on in the child's life (Tariq et al. 1999; Michel et al. 1980). In addition, it is established that cord blood immunoglobulin E level decreases with consecutive births (Karmaus et al. 2001). Devreux et al. (2002) have reported decreasing T helper proliferative responses of cord blood mononuclear cells with increasing birth order (Devreux et al. 2002). Our present findings did not reveal any relationship between the birth sequence and the prevalence of allergies. Indeed, students with sibling(s), irrespective of the age of the sibling (s), reported allergic conditions less often than students who were their parents' only children.

The connection between the prevalence of allergies and the place of residence is explained by varying exposure to air pollution and endotoxines. Air pollution contributes to the development of allergic conditions and asthma, and exposure to a high level of endotoxines prevents asthma and allergies (Tavernier et al. 2006; Braun-Fahrlander et al. 2002). Villages are characterized by lower air pollution level, higher level of endotoxins, as well as greater exposure to pathogens compared to cities. From an early age on, children in rural areas live in an environment rich in allergens such as animal hair or plant pollen (Ege et al. 2006; Braun-Fahrlander et al. 2002; Riedler et al. 2001).

The two remaining socio-economic factors discussed in the present study, i.e., mother and father's level of education were not correlated with the prevalence of allergic conditions. This could be attributed to the homogenization of living conditions and lifestyles across groups of different education levels.

In this study we reported no effect on the prevalence of allergies of factors related to estrogen level either. The first of such factors, i.e., the age at menarche, is the indicator of the endogenic level of estrogens, which in turn has a significant influence on the development of the immune system. Westergaard et al. (2003) have shown that early age at menarche is associated with a greater risk of allergic rhinitis in adulthood. However, Xu et al. (2000), having examined a group of 10,000 female subjects, do not find any relationship between age at menarche and allergies in adulthood. Interestingly, they reported that offsprings of mothers whose first period occurred at a later age suffer much less frequently from allergic conditions in adulthood. The cohort of female students investigated in the present study did not exhibit any significant difference in the prevalence of allergies depending on the onset of puberty, although students whose period of adolescence began earlier reported allergies somewhat more frequently than those whose onset of puberty was normal or late. Other analyzed markers of estrogen level were not related to the prevalence of allergies either. Still, the literature provides information that the prevalence of allergies is related to irregular menstrual periods (Galobardes et al. 2012) and reproductive history (Rangaraj and Doull 2003).

The findings concerning the effect of high estrogen level on the development of allergies are not fully unanimous. This could be caused by the fact that, rather than blood hormone levels, many studies make use of indirect indicators. It must be noted that even in the case of direct hormone tests, it is only the current level of a hormone that is being measured. What is unknown is to what extent such level reflects a high exposure to estrogen in previous periods of life critical to the development of the immune system. It is also suggested that the risk of allergy is affected to a much lesser extent by endogenic hormones than hormones present in the natural environment, mainly food (Chighizola and Meroni 2012; Daxenberger et al. 2001).

Studies on allergy risk factors are problematic in that a person throughout his life is exposed to numerous environmental factors, which are often interrelated. It is thus difficult to isolate a single factor(s) and recognize the way in which it exerts an influence. Studies using animal models may prove helpful in this respect, as they make it possible to control conditions. However, the human model of development is unique and

different from other animal species. For this reason, monitoring studies on the prevalence of allergies and factors correlated with the incidence of allergies should be constantly conducted, and meta-analyses of published data will allow researchers to identify risk factors and limit the number of allergic individuals in the future.

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

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Psychosocial Context of Differences Between Asthmatic and Diabetic Patients in Adaptation to Disease

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Abstract

A significant rise in the incidence of asthma and diabetes makes the psychosocial underpinnings of these diseases an increasingly important issue. This article examines differences in psychosocial functioning between healthy people and patients suffering from asthma and diabetes, as separate disease entities. Psychological factors seem to play a significant role particularly in the process of recovery and adaptation to the disease. Our assumption was that a time perspective, a sense of belonging, and a hope may be related to the functioning of people with chronic asthma and diabetes. The study involved a total of 90 people assigned to three groups: healthy individuals, asthmatic patients, and diabetic patients. The findings demonstrate that patients suffering from asthma have a different attitude toward the future and a sense of fatalism in the present. Yet there are no significant differences between asthma patients and healthy individuals in the sense of belonging and hope. Diabetic patients perceive the present as more fatalistic than asthmatic patients and healthy individuals, and they are less oriented at setting and achieving future goals. The finding that the type and course of the disease are associated with specific psychosocial adaptation may have functional and therapeutic implications, and thus should get psycho-clinical attention.

Keywords

Asthma • Diabetes • Health • Hope • Psychosocial functioning • Psychotherapy • Sense of belonging

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1 Introduction

Asthma and diabetes belong to chronic diseases which are of biological origin, have a

psychosomatic component, and have one or more of the following outcomes (in different constellations, resulting from the course of a particular disease):

- restrict the patients' everyday activity and psychosocial functioning and make it difficult for them to perform their social roles;
- make the patients dependent on restrictions resulting from the character of their disease, such as constant/everyday medication, a particular diet, and the use of specific equipment.

The population of asthmatic and diabetic patients has increased in recent years. The World Health Organization indicates that by 2020 the incidence of psychiatric disorders will also increase. The present article is an analysis of differences in psychosocial functioning among healthy individuals and asthmatic and diabetic patients.

The course of a chronic disease involves numerous inconveniences. Illness, like any other difficult situation, requires the patient's adjustment. Usually, in the course of a chronic disease, patients can be observed to cope differently with its symptoms. The symptoms are, for example, increased fear of the future, a sense of guilt about close family, and mild depression and despair, which all decrease the patients' motivation for recovering. From the physiological point of view, emotions are psychophysical and chemical changes, electric and hormonal changes included, which occur in the human nervous system. In return, they influence the functioning of tissues and organs, the integrity of the immune system, and the effects of many biological substances, increasing or decreasing the control of physiological processes in the human organism. A positive attitude is related to a faster recovery (De Meester 2013).

One of the emotional aspects of anxiety formation in an individual is taking a particular time perspective (Gilbert 2010). Representatives of many academic disciplines, such as physics, psychology, philosophy, and history, ponder the complexity of time. Albert Einstein was the first to assume that time is a relative value, a specific feature, and a value relative to different beings. Thus, everybody may have his own time only if it is not an illusion. Chronopsychology deals with rhythmic changes in biological processes taking place in human organisms, i.e., ultradian, circadian, and infradian cycles set by the individual's biological clock (Wilczynska 2013). The life rhythm of asthmatic and diabetic patients is determined by the specificity and cyclic nature of their respective treatment. Hence, biological cycles overlap with socially-conditioned cycles set by treatment repeated every week. They have an influence on the so-called circaseptan rhythm.

Modern research in psychology focuses on three aspects of time experiencing. They are based on the relatively independent clocks: biological clock/time, psychophysical clock/time, and existential clock/time. The individual's influence on his temporal clocks has not yet been investigated. The human ability to influence the existential perception of time has been identified relatively well. It is responsible for the formation of a time perspective and its significance for the individual's daily functioning. Zimbardo and Boyd (2009) enumerate five perspectives of time perception. They are the following: past-negative, past-positive, present-hedonistic, present-fatalistic, and future orientation. The time perspective determines the individual's hierarchy of values, creates favorable conditions for expressing related emotions, builds self-esteem and respect for others, and makes one to behave in a particular way in different situations. In the future orientation, thoughts and experience focus on ideas and expectations concerning the future, and not on stimuli provided at the present. The domination of the present perspective has an influence on intense experiencing what is happening at the present. The past orientation, in turn, is related to analyzing the meaning of past experiences. The research shows that the future perspective is related to preventive actions and pro-health behaviors, as well as to greater life satisfaction (Zimbardo and Boyd 2009).

The sense of belonging is described as the individual's personal experience, a sense of being important and appreciated, and fit for the environment (Hagerty and Patusky 2003). According to

the past psychological research, the sense of belonging is described in the following terms:

- experience of being needed, important and respected by others, medical staff and close family included (*valued involvement*);
- experience of being fit and agreeable to others despite the disease symptoms (*fit involvement*) (Kestenberg and Kestenberg 1988; Hagerty and Patusky 2003; Wilczynska 2013).

The greater sense of belonging is a symptom of lesser stress and reduced depression, and constitutes a strong predictor of good health. That is why the sense of belonging and interpersonal relations is the key variables investigated in nursing and health care research. The data collected in hospitals and other health care institutions concerning the extent to which the patient's need for belonging is satisfied (to what extent the patient feels important and respected) are used in the organization of patients' support in health care institutions to optimize the recovery process (Hagerty and Patusky 2003).

Hope, a constant personality variable, has been investigated as an indicator of motivation for recovering (Irving et al. 2004). The hope theory formulated by Snyder et al. (2000b) describes hope for success as a person's conviction that in spite of obstacles and disease-related problems he can manage. The construct of hope for success also includes another component, the ability to find solutions (Trzebiński and Zięba 2003). Persons with greater hope easily engage in preventive actions, better get used to cope with the disease, and are more persistent in taking administered medicines and applying therapeutic programs (Snyder et al. 2000a).

Suffering from asthma may give a sense of security in reactions with loved ones, and especially in the provision of care and support. A child may be rejected when healthy or manifests aspirations for autonomy, while the emotional 'reinforcement' occurs in the form of special care and attention from the mother when it is sick and helpless (Sperling 1968). Families of people suffering from asthma usually give the impression of being harmonious (Schoenhals and Bernatz 1984). In relations between members of the family, changes and open confrontation are avoided if they can result in a conflict. Members of those families declare no problems, but their unconscious fantasies are associated with problems in the sphere of autonomy and separation anxiety. Parentification is very common in those families (i.e. the child 'plays' the role of their own parents' parent) as is the acquisition by the child of the 'responsibility' for the health and lives of others: 'I use as little air as possible to leave the most for my parents. I have to strangle, so that they could breathe'.

2 Goal of Research

In the present study, it has been assumed that the time perspective, the sense of belonging, and hope may be related to the daily functioning of patients with chronic asthma and diabetes. It seems interesting to what extent the type and course of the disease, indirectly its symptoms and treatment included, may be related to the patients' psychosocial functioning in terms of the above-described dimensions. Thus, our aim was to show psychosocial differences in adaptation to disease between asthmatic and diabetic patients, and to compare the patients' daily functioning with that of healthy individuals (control group). A chronic disease and the necessity to undergo constant treatment cause a stressful situation. The patients' adaptation to their disease and the mode of treatment require changes in many areas of their daily functioning.

The aim of the research was to investigate:

- if there are significant differences in the sense of belonging and the need for belonging between asthmatic and diabetic patients, and healthy individuals;
- if there are significant differences in the perception of the Present between asthmatic and diabetic patients; and if there are any

significant differences in this respect between the patients and healthy individuals;
- if there are statistically significant differences in the orientation toward the Future between asthmatic and diabetic patients; and if there are any significant differences in this respect between the patients and healthy individuals;
- if there are significant differences in Hope between asthmatic and diabetic patients; and if there are any significant differences in this respect between the patients and healthy individuals.

It has been assumed that there are differences between asthmatic and diabetic patients due to the specificity of the respective diseases and their treatment. We have recognized that because of similar experience with medical staff in health care institutions, asthmatic and diabetic patients will have the same need for belonging and the sense of belonging, but they will differ in this respect from healthy individuals, whose need for belonging is stronger (Hagerty and Patusky 2003). Diabetic patients are supposed to express a higher degree of Present-Fatalism than asthma patients and healthy individuals due to the fact that the daily functioning of diabetic patients involves more severe restrictions (Al-kalemji et al. 2014; Petersen 2008). Asthma patients are expected to concentrate more on future than diabetic patients, because they are unable to monitor their disease course, and asthma attacks are unpredictable and troublesome. As diabetic patients have to monitor their blood glucose level daily, they can concentrate more on present events than asthmatic patients do (Present-Fatalistic) (Zimbardo and Boyd 2009). What is more, it can be expected that asthmatic patients will be more highly motivated to set goals and achieve them, as their prognoses involve more long-lasting health improvement in comparison to diabetic patients' prognoses.

3 Methodological Tools

3.1 Study Group

The study was conducted in conformity with the 1989 Declaration of Helsinki on Human Experimentation formulated by the World Medical Association and was approved by the local Ethics Committee.

Ninety persons participated in the research: 30 asthmatic patients, 30 diabetic patients, and 30 persons not suffering from any chronic somatic diseases who constituted the reference group. The first step of statistical elaboration was to compare the asthma patients with the control group, while the second step consisted of comparing the asthma and diabetic patients. The mean age in the groups was the following: asthma (30.5 ± 11.5 years), diabetes (27.4 ± 10.9 years), and controls (28.5 ± 10.2 years). Overall, there were 71 women and 19 men, about evenly ascribed to each group. All of them were informed of anonymous participation in the research. Part of the research was conducted in the hospital (asthma group), while the diabetic and control groups responded to the questionnaires *vi*a the internet.

3.2 Psychometric Tools

The research tools used in the study are the following:

- The sense of belonging and the need for belonging were assessed by means of the original tool, *Sense of Belonging Instrument* (SOBI), created by Hagerty and Patusky (1995). The tool consists of 31 statements which are evaluated by the respondents on a four-point Likert scale.

- The time perspective was investigated by means of a shortened version of *Short Zimbardo Time Perspective Inventory* (SZTPI-PL). The version consists of 15 statements (Cybis et al. 2012).
- Hope for success was measured by means of the Hope for Success Questionnaire (Łaguna et al. 2005), a Polish version of *The Hope Scale* (Snyder et al. 2000b) in which the respondents score their responses on an eight-point rating scale.

Data were given as means ± SD of raw scores tallied from the questionnaires and were statistically compared with a *t*-test if distributed normally, or with the Mann-Whitney U test in case of skewed distribution. A p-value <0.05 defined statistically significant differences.

4 Results and Discussion

We found significant differences in the present-fatalistic attitude among the three groups of subjects investigated in that the asthmatic patients showed a lower level of fatalism than that present in the diabetic patients. The latter were not different in this respect compared with the control healthy individuals. The asthmatic patients also showed a clear tendency for a better outlook for the future, surpassing even the healthy subjects, than the diabetic ones, although the difference between the two reached just borderline significance. No differences regarding the sense of belonging or the need for belonging were found between the asthmatic and diabetic patients or between either group of patients and the healthy subjects. There were no differences among the three groups of subjects regarding the expression of hope either (Table 1).

The results of this study demonstrate differences in the perception of the time perspective between asthmatic and diabetic patients. The asthmatic patients perceive the present situation in a definitely less fatalistic way than patients suffering from diabetes. The asthmatic patients also concentrate more on, and reach out toward, the future than diabetic patients. That can happen for several reasons. Firstly, asthma seems to be a disease with a better prognosis of at least temporary recovery and daily functioning improvement, which creates favorable conditions for making plans and expectations to appear (Zimbardo and Boyd 2009). It seems that the results are also related to the effectiveness of asthma treatment. Beside, asthmatic symptoms may hamper the patients' daily functioning less than diabetic symptoms do. It can also be assumed that asthmatic attacks, being spectacularly violent, make other people immediately offer help and care for the patient, which minimizes fatalistic perception of the present, and makes the patient feel better and be ready to make plans for the future. The diabetic patients, on the other hand, presented a decidedly fatalistic attitude toward the present. That could be explained by the patients' concentration on everyday impediments related to the necessity of monitoring the disease course. Diabetic symptoms are more difficult to observe and less spectacular, but at the same time they are severe and life-threatening. Because of that, the

Table 1 Psychological attitudes of asthmatic and diabetic patients compared with the healthy controls

	Asthma group ($n = 30$)	Control group ($n = 30$)	Diabetes group ($n = 30$)	p-value
Present-fatalistic	5.3 ± 2.0	5.6 ± 2.7	5.8 ± 1.9	Asthma *vs.* diabetes p = 0.02[a]
Future orientation	12.1 ± 2.4	11.5 ± 3.0	10.8 ± 3.0	Asthma *vs.* diabetes p = 0.07
Sense of belonging	56.9 ± 11.7	53.6 ± 12.8	53.7 ± 17.9	Asthma *vs.* control p = 0.32
Need for belonging	40.1 ± 5.4	39.5 ± 5.1	40.3 ± 4.9	Asthma *vs.* diabetes p = 0.92
Hope	66.5 ± 11.8	66.2 ± 9.4	64.1 ± 14.3	Asthma *vs.* diabetes p = 0.56

Data are means ± SD of raw scores; *t*-test was used for the comparison of scores for sense of belonging and Mann-Whitney *U* test was used for the remaining comparisons

[a]statistically significant difference

patients, even in a deteriorating health condition, do not get outright support and care comparable to those the asthma patients do, which could constitute the reason for the expression of a more fatalistic orientation by diabetics patients. By the same token, the future seems to them less important, as their main problem is the present health condition. The patients' ability to perceive the present world in a relatively positive way shows that they believe in other people's kindness and in positive aspects of life. In diabetes, a high score in the present-fatalistic scale may be associated with a tendency to perceive the world as unfavorable and unpredictably threatening.

Fatalism concerning the present experience observed in diabetic patients can also be related to their sense of helplessness for the future prospects. It is possible that perception of difficulties in diabetic treatment may negatively affect the patients and make their recovery more difficult. In the light of the results of the present study, one can conclude that diabetic patients, in comparison to asthmatic and healthy individuals, decidedly more often give up making future plans and concentrate on the present, which they perceive as fatalistic. According to Zimbardo and Boyd (2009) in exceptionally difficult conditions fatalism may have a realistic character. It means that such a pattern of daily functioning can be followed even by persons with a high resistance to adversities. Lack of interest in the future can be related to depression as well as to submission to the disease.

The results of the present study further show that asthmatic patients did not differ from the diabetic ones in terms of the sense of belonging or the need for belonging. Hagerty and Patusky (2003) have argued that the sense of belonging is related to the patients' need for acceptance and support at the time of illness, which is fulfilled in the relation with medical staff. It was an unexpected finding that the results of chronic disease patients' did not differ from those of healthy individuals. It is thus likely that the health care perceived by the patient gives him a sense of belonging and a feeling of being important comparable to those experienced by a healthy individual. Lack of differences among the groups studied regarding the level of hope indicates that despite the identified difficulties, all patients were able to realize their demands, to look for solutions, and to follow the therapeutic programs to the same extent. The results show that the patients' determination to take up a challenge is similar to that of healthy individuals.

The findings of the present study may be useful for psychosocial support of patients suffering from chronic diseases. In case of asthma, psychological interventions should focus on the patients' potential to set and achieve future goals, which might accelerate their recovery. In case of diabetes, the care givers and medical staff alike should psychologically support the patients by expressing an interest in therapy and everyday functioning in their own environment. The therapy should involve activities aimed at changing the patients' fatalistic attitude and building their belief that the outcome will be positive. We conclude that different chronic diseases need different strategies of psychological and social support to be employed by the patients' surrounding and medical staff.

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

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Exacerbations of Chronic Obstructive Pulmonary Disease and Quality of Life of Patients

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Abstract

Exacerbations of chronic obstructive pulmonary disease (COPD) are one of the most important factors which influence the course of disease and quality of life in COPD patients. The aim of the study was to assess the exacerbation frequency in COPD patients in relation to COPD severity and to evaluate the impact of the number of exacerbations on quality of life. The study included 445 COPD patients in all four progressive stages of the disease according to GOLD classification. The patients recorded exacerbations in diaries. Spirometry, St. George's Respiratory Questionnaire, and dyspnea score were assessed at baseline and after 12 and 24 months from enrollment. After 24 months, 261 diaries were returned. The mean number of exacerbations per year in the sequential GOLD 1–4 stages of COPD was as follows: 1.3 ± 2.1, 1.4 ± 2.0, 1.7 ± 1.8, and 3.4 ± 4.5. A statistical difference in the exacerbation frequency was noted for GOLD 4 and the remaining groups. A significant negative correlation was found between the number of exacerbations and functional status for GOLD 2 and 3 stages. We conclude that the number of exacerbations is the highest in the most severe stage of the disease. The quality of life of patients with moderate and severe COPD correlates negatively with the number of exacerbations.

Keywords

Chronic obstructive pulmonary disease • Dyspnea • Exacerbation • GOLD staging • Quality of life • Spirometry • St. George's Respiratory Questionnaire

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1 Introduction

Exacerbations of chronic obstructive pulmonary disease (COPD) significantly influence the

natural course of the disease. The Global Initiative for Obstructive Lung Disease (GOLD) defines COPD exacerbation as an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication. According to the current GOLD classification, patients are classified to a high-risk group after a single COPD-related exacerbation requiring hospitalization (GOLD 2014). On average, patients with moderate-to-severe COPD experience two to three exacerbations per year (Burge and Wedzicha 2003). However, the estimate is that as much as 50 % of all exacerbations are not reported by patients who increasingly accept the symptoms over the disease course and do not perceive them as problematic (Wedzicha and Donaldson 2003). The frequency of COPD exacerbations is known to be associated with increased severity of the disease (Halpin et al. 2012). Exacerbations are also associated with a greater annual decline in the force expiratory volume in 1 s (FEV_1) (Halpin et al. 2012; Hoogendoorn et al. 2010), particularly in smokers (Donaldson et al. 2002; Kanner et al. 2001). Studies demonstrate that in some patients the post-exacerbation spirometry results do not return to the pre-exacerbation levels. Seemungal et al. (2000) have shown that the morning peak expiratory flow (PEF) does not recover to the pre-exacerbation level within 90 days in 7.1 % of patients. The highest decline in PEF is observed in patients who report increased dyspnea, wheezing, and symptoms of cold. Additionally, COPD exacerbations have a negative influence on the patients' quality of life (Llor et al. 2008; Seemungal et al. 1998). This is particularly pronounced in patients with severe COPD (FEV_1 35–50 % predicted), admitted to hospital, and those who experience exacerbations in the autumn and winter (Miravitlles et al. 2004). The number of COPD exacerbations is a predictor of increased mortality. Singanayagam et al. (2013) have listed GOLD 4 stage of severity, age, male sex, low body mass index, cardiac failure, chronic renal failure, long-term oxygen therapy, lower limb edema, cor pulmonale, acidemia, and elevated plasma troponin level as the major prognostic factors associated with increased short-term mortality in COPD patients with acute exacerbation. The mortality rate 2 years post-exacerbation is influenced by the age, low body mass index, cardiac failure, diabetes, ischemic heart disease, cancer disease, FEV_1, long-term oxygen therapy, and the level of partial oxygen tension in the arterial blood on admission.

The aim of the present study was to assess the frequency of COPD exacerbation in relation to the stage of disease severity and to evaluate the impact of exacerbations on the health-related quality of life (HRQoL).

2 Methods

The study was approved by the Review Board of the Medical University of Warsaw (permit no. KB/207/2008). There were 445 COPD patients included into the study who were at all stages of the disease according to the GOLD classification (GOLD 2014). The mean age of the patients was 66.4 ± 9.2 years (range 38–87). Concerning the smoking habit the mean number of pack/years was 38.5 ± 22.9 (range 0–225).

The patients completed a questionnaire at the first visit, reporting the number of COPD exacerbations within the preceding 24 months, and were asked to record all ongoing exacerbations and hospitalizations in a diary. The patients underwent spirometry and a bronchial reversibility test at the study beginning – Visit 1, after 12 months – Visit 2, and 24 months – Visit 3. The test was performed according to the guidelines of the Polish Respiratory Society (Tomalak et al. 2006). Dyspnea was assessed with the modified Medical Research Council (mMRC) scale (Mahler and Wells 1988) and the patients were asked to complete the St. George's Respiratory Questionnaire (Jones et al. 1991). After 24 months 261 (58.6 %) diaries were returned and analyzed.

Normally distributed variables, as assessed by the Shapiro-Wilk test, were given as means ± SD and a skewed distribution was represented

as medians and interquartile ranges. Student's *t*-test or Wilcoxon's test was used for inter-group comparisons depending on the respective distribution of variables. Spearman's rank coefficient was employed for the assessment of correlations. Statistical significance was set at $p < 0.05$. Statistica for Windows software (StatSoft, Inc. ver. 10) was used for all data elaboration.

3 Results

According to the self-reported exacerbation rate in the 24 months preceding the study onset, the patients experienced a mean of 8.7 ± 8.0 (1–17) exacerbations. In the group of 261 patients who returned their diaries after the following 2-year study period, the exacerbation frequency per year was as follows: GOLD 1: 1.3 ± 2.1; GOLD 2: 1.4 ± 2.0; GOLD 3: 1.7 ± 1.8; GOLD 4: 3.4 ± 4.5; disease severity was assessed according to the GOLD guidelines (GOLD 2010). There were no significant differences in the exacerbation rate among the first three GOLD severity stages, but the number of exacerbations was significantly higher in GOLD 4 when compared with GOLD 1–3 stages ($p < 0.01$).

As expected, COPD severity had an impact on the quality of life domains assessed in the St. George's Questionnaire (Table 1).

We did not show any correlations between the number of exacerbations, on the one side, and the individual components of the St. George's Respiratory Questionnaire and pulmonary function tests, on the other side, in the patients at GOLD 1 and GOLD 4 stages. However we did find a number of correlations in the patients at GOLD 2 and GOLD s stages. In GOLD 2, the number of exacerbations correlated with symptoms ($r = 0.33$; $p < 0.0001$), impact of the disease on functional status ($r = 0.34$; $p < 0.0001$), and health status total score ($r = 0.32$; $p = 0.0002$) in St. George's Respiratory Questionnaire, and with pre-bronchodilator vital capacity (VC) ($r = -0.31$; $p = 0.0001$), post-bronchodilator VC ($r = -0.28$; $p = 0.0006$), pre-bronchodilator forced expiratory volume in 1 s (FEV_1) ($r = -0.2$; $p = 0.01$), and post-bronchodilator FEV_1 ($r = -0.22$; $p = 0.004$) in pulmonary function tests. In GOLD 3, the number of exacerbations correlated with symptoms ($r = 0.24$; $p = 0.01$), activity ($r = 0.32$; $p = 0.008$), impact of the disease on functional status ($r = 0.21$; $p = 0.03$), and health status total score ($r = 0.26$; $p = 0.0007$) in St. George's Respiratory Questionnaire as well as with the mMRC score ($r = 0.21$; $p = 0.03$).

An additional analysis was performed for patients with 0–1 exacerbation ($n = 190$; 72.8 %) and ≥2 exacerbations *per* year ($n = 71$; 27.2 %). The mean age was comparable in both groups. There were more women than men among those with ≥2 exacerbations per year (35.3 *vs.* 23.3 %, respectively; $p < 0.05$). The mean FEV_1 was lower in those with ≥2 exacerbations than with 0–1 exacerbations *per* year (1.2 ± 0.5 L *vs.* 1.5 ± 0.6 L; $p < 0.01$; 46.3 ± 16.7 % predicted *vs.* 53.1 ± 18.5 % predicted; $p < 0.01$, respectively) (Table 2). No differences were found in the number of hospitalizations. There were more patients treated with inhaled steroids and theophylline in the group with ≥2 exacerbations *per* year. These patients also had higher scores in

Table 1 St. George's Respiratory Questionnaire scores in relation to the GOLD stages of COPD severity

	GOLD 1	GOLD 2	GOLD 3	GOLD 4
Symptoms	46.9 ± 19.6	51.9 ± 21.7	58.1 ± 18.5**	66.2 ± 23.7***
Activity	47.4 ± 25.8	60.1 ± 20.3	67.9 ± 19.1***	80.7 ± 13.1***
Impact	25.9 ± 16.4	34.4 ± 17.1*	43.0 ± 19.0***	57.4 ± 16.3***
Total	36.6 ± 1.4	45.1 ± 15.7*	52.9 ± 16.1***	66.2 ± 13.1***

Symptoms: **$p \leq 0.01$ for GOLD 3 *vs.* GOLD 2, and ***$p \leq 0.001$ for GOLD 4 *vs.* GOLD 3
Activity: ***$p \leq 0.001$ for both GOLD 3 *vs.* GOLD 2 and GOLD 4 *vs.* GOLD 3
Impact: *$p \leq 0.05$ for GOLD 2 *vs.* GOLD 1, and ***$p \leq 0.001$ for GOLD 3 *vs.* GOLD 2 and GOLD 4 *vs.* GOLD 3
Total: *$p \leq 0.05$ for GOLD 2 *vs.* GOLD 1, and ***$p \leq 0.001$ for GOLD 3 *vs.* GOLD 2 and GOLD 4 *vs.* GOLD 3

Table 2 Comparison of post-bronchodilator spirometry in patients with 0–1 exacerbations and ≥2 exacerbations *per* year

Exacerbations	0–1/year	≥2/year	p
FEV_1 (L)	1.5 ± 0.6	1.2 ± 0.5	<0.01
FEV_1 (% pred)	53.1 ± 18.5	46.3 ± 16.7	<0.01
FVC (L)	2.9 ± 0.9	2.5 ± 0.9	<0.01
FVC (% pred)	81.0 ± 21.5	73.2 ± 2.2	<0.01

FEV_1 forced expiratory volume in 1 s; forced vital capacity

Table 3 St. George's Respiratory Questionnaire score in patients with 0–1 exacerbations and ≥2 exacerbations *per* year

Exacerbations	0–1/year	≥2/year	p
Symptoms	49.4 ± 20.4	61.2 ± 25.2	<0.001
Activity	61.7 ± 21.2	75.9 ± 17.1	<0.001
Impact	35.8 ± 18.3	53.2 ± 17.3	<0.001
Total	46.1 ± 18.8	61.6 ± 14.4	<0.001

Data are means ± SD

the quality of life domains in the St. George's Respiratory Questionnaire (Table 3).

The number of self-reported exacerbations ranged from 0 to 8 *per* year. On Visit 1, 92 patients reported 1 exacerbation (62 % hospitalizations) and 5 patients reported 8 exacerbations (60 % hospitalizations). On Visit 2 after 12 months into the study, 73 patients reported 1 exacerbation (29 % hospitalization); the maximal number of 5 exacerbations was reported only by 1 patient (100 % hospitalizations). On Visit 3 after 24 months, 48 patients reported 1 exacerbation (35 % hospitalizations); the maximal number of 6 exacerbations was reported by 1 patient again (100 % hospitalizations). No significant differences in the quality of life score in the St. George's Respiratory Questionnaire and the perception of dyspnea scale in the Medical Research Council Questionnaire were noted throughout the study period (Table 4).

4 Discussion

The present study confirmed that the risk of exacerbation increases with COPD severity and that exacerbations have an impact on patients' quality of life. Alonso et al. (2004) have shown that chronic conditions worsen quality of life. This applies not only to COPD but also to other chronic diseases. In COPD patients, quality of life is influenced not only by the disease and its severity, but also by numerous co-morbidities (Black-Shinn et al. 2014; Burgel et al. 2013). In general, the self-perceived quality of life tends to be lower in female patients with COPD (Antonelli-Incalzi et al. 2003). Quality of life quantified in the St. George's Respiratory Questionnaire, designed to measure the health-related status of patients with respiratory diseases (Black-Shinn et al. 2014), strongly correlates with the BODE index (Medinas Amoros et al. 2009) which is considered to be a reliable indicator of prognosis and mortality risk in COPD. The present findings confirmed that the quality of life assessed by St. George's Respiratory Questionnaire declines with COPD severity. However, we failed to show any effect of advancing time of disease on quality of life and perception of dyspnea during 2 years of follow-up.

The present observations on the impact of COPD severity and exacerbation rate on quality of life concur with the results of other authors. Hoogendoorn et al. (2010) have reported that the number of COPD exacerbations increased with the severity of the disease, ranging from 0.8 in mild COPD, to 2.1 exacerbations *per* year in very severe COPD. In a study by Solem et al. (2013), the number and severity of exacerbations significantly affected quality of life in severe and very severe COPD patients, and a positive correlation was revealed between the total score on the St. George's Respiratory Questionnaire and the burden of exacerbations over the past 12 months. Seemungal et al. (1998) have shown that the quality of life was significantly lower in patients with at least 3 exacerbations over the past 12 months, both treated and untreated, as compared with those who experienced fewer exacerbations. A correlation between quality of life and COPD severity and exacerbation has also been demonstrated by Jones et al. (2011). Esteban et al. (2009) have revealed that exacerbation-related hospitalization is an

Table 4 Results of St. George's Respiratory Questionnaire and Medical Research Council (mMRC) scales at the study beginning (Visit 1), after 12 months (Visit 2) and 24 months (Visit 3)

	Symptoms	Activity	Impact	Total	mMRC
Visit 1	56.2 ± 21.7	66.0 ± 21.2	40.8 ± 19.8	51.0 ± 18.0	2.1 ± 1.1
Visit 2	58.6 ± 20.8	62.2 ± 2.6	39.6 ± 20.5	49.8 ± 18.6	2.0 ± 1.0
Visit 3	58.1 ± 21.9	60.8 ± 19.6	39.5 ± 18.9	48.5 ± 17.2	2.1 ± 1.0
	NS	NS	NS	NS	NS

Data are means ± SD; *NS* non-significant

independent factor, irrespective of FEV_1, age, and co-morbidities, affecting health-related quality of life. In the present study, the number of COPD exacerbations was significantly higher in patients being at GOLD 4 stage of severity compared to those at GOLD 1–3 stages. Patients with ≥2 exacerbations *per* year had a lower FEV_1 and higher scores in all domains assessed by the St. George's Respiratory Questionnaire. Of note, the number of women in this group was higher than in the group with less frequent exacerbations, i.e. 0–1 exacerbation *per* year. That is consistent with the findings of Jenkins et al. (2012) who have found that female gender is conducive to frequent exacerbations. Müllerová et al. (2014) have also found there was a female predominance in patients who had a higher exacerbation rate. In contrast, Suissa et al. (2012) have demonstrated that men are more likely to experience exacerbation recurrence; moreover, increased risk of exacerbation-related mortality may also be related with male gender.

In the present study, the highest scores were seen in the activity domain of the St. George's Respiratory Questionnaire, irrespective of the time point and subgroup analyzed. That is an important issue as physical inactivity due to dyspnea, muscle weakness, and anxiety deepen sedentarism and cause progressive deconditioning. Troosters et al. (2013) have emphasized that loss of activity in COPD patients carries the risk of hospitalization and increases all-cause mortality. COPD patients with impaired activity are prone to depression (Iguchi et al. 2013), which may also predispose to COPD exacerbations (Laurin et al. 2012).

In conclusion, frequency of COPD exacerbations increases with the severity of the disease. Female gender seems to contribute to more frequent exacerbations. Exacerbations have an impact on the quality of life in patients with COPD.

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Influence of Sensory Stimulation on Exhaled Volatile Organic Compounds

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Abstract

The real-time exhaled volatile organic compounds (VOCs) have been suggested as a new biomarker to detect and monitor physiological processes in the respiratory system. The VOCs profile in exhaled breath reflects the biochemical alterations related to metabolic changes, organ failure, and neuronal activity, which are, at least in part, transmitted *via* the lungs to the alveolar exhaled breath. Breath analysis has been applied to investigate cancer, lung failure, and neurodegenerative diseases. There are by far no studies on the real-time monitoring of VOCs in sensory stimulation in healthy subjects. Therefore, in this study we investigated the breath parameters and exhaled VOCs in humans during sensory stimulation: smell, hearing, sight, and touch. Responses sensory stimulations were recorded in 12 volunteers using an iAQ-2000 sensor. We found significant effects of sensory stimulation. In particular, olfactory stimulation was the most effective stimulus that elicited the greatest VOCs variations in the exhaled breath. Since the olfactory pathway is distinctly driven by the hypothalamic and limbic circuitry, while other senses project first to the thalamic area and then re-project to other brain areas, the findings suggest the importance of olfaction and chemoreception in the regulation lung gas exchange. VOCs variations during sensory activation may become putative indicators of neural activity.

Keywords

Breath • Hearing • Perception • Sensory stimulation • Smell • Volatile organic compounds

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1 Introduction

Ninety nine percent of the exhaled breath matrix is composed of few compounds such as the inorganic gasses nitrogen, oxygen, and carbon dioxide, and water vapor and inert gases. The residual part consists of a mixture of many molecules: non-volatile organic compounds, e.g., leukotrienes, prostaglandins, and serotonin, and volatile organic compounds (VOCs), e.g., aldehydes, ketones, and benzene derivatives, and others (Phillips et al. 1999; Solga and Risby 2010; Mazzatenta et al. 2013a). VOCs are both exogenous and endogenous. The former are derived from the environment or human habits, e.g., smoking. The latter are a result of body metabolic processes, e.g., glucose degradation leads to the formation of acetone, cholesterol biochemical pathway produces the mevalonic acid derive isoprene, or peroxidation of fatty acids produces alkanes. Gas exchange at the alveolar-blood capillary membrane of the lung is a passive diffusion driven by concentration gradients. Following the vital gasses oxygen and carbon dioxide, molecules present in the blood can diffuse passively into the breath. In normal subjects, more than 3400 different VOCs can be detected in exhaled breath (Mazzatenta et al. 2013a). The VOCs profile in exhaled breath reflects the biochemical alterations related to metabolic changes, organ failure, or neuronal dysfunction in disease, which are, at least in part, transmitted *via* the lung to the alveolar exhaled breath, even at the very onset of disease (Mazzatenta et al. 2013a, b, c, 2015b). In recent studies VOCs analysis has been applied to neurodegenerative diseases (Ionescu et al. 2011; Tisch et al. 2013; Mazzatenta et al. 2015b) and cognition (Mazzatenta et al. 2013c). Interestingly, VOCs evaluation in healthy centenarians shows a peculiar pattern in comparison with young and senior controls (Mazzatenta et al. 2015a). Neuronal metabolism in disease induces VOCs alterations, as suggested by the studies above outlined, but there is apparent lack of studies on normal neuronal activity and VOCs release. Therefore, in the present study we investigated the exhaled VOCs in real-time during sensory stimulations.

2 Methods

This observational, non-invasive, and anonymous study included 12 volunteers, (6 males and 6 females, age range 25–32 years). The volunteers provided written informed consent and the procedure was performed in agreement with the Ethical Standards of the Helsinki Declaration for Human Experimentation. Study protocol was accepted by an institutional Review Board for Research.

Breath pattern and exhaled breath content of VOCs were recorded for 10 min in a standard controlled condition, in the morning between 10 and 11 a.m. in a VOCs free room, monitored before each subject by a control recording of the environmental air. In addition, other physical parameters (T, brightness) that could affect the VOC recording were controlled. Sensory stimulation, taking 1–3 s, were the following: citral – a volatile liquid with a lemon-like odor, hand clap, flashing lights, and hand touch. The recording system used was an iAQ-2000 (Applied Sensor, Warren, NJ) equipped with a metal oxide semiconductor (MOS) having a sensing range of 450–2000 ppm CO_2 equivalents, which is able to detect a broad range of volatile compounds (both organic and inorganic, e.g., alcohols, aldehydes, aliphatic hydrocarbons, amines, aromatic hydrocarbons, ketones, organic acids, and CO), while correlating directly with the CO_2 levels (Mazzatenta et al. 2013a, b, c, 2015a, b). The entire datasets were initially analyzed by MANOVA. Then, a series of *post-hoc* one-way ANOVA was performed. Data treatment and statistical analysis were done by Excel, Origin, and SPSS software, the α-level was set at 0.05. Data normalization was made by $\log_{10}$.

3 Results

There were no appreciable differences in breathing frequency and peak inspiratory resistance, the latter was taken as a surrogate of the volume component of breathing pattern, between the baseline level and that after consecutive sensory

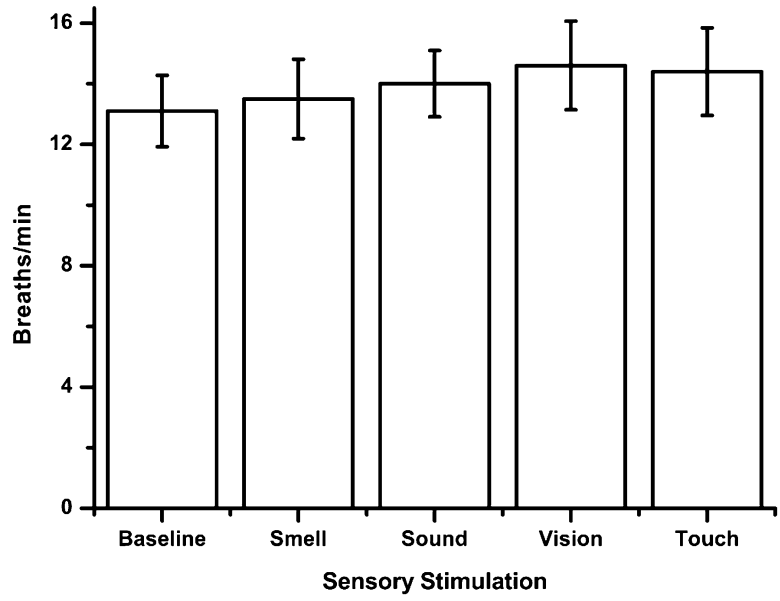

Fig. 1 Breathing frequency during sensory stimulations

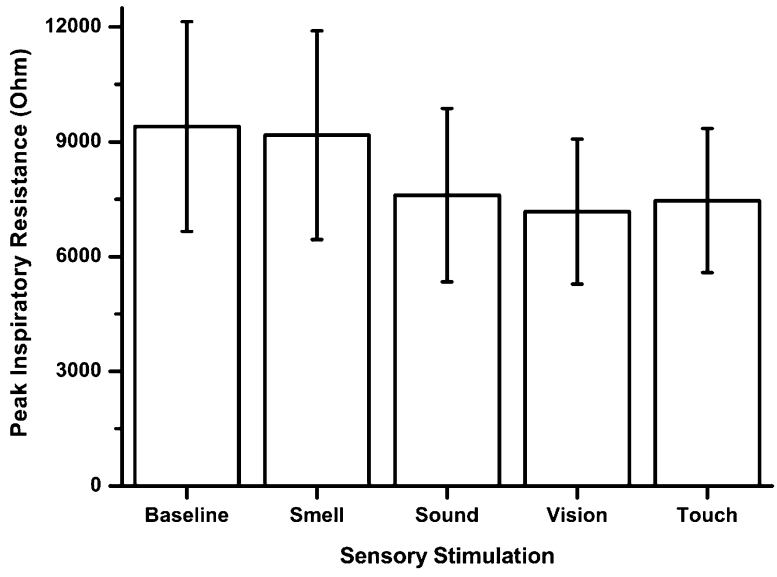

Fig. 2 Inspiratory resistance, taken as a surrogate of the volume component of respiratory pattern, during sensory stimulations

stimulations (Figs. 1 and 2). In contrast, we found substantial differences in the VOCs real-time recording between the baseline and sensory stimulation patterns $F_{(4.15)} = 285.3$, $p < 0.001$. A series of *post-hoc* one-way ANOVA analyses showed the differences at $p < 0.001$: between baseline, on the one side, and olfactory ($F_{(1.58)} = 629.5$), auditory ($F_{(1.58)} = 158.1$), visual ($F_{(1.58)} = 170.1$), and tactile ($F_{(1.58)} = 41.7$) stimulations (Fig. 3).

4 Discussion

In the present study we investigated whether sensory stimulation could affect the content of

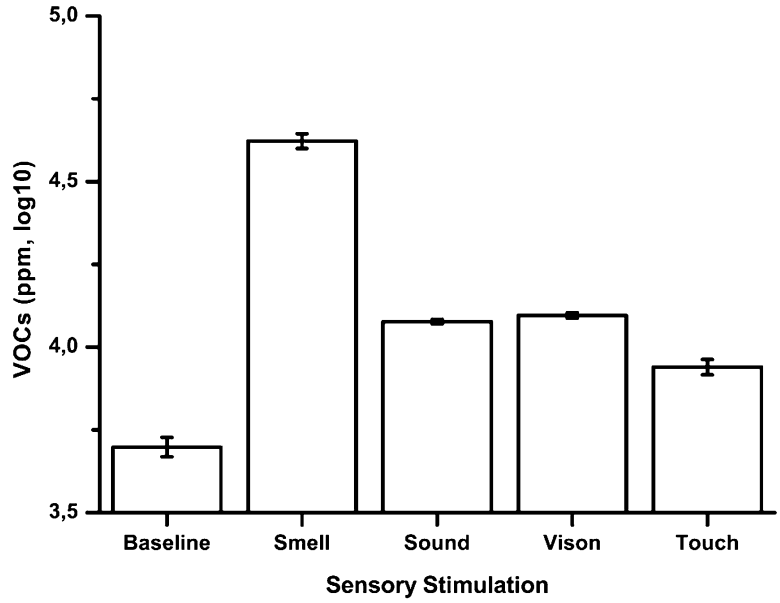

Fig. 3 Real-time exhaled volatile organic compounds (VOCs) during sensory stimulations. Stimulation of each sensory system significantly enhanced VOCs compared with the baseline level ($p < 0.001$)

VOCs in exhaled breath on the premise that the VOCs content could reflect variations in neuronal metabolism altered through transmission of sensory receptors activity to the perceptive brain areas. We found a significant increase in the total amount of VOCs exhaled during sensory stimulation. In particular, the olfactory stimulation was much effective in increasing VOCs. That effect could result from the geometry of olfactory neural circuitry which does not pass through the thalamus, but rather projects straight to the limbic system, which is contrast to the other sensory modalities investigated in the present study. The variations in exhaled VOCs content we recorded could not be confounded by altered breathing pattern, since the sensory stimulations were short in time so much that they failed to affect the breathing pattern.

In recent studies, investigation of exhaled VOCs has been applied to neurodegenerative diseases and cognitive disturbances in diabetes type 2, which highlights that these compounds vary in concentration and fingerprint in a range of pathological conditions (Ionescu et al. 2011; Tisch et al. 2013; Mazzatenta et al. 2013c, 2015b). However, the unanswered question remained of whether VOCs content could also be influenced due to activation of neuronal metabolism during peripheral sensory stimulation in healthy subjects. The present investigation provided a confirmatory answer to that issue. These findings broaden our knowledge of the link between neural metabolism and exhaled VOCs alterations. The study also highlights the olfactory function as being notably sensitive and influential in producing VOCs alterations in exhaled breath.

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

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