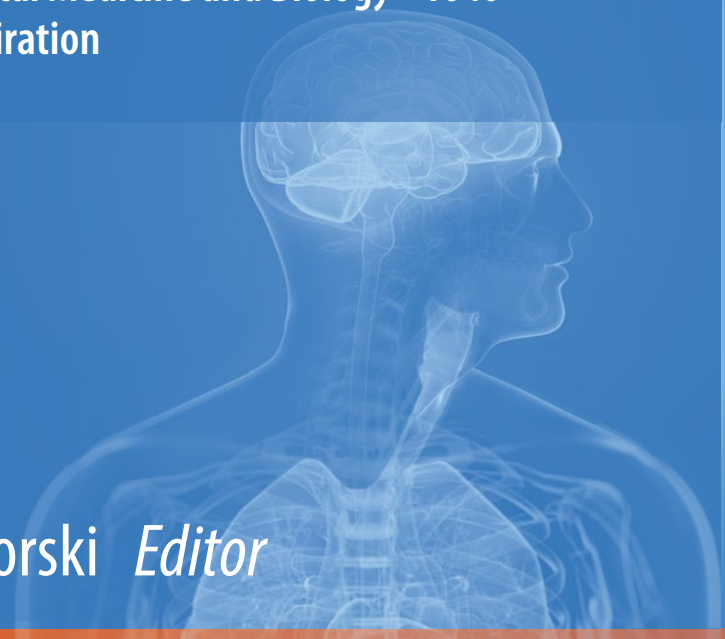
Advances in Experimental Medicine and Biology 1040
Neuroscience and Respiration



Mieczyslaw Pokorski *Editor*

# Clinical Research Involving Pulmonary Disorders

Springer

# Advances in Experimental Medicine and Biology

## Neuroscience and Respiration

Volume 1040

**Subseries Editor**
Mieczyslaw Pokorski

More information about this series at http://www.springer.com/series/13457

Mieczyslaw Pokorski
Editor

# Clinical Research Involving Pulmonary Disorders

Springer

*Editor*
Mieczyslaw Pokorski
Opole Medical School
Opole, Poland

ISSN 0065-2598 ISSN 2214-8019 (electronic)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-74091-1 ISBN 978-3-319-74092-8 (eBook)
https://doi.org/10.1007/978-3-319-74092-8

Library of Congress Control Number: 2018944153



Printed on acid-free paper

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

# Preface

The book series Neuroscience and Respiration presents contributions by expert researchers and clinicians in the multidisciplinary areas of medical research and clinical practice. Particular attention is focused on pulmonary disorders as the respiratory tract is up front at the first line of defense for organisms against pathogens and environmental or other sources of toxic or disease-causing effects. The articles provide timely overviews of contentious issues or recent advances in the diagnosis, classification, and treatment of the entire range of diseases and disorders, both acute and chronic. The texts are thought as a merger of basic and clinical research dealing with biomedicine at both molecular and functional levels and with the interactive relationship between respiration and other neurobiological systems, such as cardiovascular function, immunogenicity, endocrinology and humoral regulation. The authors focus on modern diagnostic techniques and leading-edge therapeutic concepts, methodologies, and innovative treatments in both adults and children. Practical, data-driven options to manage patients are considered.

Body functions, including lung ventilation and its regulation, are ultimately driven by the brain. However, neuropsychological aspects of 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 health is undeniable. Scientists accept a powerful psychological connection that can directly affect our quality of life and health span. Psychological approaches, which can decrease stress, can play a major role in disease therapy.

Clinical advances stemming from molecular and biochemical research are but possible if research findings are translated into diagnostic tools, therapeutic procedures, and education, effectively reaching physicians and patients. All this cannot be achieved without a multidisciplinary, collaborative, bench-to-bedside approach involving both researchers and clinicians. The role of science in shaping medical knowledge and transforming it into practical care is undeniable.

Concerning respiratory disorders, their societal and economic burden has been on the rise worldwide, leading to disabilities and shortening of life-span. Chronic obstructive pulmonary disease (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 articles published in this series will assume a leading position as a source of information on interdisciplinary medical research advancements, addressing the needs of medical professionals and allied health-care workers, and become a source of reference and inspiration for future research ideas.

I would like to express my deep gratitude to Paul Roos, Tanja Koppejan, and Cynthia Kroonen of Springer Nature NL for their genuine interest in making this scientific endeavor come through and in the expert management of the production of this novel book series.

Mieczyslaw Pokorski

# Contents

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 1–5
DOI 10.1007/5584_2017_96

Published online: 10 September 2017

# Peripheral Arterial Tonometry in Pulmonary Vasculitis

A. Falkowski, K.A. Wardyn, and K. Życińska

**Abstract**

Vascular wall inflammation in primary vasculitides results in diminished vessel dilation and finally impaired blood flow, causing multiple organs dysfunction and ultimate damage. In granulomatosis with polyangiitis (GPA), the inflammatory process concerns small and medium sized vessels and its pulmonary location is often predominant. The pivotal role in the development of that pathology plays vascular endothelium. Endothelial vasodilatory function strongly depends on the instant production and release of nitrogen oxide (NO), a potent local factor controlling vascular tonus. NO output is triggered by a variety of stimuli, especially by ischemia. The endothelial vasodilatory ability can be measured indirectly by a few of methods, one of them is peripheral arterial tonometry (PAT). The method assesses reactive hyperemia, mediated mostly by NO release, as a response to vessel occlusion. The vasodilatory reaction depends on the quality of the endothelium which deteriorates with time of GPA disease progression. The aim of the present study was to estimate a correlation between the clinical status, reflected by the disease extent index (DEI), and the vasodilatory endothelial function reflected by the index of arterial reactive hyperemia (RHI), measured by PAT in 27 patients with GPA, having a significant pulmonary involvement. We found a moderate inverse correlation between DEI and log-transformed RHI ($r = -0.46$, $p < 0.05$). The conclusion is that impaired endothelial function, as assessed by RHI-PAT, might predict the GPA progression.

**Keywords**

Endotheium • Granulomatosis • Inflammation • Polyangiitis • Pulmonary vasculitis • Vascular wall • Vasodilation • Tonometry

## 1 Introduction

According to the Chapel Hill Consensus, granulomatosis with polyangiitis (GPA) is defined as 'necrotizing granulomatous inflammation usually involving the respiratory tract, and necrotizing vasculitis affecting predominantly small-to medium-sized vessels' (Jennette 2013). There is a necrotic inflammatory process in GPA running in the vessel walls, with the accompanying infiltration of neutrophils and the presence of antineutrophil cytoplasmic (cANCA) and perinuclear antibodies (pANCA), and other inflammatory mediators such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), interleukin-1 (IL-1) and IL-6, and monocyte

A. Falkowski (✉), K.A. Wardyn, and K. Życińska
Department of Family Medicine, Internal and Metabolic Diseases, Warsaw Medical University, and Systemic Vasculitis Outpatient Clinic Czerniakowski Hospital, Warsaw, Poland
e-mail: a.falkowski.ptmr@gmail.com

chemoattractant protein-1 (MCP-1) (Lutalo and D'Cruz 2014; Wiatr and Gawryluk 2013; Lembicz et al. 2014). Granulomatous inflammation in the respiratory tract is frequent in the course of GPA. The formation of granulomata and the development of necrotizing vasculitis of small and medium vessels cause vast airway tissue lesions. Pulmonary manifestations of the morbid process in GPA could be also accompanied by renal, ocular, neural, and other organ involvement. The most frequent respiratory symptoms consist of chest pain, cough, dyspnea, inspiratory stridor, and hemoptysis. The symptoms, often times severe, are a clinical manifestation of diffuse alveolar hemorrhage caused by pulmonary capillaritis (Lally and Spiera 2015).

Vascular wall inflammation in primary vasculitides seems associated with atherosclerosis progression, resulting in multi-organ ischemic damage and dysfunction. Yet it is not regarded as a classic risk factor in cardiovascular diseases. A pivotal role in both development of atherosclerosis and vasculitis plays a local availability of endothelium-derived nitric oxide. The impaired nitric oxide-mediated vasodilation is a feature of symptomatic organ ischemia. Structural changes in the vessel wall, resulting in enhanced thickness and calcification, play a role in histological underpinnings of the process (Blum and Nahir 2013). Due to a vast vascular involvement, GPA is bound to increase the risk of myocardial and brain infarctions (Aviña-Zubieta et al. 2016).

The assessment of endothelial vasodilatory function is based on a variety of research methods, most of them are experimental, difficult to calibrate, and not standardized, between users and laboratories. That is a reason of as yet non-employment of endothelial function indices in routine clinical diagnostics. The methods are usually based on complicated invasive and time consuming procedures. The exemplary may be the intracoronary acetylcholine challenge consisting of Doppler measurement of the left coronary artery blood flow, before and after acetylcholine infusion in incremental concentrations by a catheter positioned in the artery origin (Suwaidi et al. 2000; Ludmer et al. 1986). Flow mediated dilation ultrasonography is an example of a non-invasive method that consists of the assessment of changes in the brachial artery diastolic diameter in response to a blood flow stimulus. That method, however, strongly depends of the quality of an ultrasound system and the operator's skills (Wilk et al. 2013). There is an array of other experimental methods, less frequently employed in the assessment of endothelial function, such as infrared and near-infrared imaging, electron beam computed tomography, magnetic resonance imaging, and the molecular imaging techniques.

Peripheral arterial tonometry (PAT) is an alternative for the methods outlined above. It is a non-invasive and operator independent method. PAT has been validated against the intracoronary acetylcholine challenge method, taken as a reference, and shows 82% sensitivity and 77% specificity (Bonetti et al. 2004). The method assesses digital pulsatile volume changes, using plethysmography probes mounted on fingertips, caused by vasodilation and reactive hyperemia after a transient brachial artery occlusion. There are significant associations between the brachial artery flow-mediated dilatation, expressed as percent dilatation (FMD %) and fingertip PAT expressed as log-reactive hyperemia index and cardiac mortality risk (Dahle et al. 2014).

The disability equality index (DEI) is a sum of organ and system assessment scores, and it has been designed and validated for use in patients with systemic vasculitis with granulomatosis. This complex index is highly reproducible and associates with cANCA titer, leukocyte and platelet count, and is a reliable research instrument to compare different clinical trials (Merkel et al. 2009; de Groot et al. 2001). Nonetheless, due to a clinical complexity of GPA, there is an apparent need of a simpler and reliable measure that would enable the quantitative assessment of the disease extent. The aim of the present study was to estimate of a correlation between clinical status of GPA patients, assessed by the DEI index and by the log-transformed PAT reactive hyperemia index (LnRHI) in response to occlusion of brachial artery in the arm.

## 2 Methods

The study was approved by the Ethics Committee of Warsaw Medical University in Warsaw, Poland and it was conducted in accord with the Declaration of Helsinki for Human Research of the World Medical Association. Twenty seven patients with the diagnosis of pulmonary vasculitis with granulomatosis were enrolled into the study (16 women and 11 men) of the mean age of 48.6 years (range 24–77 years). The diagnosis was based on Chapel Hill Consensus diagnostic criteria for GPA. The endothelial function was measured during routine hospitalization using peripheral arterial tonometry (EndoPAT 2000 system; Itamar Medical Co, Caesarea Ind. Park, Israel). During the test, left brachial artery was occluded and tonometric signal was recorded in most cases from the index fingertip on the same side. The measurement on other arm that remained unoccluded, closely akin to the tested arm, served as a reference for signal comparison (Fig. 1). In addition, DEI forms also were filled out by the patients. In the analysis of the results of PAT measurements we took into consideration correlations between the following variables: DEI index, log-transformed RH-PAT index (RHI), cANCA, and pANCA titrates, and LDL plasma concentration (Fig. 2). The equality of variances was verified with Levene's test and the normality assumption was evaluated using Shapiro-Wilk's test. The level of statistical significance was set at $p = 0.05$.

## 3 Results

We found a moderate inverse correlation between DEI and LnRHI indices; $r = -0.47$, $p < 0.05$ (Pearson's coefficient) (Fig. 3). There was no significant correlation between LnRHI and any of the essential biochemical markers of

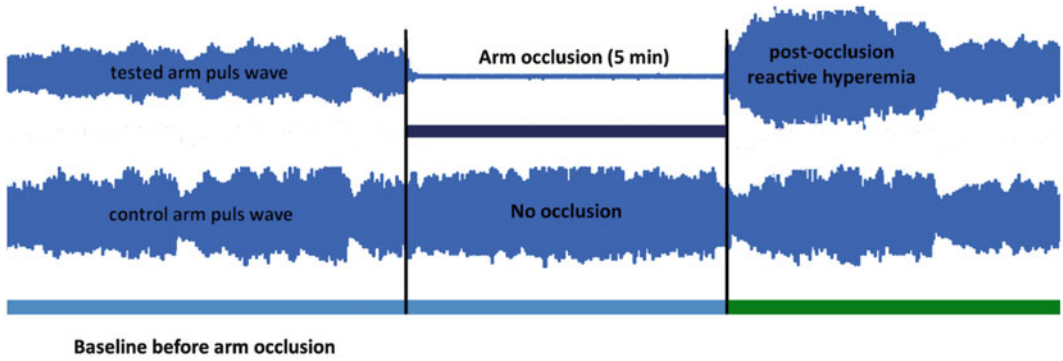



**Fig. 1** Exemplary fingertip recordings of peripheral arterial tonometry (PAT); pulse wave signals from tested arm with occlusion (*top*) and control arm without occlusion (*bottom*)

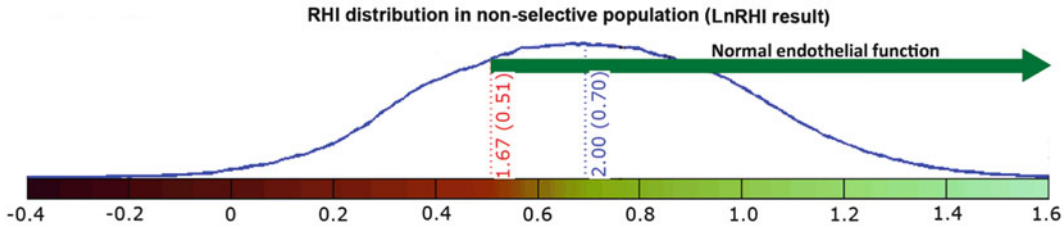



**Fig. 2** Graphical representation of digital transformation of peripheral arterial tonometry (PAT) signal. Reactive hyperemia index (RHI) of endothelial function; natural log-transformed RHI (LnRHI) in parenthesis. Norm: RHI > 1.67 (LnRHI >0.51). The right-hand (*blue*) *vertical line* corresponds to the central measure of RHI distribution

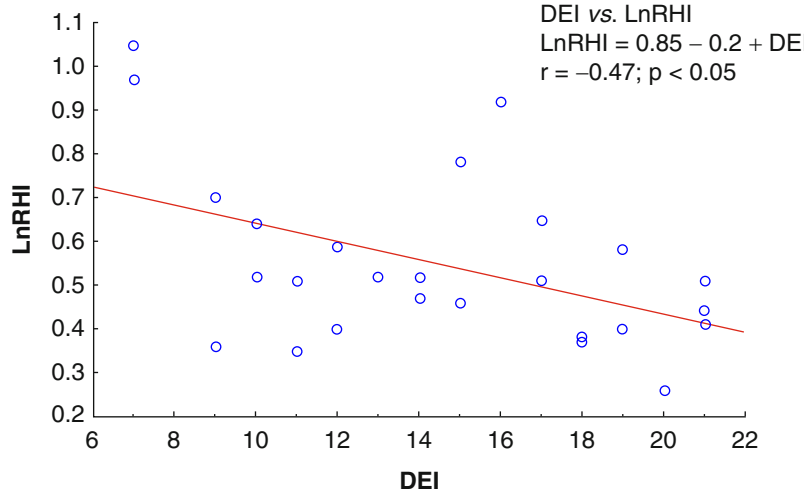



**Fig. 3** Correlation between disability equality index (*DEI*) and natural log-transformed reactive hyperemia index (*LnRHI*)

granulomatosis with polyangiitis (GPA) such as low-density lipoproteins (LDL), cANCA, or pANCA.

## 4 Discussion

GPA is a multisystem disease whose etiology needs a further exploration. Clinical and experimental data point to a number of possible pathogenic pathways and the 'endothelial trace' seems is one of them (Csernok and Gross 2013). The lack of an adequate vasodilatory response to the ischemic stimulus in small vessels has been primarily observed in conditions related to atherosclerosis, especially in coronary artery disease (CAD). Peripheral arterial tonometry has proven a valuable and reliable diagnostic tool in detecting endothelial vasodilatory dysfunction in patients with CAD. In the present study, 18.5% of the participating patients had a confirmed CAD, with no history of myocardial infarction in any of them. Nonetheless, atherosclerotic involvement in patients with GPA could bias the PAT results. The PAT methodology makes it rather impossible to separate the contribution of atherosclerosis and vasculitis to impaired endothelial vasodilation. In PAT employed in the present experiment, with brachial artery occlusion and signal recording from a fingertip, reactive hyperemia index correlated significantly with disease extent index; the latter being a synthetic assessment based on multisystem clinical symptoms reflecting disease progression. The reactive hyperemia index, however, did not correlate with any of the essential biochemical markers of GPA, including ANCA antibodies titrates. This preliminary study is a prospective cross-sectional observation. A longitudinal assessment during the course of GPA development would give a more accurate insight into the correlation between the reactive hyperemia and disease extent indices, and would better elucidate the predicting value of the former index in GPA progression. Despite the limitations above outlined, PAT seems to be a promising methodology for a clinical assessment in patients with GPA.

We conclude that impaired endothelial function reflected by reactive hyperemia in peripheral arterial tonometry may be of help in the diagnostics of GPA and in the assessment of GPA progression.

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

## References


Aviña-Zubieta JA, Mai A, Amiri N, Dehghan N, Tan JA, Sayre EC, Choi HK (2016) Risk of myocardial infarction and stroke in patients with granulomatosis with polyangiitis (Wegener's): a population-based study. Arthritis Rheumatol 68(11):2752–2759

Blum A, Nahir M (2013) Future non-invasive imaging to detect vascular plaque instability and subclinical non-obstructive atherosclerosis. J Geriatr Cardiol 10 (2):178–185

Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, Kuvin JT, Lerman A (2004) Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. J Am Coll Cardiol 44(11):2137–2141

Csernok E, Gross WL (2013) Current understanding of the pathogenesis of granulomatosis with polyangiitis (Wegener's). Expert Rev Clin Immunol 9(7):641–648

Dahle DO, Jenssen T, Holdaas H, Asberg A, Soveri I, Holme I, Mjoen G, Eide IA, Pihlstrom H, Dörje C, Halden TA, Hartmann A (2014) Uric acid and clinical correlates of endothelial function in kidney transplant recipients. Clin Transpl 28(10):1167–1176

de Groot K, Gross WL, Herlyn K, Reinhold-Keller E (2001) Development and validation of a disease extent index for Wegener's granulomatosis. Clin Nephrol 55 (1):31–38

Jennette JC (2013) Overview of the 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides. Clin Exp Nephrol 17(5):603–606

Lally L, Spiera RF (2015) Pulmonary Vasculitis. Rheum Dis Clin N Am 41(2):315–331

Lembicz M, Batura-Gabryel H, Nowicka A (2014) Granulomatosis with polyangiitis - clinical picture and review of current treatment options. Pneumonol Alergol Pol 82(1):61–73. (Article in Polish)

Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, Ganz P (1986) Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Eng J Med 315 (17):1046–1051

Lutalo PM, D'Cruz DP (2014) Diagnosis and classification of granulomatosis with polyangiitis (aka Wegener's granulomatosis). J Autoimmun 48-49:94–98

Merkel PA, Cuthbertson DD, Hellmich B, Hoffman GS, Jayne DR, Kallenberg CG, Krischer JP, Luqmani R, Mahr AD, Matteson EL, Specks U, Stone JH (2009) Comparison of disease activity measures for antineutrophil cytoplasmic autoantibody (ANCA)-associated vasculitis. Ann Rheum Dis 68(1):103–106

Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A (2000) Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 101(9):948–954

Wiatr E, Gawryluk D (2013) Primary systemic ANCA-associated vasculitis--recommendations concerning diagnosis and treatment. Pneumonol Alergol Pol 81 (5):479–491. (Article in Polish)

Wilk G, Osmenda G, Matusik P, Nowakowski D, Jasiewicz-Honkisz B, Ignacak A, Czesnikiewicz-Guzik M, Guzik J (2013) Endothelial function assessment in atherosclerosis: comparison of brachial artery flow-mediated vasodilation and peripheral arterial tonometry. Pol Arch Med Wewn 123(9):443–452

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 7–12
DOI 10.1007/5584_2017_87

Published online: 15 August 2017

# Predominance of Comorbidities in the Detriment of Daily Activity in Sarcoidosis Patients

S. Kostorz, D. Jastrzębski, M. Sikora, A. Zebrowska, A. Margas, D. Stepanik, H. Swinder, and D. Ziora

**Abstract**

Sarcoidosis may affect lung function, working ability, overall mobility, and daily activity. In the present study we performed an analysis of clinical settings in patients with sarcoidosis to disentangle its influence on daily physical activity (PA). PA assessment (number of steps per day, daily energy expenditure) was performed by accelerometry during consecutive 7 days after discharge from hospital. Thirty patients with sarcoidosis, aged 46.4 ± 10.5, were enrolled in the study. Clinical data (age, gender, steroid consumption, weight, and comorbidities), lung function tests (forced expiratory volume in one second – FEV1, forced vital capacity – FVC, and lung diffusion for carbon monoxide – $DL_{CO}$), mobility (6-minute walk test – 6 MWT) and physical performance (oxygen consumption at anaerobic threshold – $VO_2$/AT) were estimated. The mean daily PA (5214 ± 2699 steps/day) and $VO_2$max (22.3 ± 7.0 ml/kg/min) were lower when referenced to the age-group predicted values. A significant greater daily PA was observed in sarcoidosis patients without comorbidities compared with those having more than two comorbidities ($p = 0.046$). No association was found between steroid use, lung function, and 6MWT. Daily PA was associated with patients aerobic efficacy and $VO_2$max ($r = 0.38$, $p < 0.04$). The findings demonstrate a significant influence of comorbidities on sarcoidosis patients' exercise tolerance and daily PA. Special treatment considerations, including the potential impact of comorbidities, may help optimize exercise regimes, link physical activity with health, and prevent sarcoidosis complications.

**Keywords**

Accelerometry • Comorbidities • Exercise tolerance • Lung function • Physical activity • Sarcoidosis

## 1 Introduction

Sarcoidosis is a multisystem granulomatous disease with a wide range of symptoms, including fatigue, pain, reduction of muscle strength and exercise tolerance, and dyspnea. Although many

S. Kostorz, D. Jastrzębski (✉), A. Margas, D. Stepanik, H. Swinder, and D. Ziora
School of Medicine with the Division of Dentistry, Department of Lung Diseases and Tuberculosis, Medical University of Silesia, 1 Koziołka Street, 41-803 Zabrze, Poland
e-mail: darekjdr@poczta.onet.pl

M. Sikora and A. Zebrowska
Department of Physiological and Medical Sciences, The Jerzy Kukuczka Academy of Physical Education, 72A Mikolowska Street, 40-065 Katowice, Poland

organs can be affected, most common the lungs and lymph nodes are afflicted. Comorbidities are presented relatively often in sarcoid patients; in a range of 30–70% of cases (Pohle et al. 2016). The influence on lung function of sarcoidosis is well known, leading to restrictive or obstructive changes in the airways, depending on the disease stage. A diminution of working ability is common, with a different range of fatigue that also may be present at rest (Jastrzebski et al. 2015). It seems reasonable that all these changes take a toll on physical activity (PA) of sarcoidosis patients.

Physical activity monitors have been introduced into scientific and clinical practice in the last decade. These instruments are now validated, as their acceptability and usability are well assessed (Rabinovich et al. 2012). Recently, some authors have reported a diminution of PA in sarcoidosis patients (Judson 2015; Saligan 2014). However, little is known about the underlying reasons of PA impairment. In the present study we attempted to sort out the crucial factors that affect the loss of PA in sarcoidosis patients.

## 2 Methods

### 2.1 Subjects

The study was approved by the Bioethics Committee of the Medical University of Silesia, Poland (no. KNW/0022/KB1/32/12) and written informed consent was obtained from all patients. Thirty, non-smoking, consecutive patients, aged 30–60 (mean age of 46.4 ±10.5 years), with a newly diagnosed (up to 6 months) sarcoidosis (in II stage) according to the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) criteria were enrolled to this prospective, observational study (Hunninghake et al. 1999). The study was conducted at the Department of Lung Diseases and Tuberculosis of the Medical University of Silesia with cooperation of the Department of Physiological and Medical Sciences of the Jerzy Kukuczka Academy of Physical Education from January 2014 till December 2015. Only white collar, non-smoking patients were included into the study. Patients were divided into 3 groups according to the number of theirs comorbidities. Group I represented seven patients without comorbidities. Group II comprised patients with one comorbidity, i.e., with arterial hypertension (eight patients) and diabetes (three patients), and Group III comprised 12 patients with sarcoidosis and two or more comorbidities, mainly arterial hypertension, diabetes, and heart insufficiency.

### 2.2 Physiological Measurements

Spirometry was performed using a Lungtest apparatus (MES; Cracow, Poland) in accordance with the ATS/ERS guidelines (Miller et al. 2005). The obtained results of forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1) and forced expiratory volume in one second % of vital capacity (FEV1/FVC) were expressed as a percentage of predicted values according to Quanjer et al. (1993). The diffusing capacity of lung for carbon monoxide ($DL_{CO}$) was measured by a single-breath method. These results were expressed as % of predicted according to the European Community for Coal and Steel (Quanjer et al. 1993). The ergospirometry test following the Bruce protocol was also performed. $VO_2$max was measured with a gas analyzer (Metalyzer 3B-2R; Cortex Biophysik GmbH, Leipzig, Germany). Heart response was continuously monitored with a 12-lead electrocardiogram. The effort test was maintained until exhaustion. The anaerobic threshold (AT) and its relation to maximal oxygen consumption ($VO_{2max}$/AT; ml/min) were calculated according to Wasserman et al. (1999). Additionally in all patients, the 6-minute walk test (6MWT) was conducted according to the 2002 American Thoracic Society guidelines (Crapo et al. 2002).

PA was measured by actigraphy for seven consecutive days after discharge from the hospital in the patients' own environment using the ActigraphGT3x + (ActiGraph LLC, Pensacola, FL). The ActiGraph GT3X+, in conjunction with the ActiLife analysis software, delivers

objective 24-h physical activity and sleep/wake measurements including raw acceleration, activity counts, energy expenditure, physical activity, and body position. In the study we used a mean daily step count (steps/day), daily energy expenditure (kcal/day), and daily metabolic equivalents (MET). The mean values averaged over the seven consecutive days were presented.

### 2.3 Statistical Evaluation

Data are presented as means ± SD. The intergroup correlation coefficients were determined with Spearman's rank correlation coefficient. Statistical significance was set at $p < 0.05$. Statistical analysis was performed using a commercial Statistic package (StatSoft, Tulsa, OK).

## 3 Results

The demographic and clinical data of sarcoidosis patients are presented in Table 1.

Significant differences were observed among Groups I, II, and III in PA measured by steps *per* day. The number of steps made *per* day was greater in Group I (7333 ± 3265) compared with both Group II (5239 ± 2352) and Group III (3954 ± 1940), with the difference between Group I and Group III being significant at $p < 0.05$ (Fig. 1).

**Table 1** Demographic and clinical data of 30 sarcoidosis patients

| Variable | Means ±SD |
|---|---|
| BMI (kg/m$^2$) | 27.9 ± 3.5 |
| Age (yr) | 46.4 ± 10.5 |
| FEV1 (%) | 93.1 ± 16.5 |
| FVC (%) | 99.8 ± 16.6 |
| FEV1/FVC (%) | 76.6 ± 5.7 |
| $DL_{CO}$ (%) | 91.8 ± 26.7 |
| 6MWT (m) | 548.5 ± 57.0 |
| $VO_2$max (ml/kg/min) | 22.3 ± 7.0 |
| $VO_2$/AT (ml/kg/min) | 13.3 ± 4.0 |
| Work (Wat) | 189.9 ± 88.9 |
| Steps/day | 5214 ± 2699 |
| Kcal/day | 350.0 ± 285.7 |
| MET/day | 1.6 ± 0.3 |

*BMI* body mass index, *FEV1* forced expiratory volume in one second, *FVC* forced vital capacity, *FEV1/FVC* forced expiratory volume in one second % of vital capacity, *6MWT* six-minute-walk test, $DL_{CO}$ diffusion capacity, $VO_{2max}$ maximal oxygen consumption, $VO_2/AT$ oxygen consumption at anaerobic threshold, *Kcal* kilocalories, *MET* metabolic equivalent

### 3.1 Correlations of Spirometry and Exercise Tolerance

Only did two patients exceeded 10,000 steps *per* day, which is considered the minimum of daily activity. The number of steps *per* day ranged from 13835 to 2428 (the mean of 5214 ± 2699) in the entire study sample. There was no differences in the number of steps regarding gender, weight, or age.

The mean value of BMI was 27.9 kg/m$^2$. No correlation between PA and BMI was noticed ($r = 0.02$, $p = 0.90$). No correlations were observed between PA (steps/day) and spirometric variables (FEV1, FVC, and FEV1/FVC; r= -0.05, p=0.81; r = -0.16, p = 0.42; and r = 0.16, p = 0.41, respectively). Diffusion capacity was reduced ($DL_{CO}\% < 80\%$) in eight patients. No correlation between PA (steps/day) and $DL_{CO}$ was observed ($r = -0.15$, $p = 0.43$).

The mean value of distance in 6MWT was 548 ± 57 m. In nine patients, a shortened distance of <500 m was observed. We did not observed a distance under 400 m in any of the patients. There was no correlation between PA (steps/day) and distance in 6MWT ($r = 0.01$, $p = 0.62$) (Table 2).

$VO_2$max is a crucial parameter that indicates exercise capacity and varies depending on age and gender. Taking into account the variables assessed, none of the patients manifested good exercise capacity according to Wasserman et al. (1999). Seven patients had exercise capacity classified as low, 13 patients had sufficient-low physical efficiency and only 10 patients had medium exercise capacity. The mean daily PA (5214 ± 2699 steps/day) and $VO_2$max (22.3 ± 7.0 ml/kg/min) were lower compared with predicted values (Table 1). A significant correlations was observed between the number

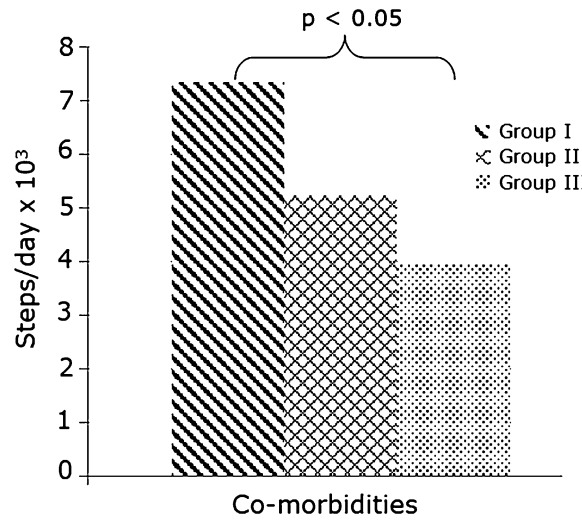



**Fig. 1** Number of steps *per* day in reference to comorbidities; $p < 0.05$ for the difference between Group I and Group III

**Table 2** Correlations between demographic data, clinical settings, and physical activity in patients with sarcoidosis

| | | Kcal | Steps | MET |
|---|---|---|---|---|
| Age | *CC* | −0.08 | −0.12 | −0.19 |
| | *SL* | 0.66 | 0.54 | 0.30 |
| BMI | *CC* | 0.25 | 0.02 | 0.14 |
| | *SL* | 0.19 | 0.90 | 0.47 |
| FVC | *CC* | −0.11 | −0.16 | 0.02 |
| | *SL* | 0.58 | 0.42 | 0.93 |
| FEV1 | *CC* | 0.04 | −0.05 | 0.06 |
| | *SL* | 0.83 | 0.81 | 0.78 |
| FEV1/FVC | *CC* | 0.14 | 0.16 | 0.09 |
| | *SL* | 0.49 | 0.41 | 0.66 |
| $DL_{CO}$ | *CC* | −0.06 | −0.15 | −0.05 |
| | *SL* | 0.76 | 0.43 | 0.82 |
| 6MWT | *CC* | 0.11 | 0.10 | 0.05 |
| | *SL* | 0.55 | 0.62 | 0.70 |
| $VO_2$max | *CC* | 0.43 | 0.38 | 0.14 |
| | *SL* | 0.02 | 0.04 | 0.48 |
| $VO_2$AT | *CC* | 0.16 | 0.002 | 0.31 |
| | *SL* | 0.39 | 0.99 | 0.09 |
| Work | *CC* | 0.01 | 0.21 | 0.09 |
| | *SL* | 0.96 | 0.27 | 0.64 |

*CC* correlation coefficient, *SL* significance level, *BMI* body mass index, *FVC* forced vital capacity, *FEV1* forced expiratory volume in one second, *FEV1/FVC* forced expiratory volume in one second % of vital capacity, $DL_{CO}$ diffusion capacity, *6MWT* six minute walk test, *Kcal* kilocalories, *MET* metabolic equivalent, $VO_{2max}$ maximal oxygen consumption, $VO_2AT$ oxygen consumption at anaerobic threshold

of steps/day and $VO_2$max ($r = 0.38$, $p = 0.04$). Also, a significantly higher maximal oxygen uptake was associated with daily energy expenditure (kcal/day) ($r = 0.43$, $p = 0.02$).

## 4 Discussion

Physical activity is defined as any bodily movement produced by the contraction of skeletal muscle that increases energy expenditure above a basal level (Caspersen et al. 1985). An objective approach to measuring physical activity consists of defining a quotidian physical activity in the place of residence, without the influence of temporary emotional state and weakness, changing place or lifestyle, or without any other external factors.

There is a consensus that physical activity is better estimated in a direct measurement by using activity monitors rather than questionnaires (Pitta et al. 2005). In the present study we used Actigraph GT3X setup that demonstrates the best correlation with the outcomes of exercise capacity and a good correlation with the measures of energy expenditure (Rabinovich et al. 2012). Patients wore the activity monitors during wakefulness for seven consecutive days. The monitors measure outputs derived directly from

accelerometer data (steps) and estimate energy expenditure outputs derived from these and other variables (i.e., from anthropometric data of the patient) using prediction equations (kcal and MET). Rabinovich et al. (2012) in their multicenter study have found a good correlation between activity monitor variables and exercise capacity in patients with COPD. In the present study, concerning patients with sarcoidosis, we found correlations between the activity monitors data (steps, kcal, and MET) and variables reflecting the exercise capacity ($VO_2$max, $VO_2$AT, and work) but not lung function tests (FVC, FEV1, and $DL_{CO}$). A limitation of our study is a small number of subjects, i.e., 30 patients in comparison to study of Rabinovich et al. (2012), who have analyzed data from 80 patients with COPD.

Several studies emphasize the association of physical activity and comorbidities (Dorenkamp et al. 2016). Multimorbidity, defined as the coexistence of two or more chronic diseases, is progressively more prevalent with age (Marengoni et al. 2011). However, a growing prevalence of patients with multiple chronic diseases is not only a result of aging and advancing medical care, but also is related to modifiable factors such as unhealthy lifestyle and behaviors. Recently, Dorenkamp et al. (2016) have presented results of SMILE cohort study in which they demonstrate that the lowest rate of physical activity manifest patients with heart diseases, respiratory diseases, and diabetes mellitus. Likewise, in the present study we found significant correlations between daily physical activity and comorbidities. In the two groups of patients with a greater number of comorbidities, physical activity was forceful reduced.

In study of Fleischer et al. (2014) comorbidities have been observed in 83% of sarcoidosis patients, within which 16.3% reported more than three comorbidities. Epidemiologic data reported by Niewiadomska et al. (2016) have estimated the frequency of comorbidity occurrence in sarcoidosis at 32%. That study is based on hospital records in the Silesian voivodeship provided by the National Health Found in Poland. In our opinion, the underestimated frequency of comorbidity occurrence in sarcoidosis patients in the Niewiadomska et al.'s study (2016) is caused by mistakes that used to be frequently seen in medical charts. Those author have reported that the most prevalent comorbidities in sarcoidosis patients were diseases of the cardiovascular system (39.5%), metabolic diseases (22.1%), chronic respiratory diseases (9.3%), and diseases of the musculoskeletal system and connective tissue (8.2%). That is consistent with the results of the present study, in which the cardiovascular and metabolic diseases were observed most frequently in this group of patients.

In conclusion, the present study adds to the knowledge on a relationship between physical activity and comorbidities in patients with sarcoidosis. In addition to the relationship of sarcoidosis and physical activity, the study demonstrates that the cluster of patients with sarcoidosis and cardiovascular or metabolic diseases reported the lowest level of physical activity. Therefore, it is important for specialists, general practitioners, or physiotherapists to support especially those groups of patients in initiating and maintaining the appropriate physical activity level. Monitoring the level of physical activity and the patient's capacity to perform this activity in sarcoidosis may help ensure a more targeted and effective medical care.

**Acknowledgments** This study was supported by grant KNW-1-093/K/6/0 from the Medical University of Silesia, Poland.

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

## References

Caspersen CJ, Powell KE, Christenson GM (1985) Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. Public Health Rep 100(2):126–131

Crapo RO, Casaburi R, Coates AL, Enright PL, MacIntyre NR, McKay RT, Johnson D, Wanger JS, Zeballos RJ (2002) ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 166:111–117

Dorenkamp S, Mesters I, Schepers J, Vos R, van den Akker M, Teijink J, de Bie R (2016) Disease combinations associated with physical activity identified: the SMILE cohort study. Biomed Res Int 2016:9053578. doi:10.1155/2016/9053578

Fleischer M, Hinz A, Brahler E, Wirtz H, Bosse-Henck A (2014) Factors associated with fatigue in sarcoidosis. Respir Care 59(7):1086–1094

Hunninghake GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R, Eklund A, Kitaichi M, Lynch J, Rizzato G, Rose C, Selroos O, Semenzato G, Sharma OP (1999) ATS/ERS/WASOG statement on sarcoidosis. American Thoracic Society/European Respiratory Society/world Association of Sarcoidosis and other granulomatous disorders. Sarcoidosis Vasc Diffuse Lung Dis 16(2):149–173

Jastrzebski D, Ziora D, Lubecki M, Zieleznik K, Maksymiak M, Hanzel J, Poczatek A, Kolczynska A, Nguyen Thi L, Zebrowska A, Kozielski J (2015) Fatigue in sarcoidosis and exercise tolerance, dyspnea, and quality of life. Adv Exp Med Biol 833:31–36

Judson MA (2015) The clinical features of sarcoidosis: a comprehensive review. Clin Rev Allergy Immunol 49 (1):63–78

Marengoni A, Angelman S, Melis S, Mangialasche F, Karp A, Garmen A, Meinow B, Fratiglioni L (2011) Aging with multimorbidity: a systematic review of the literature. Ageing Res Rev 10(4):430–439

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J (2005) Standardisation of spirometry. Eur Respir J 26:319–338

Niewiadomska E, Kowalska M, Zejda JE (2016) Comorbidity diseases in adults with diagnosed interstitial lung diseases among inhabitants of the Silesian voivodeship. Med Pr 67(6):751–763

Pitta F, Troosters T, Spruit MA, Decramer M, Gosselink R (2005) Activity monitoring for assessment of physical activities in daily life in patients with chronic obstructive pulmonary disease. Arch Phys Med Rehabil 86(10):1979–1985

Pohle S, Baty F, Brutsche M (2016) In-hospital disease burden of sarcoidosis in Switzerland from 2002 to 2012. PLoS One 11(3):e0151940. doi:10.1371/journal.pone.0151940

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC (1993) Lung volumes and forced ventilatory flows. Report working party standardization of lung function tests. European Community for steel and coal. Official statement of the European Respiratory Society. Eur Respir J Suppl 16:5–40

Rabinovich RA, Louvaris Z, Raste Y, Langer D, Van Remoortel H, Giavedoni S, Burtin C, Regueiro EM, Vogiatzis I, Hopkinson NS, Polkey MI, Wilson FJ, MacNee W, Westerterp KR, Troosters T (2012) Validity of physical activity monitors during daily life in patients with COPD. Eur Respir J 42(5):1205–1215

Saligan LN (2014) The relationship between physical activity, functional performance, and fatigue in sarcoidosis. J Clin Nurs 23(15–16):2376–2379

Wasserman K, Hansen JE, Sue DY, Casaburi R, Whipp BJ (1999) Principles of exercise testing and interpretation. Including pathophysiology and clinical applications, 3rd edn. Lippincott Williams and Wilkins, Philadelphia

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 13–21
DOI 10.1007/5584_2017_85

Published online: 25 October 2017

# Physical Functioning and Symptoms of Chronic Fatigue in Sarcoidosis Patients

K. Pilzak, A. Żebrowska, M. Sikora, B. Hall, O. Łakomy, S. Kostorz, D. Ziora, and D. Jastrzębski

**Abstract**

Scientific reports underscore the importance of measuring the health-related quality of life in sarcoidosis patients. The present study seeks to define how sarcoidosis patients' quality of life, daily physical activity, and physical performance are related to each other. Seventeen patients (mean age 46.8 ± 8.8 years) suffering from sarcoidosis completed the following questionnaires: the fatigue assessment scale (FAS), the quality of life scale (SF-36 questionnaire), and the Borg dyspnea scale. Physical activity (PA) was assessed using accelerometry. Respiratory function, consisting of forced expiratory volume in one second (FEV1), forced vital capacity (FVC), forced expiratory volume in one second as a percentage of vital capacity (FEV1/%FVC), and diffusing capacity of the lungs for carbon monoxide ($DL_{CO}$), were assessed. In addition, performance in 6-min walk test (MWT), aerobic capacity assessed from maximal oxygen uptake ($VO_2$max), and the metabolic equivalent of task (MET) were evaluated. We found that daily PA (4566 ± 2378 steps/day) and $VO_2$max (21.8 ± 5.9 ml/kg/min) were lower in sarcoidosis patients than the known predicted values in healthy age-matched individuals. There were significant inverse associations between the FAS score and 6MWT ($r = -0.62$; $p < 0.01$), and between SF-36 score and 6MWT ($r = -0.55$; $p < 0.03$). In contrast, SF-36 scores associated with fatigue and dyspnea scores ($r = 0.72$; $p < 0.001$ and $r = 0.85$; $p < 0.001$). These findings imply that sarcoidosis patients are less active compared with healthy subjects. The FAS and SF-36 scales seem to be effective tools for assessing the severity of fatigue in sarcoidosis patients.

**Keywords**

Aerobic capacity • Exercise • Fatigue • Pulmonary sarcoidosis • Quality of life respiratory function

K. Pilzak, A. Żebrowska (✉), M. Sikora, and O. Łakomy
Department of Physiological and Medical Sciences, The Jerzy Kukuczka Academy of Physical Education, 72A Mikolowska Street, 40-065 Katowice, Poland
e-mail: a.zebrowska@awf.katowice.pl

B. Hall
School of Health Sciences, University of Salford, Allerton Building, Frederick Road Campus, Salford M6 6PU, England, UK

S. Kostorz, D. Ziora, and D. Jastrzębski
School of Medicine with the Division of Dentistry, Department of Lung Disease and Tuberculosis, Medical University of Silesia, 1 Koziołka St, 41-803 Zabrze, Poland

## 1 Introduction

Sarcoidosis, a multi-organ disease, is the second most frequent pathological condition involving the respiratory system in young and middle-aged persons after asthma (De Vries et al. 2004). Its clinical picture shows the presence of granulomas in the lymph glands, lungs, eyes, skin, and in other organs. Sarcoidosis may give no symptoms, or have an acute or chronic course. The unspecific symptoms of sarcoidosis include elevated body temperature, muscle pain, general weakness, perspiration, and weight loss (Baughmann et al. 2011). The organ manifestations of respiratory sarcoidosis – the most frequent form of this condition – include the development of dyspnea and progressing circulatory and respiratory insufficiency. Other manifestations include the involvement of lung parenchyma and hilar lymph glands, erythema nodosum in the skin, fever, and joint pain and swelling. All these symptoms comprise the Löfgren syndrome (Judson 2008). Around 10% of sarcoidosis patients are diagnosed with pleural effusion and the involvement of the upper respiratory tract with airway obstruction symptoms. Spirometry shows reduced maximal expiratory flows at $MEF_{50\%FVC}$ and $MEF_{25\%FVC}$ (Radwan et al. 1999). Another element of the respiratory system affected by sarcoidosis is the pulmonary vessels. Clinical conditions that can impair breathing capacity, leading also to lower exercise tolerance and fatigue, are pulmonary hypertension, pulmonary embolism, and granulomatosis with polyangiitis. Reports from studies on sarcoidosis patients point to reduced capacity for physical work and reluctance toward mental work. The psychophysical condition of these patients significantly influences their self-assessment of health and quality of life regardless of the severity of the symptoms (De Kleijn et al. 2009; Drent et al. 1998; Ebrahim 1995). It has been demonstrated that performing the quality-of-life assessment, in addition to functional and clinical assessments, is essential to determine the efficacy of therapies for many chronic conditions, including sarcoidosis (De Vries et al. 2010; Michielsen et al. 2007; Smith et al. 1999). In view of the circumstances outlined above, the present study seeks to define how sarcoidosis patients' quality of life, daily physical activity, and physical performance are related to each another.

## 2 Methods

### 2.1 Subjects

The study protocol was approved by the Bioethics Commission of the Medical University of Silesia in Poland. All participants were familiarized with the rules and goals of the study and signed informed consent. The study was conducted at the Clinic for Pulmonary Diseases and Tuberculosis in Zabrze, Poland The study sample consisted of 17 persons (7 women and 10 men) aged 46.8 ± 8.8 years, with the mean body mass of 81.6 ± 15.1 kg (59% of them were overweight), who suffered from sarcoidosis for no longer than 4 years. All patients were diagnosed with sarcoidosis according to the criteria developed by the American Thoracic Society/World Association for Sarcoidosis ATS/ERS/WASOG (Hunninghake et al. 1999).

The inclusion criteria were as follows:

- a stable period of the disease, without infections or exacerbation in the preceding month;
- glucocorticosteroids and immunosuppressants not administered in the past;
- baseline medical examination showing no indications for treatment;
- cardiorespiratory function satisfying the requirements of a graded exercise test;
- motor function satisfying the requirements of a graded exercise test.

The exclusion criteria included:

- ischaemic heart disease, heart failure, and severe pulmonary hypertension;
- serious liver dysfunction and diabetes;

- cerebral stroke;
- addiction to cigarettes, drugs, or alcohol;
- dementia;
- motor or neurological dysfunction preventing exercise;
- reluctance or lack of consent to cooperate.

## 2.2 Assessment of Pulmonary Function

All subjects were tested for pulmonary function using a Jaeger-Masterlab spirometer (Erich Jaeger GmbH; Warzburg, Germany). Three lung function parameters were measured: forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and forced expiratory volume in one second as a percentage of vital capacity (FEV1/%FVC). The diffusing capacity of the lungs for carbon monoxide ($DL_{CO}$) was measured using a single-breath method. Results were normalized to the reference values proposed by the European Community for Coal and Steel and were presented as percentage of the predicted values (Quanjer et al. 1993).

## 2.3 Assessment of Physical Capacity

All the patients performed a graded treadmill exercise test during which their maximal oxygen uptake ($VO_2$max) and the metabolic equivalent of task (MET) were measured. To assess physical efficiency, a six-minute walk test (6MWT) was employed. The 6MWT was conducted according to the 2002 American Thoracic Society guidelines which consider the distance walked in meters (Frost et al. 2005; Gibbons et al. 2001). Heart rate (HR) was monitored and the spirometric and gasometric parameters were recorded at rest, during, and post-exercise (Ergospirometr Metalyzer 3B-2R; Cortex, Leipzig, Germany). After a three-minute warm-up on the treadmill, exercise intensity was increased according to the Bruce protocol. Participants exercised until they reached the maximum workload they could tolerate or until voluntary muscle failure. Maximal oxygen uptake ($VO_2$max) was determined based on the maximum heart rate criterion (HRmax) and the expiratory exchange ratio (RER) exceeding a value of 1.1 (ACSM 2014; Golding 2000).

## 2.4 Assessment of Physical Activity

Physical activity (PA) of patients was measured using an Actigraph GT3X+ device (Actigraph; Pensacola, FL) that monitors the number of steps and the amount of energy expended during daily physical activity. The primary indicator of PA, i.e., the number of steps/d, was enhanced by daily energy expenditure (kcal/kg/d). The device was activated after the participants were instructed on how to use it and was worn by them for 7 days, from 7 a.m. to 22 p.m. On day 7, the recorded data were downloaded for analysis by a dedicated software (Actilife 6; Actigraph, Pensacola, FL). The analysis was performed by the same investigator who supervised the duration of measurements. The energy expenditure and the number of steps obtained from the accelerometer were compared with values recommended for the age-group investigated (Tudor-Locke et al. 2002).

## 2.5 Questionnaires

All the partients were asked to complete questionnaires assessing their:

- fatigue (the Fatigue Assessment Scale – FAS) with the following score intervals: (10–21 – fatigue is not perceived); (22–34 – fatigue is perceived); (35–50 – a very strong feeling of fatigue) (De Vries et al. 2004);
- dyspnea (the modified analog Borg dyspnoea scale (0–10));
- quality of life (the SF-36 questionnaire enabling the self-assessment of quality of life with respect to the physical domain (SF-physical) and mental domain (SF-mental) divided into 8 subscales).

According to the Polish interpretation of the SF-36, the higher the composite score on the questionnaire's 0–100 scale, the lower quality of life (Tylka and Piotrowicz 2009).

### 2.6 Statistical Analysis

For the quantitative interval variables, descriptive statistics such as means, medians, standard deviations, and extreme values were calculated and then tested for normal distribution using the Shapiro-Wilk test. For the qualitative categorical variables, the number and percentage distributions were determined. Variable correlations for patients' clinical status, experienced fatigue, physical capacity, and quality of life were estimated with the use of canonical correlation analysis, multiple regression analysis, analysis of variance, and non-parametric tests (Spearman's rank correlation, Kruskal-Wallis analysis of variance and chi-square ($\chi^2$) tests of independence). A p-value $< 0.05$ defined statistical significance. Statistical analysis of the data was performed using Statistica v12.5 (StatSoft Polska, Cracow, Poland).

## 3 Results

The sarcoidosis patients' somatic characteristics and results of spirometry examinations are shown in Table 1. The mean FVC amounted to 85.9 ± 35.8%predicted. Symptoms of mild airway obstruction, with FEV1/FVC > 0.7, were recognized in five of the patients. The assessment of the patients' physical performance is shown in Table 2. $VO_2$max obtained from the graded exercise test (ExTest) was 21.8 ± 5.9 (ml/kg/min) and constituted 72.9 ± 21.8% of its predicted value; and HRmax exceeded 90% of the predicted value. The mean daily PA consisting of 4566 ± 2378 steps/day was lower than predicted. The patients were the least active on Sundays (Fig. 1). The result of their 6MWT amounted to 544.3 ± 45.5 m, which was lower than the standard 600.2 ± 102.0 m for their age-group.

**Table 1** Sarcoidosis patients' somatic characteristics and respiratory function

| Variables | Patients ($n = 17$) |
|---|---|
| Age (years) | 46.8 ± 8.8 |
| Height (m) | 1.7 ± 0.1 |
| Weight (kg) | 81.6 ± 15.1 |
| BMI (kg/m$^2$) | 28.0 ± 4.3 |
| FVC (L) | 3.3 ± 1.4 |
| FVC (%pred) | 85.9 ± 35.8 |
| FEV1 (L) | 2.4 ± 1.1 |
| FEV1 (%pred) | 78.0 ± 33.1 |
| FEV1/FVC (%) | 65.0 ± 25.4 |
| $DL_{CO}$ (%pred) | 81.1 ± 27.5 |

Data are means ±SD; *BMI* body mass index, *FVC* forced vital capacity, *FEV1* forced expiratory volume in one second, $DL_{CO}$ the diffusing capacity of the lungs for carbon monoxide

**Table 2** Sarcoidosis patients' physical performance

| Variables | Patients ($n = 17$) |
|---|---|
| $VO_2$ max (mL/kg/min) | 21.8 ± 5.9 |
| $VO_2$ max (%pred) | 72.9 ± 21.8 |
| HR max (beats/min) | 157.1 ± 23.6 |
| HR max (%pred) | 90.4 ± 13.3 |
| $VO_2$/HR max (mL/beats) | 11.5 ± 4.3 |
| $VO_2$/HR max (%pred) | 81.6 ± 25.7 |
| VE/$VO_2$ max (L/min) | 34.9 ± 7.5 |
| VE/$VO_2$ max (%pred) | 40.1 ± 6.4 |
| MET rest | 1.3 ± 0.6 |
| MET max | 6.2 ± 1.7 |
| MET max (%pred) | 72.9 ± 21.7 |

Data are means ±SD; $HR_{max}$ maximum heart rate, $VO_2$ maximal oxygen uptake, *VE* minute ventilation, *MET* the metabolic equivalent of task

The patients had a distinct, albeit moderate, perception of fatigue and dyspnea, with the scores of 29.0 ± 4.0 and 4.1 ± 2.2 points on the FAS and Borg scales, respectively. The Borg score associated with that of FAS ($r = 0.74$; $p < 0.001$) (Fig. 2).

The patients scored 62.0 ± 19.1 on the SF-36. Physical and mental domain scores (53.1 ± 19.3 and 23.5 ± 12.9, respectively) associated with each other ($r = 0.74$; $p < 0.001$), and both mental domain and fatigue scores associated inversely with 6MWT ($r = -0.51$; $p < 0.04$ and $r = -0.62$; $p < 0.01$, respectively) (Fig. 3).

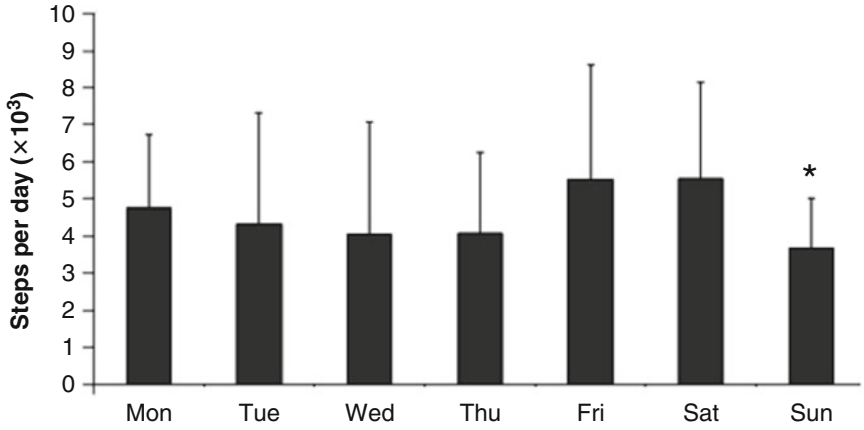



**Fig. 1** Daily physical activity as indicated by the number of steps (Data are means ±SD; *$p < 0.05$ for Sun *vs.* Sat, Fri, and Mon)

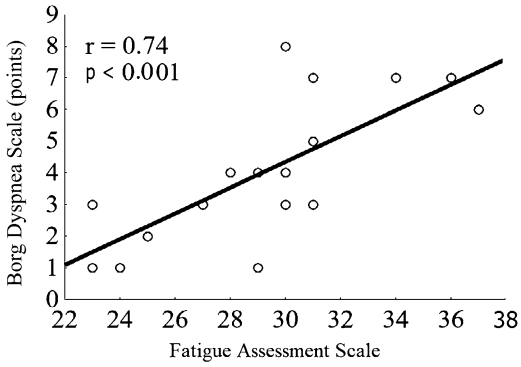



**Fig. 2** Association between fatigue assessment (FAS) and Borg dyspnea scales

The inverse associations between the performance in 6MWT, on the one side, and SF-36 score (r = −0.50; p = 0.03) and its mental domain (r = −0.51; p = 0.04), on the other side, were significant (Fig. 3). Both the Borg score of dyspnea and the FAS score of fatigue associated with the SF-36 score; r = 0.84 ($p < 0.0001$; Fig. 4) and r = 0.72 (p = 0.001; Fig. 5), respectively.

We failed to find any appreciable associations between the sarcoidosis patients' $VO_2$max and self-assessed quality of life or MET. HRmax obtained from the exercise test showed a tendency for inverse associations with both fatigue (r = −0.37) and SF-36 quality of life scores (r = −0.37) (p = 0.10).

## 4 Discussion

Fatigue associated with sarcoidosis, clinically difficult to diagnose and classify, is a recognized issue in its treatment. Nonetheless, research has failed to explain how fatigue relates to the clinical course of sarcoidosis (Jastrzębski et al. 2015; Costabel 2011). The present study was undertaken to assess the efficacy of methods measuring fatigue in sarcoidosis patients and to determine how the patients' self-assessments of fatigue, physical capacity, and performance relate to each other. We demonstrate that a poor performance in 6MWT significantly associated with fatigue and lower SF-36-assessed quality of life and its mental sphere. In addition, dyspnea significantly associated with greater fatigue and lower quality of life. The results of graded exercise and 6-min walk tests demonstrate that the assessment of fatigue is most reliable in the judgment on physical functioning in sarcoidosis patients. The patients' performance in 6MWT in the present study was akin to that reported by Alhamed (2009), who has also found that the 6MWT distance and the Borg dyspnea score are inversely correlated. The fatigue results reported in other studies also show that these patients find physical activity onerous regardless of the level of pulmonary dysfunction, the stage of treatment, or the level of immunological markers (Drent et al. 2012; De Kleijn et al. 2011).

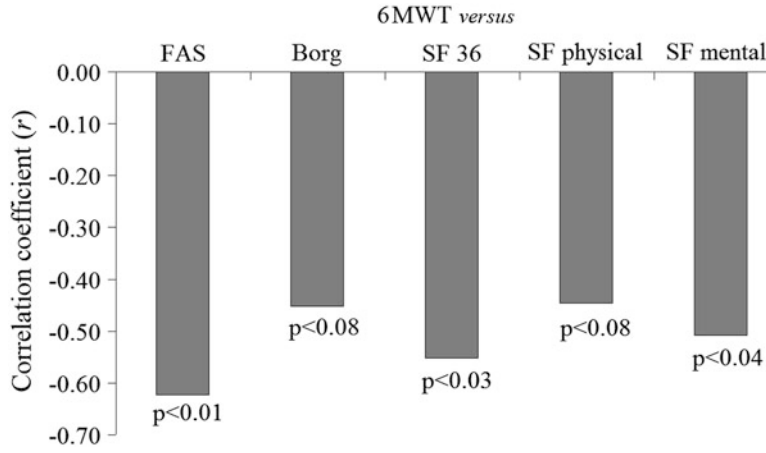



**Fig. 3** Associations the 6-min walk test (6MWT) *vs*. fatigue assessment scale (FAS), Borg dyspnea scale, and quality of life (SF-36) and its domains

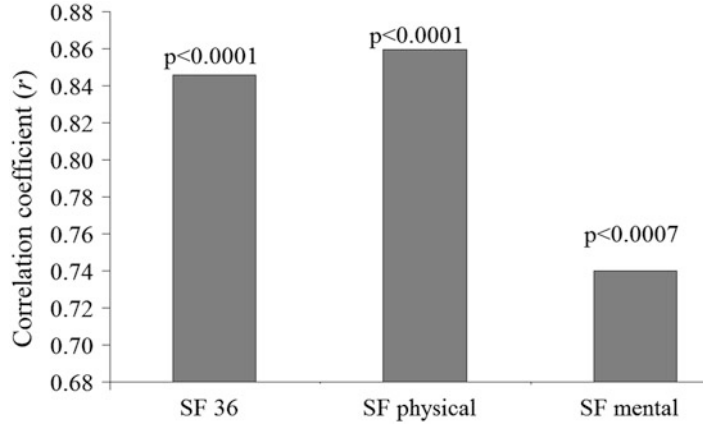



**Fig. 4** Association between SF-36 quality of life and Borg dyspnea scales

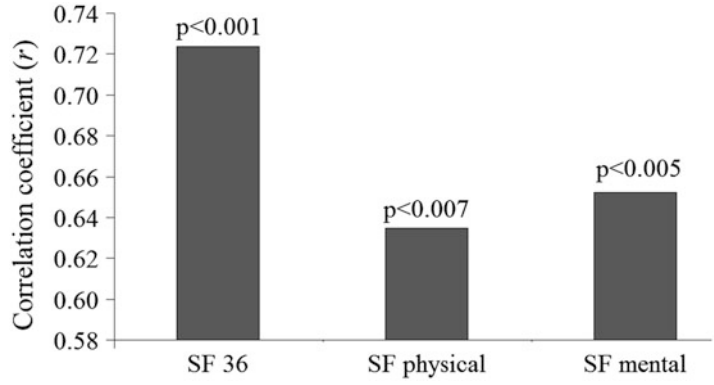



**Fig. 5** Association between SF-36 quality of life and fatigue assessment (FAS) scales

The assessments of fatigue and quality of life are effective tools in predicting the capability of physical functioning in patients suffering from sarcoidosis. We demonstrate that sarcoidosis patients had moderate aerobic insufficiency and failed to achieve the $VO_2$max levels and 6MWT performance typical of their age-group. The patients' daily physical activity was lower than recommended, as also was exercise capacity when compared with the ACSM (2014) and Golding (2000) standards of percent predicted MET. Sarcoidosis is a chronic condition associated with unsolved problems relating to its pathogenesis, treatment, and management. Studies show that a high percentage of sarcoidosis patients have reduced exercise tolerance and perceive tiredness (De Vries et al. 2004). There are no reports pointing to associations between the results of clinical tests, such as pulmonary function, radiography, or inflammatory markers – IL-2 receptors, C-reactive protein, amyloid A – and fatigue. Interestingly, the only variable in this group of patients that correlates with fatigue is a lower diffusing capacity of the lungs for carbon monoxide. The outcomes of other questionnaire surveys involving sarcoidosis patients show that the perception of fatigue, general weakness, and short breath during daily activities erodes the patient motivation for work. Dyspnea and fatigue adversely influence quality of life and, in some cases, lead to depression (Valeyre and Humbert 2012; Sharma 1999; Drent et al. 1998).

It is known that exercise training can improve health, physical capacity, and respiratory muscle function in patients with respiratory conditions, and it can alleviate their feeling of fatigue and dyspnea (Spruit et al. 2013; Langer et al. 2012; Hospes et al. 2009; Baugmann et al. 2007). The research on rehabilitation of patients with respiratory insufficiency demonstrates that accelerometry provides a precise measurement of daily physical activity. The advantage of the method is that it records data over a relatively long period and it enables a 3D analysis of motion (Lores et al. 2006; Nguyen et al. 2006; Coronado et al. 2003; Steele et al. 2003). Daily physical activity of patients with obstructive lung disease correlates with their performance in 6MWT and it is lower compared with that in control subjects (Lores et al. 2006). The association between 6MWT results, which are indicative of exercise tolerance, and self-assessed duration of daily physical activity is reported to be high and positive (Zieleźnik et al. 2015). The knowledge of physical dysfunction and lower quality of life perceived by sarcoidosis patients may be useful in setting the appropriate rehabilitative strategies for them (Lingner et al. 2015).

Michielsen et al. (2007) have studied 150 middle-aged sarcoidosis patients. More than 80% of those patients experienced fatigue and one half of them reported a reduced exercise tolerance. The main causes of lower quality of life in that study were fatigue, dyspnea, reduced physical capacity, and a joint pain. It is noteworthy that fatigue is a major predictor of quality of life and both physical and mental health. In the present study, reduced exercise tolerance also was accompanied by an enhanced perception of fatigue and a reduced quality of life, particularly in its mental dimension. Other authors have found similar associations in sarcoidosis patients, with a somehow higher score on the fatigue assessment scale than that obtained in the present study (Fleisher et al. 2014; Hinz et al. 2011).

In conclusion, the present study demonstrates a substantial reduction in physical capacity and a lower exercise tolerance in middle-aged sarcoidosis patients. Patients' physical capacity was below the standard recommended for age-matched healthy subjects. The fatigue assessment scale and the SF-36 quality of life questionnaire proved useful tools in denoting the self-perceived capability of physical and metal functioning in sarcoidosis patients.

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

## References


ACSM (2014) ACSM's guidelines for exercise testing and prescription, 9th edn. Lippincott Williams & Wilkins, Baltimore

Alhamed EH (2009) The six-minute walk test in patients with pulmonary sarcoidosis. Ann Thorac Med 4 (2):60–64

Baughmann RP, Culver DA, Judson MA (2011) A concise review of pulmonary sarcoidosis. Am J Respir Crit Care Med 183(5):573–581

Baugmann RP, Sparkman BK, Lower EE (2007) Six minute walk test and health status assessment in sarcoidosis. Chest 132(1):207–213

Coronado M, Janssens JP, de Muralt B, Terrier P, Schutz Y, Fitting JW (2003) Walking activity measured by accelerometry during respiratory rehabilitation. J Cardpulm Rehabil 23:357–364

Costabel U (2011) Skeletal muscle weakness, fatigue and sarcoidosis. Curr Probl Psychiatry 12(2):141–145

De Kleijn WP, Elfferich MD, De Vries J, Jonker GJ, Lower EE, Baugmann RP, King TE Jr, Drent M (2009) Fatigue in sarcoidosis: American versus Dutch patients. Sarcoidosis Vasc Diffuse Lung Dis 26(2):92–97

De Kleijn WP, Drent M, Vermunt JK, Shigemitsu H, De Vries J (2011) Types of fatigue in sarcoidosis patients. J Psychosom Res 71(6):416–422

De Vries J, Michielsen HJ, Van Heck GL, Drent M (2004) Measuring fatique in sarcoidosis: the fatigue assessment scale (FAS). Br J Health Psychol 9(3):279–291

De Vries J, Lower EE, Drent M (2010) Quality of life in sarcoidosis: assessment and management. Semin Respir Crit Care Med 31(4):485–493

Drent M, Wrinsberger RM, Breteler MH, Kock LM, de Vries J, Wouters EF (1998) Quality of life and depressive symptoms in patients suffering from sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 15(1):59–66

Drent M, Lower EE, De Vries J (2012) Sarcoidosis-associated fatigue. Eur Respir J 40(1):255–263

Ebrahim S (1995) Clinical and public health perspectives and applications of health-related quality of life measurement. Soc Sci Med 41(10):1383–1394

Fleisher M, Hinz A, Brahler E, Wirtz H, Bosse-Henc A (2014) Factors associated with fatigue in sarcoidosis. Respir Care 59(7):1086–1094

Frost AE, Langleben D, Oudiz R, Hill N, Horn E, McLaughlin V, Robbins IM, Shapiro S, Tapson VF, Zwicke D, DeMarco T, Schilz R, Rubenfire M, Barst RJ (2005) The 6-min walk test (6MW) as an efficacy endpoint in pulmonary arterial hypertension clinical trials: demonstration of a ceiling effect. Vasc Pharmacol 43(1):36–39

Gibbons WJ, Fruchter N, Sloan S, Levy RD (2001) Reference values for a multiple repetition 6-minute walk test in healthy adults older than 20 years. J Cardpulm Rehabil 21(2):87–93

Golding LA (2000) YMCA fitness testing and assessment manual, 4th edn. Human Kinetics, Champaign

Hinz A, Fleischer M, Brähler E, Wirtz H, Bosse-Henck A (2011) Fatigue in patients with sarcoidosis, compared with the general population. Gen Hosp Psychiatry 33 (5):462–468

Hospes G, Bossenbroek L, den Hacken NH, van Hengel P, de Greef MH (2009) Enhancement of daily physical activity increases physical fitness of outclinic COPD patients: results of an exercise counseling program. Patient Educ Couns 75(2):274–278

Hunninghake GW, Costabel U, Ando M, Baughman R et al (1999) ATS/ERS/WASOG statement on sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis 16 (2):149–173

Jastrzębski D, Ziora D, Lubecki M, Zieleźnik K, Maksymiak M, Hanzel J, Początek A, Kolczyńska A, Nguyen Thi L, Żebrowska A, Kozielski J (2015) Fatique in sarcoidosis an exercise tolerance, dyspnea, and quality of life. Adv Exp Med Biol 833:31–36

Judson MA (2008) Sarcoidosis: clinical presentation, diagnosis, and approach to treatment. Am J Med Sci 335(1):26–33

Langer D, Cebrià i Iranzo MA, Burtin C, Verleden SE, Vanaudenaerde BM, Troosters T, Decramer M, Verleden GM, Gosselink R (2012) Determinants of physical activity in daily life in candidates for lung transplantation. Respir Med 106(5):747–754

Lingner H, Großhennig A, Flunkert K, Buhr-Schinner H, Heitmann R, Tönnesmann U, van der Meyden J, Schultz K (2015) ProKaSaRe study protocol: a prospective multicenter study of pulmonary rehabilitation of patients with sarcoidosis. Adv Exp Med Biol 833:31–36

Lores V, García-Río F, Rojo B, Alcolea S, Mediano O (2006) Recording the daily physical activity of COPD patients with an accelerometer: an analysis of agreement and repeatability. Arch Broncopneumol 42 (12):627–632

Michielsen HJ, Peros-Golubicic T, Drent M, De Vries J (2007) Relationship between symptoms and quality of life in a sarcoidosis population. Respiration 74 (4):401–405

Nguyen HQ, Steele B, Benditt JO (2006) Use of accelerometers to characterize physical activity patterns with COPD exacerbations. Int J Chron Obstruct Pulmon Dis 1(4):455–460

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC (1993) Lung volumes and forced ventilatory flows. Report of working party standardization of lung function tests. European Community for steel and coal. Official statement of the European Respiratory Society. Eur Respir J Suppl 16:5–40

Radwan L, Zielonka TM, Maszczyk Z, Meleniewska-Maciszewska A, Hawryłkiewicz I (1999) Functional disturbances in patients with interstitial lung diseases without signs of restriction. Pneumonol Alergol Pol 67 (5–6):180–188. (Article in Polish)

Sharma OP (1999) Fatigue and sarcoidosis. Eur Respir J 13(4):713–714

Smith KW, Avis NE, Assman SF (1999) Distinguishing between quality of life and health status in quality of life research: a meta-analysis. Qual Life Res 8:447–459

Spruit MA, Singh SJ, Garvey C et al (2013) An official American Thoracic Society/European Respiratory Society statement: key concepts and advances in pulmonary rehabilitation. Am J Respir Crit Care Med 188 (8):e13–e64

Steele BG, Belza B, Hunziker J, Holt L, Legro M, Coppersmith J, Buchner D, Lakshminaryan S (2003) Monitoring daily activity during pulmonary rehabilitation using a triaxial accelerometer. J Cardpulm Rehabil 23:139–142

Tudor-Locke C, Ainsworth BE, Thompson RW, Matthews CE (2002) Comparison of pedometer and accelerometer measures of free-living physical activity. Med Sci Sports Exerc 34(12):2045–2051

Tylka J, Piotrowicz R (2009) Quality of life questionnaire SF-36 – Polish version. Kardiol Pol 67:1166–1169

Valeyre D, Humbert M (2012) Everything you always wanted to know about sarcoidosis... but were afraid to ask. Presse Med 41(6 Pt 2):e273–e274. doi:10.1016/j.lpm.2012.04.010

Zieleźnik K, Jastrzębski D, Ziora D (2015) Fatique in patients with inactive sarcoidosis does not correlate with lung ventilation ability or walking distance. Pilot study. Pneumon Alergol Pol 83:15–22

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 23–27
DOI 10.1007/5584_2017_95

Published online: 10 September 2017

# Adenoid Cystic Carcinoma of the Cavernous Sinus – Otolaryngological Sequelae of Therapy: Case Report

Edyta Dzięciołowska-Baran and Aleksandra Gawlikowska-Sroka

**Abstract**

A 60-year-old woman, otherwise in a good health condition, was first admitted to the hospital with a year-long tingling sensations of the right half of the face, which gradually turned into severe neuralgia corresponding to maxillary (V2) and mandibular (V3) branches of the right trigeminal nerve. MRI scans of the head revealed an unusual hyperplastic or inflammatory changes along the brain base, cavernous sinus extending toward the pterygopalatine fossa, and lateral pterygoid on the right side. Meningioma was suspected and neuralgia was treated conservatively. About 2 years later, due to severe facial and eye pain, the patient underwent decompression of trigeminal nerve roots – Janetta's surgery. The following MRI scans revealed a tumor of cavernous sinus, arousing suspicion of malignancy. Histological specimens obtained after a biopsy and then partial transnasal tumor resection yielded a diagnosis of adenoid cystic carcinoma. The patient was treated with proton radiation therapy. The therapy caused burns in the oropharyngeal soft tissues extending from the oral cavity to the throat and esophagus. The additional adverse effect of the therapy was hypoacusis and a damage to the right cornea. A radiation-induced sinusitis appeared that required surgical intervention. The patient suffered a string of further complications, including pneumonia and a transient kidney failure. In the end, the patient survived. The adenoid carcinoma in the currently 64-year-old woman is visibly reduced, but she still suffers from the trigeminal neuralgia. The patient remains under constant laryngological outpatient care as she requires a periodic cleansing of nasal cavities and hypoacusis monitoring.

**Keywords**

Adenoid cystic carcinoma • Hypoacusis • Intracranial tumor • Irradiation • Proton beam therapy • Sinusitis

## 1 Introduction

Adenoid cystic carcinoma is a rare, malignant, epithelial neoplasm in the oral and maxillofacial region. It was first described in 1856 by Billroth and primarily named cylindroma (Alleyne et al. 1996). Other terms have been applied to this tumor, e.g., adenocystic basal-cell/basaloid carcinoma, pseudoadenomatosus carcinoma, or basaloma. It usually arises within secretory glands, most commonly the major and minor salivary glands of the head and neck. Other sites of origin include the upper respiratory tract

E. Dzięciołowska-Baran (✉) and A. Gawlikowska-Sroka
Department of Anatomy, Pomeranian Medical University, 72 Powstańców Wlkopolskich Street, 70-111 Szczecin, Poland
e-mail: edybar@tlen.pl

– trachea and lungs, also lacrimal glands, mammary glands, skin, and the genital tract – vulva and prostate (Adachi et al. 2006). Adenoid cystic carcinoma is slow growing but is highly invasive and locally aggressive and has a natural and high tendency to recur. This type of tumor is prone to infiltrate neural structures and extend perineurally (Pommier et al. 2006). Delayed distant metastases are not uncommon. Intracranial localization has been regarded as rare and varies from 4% to 22% (Alleyne et al. 1996). The most common intracranial sites are the optic chiasm, Gasserian ganglion, and the trigeminal nerve roots (Adachi et al. 2006). A review of the world literature has revealed some cases of adenoid cystic carcinoma in the cavernous sinus as the primary focus of the tumor (Hayashi et al. 2014; Arsene et al. 2006; Allleyne et al. 1996; Wakisaka et al. 1990).

Symptoms of intracranial tumor invasion depend on its location and can be for a long time difficult to characterize. In the case of the carvenous sinus involvement, symptoms may initially consist of face numbness, gradually turning in facial and eye socket pain, and paresthesia often accompanied by visual disturbances such as photophobia or diplopia. At this point, patient usually is treated by a neurologist. The diagnosis is based on imaging studies, which reveal a tumor mass, but do not give an answer about its origin or nature. Meningioma is initially often suspected, to the reassurance of a physician (Arsene et al. 2006). The concern, however, is an increase in the severity of symptoms. The only way for a conclusive diagnosis is to conduct a histopathological examination. However, the risk of bleeding makes it difficult to obtain a specimen for histopathology. The main challenge at this point is to choose an appropriate form of treatment. Surgical removal of a tumor, especially its entire mass, may actually border on a miracle. Most often it is not even taken under consideration because of the bleeding risk and potential surrounding anatomical structure damage. The only possible solution left is radiotherapy, which in its conventional form can be as dangerous as surgical therapy due to possible surrounding tissue damage (Osuch-Wójcikiewicz and Bruzgielewicz 2010). Thus, proton beam therapy is steadily gaining ground. The main advantage of this treatment lies in its very high precision. This is due to the physical properties of a proton beam and the distribution of a radiation dose in tissues. The advantage of this treatment over conventional radiation derives from the fact that the maximum therapeutic dose of protons occurs at a distinct depth of about 30 cm, which leaves the patient skin out. Then, it might be possible to effectively destroy a tumor mass located, for instance, at the skull base while avoiding over-radiation of surrounding healthy tissues (Allen et al. 2012; Pommier et al. 2006; Suit et al. 2003).

The present article describes a rare case of adenoid carcinoma located primarily in the cavernous sinus and then invading the surrounding structures. A careful selection of the best possible therapy did not save the patient from a number of symptoms, ailments, and adverse effects, particularly involving the otolaryngological region. The symptoms in the presented patient are unusual to the extent of being very rare considering the proton beam radiation therapy. The case report described shows that even the most current and advanced oncological treatment method may result in adverse effects, the physicians should be aware of. Also, patients, when adequately informed, would likely easier face treatment sequelae.

## 2 Case Report

The report was approved by the Ethics Committee of Pomeranian Medical University in Szczecin, Poand. A 60-year-old patient up to this point in a generally good condition began to feel tingling, numbness, and burning sensations on the right side of the face. After a short while, pain appeared around the right eye. Symptoms gradually worsened and conventional painkillers were helpful for a short stretches of time only. These initial symptoms were suggestive of trigeminal neuralgia and the patient was directed to a neurologist. An MRI brain scan showed an intensification and thickening of the

meninx in the cavernous sinus area, penetrating into the pterygopalatine fossa and lateral pterygoid muscle. The lesion could signify meningioma or infiltration of the second and third branch of the trigeminal nerve. A conservative treatment of neuralgia was recommended with periodic monitoring and repeated imaging after a while. One and a half years later, neuralgia intensified to the point that painkillers were to no avail, and pain became unbearable. Then, microdecompression of the trigeminal nerve was performed, resulting in a short-lasting analgesic effect. The MRI repeated two and a half years after the previous one revealed an outgrowth of a tumor mass, suggestive of a malignant nature of the lesion. A transnasal partial resection of the tumor was performed through the sphenoid, ethmoid, and maxillary sinuses, which enabled the collection of specimens for a histopathological examination. Endoscopically, the lesion was described as a creamy tumor mass of white color, soft, gelatinous, and moderately bleeding. The mass was removed from the bottom of the pterygopalatine fossa, Meckel's cavity, cavernous sinus, and from the area of the orbit apex. It was relatively easy to separate the tumor mass from the meninx and nerve branches; the appearance of the tumor was unlike that of meningioma. The histopathological examination resulted in the diagnosis of adenoid cystic carcinoma. Proton beam therapy was chosen as the most optimal method of treatment. Irradiation took place in a facility in Munich, Germany and it was continued for 6 weeks. The patient received 33 doses of 2.20 Gy (a total of 72.60 Gy) to the tumor area, 33 doses of 1.80 Gy to the lymphatic nodes of I-IIIa level on the right side (a total of 59.40 Gy), and another 33 doses of 1.80 Gy to the nodes of Ib/II level on the left side (a total of 59.40 Gy).

Already before irradiation, just after tumor resection, the patient was complaining of a slight hearing loss on the right side, with a feeling of clogging in the ear. Conductive hearing loss was diagnosed and paracentesis was performed, which brought an immediate improvement. Upon the completion of irradiation, intense but transient dermatitis and xerostomia appeared, which did not entirely subsided. There was a post-radiation damage to the right cornea, with ulcer perforation, necessitating keratoplasty and cataract removal. The eye-related treatment had to be disrupted due to further otolaryngological complications. The patient experienced a post-radiation inflammation of all sinuses, with a significant increase of proinflammatory biochemical markers, very strong nose and face pain, erythema and swelling of soft tissues of the right cheek, and reappearance of the hearing loss in the course of subacute mucosal otitis media, which this time was bilateral. It is worth noting at this juncture that the patient had no problems with sinuses before radiotherapy, as confirmed by imaging studies. At about the same time, the patient started to display signs of kidney failure. The woman was extremely weak, emaciated, had mood disorders, and suffered from resounded neuralgic pains. The drainage of sinuses was performed. The patient reported a hearing improvement, but after another 2 weeks she was readmitted to the hospital because of recurrent swelling of the cheek soft tissue and sinus pains. At this point targeted antibiotic therapy commenced. A significant clinical improvement was achieved, along with a decrease in biochemical proinflammatory markers, which however failed to fully normalize. A massive mucosal swelling and hyperplasia remained to be visible in radiographs. Secretion drying in the nostrils, causing mucosal casts in nasal cavities, became a painful problem, particularly during further endoscopy attempts. Both pharyngotympanic tubes became dysfunctional and despite treatment effort, the hearing loss deepened to the extent of cutting the patient off from communication with the external environment. Some improvement in hearing became possible after application of hearing aids. The patient survived all the complications and adverse episodes. From the oncological standpoint, cancer has clearly been in significant regression.

## 3 Discussion

Adenoid cystic carcinoma as a primary tumor of the skull base occurs very rarely, especially in the cavernous sinus area. Virtually in all cases described in the literature, the diagnostic process and treatment have caused a variety of medical problems, making the prognosis of recovery highly mercurial (Hayashi et al. 2014). At the beginning of disease, when symptoms are not yet strongly pronounced and based on imaging scans, a suspicion of meningioma arises, which is a benign tumor not requiring an urgent intervention (Arsene et al. 2006). Usually, it is a reason for putting off a final histopathological verification until after the symptoms have become intense. The intensification of symptoms, however, signifies an expansion of a tumor and arouses a reasonable suspicions of a malignant change, which forces the undertaking more radical diagnostic steps. Taking a biopsy specimen in case of carvenous sinus tumor is not an easy task, since it is performed through the endonasal approach, with a high risk of hemorrhage. In the patient presented above in this report, the right sphenoid sinus was opened, right side etmoidectomy and maxillary sinus anstrostomy were performed, and the posterior wall of the sinus, along with the medial wall of the eye orbit, was lifted. Shortly after this procedure, the patient experienced the right-sided conductive hearing loss, which resolved after myringotomy. Dysfunction of the Eustachian tube is explicable by a swelling around the tube end (Osuch-Wójcikiewicz and Bruzgielewicz 2010). Proton beam therapy, considered an optimal treatment for carvenous sinus tumor, may cause a variety of complications (Parsons et al. 1994), which also came to light in the patient case presented above. While dermatitis, xerostomia, and eye damage were among the expected side effects of irradiation, a high intensity sinusitis was rather unexpected, particularly the patient had never had any sinus-related problems. A reasonable explanation for the sinusitis could be the implementation of transnasal access to the carvenous sinus. In effect, a geometrical structure of the paranasal sinuses was substantially rearranged as a result of constantly changing pressure of air flow, which led to the formation of castings of dried secretions, impairing sinus permeability and giving rise to chronic inflammation that accompanied the patient throughout the entire therapy. While in conventional radiotherapy, particularly combined with chemotherapy, hearing loss is fairly common, it seems an extremely rare occurrence in proton therapy. In the presented patient case, conductive hearing loss occurred in the course of otitis media with effusion, likely caused by dysfunctional pharyngotympanic tubes. However, isolated edema within the Eustachian tubes as a result of irradiation cannot be ruled out. Unfortunately, drainage treatment turned out insufficient and the patient had to be fitted with a hearing aid.

In conclusion, this case report presents a diagnostic process and treatment of a patient with rare adenoid cystic carcinoma of cavernous sinus. Proton beam therapy, judged the optimal treatment available, gave rise to serious otolaryngological sequelae, including conductive hearing loss, physicians should be aware of. Nonetheless, proton therapy remains an effective and recommended way to treat inoperable tumors of the skull base.

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

## References

Adachi K, Yoshida K, Ueda R, Kawase T (2006) Adenoid cystic carcinoma of the cavernous region – case report. Neurol Med Chir (Tokyo) 46:358–360

Allen AM, Pawlicki T, Dong L, Fourkal E, Buyyounouski M, Cengel K, PLastaras J, Bucci MK, Yock TI, Bonilla L, Price R, Harris EE, Konski AA (2012) An evidence based review of proton beam therapy: the report of ASTRO's emerging technology committee. Radiother Oncol 103:8–11

Alleyne CH, Bakay RE, Costigan D, Thomas B, Joseph GJ (1996) Intracranial adenoid cystic carcinoma: case report and review of the literature. Surg Neurol 45 (3):265–271

Arsene K, Ardeleanu C, Danaila L (2006) Skull base tumor invading both cavernous sinuses.Adenoid cystic

carcinoma mimicking a meningioma. Rom J Morphol Embryol 47:367–371

Hayashi Y, Iwato M, Kita D, Miyashita K, Yoshizaki T, Hamada J (2014) Adenoid cystic carcinoma in the cavernous sinus diagnosed with the endoscopic endonasal approach. Turk Neurosurg 24(5):814–818

Osuch-Wójcikiewicz E, Bruzgielewicz A (2010) Complications after radiotherapy of head and neck malignancies. Otolaryngol 9(1):1–6

Parsons JT, Bova EJ, Fitzgerald CR, Mendenhall WW, Million RR (1994) Sever dry-eye syndrom following external beam irradiation. Int J Radiat Oncol Biol Phys 30(4):775–780

Pommier P, Liebsch NJ, Dreschler DG, Lin DT, McIntyre JF, Barker FG, Adams JA, Lopes VV, Varvares M, Loeffler JS, Chan AW (2006) Proton beam radiation therapy for skull base adenoid cystic carcinoma. Arch Otolaryngol Head Neck Surg 132:1242–1249

Suit HD, Goldberg S, Niemierko A, Trofimov A, Adams J, Paganetti H, Chen G, Bortfeld T, Rosenthal S, Loeffler Y, Delaney T (2003) Proton beams to replace photon beams in radical dose treatments. Acta Oncolol 42(8):800–808

Wakisaka S, Nonaka A, Morita Y (1990) Adenoid cystic carcinoma with intracranial extension. Report of three cases Neurosurg 26:1060–1065

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 29–37
DOI 10.1007/5584_2017_103

Published online: 2 February 2018

# Tuberculosis of the Urogenital Tract in Adults in a Tertiary Referral Center

Jacek Jagodziński, Tadeusz M. Zielonka, Krystyna Peplińska, and Katarzyna Życińska

**Abstract**

The genitourinary system is the main location of extrapulmonary tuberculosis. In Poland, it occupies the third place after tuberculosis of the pleura and lymph nodes. The aim of this study was to evaluate the prevalence and characteristics of tuberculosis in the urogenital tract in adult patients in a tertiary referral center in the years 2007–2015. The retrospective study included 87 patients, 42 women and 45 men. The average age was 62 ± 15 years. Changes in the urinary tract were diagnosed in 91% of women and 64% of men. Testicular tuberculosis was found in ten men, prostate tuberculosis in five, and in individual cases tuberculosis of the epididymis, scrotum, uterus, and the fallopian tube were found. The diagnosis was confirmed by bacteriological methods in 47% of patients, by histopathological in 41%, and by molecular methods in 23% of patients. In 84% of patients urological or gynecological interventions had to be applied. Patients were burdened with a number of urological diseases or diseases affecting other systems which hampered the diagnosis of tuberculosis. Antituberculosis treatment gave good results. Urogenital tuberculosis is a multivariate disease and a standard unified approach is impossible.

**Keywords**

Diagnostics • Extrapulmonary tuberculosis • Genitourinary system • Risk factors • Tuberculosis • Urological interventions

## 1 Introduction

Tuberculosis (TB) remains one of the major health problems, causing about 9 million new cases and 1.5 million of deaths each year (WHO 2014). In 2015 in Poland, 6,430 cases of TB were diagnosed, among which 352 cases were extrapulmonary. The urogenital tract was infected in only 43 patients of the latter patients. That puts the urogenital location of lesions in the third place after pleural TB (118 cases) and lymph nodes TB (55 patients), and bones and joints in the fourth place (39 patients). A long-term evaluation of the prevalence of extrapulmonary TB in Poland indicates a

J. Jagodziński
Mazovian Center for the Treatment of Lung Diseases and Tuberculosis in Otwock, Otwock, Poland

T.M. Zielonka (✉) and K. Życińska
Department of Family Medicine, Medical University of Warsaw, 1A Banacha Street, 02-097 Warsaw, Poland
e-mail: tadeusz.zielonka@wum.edu.pl

K. Peplińska
Department of Internal Medicine and Cardiology, Solec Hospital, Warsaw, Poland

systematic decrease. The incidence of TB was 11.2% in the years 1974–1982, 8.2% in the years 2002–2010, and it is 5.5% at the present time (Rowinska-Zakrzewska et al. 2013, 2014). By contrast, in western countries the incidence of extrapulmonary TB is much higher and rising. In Great Britain, it rose from 48 to 53% in 1999–2006 (Kruijshar and Abubakar 2009), and in the entire EU from 16 to 22% in 2002–2011 (Kulchavenya 2014a). In the US and Germany it is over 20% (Forssbohm et al. 2008; CDC 2007), and in the Netherlands and Canada it is over 40% (Ellis et al. 2010; Erkens et al. 2007). Factors promoting a higher prevalence of extrapulmonary TB include HIV infection and the rising number of immigrants among patients with TB (Kruijshar and Abubakar 2009). These factors are of a minor role in Poland. The percentage of foreigners among patients suffering from TB in Poland is 0.8%, while in many western countries it exceeds 70–80% (Rowińska-Zakrzewska et al. 2014; Zammarchi et al. 2014). The situation is comparable when it comes to the coinfection of TB and HIV. In Poland there are just 0.3% of such cases, while in western countries this coinfection afflicts a couple of percentage points of patients and in some African countries over 50% of patients (WHO 2014). The women to men ratio is higher in extrapulmonary TB than in pulmonary TB, and extrapulmonary TB occurs in a younger population (Rowinska-Zakrzewska et al. 2013; Kruijshar and Abubakar 2009; Forssbohm et al. 2008). The differential diagnosis of urogenital TB may be difficult and often requires invasive methods as clinical signs and results of additional tests suggest other diseases, especially neoplasms, infections, urolithiasis, etc. Cases of urinary system TB are reported more often than those of genital system infection (Kulchavenya and Kholtobin 2015).

The aim of the present study was to evaluate the prevalence and characteristics of urogenital TB in adult patients in a tertiary hospital center in Poland in the years 2007–2015.

## 2 Methods

### 2.1 Study Design

The study was conducted in conformity with the Declaration of Helsinki for Human Experimentation of the World Medical Association. A local Bioethics Committee of the Warsaw Medical University in Poland waived the requirement for ethical permission due to a retrospective character of the study that was based on a collection of aggregated data only. The inclusion criterion was the diagnosis of urogenital TB (A18.1 in ICD-10 classification) in hospitalized patients in the Mazovian Center for the Treatment of Lung Diseases and Tuberculosis in the city of Otwock, Poland, in the years 2007–2015. Medical records evaluated concerned the hospitalization and post-hospitalization periods. Patients' characteristics are displayed in Table 1. There were 87 adult patients aged 62 ± 15 (range 18–86 years), F/M –

**Table 1** Characteristics of patients with urogenital tuberculosis

| | Women | Men | Total |
|---|---|---|---|
| Number of cases | 42 | 45 | 87 |
| Age (year) | 61 ± 15 | 64 ± 12 | 62 ± 15 |
| Single | 3 | 2 | 5 |
| Married | 24 | 37 | 61 |
| Widowed | 10 | 3 | 13 |
| Divorced | 5 | 3 | 8 |
| Warsaw | 16 | 20 | 36 |
| Small town | 19 | 15 | 34 |
| Rural area | 7 | 10 | 17 |
| Unemployed | 0 | 4 | 4 |
| Disability pension | 6 | 5 | 11 |
| Pensioners | 24 | 23 | 47 |
| Working | 11 | 13 | 24 |
| Students | 1 | 0 | 1 |
| Smokers | 7 | 16 | 23 |
| Ex-smokers | 7 | 13 | 20 |
| Non-smokers | 28 | 11 | 39 |
| Higher education | 10 | 6 | 16 |
| Secondary education | 16 | 18 | 34 |
| Professional education | 3 | 9 | 12 |
| Primary education | 10 | 9 | 19 |

42/45, evaluated. The majority of patients were married (70%), although 43% of women and only 17.5% of men were single. Forty one percent of patients lived in Warsaw, 20% in rural areas, and the remaining in small towns. The majority of patients were unemployed and only 27.5% were actively working (26% of women and 29% of men). There were 70% never-smokers among women and 26% among men; in 5 cases data on cigarette smoking were missing. Forty two patients had secondary education, 23% primary education, 20% higher education, and 15% had vocational education; in six cases data on education were missing. None of the patients were infected with HIV.

## 3 Results

### 3.1 Lesion Location

TB of the urinary system predominated (76%) and TB of the genital system was diagnosed in 24% of patients. Lesions in the urinary system were found in 91% of women and in 61% of men. In addition, testicular TB was found in ten men, prostate TB in five men, and epididymis and scrotum TB in one man. Among females, there were two cases of uteral TB, one case of ovarian TB, and one case of fallopian tube TB.

### 3.2 Diagnostic Criteria

The diagnosis of urogenital TB was most often confirmed by microbiological methods, less often by histopathological evaluation or genetic tests (Table 2). Culture was positive in 60% of females and 36% of males. Mycobacteria were most often cultured from urine samples (41 patients). The urine was also the most frequent source of positive results of genetic tests (Table 3). Such tests diagnosis TB in 23% of patients. For a morphological examination, the most often used samples were those from the testicles, urinary bladder, prostate, and kidney. Morphological examination confirmed the diagnosis of TB in 53% of men and in 29% of women. In three cases, both histopathological and genetic examinations gave positive results. In four cases, histopathological and culture tests were positive and in another three cases genetic tests and urine cultures were positive. Only in five cases (6%) the diagnosis was based on clinical signs and imaging.

**Table 2** Patients with positive result for tuberculosis

| Methods | Women (n) | Men (n) | Total (%) |
|---|---|---|---|
| Bacterial culture | 25 | 16 | 47% |
| PCR reaction | 9 | 11 | 23% |
| Histological evaluation | 12 | 24 | 41% |

**Table 3** Biological specimens used to confirm tuberculosis

| Specimen | Bacterial culture | Histological evaluation | PCR reaction |
|---|---|---|---|
| Urine | 39 | – | 17 |
| Testicle | – | 10 | 2 |
| Wound secretion – surgery of scrotum | 1 | – | – |
| Epididymis | – | 1 | 1 |
| Prostate | – | 5 | – |
| Bladder | – | 9 | – |
| Kidney | – | 6 | – |
| Ovaries | – | 1 | – |
| Oviduct | – | 1 | – |
| Uterus | – | 2 | – |
| Sperm | 1 | – | – |

### 3.3 Urological and Gynecological Interventions

In 79% of patients, a variety of urological or gynecological interventions were necessary (Table 4). These interventions were necessitated by the presence of alterations in the urogenital system, consequences of TB, or concurrent diseases located in this anatomic region. Orchiectomy (10 cases) and nephrectomy (7 cases) were performed most commonly. Neoplasia was suspected in all orchiectomy cases, but histopathologic examinations confirmed that the testicular

**Table 4** Urological and gynecological procedures performed in patients with urogenital tuberculosis

| Procedures | Women (n) | Men (n) | Total |
|---|---|---|---|
| Renal biopsy | 1 | 1 | 2 |
| Nephrectomy | 6 | 1 | 7 |
| Nephrostomy | 6 | 1 | 7 |
| Catheter JJ/DJ | 9 | 2 | 11 |
| Cystoscopy | 7 | 6 | 13 |
| Cystoprostatectomy | 0 | 1 | 1 |
| Cystectomy | 1 | 0 | 1 |
| Prostatectomy | 0 | 1 | 1 |
| TURT/TURP | 3 | 6 | 9 |
| URSL | 4 | 2 | 6 |
| Orchiectomy | 0 | 10 | 10 |
| Epididymectomy | 0 | 1 | 1 |
| Curettage of uterus | 2 | 0 | 2 |
| Hysterectomy | 2 | 0 | 3 |
| Total | 41 | 32 | 73 |

*TURT* Transurethral resection of tumor, *TURP* Transurethral resection of the prostate, *URSL* Ureteroscopic lithotripsy

lesions were caused by TB. Nephrectomy was performed due to a kidney tumor (3 cases), pyonephrosis (2 cases), and kidney cirrhosis (1 case). In two patients, TB of the urinary system was diagnosed in patients in whom nephrectomy was performed due to cancer. Transurethral removal of a tumor or prostate was often performed. Cystoscopy was performed in 13 patients with suspected urinary bladder tumor or chronic cystitis. In several patients, the placement of double-sided bladder loop and renal pelvic loop catheters was required due to urethral strictures or urinary lithiasis. In several other patients, nephrostomy was performed due to hydronephrosis. In six patients, urinary stones were removed endoscopically. In single cases, epididymectomy, cystoprostatectomy, prostatectomy, cystectomy, hysterectomy, or uteral curettage were performed.

## 3.4 Differential Diagnosis

Differential diagnosis was complicated by the coexistence of other urinary system diseases, which sometimes delayed the diagnosis of urogenital TB. Kidney stones were diagnosed in 15 patients, large kidney cysts in four patients, and polycystic kidney, lack of kidney, kidney cirrhosis, and urinary bladder cirrhosis were diagnosed in one patient each. A urethral stricture was observed in nine patients and bladder cancer in another nine patients. The BCG was instilled in the urinary bladder because of cancer in five patients. A post-nephrectomy diagnosis of coexisting kidney cancer and tuberculosis was made in two patients. Prostate cancer was detected in two patients. Twenty five patients complained of chronic cystitis. One patient was after a kidney transplant due to chronic kidney disease. Two female patients reported bleeding from the reproductive tract.

## 3.5 Comorbidities

The most common comorbidities accompanying the urogenital TB were arterial hypertension (55%), diabetes mellitus (31%), and coronary artery disease (25%). Heart failure was present in 13% of patients, past myocardial infarction in 5%, chronic kidney disease in 16%, and thyroid gland diseases in 13% of patients. Single cases of asthma, chronic obstructive pulmonary disease, rheumatoid arthritis, cholecystolithiasis, chronic pancreatitis, gastric ulceration, past stroke and dementia were recorded. Three men and three women had a history of previously treated TB. Pulmonary lesions that might have been caused by active TB were detected in 15 patients. However, sputum culture was negative for TB in those cases.

## 3.6 Treatment and Disease Course

There were no cases of multidrug resistant TB among the patients with urogenital TB. Antimycobacterial treatment was initiated in the hospital in 79 patients. The standard in-hospital treatment consisted of 4 or 3 drugs: rifampicin (RMP), isoniazid (INH), pyrazinamide (PRZ), and ethambutol (EMB) given for 2 months, and then 2 drugs (INH + RMP) for 4 months in out-hospital

setting. Drug intolerance appeared in one woman and it forced treatment discontinuation. One patient had died before the commencement of treatment. In the patient suffering from urinary bladder cancer, who was treated with BCG instilled into the bladder, cultures turned out positive for *Mycobacterium bovis*. In this case, antituberculous treatment was not started. In another patient suffering from prostate cancer, histopathological changes were typical for TB, but the decision was made to delay the antituberculous treatment due to an overall poor patient's condition. In two cases, TB was confirmed by culture after the patients had been discharged, so no antituberculous in-hospital treatment could be initiated. In two other cases, antituberculous treatment was postponed and the patients remained under ambulatory observation due to a discrepancy between the histopathology results and clinical signs.

## 4 Discussion

Patients suffering from genitourinary TB have some different features than those with pulmonary TB. In the present study, the men-to-women ratio was close to one, amounting to 1.07, which implies that male sex is not a predisposing factor for extrapulmonary TB in adults. For comparison, this ratio is 2.26 in patients with pulmonary TB in Poland (Rowińska-Zakrzewska et al. 2014). Some authors emphasize a higher incidence of genitourinary TB in men than women (de Daher et al. 2013; Figueiredo et al. 2008; Benchekroun et al. 1998). In contrast, others demonstrate a greater incidence in women than men (Mazza-Stalder et al. 2012; García-Rodríguez et al. 2011). The discrepancy may stem from not taking into account the location of TB lesion in the urinary system. It is postulated that a higher incidence of illnesses with urine stasis in females promotes the accumulation of mycobacteria in the urothelium (Kulchavenya 2014b). Considering just the renal involvement, the male-to-female ratio was 0.74 in the present study.

The mean age of patients was $62 \pm 15$ years in the present study. This disagrees with reports that extrapulmonary TB occurs more often in younger groups (Rowinska-Zakrzewska et al. 2013; Figueiredo et al. 2008). It is probable that in other countries extrapulmonary TB occurs more often in HIV-infected, immunocompromised, and usually younger persons (Shivakoti et al. 2017). There were no HIV-infected patients in the present study, which speaks for the urogenital TB being an endogenic infection. Patients with pulmonary TB, treated in the same center at the same time, were junior to those with genitourinary TB. Two thirds of the former were below 60 years of age, with the average around 52 years (Błachnio et al. 2014; Jagodziński et al. 2012). In a Japanese study, in which there were no HIV-coinfections either, the peak incidence of urogenital TB was in persons of 50–60 years of age (Nakane et al. 2014).

There are demographic and social differences among those suffering from genitourinary TB and pulmonary TB in Poland. The number of people with genitourinary TB is much higher in cities than that in rural areas; with a ratio of 4.12. An open question remains of whether the incidence of urogenital TB is truly lower in small towns and rural areas or detection is better in big cities, usually much better equipped with hospitals and diagnostic systems. For comparison, this ratio amounts only to 1.55 for pulmonary TB (Rowińska-Zakrzewska et al. 2014). In the present study we show that the majority of patients suffering from genitourinary TB were pensioners (66.5%), some were professionally active (27.5%), and some were unemployed (5%). That is in contrast to a report from China that has shown that genitourinary TB occurs more often in the unemployed (Huang et al. 2013). Among patients with pulmonary TB in the Mazovian voivodship in Poland, those professionally active, pensioners, and unemployed accounted for 26%, 17%, and 57% in the male group and 29%, 43%, 28% in the female group, respectively. In the present study, the majority of patients were married; 82% of men and 57% of women, which again is in contrast to pulmonary TB where the majority is single; 57% of men and

60% of women (Błachnio et al. 2014; Jagodziński et al. 2012). On the other hand, akin to a study of Ali and Abdallah (2012) who have reported on a link between genital TB and education, we noted that persons with higher education accounted for 20%, with a secondary education for 42%, and those with primary and vocational education for 38% of the patients. The number of active smokers among the present patients with urogenital TB was rather modest; 16% of women and 36% of men. That is in accord with the notion that smoking is not a major risk factor for the development of extrapulmonary TB (Lin et al. 2009; García-Rodríguez et al. 2011).

Urinary TB predominated in the present study group (76%), accounting for 64% male and 88% female patients. TB of the urinary tract is easier to recognize than genital involvement, because the diagnostic basis is a urine culture. Urine is easily collectable, compared with sputum from the lungs, and the examination can be repeated many times. Urine is also the best specimen for obtaining positive genetic results. The ease of obtaining the material for bacteriological testing is conducive to TB detection in the urinary tract. In other reports, TB location in the kidney and urinary tract has been found significantly more frequent than in the genitals (Nakane et al. 2014; Kulchavenya 2013). Mycobacteria carried with the blood, or their fragments after disintegration, enter the kidneys and are excreted in urine. That is evidenced by successful attempts to search for genetic material in the urine of patients suffering from active pulmonary TB (Heydari et al. 2014). That also explains why blood-filtering kidneys can disseminate the blood-borne TB infection throughout the body (Merchant et al. 2013). In the present study, positive cultures were obtained in 47% of patients; the percentage closely akin to that in a Chinese study conducted in a group of 239 patients (Huang et al. 2013). However, a meta-analysis of multi-country studies has demonstrated the high 64% rate of bacteriologically confirmed genitourinary TB (Figueiredo et al. 2008).

In the present study, histopathological diagnosis of urogenital TB was made in half of the men and a quarter of the women. However, the testicle, epididymis, prostate, and scrotum are much more easily accessible for collection of histopathological specimens than ovaries, fallopian tubes, or uterus. That may explain a more frequent detection of genital TB in men (36%) than women (12%). Genital TB is a chronic pathology, which can give hardly any symptoms, and is thus underdiagnosed, albeit it often causes infertility (Mahajan et al. 2016; Kulchavenya 2014b). The diagnosis also is difficult due to a low mycobacterial count. A good diagnostic yield results provides a combined use of the interferon gamma release assay (TB blood test), bacteriological, and endometrial biopsy results.

A growing role of genetic testing in the diagnosis of TB of the urinary system is drawing attention. In the present study, nearly a quarter of patients came out positive on genetic tests. The role of these tests will certainly grow in the diagnosis of extrapulmonary TB, because the time to establish the diagnosis is short and the genetic testing, particularly when combined with a histopathological assessment, gives practically a certainty of the infection (Portillo-Gomez et al. 2000). Sensitivity of genetic tests in urine ranges from 25 to 93% and specificity is reaches 95–100% (de Daher et al. 2013). It is essential to introduce genetic tests into the routine diagnostics of both pulmonary and extrapulmonary TB. That would also limit the unnecessary use of toxic anti-mycobacterial treatment.

A small percentage (6%) of diagnosis of urogenital TB is established on the basis of a clinical picture and imaging studies. In case of pulmonary TB, this percentage is significantly higher and reaches 26.4% in Poland (Rowińska-Zakrzewska et al. 2014). The open question remains of whether this is due to an increased detection of pulmonary TB or insufficient diagnosis of urogenital TB. A Japanese study shows that the cases of urogenital TB, second in

frequency to pulmonary TB, amount to 31% of all TB patients (Nakane et al. 2014). A multi-country meta-analysis puts the prevalence of urogenital TB at about 37% (Figueiredo et al. 2008). The prevalence of urogenital diagnosis of 7% in Poland strongly suggests the possibility of its being underdiagnosed. Renal TB is rarely accompanied by pulmonary TB lesions as unraveled in radiographs (de Daher et al. 2013).

Almost all (80%) patients with genitourinary TB require specialized urological care. Gupta et al. (2006) has reported that the use of more than one procedure *per* each of the 241 patients with genitourinary TB, including the collection of specimens for histopathology, with a total of 248 interventions, involving 33 endoscopic, 87 ablative, and 128 reconstructive procedures. In many cases, interventions were required to maintain patency of the urinary tract disordered by TB or were due to a coexisting neoplasm; the latter was noted in 15% of patients. Nakane et al. (2014) have demonstrated a high incidence of urinary tract stenosis (53%), and much fewer cases of pelvic deformations (11%), kidney cirrhosis (8%), calcification (5%), and malignant neoplasms (1%). In that study urological interventions, such as removal of a kidney, testicle, or epididymis, are performed quite often. A retrospective evaluation demonstrates that congenital anomalies of the urinary tract, chronic kidney disease, kidney stones, renal cysts, and renal transplantation all increase the risk of TB in the genitourinary tract (Kulchavenya 2013). The diagnosis in most cases is set in a few months or even 1 year after onset of first genitourinary symptoms (Altiparmak et al. 2015). Diseases that mask urogenital TB and delay the diagnosis include cancer, urinary tract stones, non-tubercular urinary tract infections, and chronic kidney disease (Kulchavenya and Kholtobin 2015).

In the present study, the diagnosis of testicular TB was established after the morphological assessment of a removed testicle due to suspected cancer. Likewise, in case of uterine lesions the diagnosis was not firmly established until after a hysterectomy due to suspected cancer. Nowadays, cancer is often the first diagnosis, which sometimes leads to neglecting further diagnostic efforts, particularly in the elderly patients with multiple comorbidities. Then, autopsy unravels the infection-related background, indicates that appropriate treatment could have saved the patient (Rowińska-Zakrzewska et al. 1995). Advancements in the imaging methods to detect tumors should not lead to discontinuation of invasive procedures to establish a diagnosis based on the histopathological examination. A relative rarity of autopsies in Poland and a probable fear of legal claims when the autopsy reveals a different disease background leads to the confirmation of wrongly diagnosed cancer without a *post mortem* verification.

It is particularly difficult to diagnose mycobacteria in people after intravesical BCG treatment in the course of bladder cancer. During this treatment patients may become infected with BCG mycobacteria (Uno et al. 2014; Chowdury and Dey 2013). A similar case occurred in one of the patients in the present study group. While the diagnosis of urogenital TB can be troublesome, treatment is relatively simple and no different from that of pulmonary TB. If no drug-resistant mycobacterial infection is detected, anti-tuberculosis treatment is effective and gives good results. Unfortunately, post-tubercular lesions may remain permanently, which raises the need for later urological interventions. Nakane et al. (2014) have reported the treatment modalities used in a group of 355 patients with urogenital TB. Fifty five percent of those patients received pharmacological treatment alone, 38.5% received pharmacological plus surgical treatment, and 5.5% underwent surgical treatment alone.

In conclusion, urogenital tuberculosis is an important diagnostic problem because it occurs in elderly patients with multiple diseases that coexist in the urinary system and in other organs. This may delay diagnosis and falsely lower the disease incidence.

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

## References


Ali A, Abdallah T (2012) Clinical presentation and epidemiology of female genital tuberculosis in eastern Sudan. Int J Gynaecol Obstet 8:236–238

Altiparmak MR, Trabulus S, Balkan II, Yalin SF, Denizli N, Aslan G, Doruk HE, Engin A, Tekin R, Birengel S, Cetin BD, Arslan F, Turhan V, Mert A (2015) Urinary tuberculosis: a cohort of 79 adult cases. Ren Fail 37:1157–1163

Benchekroun A, Lachkar A, Soumana A, Farih M, Belahnech Z, Marzouk M, Faik M (1998) Urogenital tuberculosis. 80 cases. Ann Urol 32:89–94

Błachnio M, Zielonka TM, Błachnio A, Jagodziński J (2014) Socio-economic status and the duration of pulmonary tuberculosis symptoms in women treated at the Mazovian Treatment Centre of Tuberculosis and Lung Diseases in Otwock. Pneumonol Alergol Pol 82 (1):3–9.

CDC (2007) Reported tuberculosis incidence – United States, 2006. MMWR Wkly 56(11):245–250

Chowdury AR, Dey RK (2013) Penile tuberculosis following intravesical Bacille Calmette-Guérin immunotherapy. Indian J Urol 29:64–66

de Daher EF, da Silva G, Barros E (2013) Renal tuberculosis in the modern era. Am J Trop Med Hyg 88:54–64

Ellis E, Gallant V, Scholten D, Dawson K, Phypers M (2010). Tuberculosis in Canada 2009 – pre-release. 2010, Ottawa: Public Health Agency of Canada. http://www.phac-aspc.gc.ca/tbpc-latb/pubs/tbcan08pre/index-eng.ph. Accessed on 12 Oct 2016

Erkens CGM, Kalisvaart NA, Slump E, Sebek M, van Soolingen D (2007) Tuberculose in the Nederland 2005. Surveillancerapport. KNCV Tuberculosefonds, Den Haag

Figueiredo AA, Lucon AM, Junior RF, Srougi M (2008) Epidemiology of urogenital tuberculosis worldwide. Int J Urol 15:827–832

Forssbohm M, Zwahlen M, Loddenkemper R, Rieder HL (2008) Demographic characteristics of patients with extrapulmonary tuberculosis in Germany. Eur Respir J 31:99–105

García-Rodríguez J, Álvarez-Díaz H, Lorenzo-García M, Mariño-Callejo A, Fernández-Rial Á, Sesma-Sánchez P (2011) Extrapulmonary tuberculosis: epidemiology and risk factors. Enferm Infec Microbiol Clin 29:502–509

Gupta NP, Kumar R, Mundada OP, Aron M, Hemal AK, Dogra PN, Seth A (2006) Reconstructive surgery for the management of genitourinary tuberculosis: a single center experience. J Urol 175:2150–2154

Heydari AA, Movahhede Danesh MR, Ghazvini K (2014) Urine PCR evaluation to diagnose pulmonary tuberculosis. Jundishapur J Microbiol 7:e9311

Huang H, Li X, Jin J (2013) Epidemiology and clinical features of renal tuberculosis: 239 cases report. Beijing Da Xue Xue Bao 45:600–604

Jagodziński J, Zielonka TM, Błachnio M (2012) Socio-economic status and duration of TB symptoms in males treated at the Mazovian Treatment Centre of Tuberculosis and Lung Diseases in Otwock. Pneumonol Alergol Pol 80:533–540.

Kruijshar ME, Abubakar I (2009) Increase in extrapulmonary tuberculosis in England and Wales 1999–2006. Thorax 64:1090–1095

Kulchavenya E (2013) Best practice in the diagnosis and management of urogenital tuberculosis. Ther Adv Urol 5:143–151

Kulchavenya E (2014a) Extrapulmonary tuberculosis: are statistical reports accurate? Ther Adv Infect Dis 2:61–70

Kulchavenya E (2014b) Urogenital tuberculosis: definition and classification. Ther Adv Infect Dis 2:117–122

Kulchavenya E, Kholtobin D (2015) Diseases masking and delaying the diagnosis of urogenital tuberculosis. Ther Adv Urol 7:331–338

Lin J, Lai C, Chen Y, Lee S, Tsai S, Huang C, Chung HC, Liang SH, Lin HH (2009) Risk factors for extrapulmonary tuberculosis compared to pulmonary tuberculosis. Int J Tuberc Lung Dis 13(5):620–625

Mahajan N, Naidu P, Deep Kaur S (2016) Insight into the diagnosis and management of subclinical genital tuberculosis in women with infertility. J Hum Reprod Sci 9:135–144

Mazza-Stalder J, Nicod L, Janssens J (2012) Extrapulmonary tuberculosis. Rev Mal Respir 29:566–578

Merchant S, Bharati A, Merchant N (2013) Tuberculosis of the genitourinary system – urinary tract tuberculosis: renal tuberculosis. Part I. Indian J Radiol Imaging 23:46–63

Nakane K, Yasuda M, Deguchi T, Takahashi S, Tanaka K, Hayami H, Hamasuna R, Yamamoto S, Arakawa S, Matsumoto T (2014) Nationwide survey of urogenital tuberculosis in Japan. Int J Urol 21:1171–1177

Portillo-Gomez L, Morris SL, Panduro A (2000) Rapid and efficient detection of extrapulmonary *Mycobacterium tuberculosis* by PCR analysis. Int J Tuberc Lung Dis 4:361–370

Rowinska-Zakrzewska E, Korzeniewska-Kosela M, Roszkowski-Sliz K (2013) Extrapulmonary tubrculosis in Poland 1974–2010. Pneumonol Alergol Pol 81:121–129.

Rowińska-Zakrzewska E, Korzeniewska-Koseła M, Roszkowski-Śliż K (2014) The epidemiological situation of tuberculosis in Poland: Part I. According to notification rates, the incidence of tuberculosis varies in different regions of Poland: is this true? Pneumonol Alergol Pol 82:342–348.

Rowińska-Zakrzewska E, Szopiński J, Remiszewski P, Szymańska D, Miller M, Pawlicka L, Zwolska-Kwiek Z (1995) Tuberculosis in the autopsy material: analysis of 1500 autopsies performed between 1972 and 1991 in the Institute of Tuberculosis and Chest Diseases, Warsaw, Poland. Tubercle Lung Dis 76:349–354

Shivakoti R, Sharma D, Mamoon G, Pham K (2017) Association of HIV infection with extrapulmonary tuberculosis: a systematic review. Infection 45 (1):11–21

Uno T, Numazaki S, Kato N, Egawa S (2014) Tuberculous epididymitis after Bacillus Calmette-Guérin intravesical therapy: a case report. Hinyokika Kiyo 60:291–294

WHO (2014) Global tuberculosis control: surveillance, planning, financing. WHO report 2014. World Health Organization, Geneva. ISBN:978 92 4 156480 9

Zammarchi L, Bartalesi F, Bartoloni A (2014) Tuberculosis in tropical areas and immigrants. Mediterr J Hematol Infect Dis 6:e2014043

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 39–46
DOI 10.1007/5584_2017_101

Published online: 6 October 2017

# Therapeutic Efficacy of Mandibular Advancement Devices in Patients with Severe Sleep Apnea: A Preliminary Report

M. Wojda, J. Kostrzewa-Janicka, P. Bieleń, P. Jurkowski, and E. Mierzwińska-Nastalska

**Abstract**

Obstructive sleep apnea (OSA) is defined as episodes of upper airway obstructions during sleep. The method of choice in conservative treatment of OSA is the use of devices that produce positive airway pressure (CPAP). In patients with mild-moderate form of OSA, intraoral mandibular advancement devices (MAD) are applied. The aim of the study was to evaluate the therapeutic efficacy of MAD in patients with severe OSA who were unable to use CPAP. In this preliminary study three patients from a group of 25 severe OSA sufferers who failed to use CPAP, were included. The three patients underwent a triple polysomnographic (PSG) investigation: on CPAP, before MAD treatment, and during MAD. The patients completed the Epworth Sleepiness Scale questionnaire twice. We found significant improvements of subjective symptoms and PSG-evaluated variables after application of MAD in all three patients in comparison with both baseline and CPAP-recorded data. We conclude that MAD can be an alternative treatment option for severe OSA patients who are non-compliant with CPAP therapy.

**Keywords**

Airway collapse • Mandibular advancement appliance • Sleep disordered breathing • Treatment effectiveness • Upper airways

## 1 Introduction

The main feature of obstructive sleep apnea (OSA) are recurrent episodes of complete or partial upper airway obstruction that are occasionally associated with snoring, The underlying cause is mandibular muscle relaxation with a retraction of the mandible and tongue, which leads to the upper airway obstruction. OSA is defined as a sleep disorder manifest by obstruction episodes exceeding 10 s, including a decrease in upper airway airflow $\geq$50%, and a decrease in arterial oxygen saturation $\geq$ 3%, occurring $\geq$10 times *per* hour of sleep (AASM

M. Wojda, J. Kostrzewa-Janicka (✉), P. Jurkowski, and E. Mierzwińska-Nastalska
Department of Prosthodontics, Medical University of Warsaw, 59 Nowogrodzka Street, Bldg.XIa, Warsaw 02-006, Poland
e-mail: jolanta.kostrzewa-janicka@wum.edu.pl

P. Bieleń
Fourth Clinic of Lung Diseases, Institute of Tuberculosis and Lung Diseases, Warsaw, Poland

1999). OSA belongs to the group of sleep disordered breathing, making up 90% of all forms of apnea. The prevalence of OSA is rather high and is age-dependent (Young et al. 1993). In the 20–44 years old men, with the apnea-hypopnea index (AHI) $\geq$ 10 events/h as a diagnostic criterion, the prevalence of OSA is 3.2%, but in the age range of 61 to 100 years it increases to as much as 18.1% (Bixler et al. 1998). In women, the prevalence is estimated at about half of that outlined above in men.

Polysomnography (PSG) is a gold standard test for OSA diagnosis due to its ability to identify parameters described in the OSA definition. The accompanying daytime sleepiness (EDS) is routinely measured by the Epworth sleepiness scale (ESS). The scale consists of 8 questions about the probability of falling asleep in different life situations (Johns 1991). According to the American Academy of Sleep Medicine Task Force Report (AASM 1999), OSA is recognized on the basis of the following specific diagnostic criteria: AHI $\geq$ 5/h of sleep, excessive daytime sleepiness, or at least two other symptoms from among the following: suffocation during sleep, recurrent arousal from sleep, sleep providing no relaxation, daily fatigue, and daytime attention lapses. Three stages of OSA severity are considered: mild – AHI <15 events/h of sleep and the patient's involuntary daytime sleepiness during activities requiring little attention; moderate – AHI >15 < 30 events/h and involuntary sleepiness during activities requiring some attention; and severe – AHI < 30 events/h and involuntary sleepiness during activities requiring distinct attention.

Ignoring OSA treatment may result in cardiovascular complications, endocrine system disorders, and in metabolic and psychoemotional consequences (Young et al. 2002). The method of choice in conservative treatment of OSA is the use of devices that produce positive airway pressure (CPAP). In patients with mild-moderate form of OSA and in those with severe OSA who are non-compliant with CPAP therapy, intraoral mandibular advancement devices (MAD) are used (Standards of Practice Committee of American Sleep Disorders Association 1995). The aim of the present study was to evaluate therapeutic efficacy of MAD in patients with severe OSA, in whom CPAP could not be used.

## 2 Methods

The study design has been approved by a local Ethics Committee of Warsaw Medical University in Poland (permit no. KB/65/2015). The study included three male patients with severe OSA who were non-complaint with CPAP therapy, selected from 25 patients referred from Clinic of Respiratory Sleep Disorders to Department of Prosthodontics. The patients' age ranged from 38 to 61 years, body mass index ranged from 30.8 to 32.4 kg/m$^2$, and the AHI ranged from 46.8 to 57.8 episodes/h. They were investigated on an outpatient basis after being referred to participate in the study by the Clinic of Respiratory Sleep Disorders of the Institute of Tuberculosis and Lung Diseases in Warsaw, Poland. The diagnosis of severe OSA was set on the basis of polysomnographic (PSG) records, Epworth sleepiness scale (ESS), and clinical signs and symptoms. Dental examinations were performed in the Department of Prosthodontics of Warsaw Medical University in Poland. These examinations provided the information on the number and condition of teeth, the condition of paradontium, the presence of temporomandibular disorders, and the possibility of applying MAD. Inclusion and exclusion criteria are summarised in Table 1.

The therapeutic approach consisted of the use of an Silensor-el appliance holding the mandible in a protruded position (ERKODENT® Erich Kopp GmbH; Pfalzgrafenweiler, Germany). The appliance is composed of splints for the upper and lower dental arches connected. The lower jaw is held in a predetermined advanced position by two connectors that are bilaterally fixed to the splint. That counteracts the narrowing of air passage while maintaining jaw movements without falling back of the lower jaw. The result is a reduction in pharyngeal obstructive episodes. To produce a patient-specific device, impressions of the upper and

**Table 1** Inclusion/exclusion criteria of the study subjects

| Inclusion criteria | Exclusion criteria |
|---|---|
| Age > 18 years | Medical |
| AHI > 30 episodes/h – severe OSA | Respiratory or sleep disorder other than OSA |
| ESS >10 | Medication intake that could influence respiration or sleep; |
| At least 8 teeth in the upper and lower jaws | Periodic limb movement disorder; |
| Lack of periodontal problems | BMI >40; |
| Lack of temporomandibular disorders | Previous treatment with CPAP or MAD; |
| | Reversible morphological upper airway abnormalities; |
| | Other medical conditions (e.g. psychiatric disorders). |
| | Dental |
| | Temporomandibular disorders; |
| | Untreated periodontal problems; |
| | Lack of retention possibility for an oral appliance. |

lower dental arches are to be taken, using alginate mass, and registered in the constructive bite. The constructive bite in the advanced mandibular position at 60% of maximum protrusion, after obtaining relevant space between incisive teeth, was fixed using the George Gauge Bite Registration (Scheu-Dental; GmbH, Iserlohn, Germany). This device is composed of a measurement part equipped with a notch for lower incisors and two interchangeable occlusal inserts of different thickness with notches for the upper incisors. On the lateral segments of occlusal inserts, registration material was placed to register the fixed mandible position against the maxilla. Splints were covering the occlusal surfaces, incisal edges of all teeth, vestibular surfaces in three fourth of their height, and the lingual surface passing onto mucous membrane of the right-sided ridge of the oral cavity.

Patients were scheduled for control visits and three repeat PSG examinations before starting the trial with MAD and CPAP devices. They also completed the ESS questionnaire twice. The first control visit was scheduled one week after providing the patient with mandibular advancement device. The device placement was corrected to mitigate excessive pressure and to improve the comfort of MAD use as required.

The efficacy of MAD treatment was assessed one month after its adaptation. At that time, patients completed a set of ESS questionnaires again and had a repeat PSG examination while on MAD. To compare the effectiveness of MAD and CPAP, patients were subjected to PSG examination while on CPAP therapy as well. CPAP was being used by patients for 7–10 days prior to the PSG examination, which was conducted at the same place and conditions like those during the classification process.

## 3 Results

PSG examination results show improvements in OSA features after MAD and CPAP treatments compared with the baseline level before treatment (Tables 2, 3 and 4). The AHI, number of central, obstructive and mixed apneas, and the sum of all apneas all decreased in the three patients investigated. These improvements were accompanied by minimal and mean $SaO_2$ values maintained at a higher level during sleep. Althought both MAD and CPAP treatments led to distinct improvements in OSA signs and symtoms, CPAP apparently was more effective. Providing that the AHI value below 10 episodes/h, adopted as the cut-off level for the OSA diagnosis, is taken as a criterion allowing for assessing the method used as an effective one, then this value was achieved in two out of the three patients treated with both MAD and CPAP. Yet, AHI was about 10 fold lower in case of CPAP treatment (Table 2). In the remaining patient, AHI although appreciably decreased, was still maintained above 10 episodes/h; after CPAP being about half of that after MAD

**Table 2** Apnea/hypopnea index (AHI), minimum oxygen saturation, and mean oxygen saturation before and after mandibular advancement appliances (MAD) and positive airway pressure (CPAP) treatments

| | Patient 1 | | | Patient 2 | | | Patient 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | MAD | CPAP | Before | MAD | CPAP | Before | MAD | CPAP |
| AHI (episodes/h) | 54.5 | 23.4 | 13.5 | 46.8 | 5.2 | 0.5 | 57.8 | 9.4 | 0.8 |
| Minimal $SaO_2$ (%) | 70 | 74 | 79 | 73 | 86 | 94 | 72 | 85 | 91 |
| Mean $SaO_2$ (%) | 93 | 94 | 95 | 94 | 95 | 96 | 92 | 94 | 95 |

**Table 3** Number of apnea episodes and snoring time before and after mandibular advancement appliances (MAD) and positive airway pressure (CPAP) treatments

| | Patient 1 | | | Patient 2 | | | Patient 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | MAD | CPAP | Before | MAD | CPAP | Before | MAD | CPAP |
| No. of central apneas/6-h sleep | 82 | 81 | 45 | 56 | 19 | 0 | 7 | 10 | 4 |
| No. of obstructive apneas/6-h sleep | 266 | 31 | 30 | 109 | 0 | 1 | 257 | 2 | 0 |
| No. of mixed apneas/6-h sleep | 7 | 4 | 2 | 5 | 0 | 0 | 2 | 0 | 0 |
| Snoring time (min) | 53 | 31 | 29 | 45 | 199 | 71 | 134 | 273 | 20 |

**Table 4** Epworth sleepiness scale (ESS) before and after mandibular advancement appliances (MAD) and positive airway pressure (CPAP) treatments

| | Patient 1 | | | Patient 2 | | | Patient | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before | MAD | CPAP | Before | MAD | CPAP | Before | MAD | CPAP |
| ESS (points) | 16 | 12 | – | 11 | 6 | – | 14 | 13 | – |

treatment. Interestingly, in this patient both treatments equally decreased the number of obstructive apneas; however CPAP effectively slashed central apneas in half whereas MAD remaiend here without effect (Table 3). Concerning the snoring time, treatment effects were equivocal. It decreased in two patientst aftert CPAP and in one after MAD. MAD increased the snoring time in two patients and CPAP in one patient.

The ESS score also decisevely decreased after MAD therapy. The improvement in subjective symptoms was, however, smaller in proportion compared with that in AHI (Table 4).

## 4 Discussion

The Polish Society of Lung Diseases recommends the initiation of MAD treatment in patients with snoring and mild form of OSA that persist despite behavioral therapy. The American Academy of Sleep Medicine has recommended the use of dental devices in patients with primary snoring (without OSA) and mild form of OSA, with a simultaneous attempt to reduce risk factors for sleep apnea. It also recommends to use dental devices in moderate and severe forms of OSA in patients who do not tolerate CPAP, and those who do not consent to the use of CPAP and are not classified for surgical treatment (AASM 1999). Patients with severe OSA who undertake dental-related treatment most often report intolerance to CPAP treatment due to nasal injuries or complains emanating from the upper airways.

The presence of a minimum of eight stable teeth in the maxilla and mandible, the possibility of fixing the constructive bite with the mandible position at 50–75% of a maximal protrusion, the space of 3–5 mm between incisors allowing the user to breathe freely through the mouth, and the healthy parodontium are preconditions to make an attempt to treat OSA with MAD (Johal and Bottegal 2001). The greater the mandible protrusion the higher the device efficacy, but tolerance is worse (Petelle et al. 2002). Studies

aimed at assessing the relationship between therapeutic effectiveness and a degree of mandible protrusion reveal that a degree of protrusion is a crucial element that improves the patient condition (Petri et al. 2008; Blanco et al. 2005). Some authors opine that fixation of the mandible position at 70% of maximal protrusion presents a compromise between benefits and side effects the device may provide (Bernhold and Bondemark 1998). In the present study, we fixed the maximum of mandible protrusion at 60% to achieve a better tolerance of the MAD device and to avoid adverse effects, e.g., pain in the temporomandibular joints (Mehta et al. 2001), alterations in the overjet and overbite, disorders in Angle's classification, or changes in the upper incisor angle relative to the cranial base (1/NS) and the angle between *Sella* - *Nasion* - *Supramentale* (SNB) (Fritsch et al. 2001). As to the vertical dimension of the constructive bite it is thought that it should remain at the minimal level in view of the fact that the increase in the vertical dimension through mandible opening displaces the tongue down and backward decreasing the airway patency (Pitsit et al. 2002). The application of MAD devices used in the present study facilitates the mandible opening preventing its retruded displacement. However, the lack of an appropriate number of teeth and a bad condition of the parodontium in OSA patients referred to MAD treatment appeared the reason for exclusion of a large number of subjects from the study.

The literature provides numerous reports that confirm the efficacy of MAD and CPAP and compare the effectiveness of both appliances (Kostrzewa-Janicka et al. 2017; Sharples et al. 2016; Gagnadoux et al. 2009; Hoffstein 2007; Fergusson et al. 1996). However, those studies focused on the comparison of the appliances in patients with mild and moderate forms of OSA. Ferguson et al. (1996) have revealed diminished snoring and sleepiness, and a lower AHI after MAD application, without sleep quality improvement, akin to CPAP application. Those authors found that in 12 (48%) out of the 25 patients with mild-moderate OSA treatment with MAD was successful, with a reduction in AHI below 10 episodes/h, compared to the treatment success in 13 (62%) out of the 21 patients with CPAP. However, patients' compliance and satisfaction with therapy were lower, and side effects more common in patients on CPAP. The authors conclude that MAD is more satisfactory for patients, although CPAP decreases AHI to a greater extent. A review article by Hoffstein (2007) also shows a greater reduction in AHI while using CPAP than MAD treatment, especially in patients with a peripheral component in the OSA pathogenesis, but MADs are better tolerated by patients. Similar conclusions have been drawn by Okuno et al. (2014). Smith and Stradling (2002) have shown both methods as equally effective in the OSA treatment. Nevertheless, a survey carried out by McGown et al. (2001) confirms a better acceptance of MAD than CPAP appliances by patients due to a greater comfort and ease of use of the former. Studies carried out by Aarab et al. (2011) have shown that the difference between the initial and outcome AHI for CPAP treatment is 20 episodes/h, whereas this difference for MAD is 15 episodes/h. These differences in the AHI outcome values were insignificant, but they were significant when compared with the group of patients using placebo splints. Likewise, excess daytime sleepiness appreciably and comparably decreased after treatment with both types of devices. All that demonstrates an overall equal worth of both methods.

The clinical trials above outlined show improvements after applying both MAD and CPAP treatment methods. However, assuming that therapeutic efficacy would be acceptable when the AHI value drops below 5 episodes/h, then MADs would not be recommended as a primary treatment method for OSA patients (Carra et al. 2012). However, it seems likely that it could be hardly possible to decrease the AHI value below 5 episodes/h when central apneas substantially contribute to it, as was the

case the patients of the present study. Phillips et al. (2013) have suggested a graded approach to therapeutic efficacy of different methods of OSA treatment. The efficacy would be assumed complete when AHI is reduced to fewer than 5 episode/h, and partial when AHI is reduced to less than 50% of the initial AHI value, yet remaining above 5 episodes/h, seemingly also a desirable therapeutic result. Otherwise, treatment failure should be conceded. Adopting the criteria above outlined to the present study, we would state that MAD was a partially effective treatment of severe OSA.

The literature indicates the persistence of positive effects and maintained improvement in OSA symptoms after application of both therapeutic methods during a two-year follow-up (Doff et al. 2013). These results encourage medical specialists to consider MAD application as a beneficial alternative to CPAP therapy for patients with mild-moderate or even severe form of OSA, despite CPAP treatment being considered by far the method of choice. Of note, both methods are capable of enhancing the arterial oxygen saturation, although CPAP apparently has here an edge over MAD (Doff et al. 2013), which also is in line with the present findings. In contrast, Barnes et al. (2004) have failed to demonstrate an increase in $SaO_2$, which would extend beyond the effect of placebo, in MAD therapy. Tan et al. (2002) have noted a highly significant decrease in the percentage of desaturation after CPAP application, from $7.1 \pm 2.7$ to $3.3 \pm 1.6\%$, while the difference was insignificant after MAD application, a post-treatment drop to $4.8 \pm 2.7\%$.

There is a difference between subjective and instrumental assessments of CPAP and MAD therapies. Mehta et al. (2001) have found good toleration of MAD therapy in 90% of the 24 OSA patients studied, diminished apnea/hypopnea index below 5 episodes/h in 37.5% of patients, and adopting the AHI value below 10 episodes/h as indicating the desirable therapeutic effect, the group of properly treated patients increased to 54%. Almost 70% of patients reported a reduction in the frequency and volume of snoring and 92% of them reported improvements in sleep quality. Similar results have been obtained by Gotsopoulos et al. (2002) who indicate a substantial subjective improvement in patients after MAD treatment, compared with patients of the control group who used inactive occlusion splints. Nonetheless, those authors have found no real objective improvements after MAD treatment. A notable exception in disparity between subjective and objective measures is ESS score that decreases in all studies on the subject, along with a subjective feeling of less sleepiness, with no major differences between the MAD and CPAP methods. The findings obtained in the presented study somehow contrast with the above outlined in that patients with severe OSA on MAD therapy reported less subjective improvement than could be judged from objective measures recorded in PSG examination.

Based on the available literature it can be inferred that CPAP treatment is most effective therapy leading to a decrease in AHI in patients with mild-moderate forms of OSA. However, the disease has many faces and there are considerable differences in the patients' reaction to the treatment applied, which are attributable to various factors. For one thing, there is a substantial variability in the strength and duration of apnea-hypopnea episodes from night to night, particularly before treatment commencement, which makes the assessment difficult in a single PSG evaluation (Aarab et al. 2009). Secondly, morphological structures of the craniofacial and upper airway regions differ from patient to patient. Lastly, time course of morbidity and treatment are also different. Despite a very limited number of patients in the present study, we believe we may conclude that mandible advancement devices hold promise as an alternative therapeutic method that provides subjective and objective improvements in patients suffering from severe OSA who do not tolerate CPAP. Our preliminary findings confirm a positive subjective and objective effects of mandible advancement devices in such patients.

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

## References


Aarab G, Lobbezzo F, Hamburger HL, Naeije M (2009) Variability in the apnea- hypopnea index and its consequences for diagnosis and therapy evaluation. Respiration 77:32–37

Aarab G, Lobbezzo F, Hamburger HL, Naeije M (2011) Oral appliance therapy versus nasal continuous positive airway pressure in obstructive sleep apnea; a randomized, placebo-controlled trial. Respiration 81:411–419

AASM (1999) American Academy of sleep medicine task force report. Sleep- related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. Sleep 22 (5):667–689

Barnes MR, McEvoy D, Banks S, Tarquinio N, Murray CG, Vowles N, Pierce RJ (2004) Efficacy of positive airway pressure and oral appliance in mild to moderate obstructive sleep apnea. Am J Respir Crit Care Med 170:656–664

Bernhold M, Bondemark L (1998) A magnetic appliance for treatment of snoring patients with and without obstructive sleep apnea. Am J Orthod Dentofac Orthop 113(2):144–155

Bixler EO, Vgontzas AN, Ten Have T, Tyson K, Kales A (1998) Effects of age on sleep apnea in men: I. Prevalence and severity. Am J Respir Crit Care Med 157:144–148

Blanco J, Zamarron C, Abeleira Pazos MT, Lamela C, Suarez Quintanilla D (2005) Prospective evaluation of an oral appliance in the treatment of obstructive sleep apnea syndrome. Sleep Breath 9:20–25

Carra MC, Bruni O, Huynh N (2012) Topical review: sleep bruxism, headaches, and sleep-disordered breathing in children and adolescents. J Orofac Pain 26(4):267–276

Doff MH, Hoekema A, Wijkstra PJ, van der Hoeven JH, Huddleston Slater JJ, de Bont LG, Stegenda B (2013) Oral appliance versus continuous positive airway pressure in obstructive sleep apnea syndrome: a 2-year follow-up. Sleep 36(9):1289–1296

Fergusson KA, Ono T, Lowe AA, Keenan SP, Fleetham JA (1996) A randomized crossover study of an oral appliance vs nasal-continuous positive airway pressure in the treatment of mild-moderate obstructive sleep apnea. Chest 109(5):1269–1275

Fritsch KM, Iseli A, Russi EW, Bloch KE (2001) Side effects of mandibular advancement devices for sleep apnea treatment. Am J Respir Crit Care Med 164 (5):813–818

Gagnadoux F, Fleury B, Vielle B, Petelle B, Meslier N, N'Guyen XL, Trzepizur W, Racineux JL (2009) Titrated mandibular advancement versus positive airway pressure for sleep apnoea. Eur Respir J 34:914–920

Gotsopoulos H, Chen C, Quian J, Cistulli PA (2002) Oral appliance therapy improves symptoms in obstructive sleep apnea. Am J Respir Crit Care Med 166:743–748

Hoffstein V (2007) Review of oral appliance for treatment of sleep-disordered breathing. Sleep Breath 11:1–22

Johal A, Bottegal JM (2001) Current principles in the management of obstructive sleep apnea with mandibular advancement appliances. Br Dent J 190 (10):532–536

Johns MW (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 31:1551–1558

Kostrzewa-Janicka J, Śliwiński P, Wojda M, Rolski D, Mierzwińska-Nastalska E (2017) Mandibular advancement appliance for obstructive sleep apnea treatment. Adv Exp Med Biol 944:63–71

McGown AD, Makker HK, Battagel JM, Grant HR, Spiro SG (2001) Long term use of mandibular advancement splints for snoring and obstructive sleep apnea: a questionnaire survey. Eur Respir J 17(3):462–466

Mehta A, Qian J, Petocz P, Darendeliler MA, Cistulli PA (2001) A randomized, controlled study of a mandibular advancement splint for obstructive sleep apnea. Am J Respir Crit Care Med 163:1457–1461

Okuno K, Sato K, Arisaka T, Hosohama K, Gotoh M, Taga H (2014) The effect of oral appliances that advanced the mandible forward and limited mouth opening in patients with obstructive sleep apnea: a systematic review and meta-analysis of randomised controlled trials. J Oral Rehabil 41:542–554

Petelle B, Vincent G, Gagnadoux F, Rakotonanahary B, Meyer B, Fleury B (2002) One-night mandibular advancement titration for obstructive sleep apnea syndrome. Am J Respir Crit Care Med 165 (8):1150–1153

Petri N, Svanholt P, Solow B, Wildschiodtz G, Winkel P (2008) Mandibular advancement appliance for obstructive sleep apnea: results of a randomised placebo controlled trial using parallel group design. J Sleep Res 17:221–229

Phillips CL, Grunstein RR, Darendeliler MA, Mihailidou AS, Srinivasan VK, Yee BJ, Marks GB, Cistulli PA (2013) Health outcomes of continuous positive airway pressure versus oral appliance treatment for obstructive sleep apnea: a randomized controlled trial. Am J Respir Crit Care Med 187(8):879–887

Pitsit AJ, Darendeliler M, Gotsopoulos H, Petocz P, Cistulli PA (2002) Effect of vertical dimension on efficacy of oral appliance therapy in obstructive sleep apnea. Am J Respir Crit Care Med 166 (6):860–864

Sharples LD, Clutterbuck-James AL, Glover MJ, Bennett MS, Chadwick R, Pittman MA, Quinnell TG (2016) Meta-analysis of randomised controlled trials of oral mandibular advancement devices and continuous positive airway pressure for obstructive sleep apnoea-hypopnoea. Sleep Med Rev 27:108–124

Smith DM, Stradling JR (2002) Can mandibular advancement devices be a satisfactory substitute for short term use in patients on nasal continuous positive airway pressure? Thorax 579(4):305–308

Standards of Practice Committee of American Sleep Disorders Association (1995) Practice parameters for

the treatment of snoring and obstructive sleep apnea with oral appliance. Sleep 18:511–513

Tan YK, L'Estrange PR, Luo YM, Smith C, Grant HR, Simonds AK, Spiro SG, Battagel JM (2002) Mandibular advancement splints and continuous positive airway pressure in patients with obstructive sleep apnoea: a randomized cross-over trial. Eur J Orthod 24:239–249

Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S (1993) The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 328:1230–1235

Young K, Peppard PE, Gottlieb DJ (2002) Epidemiology of obstructive sleep apnea. Am J Respir Crit Care Med 165(9):1217–1239

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 47–62
DOI 10.1007/5584_2017_84

Published online: 12 August 2017

# Effectiveness of Healthcare Coordination in Patients with Chronic Respiratory Diseases

Donata Kurpas, Katarzyna Szwamel, Dorota Lenarcik, Marika Guzek, Artur Prusaczyk, Paweł Żuk, Jolanta Michalowska, Agnieszka Grzeda, and Bożena Mroczek

**Abstract**

Coordination of healthcare effectively prevents exacerbations and reduces the number of hospitalizations, emergency visits, and the mortality rate in patients with chronic respiratory diseases. The purpose of this study was to determine clinical effectiveness of ambulatory healthcare coordination in chronic respiratory patients and its effect on the level of healthcare services as an indicator of direct medical costs. We conducted a retrospective health record survey, using an online database of 550 patients with chronic respiratory diseases. There were decreases in breathing rate, heart rate, and the number of cigarettes smoked *per* day, and forced vital capacity (FVC) and forced expired volume in 1 s (FEV1) increased after the implementation of the coordinated healthcare structure. These benefits were accompanied by increases in the number of visits to the pulmonary outpatient clinic ($p < 0.001$), diagnostic costs ($p < 0.001$), and referrals to other outpatient clinics ($p < 0.003$) and hospitals ($p < 0.001$). The advantageous effects of healthcare coordination on clinical status of respiratory patients above outlined persisted over a 3-year period being reviewed.

**Keywords**

Chronic diseases • General practice • Healthcare • Healthcare expenditure • Healthcare programs • Healthcare services • Respiratory Tract Diseases

## 1 Introduction

During the twenty-first century, the coordination and integration of care for patients with chronic conditions has become an important issue in health policies around the world (Chan et al.

D. Kurpas (✉) and K. Szwamel
Department of Family Medicine, Wroclaw Medical University, 1 Syrokomli St., 51-141Wroclaw, Poland

Opole Medical School, 68 Katowicka Street, 45-060 Opole, Poland
e-mail: dkurpas@hotmail.com

D. Lenarcik, M. Guzek, A. Prusaczyk, P. Żuk, J. Michalowska, and A. Grzeda
Medical and Diagnostic Center, 2 Kleeberg Street, 08-110 Siedlce, Poland

B. Mroczek
Department of Humanities in Medicine, Pomeranian Medical University, 11 Generała Chlapowskiego Street, 70-204 Szczecin, Poland

2012). Chronic respiratory diseases, especially asthma and COPD, often coexist with other chronic diseases such as heart failure, arrhythmias, hypertension, osteoporosis, metabolic syndrome and diabetes, gastroesophageal reflux disease, allergic rhinitis, nasal polyposis, bronchiectasis, anxiety, and depression, which may significantly impact patient outcomes and even increase mortality (GOLD 2017; Prosser et al. 2010; Bisaccioni et al. 2009). Episodes of primary disease exacerbation accompanied by chronic disease result in a frequent rehospitalization of patients with chronic respiratory diseases (Bashir et al. 2016; Hijjawi et al. 2015; Pooler and Beech 2014; Fan et al. 2012; Chan et al. 2011). The coordination of services is often inadequate, especially during exacerbations; that is why an important aspect of care to this group of patients is ensuring that the correct therapy is provided at the right time (ZuWallack and Nici 2010).

Disease management programs and integrated care programs that contain coordination components such as the self-management education (asthma triggers, breathing and inhaler use techniques), case management, clinical evaluation by nurse practitioners, onsite pulmonary function testing, psychosocial assessment by social workers, optimization of evidence-based medication, and information and support from case managers can effectively prevent exacerbations, reduce the number of hospitalizations and emergency visits, and even decrease mortality among patients with chronic respiratory diseases (George et al. 2016; Jain et al. 2014; Moullec et al. 2012; Rice et al. 2010; Casas et al. 2006). Coordination is increasingly recognized as a necessary element of high-quality, patient-centered care (Liss et al. 2011). Patients with chronic diseases are some of the major users of health care services, and they require long-term, comprehensive management from a variety of health care professionals in multiple settings (Napolitano et al. 2016; Katz et al. 2014; Chan et al. 2012). Care coordination is probably the best and preferred solution for addressing the healthcare-related frustrations in patient – provider communication experienced by individuals with chronic conditions. Finally, it is a reflection of the collaboration quality between primary care and other levels of healthcare, as much as a guarantee of medical decision optimization (Smith et al. 2017). Effective care coordination occurs when a team of professionals (physicians, nurse, community health worker, and social workers) organizes a patient's care activities and shares information about the patient's care to achieve better health outcomes. In a coordinated healthcare system, actions associated with patient care are organized deliberately, and all participants in the therapeutic process share information on patients. This enables the prediction of patient's needs and preferences, which results in safer and more effective care (Agency for Healthcare Research and Quality 2016). The system of coordinated care is not only based on the involvement and enhanced role of patients, but also on the active participation of their families, caregivers, and wider communities (Stein 2016).

Numerous studies have confirmed a positive influence of coordination and integration on healthcare results, satisfaction of patients and healthcare providers, and on cost-efficiency in chronic respiratory diseases (Moreo et al. 2017; Bashir et al. 2016; George et al. 2016; Kruis et al. 2013; Moullec et al. 2012; Niesink et al. 2007). For example, Casas et al. (2006) have indicated that an integrated care intervention, consisting of an individually tailored care plan upon discharge shared with the primary care team, as well as access to a specialized nurse case manager through a web-based call center, effectively prevent hospitalizations for exacerbations in COPD patients. According to Koff et al. (2009), proactive integrated care consisting of disease-specific education, teaching self-management techniques, enhanced communication with coordinators, and remote home monitoring has the potential to improve outcomes in COPD patients through the effects of self-management, as well as early detection and treatment of exacerbations. These authors demonstrate that this kind of care dramatically improves quality of life in COPD patients, as compared to patients without the intervention, i.e., receiving the usual care. Previous studies have also shown a positive

influence of care coordination and integration programs, as much as transitional care programs, on indicators of care in patients with neurological conditions and their caregivers (Kitzman et al. 2017), in frail older adults (Berglund et al. 2015), and in patients with chronic medical and mental health comorbidities (Jackson et al. 2015).

One aspect of care coordination is a continuity of care, as measured by the continuity of care index. In a study of Hussey et al. (2014) in COPD patients, higher levels of this index have been associated with lower odds of hospitalization, emergency department visits, and complications. These authors also show the effectiveness of continuity of care (COC) in patients with diabetes mellitus and congestive heart failure. Higher levels of COC in that study may be due the fact that interactions with the same provider over time reinforce interpersonal continuity, help deliver information that is easy to understand, and are significantly associated with a better patient – provider communication (Smith et al. 2017; Aseltine et al. 2016).

Despite extensive data confirming the effectiveness of coordinated care, the present model of financing primary healthcare in Poland does not motivate family physicians to take on the role of real coordinators of patient care. The effect of a capitation fee results in the attempts to reallocate direct medical costs to higher healthcare levels, quality of healthcare is generally perceived as low, and its clinical effectiveness is not assessed. A widespread nature of chronic respiratory diseases (Burney et al. 2015), together with the multimorbidity (GOLD 2017; Prosser et al. 2010; Bisaccioni et al. 2009) and low quality of life in this group of patients (Collar 2014; Ross et al. 2013), encourage the coordination of medical services in order to improve their quality and to measure their clinical effectiveness. Studies of the effectiveness of coordination of outpatient care provided for patients with chronic respiratory diseases are scant, and their outcomes are ambiguous.

The purpose of the present study was to determine the clinical effectiveness of care coordination in patients with chronic respiratory diseases within ambulatory healthcare and its effect on the level of healthcare services as an indicator of direct medical costs.

## 2 Methods

The research was performed in accord with the Declaration of Helsinki for Human Research of the World Medical Association and was approved by the Bioethics Commission of the Medical University in Wroclaw, Poland; approval no. KB-422/2014.

### 2.1 Study Protocol and Participants

We draw on an online database to conduct a retrospective health record survey. We analyzed both paper and electronic versions of the medical histories of patients receiving treatment in the pulmonology outpatient clinic between 2006 and 2016. Statistical variables, including the number of patient visits, the number of referrals to hospital and outpatient clinics, and the range of diagnostic procedures were generated from the clinic's computer system. We assessed clinical indicators from the time prior to the implementation of healthcare coordination in 2006–2011 in the pulmonology outpatient clinic, and during three successive visits at no less than annual intervals from 2012 to 2016, which that took place following the implementation of healthcare coordination. We considered the earliest visits (V0) before a period in which some elements of coordination were implemented, and the next three visits from 2012 (V1, first visit; V2, second; and V3, third visit). Before any elements of coordination had been implemented, only some visits involved spirometry. In the study, we only analyzed those visits during which spirometry was performed.

The main inclusion criteria were as follows: at least 18 years old; diagnosis of at least one chronic respiratory disease; having visits before and after implementing the coordination management plan in a pulmonary outpatient clinic, including: V0 and three successive visits at annual intervals (no less than one year, and no

longer than 18 months); and spirometry performed during visits.

## 2.2 Study Setting

The research was conducted at the Medical and Diagnostic Center in the city of Siedlce, Poland, where the following elements of coordination in the pulmonology outpatient clinic were implemented: development of an operation system in outpatient clinics and implementation of new technologies (combining the management system of the clinic with the laboratory system, access to the descriptions of radiological examinations, access to patients' medical histories, and the results of physical examinations performed by other specialists); health education provided for patients by nurses, covering issues such as training on administering inhalation agents, respiratory rehabilitation techniques, and the methods for giving up smoking; referring patients from the pulmonology outpatient clinic to a cardiologist and a vascular surgeon; performing spirometry during every visit (working with the nursing team responsible for spirometry); planning check-ups in the pulmonology outpatient clinic (patients not showing exacerbation of the disease returned in 6 months, while those showing exacerbation returned in 3 months).

## 2.3 Study Size

The Medical and Diagnostic Center provides services for urban and rural areas within two voivodeships; namely, in eight counties in the Mazowieckie Voivodeship and one county in the Lubelskie voivodeship. This region area is populated by 1,081,433 residents, of whom 514,292 (47.6%) live in urban areas and 567,141 (52.4%) live in rural areas. To estimate the population of patients with pulmonary diseases in the region, we used information from a statistical yearbook (Central Statistical Office of Poland 2016; Health Status of Population in Poland in 2014). Based on data from the Central Statistical Office of Poland, we considered pulmonary diseases that include asthma (also allergic asthma), chronic bronchitis, chronic obstructive pulmonary disease, and pulmonary emphysema. The percentage of people with pulmonary diseases in the population of Poland was 7.2%. Hence, the estimated number of patients in the region analyzed was 77,863 ($= 0.072 \times 1{,}081{,}433$). We collected the data of 550 patients. Knowing the size of a sample, we could estimate the fraction $P$, whose real value in the population was 0.3, accurate to $\pm 0.04$, and thus $0.26 < P < 0.34$ with probability of 0.95.

## 2.4 Sociodemographic Structure of Study Group

The tables below illustrate the structure of the overall group of patients (Table 1) and their diagnoses according to the ICD-10 (Table 2). The median age was 71 years (min–max: 28–96 years). The majority of patients (63.1%, 347) lived in urban areas with populations of 20,000–99,000; 36.9% (203) of patients lived in rural areas; 2.2% (12) lived in urban areas with populations below 20,000; and 0.7% (4) lived in urban areas with populations of 200,000 or more.

## 2.5 Statistical Analysis

The distribution of continuous variables was determined using the Shapiro–Wilk normality test. The vast majority of variables did not have normal distribution. Non-parametric tests were therefore employed in further analysis. Firstly, the differences between the distributions of particular variables between consecutive visits were determined using the Friedman test, with an alternative hypothesis that the distribution during at least one visit differed from others. Next, the Wilcoxon test was applied to show between which visits the differences were observed. The differences in the distribution of a given categorical variable between successive visits were verified using the chi-square test. R v3.1.3 (for Mac OS X 10.11.5) statistical software was used for all the analyses. The significance level was set at 0.05.

**Table 1** Structure of the study sample: sex, age, and place of residence

| Sex | Age (year) | Urban residence | | Rural residence | |
|---|---|---|---|---|---|
| | | n | % | n | % |
| Women | 20–29 | 0 | 0.0 | 1 | 0.5 |
| | 30–39 | 3 | 0.9 | 2 | 1.0 |
| | 40–49 | 12 | 3.5 | 7 | 3.4 |
| | 50–59 | 14 | 4.0 | 8 | 3.9 |
| | 60–69 | 66 | 19.0 | 45 | 22.2 |
| | 70–79 | 67 | 19.3 | 40 | 19.7 |
| | 80+ | 49 | 14.1 | 21 | 10.3 |
| Men | 20–29 | 0 | 0.0 | 0 | 0.0 |
| | 30–39 | 1 | 0.3 | 1 | 0.5 |
| | 40–49 | 6 | 1.7 | 7 | 3.4 |
| | 50–59 | 5 | 1.4 | 7 | 3.4 |
| | 60–69 | 42 | 12.1 | 17 | 8.4 |
| | 70–79 | 60 | 17.3 | 36 | 17.7 |
| | 80+ | 22 | 6.3 | 11 | 5.4 |
| Total | | 347 | 100.0 | 203 | 100.0 |

**Table 2** Most common pulmonary and non-pulmonary diagnoses during four visits

| V0 | | | V1 | | | V2 | | | V3 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ICD-10 | n | % | ICD-10 | n | % | ICD-10 | n | % | ICD-10 | n | % |
| Pulmonary diagnoses | | | | | | | | | | | |
| J45 | 236 | 42.7 | J45 | 301 | 54.4 | J45 | 290 | 52.4 | J45 | 221 | 40.0 |
| J44 | 206 | 37.3 | J44 | 239 | 43.2 | J44 | 236 | 42.7 | J44 | 182 | 32.9 |
| J42 | 36 | 6.5 | J42 | 39 | 7.1 | J47 | 39 | 7.1 | J42 | 33 | 6.0 |
| J47 | 30 | 5.4 | J47 | 35 | 6.3 | J42 | 37 | 6.7 | J47 | 29 | 5.2 |
| J43 | 12 | 2.2 | J98 | 13 | 2.4 | J98 | 19 | 3.4 | J30 | 19 | 3.4 |
| J40 | 8 | 1.4 | J30 | 11 | 2.0 | J30 | 17 | 3.1 | J43 | 8 | 1.4 |
| J30 | 5 | 0.9 | J43 | 11 | 2.0 | J43 | 10 | 1.8 | J98 | 8 | 1.4 |
| J84 | 4 | 0.7 | J84 | 6 | 1.1 | J84 | 7 | 1.3 | J22 | 4 | 0.7 |
| J98 | 4 | 0.7 | J40 | 4 | 0.7 | J06 | 4 | 0.7 | J33 | 4 | 0.7 |
| J26 | 2 | 0.4 | J06 | 3 | 0.5 | J32 | 4 | 0.7 | J84 | 4 | 0.7 |
| Non-pulmonary diagnoses | | | | | | | | | | | |
| R05 | 60 | 10.8 | B90 | 15 | 2.7 | F17 | 24 | 4.3 | F17 | 23 | 4.2 |
| R06 | 40 | 7.2 | F17 | 12 | 2.2 | G47 | 18 | 3.3 | G47 | 21 | 3.8 |
| F17 | 15 | 2.7 | R05 | 12 | 2.2 | B90 | 15 | 2.7 | B90 | 13 | 2.4 |
| R91 | 14 | 2.5 | R06 | 11 | 2.0 | K21 | 8 | 1.4 | K21 | 12 | 2.2 |
| B90 | 8 | 1.4 | G47 | 8 | 1.4 | D86 | 6 | 1.1 | D86 | 6 | 1.1 |
| G47 | 6 | 1.1 | D86 | 5 | 0.9 | R91 | 6 | 1.1 | K44 | 5 | 0.9 |
| D86 | 4 | 0.7 | R91 | 4 | 0.7 | I50 | 5 | 0.9 | L29 | 5 | 0.9 |
| Z03 | 3 | 0.5 | Z03 | 4 | 0.7 | R05 | 5 | 0.9 | L30 | 4 | 0.7 |
| C34 | 2 | 0.4 | C34 | 3 | 0.5 | K44 | 4 | 0.7 | C34 | 3 | 0.5 |
| D38 | 2 | 0.4 | K21 | 3 | 0.5 | R06 | 4 | 0.7 | D38 | 2 | 0.4 |

*ICD-10* disease codes from International Classification of Diseases, revision 10, *V0* visit before 2012; *V1* visit at least 1 year following V0, *V2* visit at least 2 years following V0, *V3* visit at least 3 years following V0

## 3 Results

### 3.1 Clinical Indicators Before and After Coordination Implementation

There was no change in the mean weight of participants between visits ($p = 0.560$). The mean number of years of smoking did change between visits ($p = 0.010$), but we did not manage to find out for which visits this difference was statistically significant. The number of cigarettes smoked per day changed depending on the visit ($p = 0.018$). A significant difference was observed between the first and the second visit ($p = 0.035$), with mean values of 19.4 and 18.7, respectively.

Breathing rate per minute depended on the visit ($p < 0.001$). Significant differences were observed between every pair of visits ($p < 0.001$). The mean breathing rate decreased from visit to visit. Pulse oximetry depended on the visit ($p = 0.013$). Significant differences were observed between V2 and V3 ($p = 0.047$), with mean values of 96.4 and 96.6, respectively. Heart rate depended on the visit ($p < 0.001$). Only V0 significantly differed from the other visits ($p < 0.001$). V1, V2, and V3 did not differ from each other. Neither diastolic nor systolic blood pressure depended on the visit ($p = 0.334$ and $p = 0.553$, respectively).

FVC measurements, both absolute magnitude and percent of predicted, depended on the visit ($p < 0.001$). In both cases, the mean values significantly increased from visit 0 to visit 3: from 2.54 to 2.82 L and from 78.8 to 95.5% predicted, respectively. The FEV1 in the absolute terms did not depend on the visit ($p = 0.357$), while FEV1% predicted did ($p = 0.037$). Only did V0 significantly differ from V2 and V3 ($p = 0.029$ and $p = 0.001$); the mean values were 73.1, 74.9, and 77.5%predicted, respectively. Both FEV1/FVC and FEV1/FVC % predicted depended on the visit ($p < 0.001$). In both cases, the mean values significantly decreased from visit 0 to visit 3; from 73.5 to 65.2% and from 94.6 to 85.0%predicted value (Table 3).

The distribution of lung sound was the only discrete variable that depended on the visit ($p < 0.001$). The percentage of normal lung sound decreased from 38.2% during V0 to 11.9% during V3. Other variables had similar distributions for each visit (Table 4).

### 3.2 Medical Services Before and After Implementation of Coordination

The mean number of diagnostic examinations increased from 5.1 before to 11.4 after the implementation of coordination and the number of visits in the pulmonary outpatient clinic increased from 5.8 to 9.0, respectively ($p < 0.001$ for both). The mean number of visits to primary care physicians did not change ($p = 0.080$): 36.4 before and 32.8 after implementation. The mean number of referrals from the pulmonary outpatient clinic increased from 1.3 before to 2.2 after implementation; from the pulmonary outpatient clinic to hospital from 0.9 before to 1.6 after implementation; from the pulmonary outpatient clinic to other specialist outpatient clinic from 0.4 before to 0.6 after implementation ($p < 0.001$ for all). The detailed data on the medical services before and after the implementation of coordination are displayed in Table 5.

## 4 Discussion

Improving the quality of care and reducing costs require a strong focus on the early recognition and management of functional impairment for patients living with chronic diseases (Mulpuru et al. 2017). Health care systems must move from fragmented, single-disease-centered services to a seamless integration of care addressing the psychological, medical, social, and cognitive needs of patients with chronic respiratory diseases (Williams et al. 2016). An unplanned readmission of chronically ill patients has a huge impact on the public healthcare system (Chan et al. 2011). A reduced frequency of exacerbations is beneficial for both patients, as it

**Table 3** Continuous variables in patients with chronic respiratory diseases

| Variables | Visit | n | M | SD | Q.25% | Median | Q.75% | Min | Max | S-W test - p | F test - p | | | | W test – p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Height (cm) | | 553 | 164.8 | 8.7 | 158.0 | 164.0 | 170.0 | 78.0 | 192.0 | <0.001 | – | – | – | – | – |
| Weight (kg) | 0. | 506 | 78.0 | 16.8 | 66.2 | 75.0 | 87.0 | 45.0 | 183.0 | <0.001 | 0.560 | – | V0 | V1 | V2 |
| | 1. | 529 | 78.6 | 17.4 | 67.0 | 77.0 | 88.0 | 40.0 | 177.0 | <0.001 | | V1 | 1 | – | – |
| | 2. | 481 | 78.4 | 16.3 | 67.0 | 77.0 | 87.0 | 42.0 | 158.0 | <0.001 | | V2 | 1 | 1 | – |
| | 3. | 356 | 78.8 | 16.2 | 67.0 | 78.0 | 87.0 | 45.0 | 133.0 | <0.001 | | V3 | 1 | 1 | 1 |
| BMI (kg/m$^2$) | 0. | 506 | 28.8 | 7.0 | 24.8 | 28.2 | 31.2 | 17.1 | 128.2 | <0.001 | 0.600 | – | V0 | V1 | V2 |
| | 1. | 529 | 29.0 | 7.1 | 25.1 | 28.2 | 31.6 | 14.8 | 124.9 | <0.001 | | V1 | 1 | – | – |
| | 2. | 481 | 29.0 | 7.2 | 24.9 | 28.1 | 31.6 | 16.7 | 129.8 | <0.001 | | V2 | 1 | 1 | – |
| | 3. | 356 | 29.0 | 5.2 | 25.3 | 28.5 | 32.0 | 17.3 | 128.2 | <0.001 | | V3 | 1 | 1 | 1 |
| Years smoking | 0. | 76 | 28.7 | 13.6 | 20.0 | 30.0 | 40.0 | 1.00 | 62.0 | 0.088 | 0.010 | – | V0 | V1 | V2 |
| | 1. | 80 | 29.6 | 13.8 | 20.0 | 30.0 | 40.0 | 1.00 | 60.0 | 0.032 | | V1 | 0.34 | – | – |
| | 2. | 72 | 30.0 | 14.0 | 20.0 | 30.0 | 40.0 | 1.00 | 60.0 | 0.044 | | V2 | 0.32 | 0.19 | – |
| | 3. | 56 | 28.7 | 13.0 | 20.0 | 30.0 | 40.0 | 1.00 | 55.0 | 0.058 | | V3 | 0.32 | 0.29 | 0.29 |
| Cigarettes smoked (per day) | 0. | 94 | 19.8 | 9.9 | 15.0 | 20.0 | 20.0 | 4.0 | 60.0 | <0.001 | 0.018 | – | V0 | V1 | V2 |
| | 1. | 94 | 19.4 | 11.0 | 10.0 | 20.0 | 20.0 | 3.0 | 60.0 | <0.001 | | V1 | 1.000 | – | – |
| | 2. | 85 | 18.7 | 10.1 | 10.0 | 20.0 | 20.0 | 3.0 | 50.0 | <0.001 | | V2 | 0.325 | 0.04 | – |
| | 3. | 65 | 19.6 | 9.9 | 20.0 | 20.0 | 20.0 | 2.0 | 50.0 | <0.001 | | V3 | 1.000 | 1.000 | 1.000 |
| Breathing rate (per minute) | 0. | 152 | 19.6 | 8.4 | 18.0 | 18.0 | 20.0 | 14.0 | 78.0 | <0.001 | <0.001 | – | V0 | V1 | V2 |
| | 1. | 412 | 18.1 | 6.0 | 16.0 | 18.0 | 19.0 | 0.00 | 78.0 | <0.001 | | V1 | 0 | – | – |
| | 2. | 451 | 16.7 | 5.7 | 15.0 | 16.0 | 17.0 | 6.00 | 78.0 | <0.001 | | V2 | 0 | 0 | – |
| | 3. | 343 | 16.0 | 5.1 | 15.0 | 15.0 | 16.0 | 14.00 | 75.0 | <0.001 | | V3 | <0.001 | <0.001 | <0.001 |
| Pulse oximetry ($SO_2$%) | 0. | 240 | 96.5 | 2.0 | 96.0 | 97.0 | 98.0 | 78.0 | 99.0 | <0.001 | 0.013 | – | V0 | V1 | V2 |
| | 1. | 443 | 96.1 | 2.7 | 96.0 | 97.0 | 98.0 | 65.0 | 99.0 | <0.001 | | V1 | 0.426 | – | – |
| | 2. | 470 | 96.4 | 1.7 | 96.0 | 97.0 | 98.0 | 78.0 | 99.0 | <0.001 | | V2 | 0.052 | 0.708 | – |
| | 3. | 352 | 96.6 | 1.3 | 96.0 | 97.0 | 98.0 | 90.0 | 99.0 | <0.001 | | V3 | 0.275 | 0.184 | 0.047 |
| Heart rate (beats per min) | 0. | 351 | 77.4 | 4.7 | 76.0 | 78.0 | 80.0 | 65.0 | 98.0 | <0.001 | <0.001 | | V0 | V1 | V2 |
| | 1. | 453 | 75.5 | 7.1 | 70.0 | 78.0 | 78.0 | 49.0 | 110.0 | <0.001 | | V1 | <0.001 | – | – |
| | 2. | 465 | 75.1 | 8.4 | 70.0 | 76.0 | 78.0 | 16.0 | 110.0 | <0.001 | | V2 | <0.001 | 0.21 | – |
| | 3. | 344 | 75.6 | 7.3 | 70.0 | 75.0 | 80.0 | 52.0 | 110.0 | <0.001 | | V3 | <0.001 | 0.58 | 0.58 |
| Systolic RR (mmHg) | 0. | 110 | 125.6 | 12.1 | 120.0 | 120.0 | 130.0 | 105.0 | 170.0 | <0.001 | 0.334 | | V0 | V1 | V2 |
| | 1. | 323 | 127.0 | 10.8 | 120.0 | 125.0 | 135.0 | 100.0 | 160.0 | <0.001 | | V1 | 1.00 | – | – |
| | 2. | 445 | 126.6 | 10.5 | 120.0 | 130.0 | 130.0 | 100.0 | 170.0 | <0.001 | | V2 | 1.00 | 1.00 | – |
| | 3. | 353 | 125.8 | 8.7 | 120.0 | 130.0 | 130.0 | 100.0 | 160.0 | <0.001 | | V3 | 0.20 | 0.11 | 0.29 |

(continued)

**Table 3** (continued)

| Variables | Visit | n | M | SD | Q.25% | Median | Q.75% | Min | Max | S-W test - p | F test - p | | | | W test – p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diastolic RR (mmHg) | 0. | 110 | 77.8 | 6.5 | 70.0 | 80.0 | 80.0 | 60.0 | 110.0 | <0.001 | 0.553 | | V0 | V1 | V2 |
| | 1. | 323 | 80.8 | 4.4 | 80.0 | 80.0 | 80.0 | 60.0 | 801.0 | <0.001 | | V1 | 1.00 | – | – |
| | 2 | 445 | 78.3 | 5.0 | 80.0 | 80.0 | 80.0 | 60.0 | 110.0 | <0.001 | | V2 | 1.00 | 1.00 | – |
| | 3. | 353 | 77.7 | 5.1 | 80.0 | 80.0 | 80.0 | 40.0 | 100.0 | <0.001 | | V3 | 1.00 | 0.62 | 0.23 |
| FVC (L) | 0. | 552 | 2.54 | 0.90 | 1.94 | 2.42 | 3.05 | 0.55 | 6.92 | <0.001 | <0.001 | | V0 | V1 | V2 |
| | 1. | 547 | 2.61 | 0.97 | 1.90 | 2.51 | 3.20 | 0.62 | 6.74 | <0.001 | | V1 | 0.0001 | – | – |
| | 2. | 490 | 2.69 | 1.00 | 1.99 | 2.57 | 3.30 | 0.73 | 7.67 | <0.001 | | V2 | <0.001 | <0.001 | – |
| | 3. | 355 | 2.82 | 1.00 | 2.09 | 2.68 | 3.45 | 0.98 | 7.72 | <0.001 | | V3 | <0.001 | <0.001 | <0.001 |
| FVC (%predicted) | 0. | 551 | 78.8 | 25.1 | 64.0 | 82.0 | 95.8 | 1.8 | 142.0 | <0.001 | <0.001 | – | V0 | V1 | V2 |
| | 1. | 547 | 82.1 | 26.4 | 68.1 | 84.0 | 100.0 | 1.9 | 146.0 | <0.001 | | V1 | <0.001 | – | – |
| | 2. | 490 | 85.8 | 27.4 | 70.6 | 87.0 | 103.0 | 1.8 | 155.5 | <0.001 | | V2 | <0.001 | <0.001 | – |
| | 3. | 356 | 95.5 | 24.5 | 79.9 | 95.9 | 112.0 | 35.3 | 174.0 | 0.338 | | V3 | <0.001 | <0.001 | <0.001 |
| FEV1 (L) | 0. | 552 | 1.9 | 0.7 | 1.4 | 1.8 | 2.3 | 0.5 | 5.6 | <0.001 | 0.357 | – | V0 | V1 | V2 |
| | 1. | 548 | 1.9 | 0.8 | 1.3 | 1.8 | 2.3 | 0.3 | 5.7 | <0.001 | | V1 | 1.00 | – | – |
| | 2. | 490 | 1.9 | 0.8 | 1.3 | 1.8 | 2.3 | 0.3 | 5.9 | <0.001 | | V2 | 1.00 | 1.0 | |
| | 3. | 357 | 1.9 | 1.1 | 1.3 | 1.7 | 2.3 | 0.5 | 18.2 | <0.001 | | V3 | 1.00 | 1.0 | 0.38 |
| FEV1 (%predicted) | 0. | 549 | 73.1 | 25.6 | 58.1 | 73.0 | 91.0 | 0.2 | 136.6 | <0.001 | 0.037 | – | V0 | V1 | V2 |
| | 1. | 548 | 73.5 | 27.2 | 55.4 | 74.0 | 91.8 | 1.3 | 146.0 | 0.001 | | V1 | 0.309 | – | – |
| | 2. | 490 | 74.9 | 27.2 | 57.2 | 75.5 | 93.5 | 1.5 | 154.0 | 0.002 | | V2 | 0.029 | 0.075 | – |
| | 3. | 357 | 77.5 | 24.9 | 62.0 | 77.9 | 96.0 | 2.1 | 148.0 | 0.082 | | V3 | 0.001 | 0.071 | 0.309 |
| FEV1/FVC (%) | 0. | 548 | 73.5 | 14.0 | 65.2 | 75.0 | 81.3 | 25.1 | 183.2 | <0.001 | <0.001 | – | V0 | V1 | V2 |
| | 1. | 542 | 71.2 | 14.4 | 63.0 | 72.9 | 81.0 | 1.4 | 120.5 | <0.001 | | V1 | 0.001 | – | – |
| | 2. | 485 | 69.9 | 13.2 | 61.0 | 70.1 | 79.1 | 27.5 | 122.3 | 0.0751 | | V2 | <0.001 | <0.001 | – |
| | 3. | 357 | 65.2 | 13.9 | 57.0 | 66.8 | 74.0 | 0.0 | 101.5 | <0.001 | | V3 | <0.001 | <0.001 | <0.001 |
| FEV1/FVC (%predicted) | 0. | 548 | 94.6 | 18.2 | 84.0 | 97.0 | 105.7 | 34.0 | 238.1 | <0.001 | <0.001 | – | V0 | V1 | V2 |
| | 1. | 542 | 92.4 | 17.9 | 81.7 | 94.3 | 105.0 | 11.3 | 159.0 | <0.001 | | V1 | 0.014 | – | – |
| | 2. | 483 | 91.3 | 33.0 | 79.0 | 91.0 | 102.3 | 10.8 | 703.0 | <0.001 | | V2 | <0.001 | <0.001 | – |
| | 3. | 357 | 85.0 | 16.8 | 75.0 | 86.0 | 96.0 | 0.0 | 144.6 | 0.0001 | | V3 | <0.001 | <0.001 | <0.001 |

*n* number of subjects, *M* mean, *SD* standard deviation, *S–W test* Shapiro–Wilk normality test, *F test* Friedman's test (to verify if the distributions from various groups are the same across repeated measures), *W test* pairwise Wilcoxon rank sum test (calculate pairwise comparisons between group levels with corrections for multiple testing), *V0* visit before 2012, *V1* visit at least 1 year following V0, *V2* visit at least 2 years following V0, *V3* visit at least 3 years following V0

**Table 4** Discrete variables in the patients with chronic respiratory diseases

| Variable | Category | V0 | | V1 | | V2 | | V3 | | Pearson's | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | n | % | $Chi^2$ test | |
| Smoking at present | No | 287 | 74.0 | 316 | 77.1 | 314 | 80.3 | 245 | 81.7 | $Chi^2$ | 8.68 |
| | Yes | 91 | 23.5 | 85 | 20.7 | 66 | 16.9 | 48 | 16.0 | df | 6 |
| | Passive | 10 | 2.6 | 9 | 2.2 | 11 | 2.8 | 7 | 81.7 | p | 0.19 |
| Smoking in the past | No | 119 | 38.3 | 125 | 37.5 | 124 | 38.3 | 92 | 36.2 | $Chi^2$ | 1.01 |
| | Yes | 172 | 55.3 | 191 | 57.4 | 180 | 55.6 | 147 | 57.9 | df | 6 |
| | Passive | 20 | 6.4 | 17 | 5.1 | 20 | 6.2 | 15 | 36.2 | p | 0.98 |
| Lung sounds | Normal | 187 | 38.2 | 122 | 23.6 | 61 | 12.7 | 42 | 11.9 | $Chi^2$ | 119.11 |
| | Changes | 303 | 61.8 | 396 | 76.4 | 419 | 87.3 | 312 | 88.1 | df | 3 |
| | | | | | | | | | | p | <0.001 |
| Cough | No | 105 | 23.6 | 94 | 19.0 | 96 | 20.5 | 85 | 24.1 | $Chi^2$ | 4.65 |
| | Yes | 340 | 76.4 | 400 | 81.0 | 372 | 79.5 | 267 | 75.9 | df | 3 |
| | | | | | | | | | | p | 0.19 |

Pearson's chi-squared test for count data, including chi-squared contingency table tests and goodness-of-fit tests, *V0* visit before 2012, *V1* visit at least 1 year following V0, *V2* visit at least 2 years following V0, *V3* visit at least 3 years following V0

improves their quality of life and slows down the progression of the disease, and for the healthcare system, as it cuts the costs of treatment (Śliwiński et al. 2014). In the present study we tried to establish the clinical effectiveness of care coordination in patients with chronic respiratory diseases within outpatient healthcare, which the basic components of care coordination were introduced to. Implementing the elements of coordinated care in the pulmonary outpatient clinic involved standardizing medical documentation, dividing work among physicians and nurses, making use of the computer system, ensuring comprehensive services, and improving the organization of visits by adjusting the elements of education, taking medical history, physical examination, and diagnosis, all in consideration with patients' individual needs.

Most patients in the present research had diagnoses of asthma and COPD. These data correspond with the global statistics, reflecting the widespread nature of these diseases (Burney et al. 2015), which confirms that the study sample was representative of the general population. Our study shows that the introduction of basic components of coordination to outpatient specialist care improves clinical indicators, which are objective and available within primary healthcare, and keeps the patients' condition stable. We demonstrate a significant decline in the mean breathing rate from visit to visit; from 19.6 before implementing care coordination to 16.0 breath per min during the last visit considered in the study. The outcomes show that the pulse oximetry results remained within normal limits for a several year-long period.

Spirometry is another noninvasive and widely available objective measure that is used to identify such changes in the respiratory system as limited air flow in patients with chronic respiratory diseases (GOLD 2017). In the present study, the mean FVC values increased from visit to visit, which may have been a consequence of introducing elements of care coordination, such as the individual approach to patients, patients' understanding of, and complying with, the instructions, and the proper technique for performing spirometry.

FEV1 is an important lung function variable on the population level in predicting the clinical outcomes such as mortality and hospitalization (GOLD 2017). A commonly accepted way to determine the degree of obstruction is the classification based on the FEV1 as a percentage of the predicted value. In the study of Jain et al. (2014) the influence of the Pulmonologist-led Chronic Lung Disease Program for patients with severe asthma and COPD on healthcare utilization and predictors of its effectiveness was investigated. Those authors have demonstrated that the mean

**Table 5** Number of medical services in the patients with chronic respiratory diseases

| Service | Coordination | n | M | SD | Q.25% | Median | Q.75% | Min | Max | S–W test - p | W test - p |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diagnostic examinations in pulmonary outpatient clinic | | 550 | 16.5 | 8.2 | 10.0 | 15.0 | 21.0 | 2.0 | 56.0 | < 0.001 | – |
| | Before | 550 | 5.1 | 5.3 | 1.0 | 3.0 | 7.0 | 1.0 | 28.0 | < 0.001 | < 0.001 |
| | After | 550 | 11.4 | 5.4 | 7.0 | 11.0 | 15.0 | 1.0 | 32.0 | < 0.001 | |
| Visits to pulmonary outpatient clinic | | 550 | 14.8 | 8.2 | 9.0 | 13.0 | 20.0 | 2.0 | 48.0 | < 0.001 | – |
| | Before | 550 | 5.8 | 6.0 | 1.0 | 3.0 | 8.0 | 1.0 | 32.0 | < 0.001 | < 0.001 |
| | After | 550 | 9.0 | 4.5 | 6.0 | 9.0 | 11.0 | 1.0 | 26.0 | < 0.001 | |
| Visits to primary care physicians | | 219 | 69.2 | 38.2 | 41.0 | 63.0 | 87.0 | 8.0 | 221.0 | < 0.001 | – |
| | Before | 219 | 36.4 | 26.9 | 15.0 | 32.0 | 51.5 | 1.0 | 189.0 | < 0.001 | 0.081 |
| | After | 219 | 32.8 | 19.9 | 20.0 | 28.0 | 40.0 | 1.0 | 157.0 | < 0.001 | |
| Referrals to hospital and other specialist outpatient clinics | | 550 | 3.6 | 1.5 | 2.0 | 3.0 | 4.0 | 2.0 | 7.0 | < 0.001 | – |
| | Before | 550 | 1.3 | 0.6 | 1.0 | 1.0 | 2.0 | 1.0 | 3.0 | < 0.001 | < 0.001 |
| | After | 550 | 2.2 | 1.4 | 1.0 | 2.0 | 3.0 | 1.0 | 6.0 | < 0.001 | |
| Referrals from pulmonary outpatient clinic to hospital | | 550 | 2.5 | 1.7 | 1.0 | 2.0 | 4.0 | 0 | 6.0 | < 0.001 | – |
| | Before | 550 | 0.9 | 0.7 | 0.5 | 1.0 | 1.0 | 0 | 3.0 | < 0.001 | 0.001 |
| | After | 550 | 1.6 | 1.5 | 0 | 1.0 | 3.0 | 0 | 5.0 | < 0.001 | |
| Referrals from pulmonary outpatient clinic to other specialist outpatient clinics | | 550 | 1.0 | 0.9 | 0 | 1.0 | 1.0 | 0 | 3.0 | < 0.001 | – |
| | Before | 550 | 0.4 | 0.5 | 0 | 0 | 1.0 | 0 | 2.0 | < 0.001 | 0.003 |
| | After | 550 | 0.6 | 0.7 | 0 | 1.0 | 1.0 | 0 | 2.0 | < 0.001 | |
| Referrals from primary care physicians to hospital and specialist outpatient clinics | | 219 | 8.2 | 5.2 | 4.8 | 6.5 | 10.3 | 3.0 | 24.0 | 0.001 | – |
| | Before | 219 | 3.3 | 2.2 | 1.8 | 3.0 | 4.0 | 1.0 | 9.0 | 0.001 | 0.049 |
| | After | 219 | 4.9 | 3.8 | 2.0 | 3.5 | 6.3 | 1.0 | 15.0 | 0.009 | |
| Referrals from primary care physicians to hospital | | 219 | 0.9 | 1.2 | 0 | 0 | 1.3 | 0 | 4.0 | < 0.001 | – |
| | Before | 219 | 0.2 | 0.4 | 0 | 0 | 0 | 0 | 1.0 | < 0.001 | 0.014 |
| | After | 219 | 0.7 | 1.0 | 0 | 0 | 1.0 | 0 | 3.0 | < 0.001 | |
| Referrals from primary care physicians to specialist outpatient clinics | | 219 | 7.3 | 5.1 | 4.0 | 6.0 | 8.3 | 2.0 | 23.0 | < 0.001 | – |
| | Before | 219 | 3.1 | 2.1 | 1.8 | 3.0 | 4.0 | 1.0 | 23.0 | 0.002 | 0.209 |
| | After | 219 | 4.2 | 3.7 | 2.0 | 3.0 | 5.3 | 0 | 9.0 | 0.001 | |

*n* number of subjects, *M* mean, *SD* standard deviation, *Q* quartile, *S–W test* Shapiro–Wilk normality test, *W test* pairwise Wilcoxon rank sum test (calculate pairwise comparisons between group levels with corrections for multiple testing)

FEV1 improved from 1.78 ± 0.80 to 1.82 ± 0.77 L ($p = 0.445$). In the present study, FEV1 was not significantly dependent on the visit. Nevertheless, we observed an upward tendency in all mean percentage values of the predicted FEV1 from visit to visit, with just V0 significantly different from V2 and V3. These values were in a range from 73.1 during V0 to 77.5%predicted during V3 and if the analysis is superficial, they might be perceived as being low. It should be remembered, however, that taking 80% of the predicted value as a lower end of normal limit is not optimal. The predicted values recommended by the European Respiratory Society are intended for women of 145–180 cm and men of 155–195 cm in height, belonging to the Caucasian population aged 18–70. When these values are used in the 70+ population, they need to be extrapolated. In the present study, people aged 70+ constituted 55.3% of the sample (306 subjects, including 177 women and 129 men).

Another parameter that we considered was the Tiffeneau-Pinelli index. According to the Global Initiative for Asthma guidelines, the $FEV_1/FVC$ ratio should be above 0.75–0.80 in adults. The criterion for obstruction proposed by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) is a Tiffeneau-Pinelli index below 0.7 (70%) (GOLD 2017; Vogelmeier et al. 2017). However, it should not be forgotten that an FEV1/FVC ratio of 70% is not a real lower limit, and can only be applied as a criterion in screening or examinations performed in order to qualify patients for further diagnosis. In the case of our sample, 86.4% of which consisted of people aged 60+, it seems reasonable to take 65% of the index as the cut-off level in interpreting the results; the threshold proposed by Falaschetti et al. (2004) for people of advanced age. Our results show that the Tiffeneau-Pinelli index decreased after care coordination had been implemented. However, taking into account the participants age and the problems of keeping patients with chronic respiratory diseases stable for longer times, the results obtained can be regarded as relatively positive, as the values recorded during all visits did fall below 65%.

A study of Wróblewska et al. (2015) has confirmed the presence of an inverse association between FEV1 and patients' age ($r = -0.18$, $p = 0.009$) or the number of days of hospitalization ($r = -0.16$, $p = 0.022$). Those authors also show that the highest FEV1/FVC values were observed in non-smokers. In the present study, concomitant non-pulmonary diseases included mental and behavioral disorders caused by smoking. Kohansal et al. (2009) have demonstrated that cigarette smokers have a higher prevalence of respiratory symptoms and lung function abnormalities, a greater annual rate of decline in FEV1, and a much greater COPD mortality rate than non-smokers. Thomsen et al. (2013) have indicated that, compared to smokers with COPD, never-smokers with chronic airflow limitation have fewer symptoms, milder disease, and a lower burden of systemic inflammation. A study of Rezaei et al. (2016), conducted in 1271 patients with lung cancer (n = 415), COPD (n = 427), and ischemic heart diseases (n = 429) in Iran, has shown that the mean lengths of hospital stay for current smokers, former smokers, and non-smokers were 9.4 ± 8.4, 7.3 ± 5.3, and 6.0 ± 5.0 days, respectively. Moreover, probabilities of the length of hospital stay for current and former smokers were 56% and 21%, and 48% and 15% greater, respectively, than those for non-smokers. Faulkner et al. (2006) have shown that smoking cessation is the single most effective and cost-effective way to reduce the risk of developing COPD and stop its progression. Li et al. (2012) have conducted an interesting study in Japan, which shows that smoking cessation reverses the excess risk of COPD mortality to a level similar to that observed among men who have never smoked. In a study of George et al. (2016), patients were enrolled in a smoking cessation program and counseled on quitting smoking through telephone case management. The authors suggest that not only the telephone management but also the introduction of other components of a disease management program into the model of

care, such as timely follow up with physicians, optimization of medications, self-management of the condition, and timely treatment for exacerbation i the hospital, would have a synergistic effect on survival. In the present study, after the implementation of care coordination, we observed a positive change consisting of a gradual decline in the number of smokers from visit to visit. Still, these results were statistically insignificant. We also demonstrated that the number of cigarettes smoked per day decreased from V0 to V2. Paradoxically, however, this number increased during V3. This fact, like a decline in the Tiffeneau-Pinelli index, can be explained by a profound influence of patient-dependent factors on the results of therapy. Hijjawi et al. (2015) have indicated that early readmissions among patients with COPD are related to patient factors, such as alcohol abuse and advanced disease, rather than system or provider factors. In a multivariate analysis, the authors show the odds ratio of 2.17 for readmission in 30 days (95% CI, 1.16–4.09) in patients with alcohol abuse, and of 2.52 (95% CI, 1.18–5.38) for those getting supplemental oxygen. In a study of Omachi et al. (2013), poor health literacy is associated with a greater COPD severity, greater COPD helplessness, worse respiratory-specific health-related quality of life, and higher odds of COPD-related emergency health care utilization.

In the present study, we also investigated the influence of care coordination on the level of healthcare services as an indicator of direct medical costs. Bashir et al. (2016) has suggested that the factors influencing COPD readmissions are complex and poorly understood. But another study conducted in patients with chronic respiratory diseases showed that the highest index of healthcare services was found in men, elderly patients, and in those having a partner, from rural areas, with lower levels of disease acceptance, with low levels of satisfaction with QoL, with low levels of satisfaction with quality of health state, and with higher somatic indices (Kurpas et al. 2015). Koff et al. (2009) have suggested that proactive integrated care systems may decrease healthcare costs. However, an integrated care program for patients with COPD, conducted by Boland et al. (2015), which mainly included professionally directed interventions, was not cost-effective in primary care. In a study of George et al. (2016), readmission and hospital days were greater for the program patients (0.36 *vs*. 0.17 per person year) than for control patients (2.19 *vs*. 1.88 per person year). The present findings correspond indirectly with the results of those studies. We demonstrate a substantial increase in the number of referrals to hospital after coordination was implemented. Moreover, we noticed that the number of referrals to the pulmonary outpatient clinic, where elements of coordination had been introduced, also increased. We additionally noted an increase in the number of diagnostic examinations ordered, and in the number of referrals from the pulmonary outpatient clinic to other specialist clinics. That leads us to conclude that implementing the elements of care coordination entails an increase in the number of services, which in turn raises the costs of medical care. This relationship requires further investigation over a longer time period.

Importantly, despite the increase in the number of referrals in the present study, the number of visits to the pulmonary outpatient clinic did not significantly change; in fact there was a decreasing trend in these visits. That suggests that the number of patients with chronic respiratory diseases requiring consultation with a pulmonologist most probably increased, but owing to coordination, healthcare professionals managed to maintain the condition of patients under pulmonary care sufficiently stable not to increase the number of visits. Further research into these aspects, conducted in a greater sample, is needed to confirm that coordination contributes to a decrease in the number of visits.

In the present study, new technologies were implemented in pulmonary outpatient clinic, resulting in (1) better access of healthcare professionals to laboratory test results, descriptions of radiological examinations, medical histories, and results of physical examinations performed by other specialists; (2) the possibility of referring patients from the pulmonary outpatient clinic directly to other

specialists; and (3) cooperation with nursing staff and patient education. In fact, we demonstrate that we can achieve clinical effectiveness in patients with chronic respiratory diseases, by introducing the most basic elements of care coordination. A systematic review of 26 randomized studies (Kruis et al. 2013), involving 2997 patients with COPD from 11 different countries and covering a period of 3–12 months, has shown that patients treated with an integrated disease management program, in which several healthcare providers collaborated, have a significant improvement in quality-of-life scores, decrease in hospitalization duration by nearly 4 days, and a clinically relevant improvement of 44 m in 6 min walking distance, compared to control subjects. Moullec et al. (2012) have also conducted a retrospective longitudinal cohort study between 2004 and 2006 in the province of Quebec, Canada, showing that an integrated care program combining self-management education and case-management decreases the rate of COPD-related hospitalizations, particularly among women. A review of Niesink et al. (2007) have included ten randomized-controlled trials of a 10-year observation, comparing chronic disease management with routine care in COPD patients. The authors show positive outcomes in one or more domains of the quality-of-life instruments. Moreo et al. (2017) have indicated that care coordination is especially important in pulmonary arterial hypertension and idiopathic pulmonary fibrosis, which are relatively rare diseases with non-specific symptoms and many co-morbidities. Those authors opine that, in coordinating care for patients with complex pulmonary diseases like those, case managers across practice settings can play a key role in improving workflow processes and communication, coordinating care and transitions of care for patients with co-morbidities, providing patient and caregiver education, and advancing the care plan. A study of Jain et al. (2014), conducted in 106 patients with COPD, has shown that there is a significant decrease in the mean respiratory-related emergency room visits, mean hospitalizations, and 30-day rehospitalizations during 1-year time of follow-up after enrollment into the pulmonologist-led chronic lung disease program. In a study of Bashir et al. (2016), conducted between January and October 2013 in 461 COPD patients, post-hospital care coordination for transition of care from hospital to the community results in a 4.3% reduction in the 30-day all-cause readmission rate.

The studies above outlined show that the introduction of complex elements of care coordination enables the achievement of clinical effectiveness, as assessed by the number of hospital stays or admissions to hospital emergency department, and by the death rate. Unfortunately, these indicators cannot be applied to healthcare systems in the countries of central and Eastern Europe, which lack the centralized electronic flow of information concerning medical services.

The present study is the first of a kind concerning outpatient care provided within healthcare systems in central and Eastern Europe. The results can be generalized, as the representativeness of the sample is confirmed by the fact that the most frequent diagnoses in the study sample correspond with those in the general population of Poland. The strength of the study also lies a large sample size and the inclusion of all available indicators of care. It should be emphasized that the study was carried out in a healthcare system in which coordination on the central level has not yet been implemented.

A relatively short period of observation in the study is a limitation. Nor did we take into account the quality of life, including its psychological and social functioning, and the environmental aspects. Our research, however, is a naturalistic study based mainly on currently available data derived from a retrospective analysis. Only was a single pulmonary outpatient clinic examined, with a truly functioning system of coordinated care, due to a shortage of such centers in Poland.

Future research should involve a prospective analysis of the effectiveness of coordinated healthcare and should include a longer period of observation, with a greater number of pulmonary outpatient clinics than the current study. It is also recommended to extend the research with quality-of-life indicators to patients with chronic

respiratory diseases. It would be useful to conduct a comparative analysis of care effectiveness indicators in the same time intervals, before and after the implementation of care coordination.

## 5 Conclusions

Care coordination over a 3-year period consisted of basic components that did affect the clinical status of patients with chronic respiratory diseases. An increase in direct medical costs should be considered within the coordination management plan at the ambulatory level of healthcare. The introduction of centralized electronic flow of information concerning medical services would make it possible to calculate indicators of effectiveness, such as the number of rehospitalizations, the number of admissions to hospital emergency departments, and the death rate. The use of additional elements of coordination in healthcare systems, such as case management, psychosocial assessment by a social worker, a care planning meeting for older individuals returning home directly after visiting the emergency department, clinical evaluation by specialized nurses, optimization of evidence-based medications, and disease management programs in which several healthcare providers collaborate, lead to improvements in patient outcomes.

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

## References

Agency for Healthcare Research and Quality (2016) Care coordination. Rockville, MD. http://www.ahrq.gov/professionals/prevention-chronic-care/improve/coordination/index.html. Accessed on 4 June 2017

Aseltine RH, Sabina A, Barclay G, Graham G (2016) Variation in patient–provider communication by patient's race and ethnicity, provider type, and continuity in and site of care: an analysis of data from the Connecticut health care survey. SAGE Open Med 4:2050312115625162

Bashir B, Schneider D, Naglak MC, Churilla TM, Adelsberger M (2016) Evaluation of prediction strategy and care coordination for COPD readmissions. Hosp Pract 44(3):123–128

Berglund H, Hasson H, Kjellgren K, Wilhelmson K (2015) Effects of a continuum of care intervention on frail older persons' life satisfaction: a randomized controlled study. J Clin Nurs 24(7–8):1079–1090

Bisaccioni C, Aun MV, Cajuela E, Kalil J, Agondi RC, Giavina-Bianchi P (2009) Comorbidities in severe asthma: frequency of rhinitis, nasal polyposis, gastroesophageal reflux disease, vocal cord dysfunction and bronchiectasis. Clinics (Sao Paulo) 64(8):769–773

Boland MR, Kruis AL, Tsiachristas A, Assendelft WJ, Gussekloo J, Blom CM, Chavannes NH, Rutten-van Mölken MP (2015) Cost-effectiveness of integrated COPD care: the RECODE cluster randomised trial. BMJ Open 5(10):e007284. doi:10.1136/bmjopen-2014-007284

Burney P, Jarvis D, Perez-Padilla R (2015) The global burden of chronic respiratory disease in adults. Int J Tuberc Lung Dis 19(1):10–20

Casas A, Troosters T, Garcia-Aymerich J, Roca J, Hernández C, Alonso A, del Pozo F, de Toledo P, Antó JM, Rodríguez-Roisín R, Decramer M, Members of the CHRONIC Project (2006) Integrated care prevents hospitalizations for exacerbations in COPD patients. Eur Respir J 28:1130–1230

Central Statistical Office of Poland (2016) Area and population in the territorial profile in 2016. http://stat.gov.pl/obszary-tematyczne/ludnosc/ludnosc/powierzchnia-i-ludnosc-w-przekroju-terytorialnym-w-2016-r-,7,13.html. Accessed on 3 June 2017

Chan FW, Wong FY, Yam CH, Cheung WL, Wong EL, Leung MC, Goggins WB, Yeoh EK (2011) Risk factors of hospitalization and readmission of patients with COPD in Hong Kong population: analysis of hospital admission records. BMC Health Serv Res 11:186. doi:10.1186/1472-6963-11-186

Chan CL, You HJ, Huang HT, Ting HW (2012) Using an integrated COC index and multilevel measurements to verify the care outcome of patients with multiple chronic conditions. BMC Health Serv Res 12:405. doi:10.1186/1472-6963-12-405

Collar JM (2014) Asthma and COPD in Spain: quality of life and health consumption resources. Value Health 17(7):A601. doi:10.1016/j.jval.2014.08.2083

Falaschetti E, Laiho J, Primatesta P, Purdon S (2004) Prediction equations for normal and low lung function from the health survey for England. Eur Respir J 23:456–463

Fan VS, Gaziano JM, Lew R, Bourbeau J, Adams SG, Leatherman S, Thwin SS, Huang GD, Robbins R, Sriram PS, Sharafkhaneh A, Mador MJ, Sarosi G, Panos RJ, Rastogi P, Wagner TH, Mazzuca SA, Shannon C, Colling C, Liang MH, Stoller JK, Fiore L, Niewoehner DE (2012) A comprehensive care management program to prevent chronic obstructive pulmonary disease hospitalizations: a randomized, controlled trial. Ann Intern Med 156 (10):673–683

Faulkner MA, Lenz TL, Stading JA (2006) Cost-effectiveness of smoking cessation and the implications for COPD. Int J Chron Obstruct Pulmon Dis 1:279–287

George PP, Heng BH, Lim TK, Abisheganaden J, Ng AW, Verma A, Lim FS (2016) Evaluation of a disease management program for COPD using propensity matched control group. J Thorac Dis 8(7):1661–1671

GOLD (2017) Global initiative for asthma. Global strategy for Asthma Management and Prevention. http://ginasthma.org/2017-gina-report-global-strategy-for-asthma-management-and-prevention/. Accessed on 4 June 2017

Health status of population in Poland (2014) http://stat.gov.pl/obszary-tematyczne/zdrowie/zdrowie/stan-zdrowia-ludnosci-polski-w-2014-r-,6,6.html#. Accessed on 3 June 2017

Hijjawi SB, Abu Minshar M, Sharma G (2015) Chronic obstructive pulmonary disease exacerbation: a single-center perspective on hospital readmissions. Postgrad Med 127(4):343–348

Hussey PS, Schneider EC, Rudin RS, Fox DS, Lai J, Pollack CE (2014) Continuity and the costs of care for chronic disease. JAMA Intern Med 174:742–748

Jackson C, DuBard A, Swartz M, Mahan A, McKee J, Pikoulas T, Moran K, Lancaster M (2015) Readmission patterns and effectiveness of transitional care among medicaid patients with schizophrenia and medical comorbidity. N C Med J 76(4):219–226

Jain VV, Allison R, Beck SJ, Jain R, Mills PK, McCurley JW, Van Gundy KP, Peterson MW (2014) Impact of an integrated disease management program in reducing exacerbations in patients with severe asthma and COPD. Respir Med 108(12):1794–1800

Katz A, Martens P, Chateau D, Bogdanovic B, Koseva I (2014) Do primary care physicians coordinate ambulatory care for chronic disease patients in Canada? BMC Fam Pract 15:148. doi:10.1186/1471-2296-15-148

Kitzman P, Hudson K, Sylvia V, Feltner F, Lovins J (2017) Care coordination for community transitions for individuals post-stroke returning to low-resource rural communities. J Community Health 42 (3):565–572

Koff PB, Jones RH, Cashman JM, Voelkel NF, Vandivier RW (2009) Proactive integrated care improves quality of life in patients with COPD. Eur Respir J 33 (5):1031–1038

Kohansal R, Martinez-Camblor P, Agusti A, Buist AS, Mannino DM, Soriano JB (2009) The natural history of chronic airflow obstruction revisited: an analysis of the Framingham offspring cohort. Am J Respir Crit Care Med 180(1):3–10

Kruis AL, Smidt N, Assendelft WJ, Gussekloo J, Boland MR, Rutten-van Mölken M, Chavannes NH (2013) Integrated disease management interventions for patients with chronic obstructive pulmonary disease. Cochrane Database Syst Rev 10(10):CD009437

Kurpas D, Bujnowska-Fedak MM, Athanasiadou A, Mroczek B (2015) Factors influencing utilization of primary health care services in patients with chronic respiratory diseases. Adv Exp Med Biol 866:71–81

Li Y, Yamagishi K, Yatsuya H, Tamakoshi A, Iso H (2012) Smoking cessation and COPD mortality among Japanese men and women: the JACC study. Prev Med 55:639–643

Liss DT, Chubak J, Anderson ML, Saunders KW, Tuzzio L, Reid RJ (2011) Patient-reported care coordination: associations with primary care continuity and specialty care use. Ann Fam Med 9(4):323–329

Moreo K, Lattimer C, Lett JE, Heggen-Peay CL, Simone L (2017) Integrated transitions of care for patients with rare pulmonary diseases. Prof Case Manag 22 (2):54–63

Moullec G, Lavoie KL, Rabhi K, Julien M, Favreau H, Labrecque M (2012) Effect of an integrated care programme on re-hospitalization of patients with chronic obstructive pulmonary disease. Respirology 17(4):707–714

Mulpuru S, McKay J, Ronksley PE, Thavorn K, Kobewka DM, Forster AJ (2017) Factors contributing to high-cost hospital care for patients with COPD. Int J Chron Obstruct Pulmon Dis 12:989–995

Napolitano F, Napolitano P, Garofalo L, Recupito M, Angelillo IF (2016) Assessment of continuity of care among patients with multiple chronic conditions in Italy. PLoS One 11(5):e0154940. doi:10.1371/journal.pone.0154940

Niesink A, Trappenburg JC, de Weert-van Oene GH, Lammers JW, Verheij TJ, Schrijvers AJ (2007) Systematic review of the effects of chronic disease management on quality-of-life in people with chronic obstructive pulmonary disease. Respir Med 101 (11):2233–2239

Omachi TA, Sarkar U, Yelin EH, Blanc PD, Katz PP (2013) Lower health literacy is associated with poorer health status and outcomes in chronic obstructive pulmonary disease. J Gen Intern Med 28(1):74–81

Pooler A, Beech R (2014) Examining the relationship between anxiety and depression and exacerbations of COPD which result in hospital admission: a systematic review. Int J Chron Obstruct Pulmon Dis 9:315–330

Prosser R, Carleton B, Smith A (2010) The comorbidity burden of the treated asthma patient population in British Columbia. Chronic Dis Can 30(2):46–55

Rezaei S, Akbari Sari A, Arab M, Majdzadeh R, Shaahmadi F, Mohammadpoorasl A (2016) The association between smoking status and hospital length of stay: evidence from a hospital-based cohort. Hosp Pract 44(3):129–132

Rice KL, Dewan N, Bloomfield HE, Grill J, Schult TM, Nelson DB, Kumari S, Thomas M, Geist LJ, Beaner C, Caldwell M, Niewoehner DE (2010) Disease management program for chronic obstructive pulmonary disease: a randomized controlled trial. Am J Respir Crit Care Med 182(7):890–896

Ross JA, Yang Y, Song PX, Clark NM, Baptist AP (2013) Quality of life, health care utilization, and control in older adults with asthma. J Allergy Clin Immunol Pract 1(2):157–162

Śliwiński P, Górecka D, Jassem E, Pierzchała W (2014) Polish respiratory society guidelines for chronic obstructive pulmonary disease. Pneumonol Alergol Pol 82(3):227–263

Smith ML, Bergeron CD, Adler CH, Patel A, Ahn S, Towne SD Jr, Bien M, Ory MG (2017) Factors associated with healthcare-related frustrations among adults with chronic conditions. Patient Educ Couns 100(6):1185–1193

Stein KV (2016) Integrated care around the world. Examples to help improve (primary) health care in Poland. http://akademia.nfz.gov.pl/wp-content/uploads/2016/12/Raport_Opieka_koordynowana_PL.pdf. Accessed on 12 Apr 2017

Thomsen M, Nordestgaard BG, Vestbo J, Lange P (2013) Characteristics and outcomes of chronic obstructive pulmonary disease in never smokers in Denmark: a prospective population study. Lancet Respir Med 1 (7):543–550

Vogelmeier CF, Criner GJ, Martinez FJ, Anzueto A, Barnes PJ, Bourbeau J, Celli BR, Chen R, Decramer M, Fabbri LM, Frith P, Halpin DM, López Varela MV, Nishimura M, Roche N, Rodriguez-Roisin R, Sin DD, Singh D, Stockley R, Vestbo J, Wedzicha JA, Agusti A (2017) Global strategy for the diagnosis, management, and prevention of chronic obstructive lung disease 2017 report: GOLD executive summary. Eur Respir J 49(3). doi:10.1183/13993003.00214-2017

Williams L, Wilcox D, ZuWallack R, Nici L (2016) Integrated care: what does this mean for the COPD patient? Chron Obstruct Pulmon Dis 1:18. doi:10.21767/2572-5548.100018

Wróblewska I, Oleśniewicz P, Kurpas D, Sołtysik M, Błaszczuk J (2015) Analysis of spirometry results in hospitalized patients aged over 65 years. Clin Interv Aging 10:1071–1076

ZuWallack R, Nici L (2010) Integrated care of the COPD patient: a pulmonary rehabilitation perspective. Breathe 6(4):313–319

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 63–71
DOI 10.1007/5584_2017_100

Published online: 25 October 2017

# Two-Year Follow-Up of Fall Prediction Among Older Adults in an Independent-Living Community

Oz Zur, Yitshal Berner, Yair Ohel, and Eli Carmeli

**Abstract**

Adults over the age of 70 are at risk of falls. Early detection of risk of falls can suggest early interventions. In this study, we attempted to determine valid clinical tests that can differentiate older individuals who are at risk of falling. Older adults from an independent-living community volunteered to participate in this descriptive, cohort study. They were administered the Berg Balance Scale (BBS), Zur Balance Scale (ZBS), Head Shaking Nystagmus Head Impulse Test, Dynamic Visual Acuity, and the Hallpike maneuver for evaluating benign paroxysmal positional vertigo (BPPV); a questionnaire including sociodemographics and a health characteristics survey. Multivariate analysis indicated that a ZBS score < 51, previous fall, and number of medications strongly predict falls in older adults. ZBS score, BBS score, Hallpike maneuver, number of medications, deficit of vestibular ocular reflex, along with positive ZBS score and past fall differentiate between fallers and non-fallers. ZBS <51, taking >6 medications, and history of falls were a benchmark for high-risk of falling.

**Keywords**

Aging • Balance • Falls • Vestibular ocular reflex • Vestibular system

## 1 Introduction

The incidence of vestibular dysfunction among people 70 years of age or older is 68.7%, and it is 84.8% for those over 80 years (Agrawal et al. 2009). It is well-established that frailty leads to increased risk for falls that are a major public health concern in older adults (Fried et al. 2001). The annual incidence of falls is about 33% among community dwelling adults over

O. Zur (✉)
The Israeli Center for Dizziness and Balance Disorders, 142 Ahuza Street, Ra'anana 43100, Israel

Department of Physical Therapy, Ben Gurion University of the Negev, Beer Sheva 84105, Israel
e-mail: zurbalance@gmail.com

Y. Berner
Department of Geriatric Medicine, Meir Medical Center, Kfar Saba 44281, Israel

Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel

Y. Ohel
Department of Physical Therapy, Carmel Medical Center, Haifa 34988, Israel

E. Carmeli
Department of Physical Therapy, University of Haifa, 199 Aba Khoushy Avenue, Haifa 34988, Israel

65 years of age (Agrawal et al. 2009). Falls are the main causes of injury, disability, institutionalization, and might be the leading cause of accidental death and nonfatal injuries among older people (de Castro et al. 2015). Every 36 h, an older person experiences a fall leading to a hip fracture (Israel Central Bureau of Statistics 2015). In 2015, Israel had a population of eight million people, including about 800,000 elders. Translating that into practical data, a fall is an expensive event for the patient's family and for the national economy. Falls can be due to intrinsic factors, such as medications associated with orthostatic hypotension. Diseases, including congestive heart failure, vertigo, and diabetes mellitus increase the risk of falls as well. Extrinsic factors related to falling include stairs, physical obstacles, and demanding environments. Among the many causes of falls among the elderly, the vestibular system, which is located in the inner ear and includes the vestibular-ocular reflex (VOR) and the vestibulo-spinal reflex (VSR), plays an essential role (Zur et al. 2006).

The Berg Balance Scale (BBS) is considered a 'gold standard' test and is often used to predict falls. The BBS consists of 14 items that primarily assess transfers and static standing balance, but it includes a limited number of dynamic activities (Berg 1989). Examples of BBS items include sitting balance, sit-to-stand, standing with eyes open and closed, turning 360°, bending forward to pick something up from the floor and single-leg stance. BBS items are rated 0–4 based on performance quality, with a total score ranging from 0 to 56. A score less than 45 is a predictor for falls (Muir et al. 2008). Yet, performance of daily living activities predicts 43% of a subject's score (Bogle-Thorbahn and Newton 1996). The BBS is not a valid test for vestibular disorders.

The Zur Balance Scale (ZBS) is a new balance test that focuses on the vestibular system. The scale challenges the visual and the somatosensory systems and it evaluates the balance ability with 10 test conditions and head movements. A total score ranges from 0 to 100. The scale is safe and easy to administer after a minimal amount of training (Zur et al. 2016). The ability to predict falls with a screening tool would be very valuable in that the effective interventions could be undertaken to minimize the risk of falls (Muir et al. 2008). Therefore, the present follow-up study seeks to determine who would be at increased risk for falls and what clinical test could best predict the likelihood of one or multiple future falls.

## 2 Methods

This study was approved by the Institutional Review Board of Maccabi Health Maintenance Organization (permit no. 14/2014). All participants provided written informed consent.

### 2.1 Study Protocol

This was a prospective cohort study. Participants were recruited from an independent-living community in Israel. Data were collected on participants' health conditions (ICD-10-CM), prescribed medications, and changes in health status over 2 years (from April 2014 through March 2016). The inclusion criteria were age 70 years or over and the ability to walk independently, with or without a cane. Sociodemographic and health status data were taken from the on-site clinic, including birth date, sex, fall history, fall-related injury, weekly physical exercise activity, social activity, diseases, surgeries, and medications. The exclusion criteria were the following: using an assistive device for walking, a static visual deficit (i.e., unable to read at least the first five lines in the Snellen eye chart, even with vision correction), cognitive deficit (mini-mental state examination score less than 24), any neurological condition (such as Parkinson's disease or cerebrovascular accident), or acute orthopedic condition (such as hip fracture or joint pain).

### 2.2 Outcome Measures

The objective of this study was to define the ability to predict future falls based on previously

collected data on the characteristics of older individuals who had fallen over a past period of 6 months. We followed them for the additional 2 years to evaluate the accuracy of the prediction of future falls. The on-site medical clinic at the independent living community maintains strict records of fall episodes. The data are routinely recorded in the computer system and periodically reviewed by the in-house physician. Participants were grouped according to the number of falls (zero, one, or two or more) experienced during the two-year study period. We used the outcome measures detailed below.

#### 2.2.1 Clinical Balance Tests

Two clinical balance tests were administered: Zur Balance Scale (ZBS) and Berg Balance Scale (BBS). The ZBS scores were dichotomized to $<51$ or $\geq 51$ (ZBS_51), and the BBS scores were dichotomized to the known standard of $<45$ or $\geq 45$ (BBS_45).

#### 2.2.2 Clinical Vestibulo-Ocular Reflex Function and Positional Tests

Deficit of vestibulo-ocular reflex function (VORf) was defined as a positive result in at least one of the following clinical tests: head shaking nystagmus (HSN) (Kamei 1988), head impulse test (HIT) (Halmagyi and Curthoys 1988), and dynamic visual acuity (DVA) (Herdman et al. 1998). The Hallpike maneuver for diagnosing benign paroxysmal positional vertigo (Dix and Hallpike, 1952) was also administered as an additional test for dizziness related to positional vertigo.

#### 2.2.3 Questionnaires

The following questionnaires were administered: Activities-Specific Balance Confidence (ABC), which is a self-estimate of one's ability to maintain balance in different situations (Powell and Myers 1995); University of California Los Angeles Dizziness Questionnaire – Severity (UCLA-DQ-S), which is a self-estimate of the severity of dizziness/vertigo, and University of California Los Angeles Dizziness Questionnaire – Affect (UCLA-DQ-A) to assess the affective effects of dizziness on daily functioning (Honrubia et al. 1996); and Short Anxiety Screening Test (SAST) to assess the anxiety level (Sinoff et al. 1999).

### 2.3 Data Analysis

Univariate analysis was performed to examine the association of all potential predictor variables with the primary outcome measures in relation to a single fall and two or more falls in the preceding 2 years. Continuous variables that follow a normal distribution were reported means $\pm$SD and compared using one-way analysis of variance. Continuous variables that do not follow a normal distribution were reported by median and interquartile range and compared using the Kruskal-Wallis test. Categorical variables were reported as relative frequencies and compared using the Pearson chi-squared test or Fisher exact test. When outcomes of the overall test were significant, pairwise comparisons were performed. The false discovery rate method was used for adjusting the significance level.

Multivariate logistic regression was used to identify the independent predictors of single, multiple, and all falls in the preceding 2 years, by considering candidate variables with p-values $\leq 0.05$ in the univariate analysis or if the variable was thought to be clinically relevant. A p-value $\leq 0.05$ was considered significant. Statistical analysis was performed using SAS for Windows v9.4.

## 3 Results

Seventy-five (24%) of the 315 residents volunteered to participate in the study. There were 60 (79%) women. The participants had lived in the independent living community for a mean of $3.0 \pm 1.5$ years. Their mean age was $83 \pm 5$ (range 71–97 years). They had an average of $12 \pm 3$ years of education and were engaged in sport activities for a median of 3 h a week. Table 1 demonstrates all variables that prospectively related to the risk of falling.

**Table 1** All falls during the last 2 years

| Variable | No falls ($n = 57$) | One fall ($n = 18$) | p-value |
|---|---|---|---|
| Age, years (mean ± SD) | 83.2 (5.1) | 83.6 (4.9) | 0.81 |
| Sex, n (%) | | | 0.58 |
| Female | 44 (77%) | 15 (83%) | |
| Male | 13 (23%) | 3 (17%) | |
| No. of diseases, median (Q1, Q3) | 5.0 (3.0, 5.0) | 5.0 (4.0, 7.0) | 0.060 |
| No. of medications, median (Q1, Q3) | 6.0 (5.0, 7.0) | 7.0 (5.0, 9.0) | 0.031 |
| Weekly hours of physical activity, median (Q1, Q3) | 3.0 (2.0, 5.0) | 3.0 (0.0, 6.0) | 0.670 |
| Past fall, n (%) | | | <0.001 |
| Yes | 55 (96%) | 12 (67%) | |
| No | 2 (4%) | 6 (33%) | |
| ABC | 1,076 (371) | 934 (321) | 0.150 |
| SAST, median (Q1, Q3) | 18.0 (15.0, 21.0) | 17.5 (15.0, 21.0) | 0.790 |
| UCLA-DQ-S, median (Q1, Q3) | 3.0 (2.0, 6.0) | 3.5 (2.0, 6.0) | 0.840 |
| UCLA-DQ-A, median (Q1, Q3) | 10.0 (6.0, 14.0) | 10.0 (8.0, 13.0) | 0.600 |
| BBS_45, n (%) | | | 0.210 |
| ≥45 | 51 (93%) | 14 (82%) | |
| <45 | 4 (7%) | 3 (18%) | |
| ZBS_51, n (%) | | | 0.001 |
| ≥51 | 35 (74%) | 5 (29%) | |
| <51 | 12 (26%) | 12 (71%) | |
| VORf, n (%) | | | 0.120 |
| Negative | 21 (38%) | 3 (18%) | |
| Positive | 34 (62%) | 14 (82%) | |
| VORf_ZBS, n (%) | | | 0.005 |
| No reflex, ZBS ≥51 | 11 (24%) | 2 (13%) | |
| Reflex, ZBS ≥51 | 22 (49%) | 3 (19%) | |
| No reflex, ZBS <51 | 5 (11%) | 1 (6%) | |
| Reflex, ZBS <51 | 7 (16%) | 10 (63%) | |
| Hallpike maneuver, n (%) | | | 0.680 |
| Negative | 47 (85%) | 13 (81%) | |
| Positive | 8 (15%) | 3 (19%) | |

*ABC* Activities-Specific Balance Confidence, *SAST* Short Anxiety Screening Test, *UCLA-DQ-A* University of California at Los Angeles Dizziness Questionnaire - Affect, *UCLA-DQ-S* University of California at Los Angeles Dizziness Questionnaire - Severity, *VORf* Vestibulo-Ocular Reflex Function, *BBS* Berg Balance Scale, *ZBS* Zur Balance Scale

## 3.1 Fall History

Eighteen of the 75 participants (24%) had fallen during the 2 years of the study. Seven fell once and 11 fell more than once. Three parameters were significantly different in the all-falls group compared with no-falls group: number of prescribed medications, history of falls, and a Zur Balance Scale score less than 51%. Although the ABC questionnaire results were not significant, the scores were lower among all those who had fallen (n = 18). The oculomotor examination for assessing the VORf indicated significant changes only when the ZBS score was <51 ($p < 0.005$). VORf alone, gender, and the BBS balance test with a score under 45% did not demonstrate a difference between fallers and non-fallers.

Table 2 demonstrates the best variables for predicting falls in multivariate logistic regression analysis. The regression model shows the effects

**Table 2** Results of regression analysis for all falls

| Parameter | Category | Odds ratio | 95% Wald lower CI | 95% Wald upper CI | Log odds ratio | Standard error | p-value |
|---|---|---|---|---|---|---|---|
| Intercept | | | | | −4.21 | 1.22 | **<0.001** |
| ZBS_51 | <51 *vs.* ≥51 | 4.9 | 1.20 | 20.04 | 1.59 | 0.72 | **0.027** |
| Past falls | 1 *vs.* 0 | 7.0 | 1.03 | 47.69 | 1.95 | 0.98 | **<0.047** |
| No. of medications | | 1.4 | 1.03 | 1.80 | 0.31 | 0.14 | **<0.029** |

*ZBS* Zur Balance Scale

**Table 3** Single fall during the last 2 years

| Variable | No fall ($n = 57$) | Single fall ($n = 7$) | p-value |
|---|---|---|---|
| Age, years (mean ± SD) | 83.2 (5.1) | 84.7 (5.5) | 0.470 |
| ABC | 1,076 (371) | 1,029 (303) | 0.750 |
| No. of medications, median (Q1, Q3) | 6.0 (5.0, 7.0) | 6.0 (5.0, 9.0) | 0.560 |
| No. of diseases, median (Q1, Q3) | 5.0 (3.0, 5.0) | 6.0 (3.0, 7.0) | 0.370 |
| Past fall, n (%) | | | **0.010** |
| No | 55 (96%) | 5 (71%) | |
| Yes | 2 (4%) | 2 (29%) | |
| ZBS_51, n (%) | | | **0.015** |
| ≥51 | 35 (74%) | 2 (29%) | |
| <51 | 12 (26%) | 5 (71%) | |
| VORf, n (%) | | | 0.300 |
| Negative | 21 (38%) | 1 (17%) | |
| Positive | 34 (62%) | 5 (83%) | |
| VORf_ZBS, n (%) | | | **0.038** |
| No VORf_ZBS ≥51 | 11 (24%) | 1 (17%) | |
| VORf_ZBS ≥51 | 22 (49%) | 1 (17%) | |
| No VORf_ZBS <51 | 5 (11%) | 0 (0%) | |
| VORf_ZBS <51 | 7 (16%) | 4 (67%) | |
| Hallpike, n (%) | | | **0.012** |
| Negative | 47 (85%) | 2 (40%) | |
| Positive | 8 (15%) | 3 (60%) | |

*ABC* Activities-Specific Balance Confidence, *VORf* Vestibulo-Ocular Reflex Function, *ZBS* Zur Balance Scale

of ZBS score <51, past falls, and the number of medications as significant predictors. Those who scored <51 on the ZBS had 4.9 greater likelihood of falling compared to those who scored 51 or more. A participant who had a past fall was 7 times more likely to fall again than one who did not. A number of medications, increasing to more than 6, was associated with increased likelihood of falling.

### 3.1.1 Single Fall During Two Years

Characterization of a single fall is shown in Table 3. Seven participants (9.3%) among the 75 had fallen once in the last 2 years, while 57 (76%) had no report from the nurse about falling. A past fall ($p < 0.010$), ZBS score < 51 ($p < 0.015$) and a positive Hallpike maneuver, indicative of a positional vertigo, were significantly different between fallers and non-fallers in the single fallers group by chi-squared ($p < 0.02$). VORf alone was not significant ($p = 0.30$); however, when combined with ZBS it turned out significant ($p < 0.038$). Persons who fell had a trend toward lower scores on the ABC questionnaire, which implies lower confidence and self-esteem regarding balance than the non-fallers had. The numbers of medications

and of diseases did not differ significantly between the groups.

A multivariate logistic regression model was applied to the data to analyze the independent relationship between ZBS score < 51, positive Hallpike, and past falls simultaneously. As seen from Table 3, ZBS score ($p < 0.015$) and past fall ($p < 0.010$) were significant predictors of a fall. However, significance was lost under multivariate logistic regression model analysis. A possible explanation is a low proportion of single falls (9%) in the population (7 persons out of the 75). The Hallpike maneuver result was the only predictor of a single fall.

### 3.1.2 Multiple Falls During Two Years

Eleven (14.6%) out of the 75 participants had fallen at least twice during the last two years. These falls were recorded in the nurse's report. Five parameters predicted multiple falls: number of medications taken ($p = 0.015$), past falls ($p < 0.001$), a combination of VORf deficit with ZBS score < 51 ($p = 0.027$), ZBS <51 ($p < 0.007$), and BBS score < 45 ($p < 0.049$) (Table 4). Persons who fell had somewhat lower scores than those who did not fall (874 ± 331 *vs.* 1,076 ± 371, respectively) on the ABC questionnaire ($p = 0.100$), implying a trend toward less confidence and self-esteem to maintain balance. *Post hoc* tests revealed that the predictive effect on multiple falls of a combination of VORf deficit and ZBS score was due to significant differences in the proportion of subjects who had ZBS score <51 and VORf deficit (60% experienced a fall) compared to subjects who had ZBS score >51 and VORf deficit (11% fell), p = 0.027. That indicates that the ZBS score predicted a fall and not the VORf deficit.

Table 5 shows the best parameters for predicting two falls or more. The model shows the effects of past falls and number of medications as significant predictors. A participant who had a past fall was 8.9 times more likely to fall again than the one who did not fall. More medications were associated with

**Table 4** Two or more falls during the last 24 months

| Variable | No falls (n = 57) | More than one fall (*n = 11*) | p-value |
|---|---|---|---|
| Age, years (mean ± SD) | 83.2 (5.1) | 82.8 (4.6) | 0.800 |
| ABC | 1,076 (371) | 874 (331) | 0.100 |
| No. of medications, median (Q1, Q3) | 6.0 (5.0, 7.0) | 8.0 (6.0, 10.0) | **0.015** |
| Past fall, n (%) | | | **<0.001** |
| No | 55 (96%) | 7 (64%) | |
| Yes | 2 (4%) | 4 (36%) | |
| BBS_45, n (%) | | | **0.049** |
| ≥45 | 51 (93%) | 8 (73%) | |
| <45 | 4 (7%) | 3 (27%) | |
| ZBS_51, n (%) | | | **0.007** |
| ≥51 | 35 (74%) | 3 (30%) | |
| <51 | 12 (26%) | 7 (70%) | |
| VORf, n (%) | | | 0.200 |
| Negative | 21 (38%) | 2 (18%) | |
| Positive | 34 (62%) | 9 (82%) | |
| VORf_ZBS, n (%) | | | **0.027** |
| No VORf, ZBS ≥51 | 11 (24%) | 1 (10%) | |
| VORf, ZBS ≥51 | 22 (49%) | 2 (20%) | |
| No VORf, ZBS <51 | 5 (11%) | 1 (10%) | |
| VORf, ZBS <51 | 7 (16%) | 6 (60%) | |

*ABC* Activities-Specific Balance Confidence, *VORf* Vestibulo-Ocular Reflex Function, *ZBS* Zur Balance Scale, *BBS* Berg Balance Scale

**Table 5** Regression analysis for multiple falls

| Parameter | Category | Odds ratio | 95% Wald lower CI | 95% Wald upper CI | Log odds ratio | Standard error | p-value |
|---|---|---|---|---|---|---|---|
| Intercept | | | | | −5.01 | 1.53 | 0.001 |
| ZBS_51 | <51 *vs.* ≥51 | 3.9 | 0.69 | 21.76 | 1.36 | 0.88 | 0.123 |
| Past falls | Yes *vs.* no | 8.9 | 1.04 | 76.70 | 2.188 | 1.1 | **0.046** |
| No. of medications | | 1.4 | 1.01 | 1.99 | 0.347 | 0.17 | **0.045** |

*VORf* Vestibulo-Ocular Reflex Function, *ZBS* Zur Balance Scale

increased likelihood of falling (OR 1.4). The ZBS was a borderline significant risk factor (OR 3.9) for multiple falls in this group.

## 4 Discussion

The objective of this prospective, cohort study was to determine the parameters that predict falls among the elderly living independently in the community. The three main parameters determined were the clinical ZBS test, a fall in the past 6 months, and taking several medications. The ZBS rather than the BBS was found a better clinical test to assess balance disorders and to be a predictor of future falls in the elderly with mild-to-moderate balance disorders. A low ZBS score of 50 points or less is an expression of dysfunctional integration of three sensory systems. In a previous study, the ZBS was found to be as good as the BBS as a retrospective predictor of future falls (Zur et al. 2016). The advantages of the ZBS, compared to the BBS, are that it is quicker to administer and does not have a ceiling effect. The ZBS is sensitive for mild-to-moderate balance dysfunctions.

A history of a fall in the past 6 months is an important parameter related to predicting future falls. A previous fall increases the risk for the next fall by 9-fold. It is essential to ask patients about their fall history to learn about the risk of falling. The number of prescribed medications was an additional predictor of multiple falls. Jyrkkä et al. (2011) have reported that at least 10 medications are related to increased risk. However, we found that taking more than 6 medications increased the risk of falling by 1.36-fold compared to taking fewer medications. The situation is certainly much more complex than the number of prescribed medications, because each medication influences another.

We administered several clinical examinations to determine which one could differentiate older individuals at risk of falling. We used the Hallpike maneuver for vestibular function and three oculomotor examinations to assess the function of vestibular ocular reflex: the Head Impulse, Head Shaking, and Dynamic Visual Acuity tests. We used the BBS and ZBS to assess balance. In a retrospective study of 169 independent older adults, Zur et al. (2006) found a significant difference between fallers and non-fallers related to VORf deficit. In the present study, we hypothesized that VORf deficit would be one of the main parameters to predict the next fall, but it was not. One explanation might be a small sample size, which was less than half that of the previous study outlined above (Zur et al. 2006). Secondly, we believe that older adults unconsciously learn to compensate for chronic VORf deficit by walking slowly, moving their eyes instead of their head and walking either with a stiff ankle strategy or stiff neck strategy (Collins and De Luca 1993). Although, VORf with ZBS <51 was significantly different between the groups (Table 1; $p < 0.005$), the VORf alone was not included in the regression. We believe that a larger population and more accurate examination data, for example, with video head impulse test, could demonstrate the differences.

The Hallpike maneuver demonstrated significant differences between the groups related to a single fall (Table 3; $p < 0.012$). This information is important for patients with benign paroxysmal positional vertigo (Herdman 1994; Epley 1992; Semont et al. 1988). The ZBS and BBS scores differed significantly between multiple fallers and non-fallers. The BBS is very well-known, but it was established for neurology patients and others with severe balance dysfunction (Muir et al. 2008). For independent people who walk easily without assistive devices, the tasks are too easy, and many will score high even though they have poor balance. This means that there is a ceiling effect and the scale is not suitable for these individuals. The ZBS is more difficult for patients with severe imbalance, but suitable for people with mild-to-moderate balance disorders.

To determine which questionnaire would demonstrate the difference between fallers and non-fallers better, we used the ABC, SAST, and UCLA-DQ questionnaires. The results were not significantly different between the groups. Only the ABC demonstrated a trend. The fallers had lower self-confidence than the non-fallers did (58% *vs*. 67%, respectively).

The weakness of the current study lies with the outcome measures used, which were clinical tests, rather than laboratory assessments, such as video head impulse test and force plate for sway velocity. Another limitation relates to actual reports of falls. A fall has a negative connotation; therefore, it is likely that fewer falls were reported than occurred. A low frequency of falls might suggest that falls with injuries were mostly captured and some minor falls might have been missed, documented improperly, or were not reported due to embarrassment.

## 5 Conclusions

Early detection of balance disorders and the ability to predict the risk of falling are very important for rehabilitation. It is well-known that vestibular and balance training could prevent or postpone the next fall (Yang et al. 2012). One must keep in mind that individuals who engage in risky health behaviors often lack motivation to seek help (Dale and Lee 2016). Yet, health care providers need to emphasize primary prevention for health promotion. Fall prevention is one of the most important health issues for older adults. The number of prescribed medications, history of falls, and the ZBS score were found to be appropriate parameters for detecting risk for falling.

We believe that the examination of vestibulo-ocular reflex function for dizziness and the Zur Balance Scale should be included in the routine assessments for balance disorders among older adults. However, when severe balance deficits are present, the Berg Balance Scale should be the first choice.

**Acknowledgments** The authors would like to thank the staff Beit Ildan, especially Masi Oren and Bruria Geraff. Our thanks also go to Faye Schreiber for editing the manuscript and providing helpful comments, Ms. Dana Hadar for statistical analysis, and Yak ir Ariam for help with ZBS testing.

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

## References

Agrawal Y, Carey JP, Della Santina CC, Schubert MC, Minor LB (2009) Disorders of balance and vestibular function in US adults: data from the National Health and Nutrition Examination Survey, 2001-2004. Arch Intern Med 169:938–944

American ICD-10-CM diagnosis codes. http://www.icd10data.com. Accessed 30 Oct 2016

Berg K (1989) Measuring balance in the elderly: preliminary development of an instrument. Physiother Can 41:304–311

Bogle-Thorbahn LD, Newton RA (1996) Use of the berg balance test to predict falls in elderly persons. Phys Ther 76:576–583

Collins JJ, De Luca CJ (1993) Open-loop and closed-loop control of posture: a random-walk analysis of center-of-pressure trajectories. Exp Brain Res 95:308–318

Dale H, Lee A (2016) Behavioural health consultants in integrated primary care teams: a model for future care. BMC Fam Pract 17:97

de Castro V, Mokoroa O, Artieda J, Muniozguren N, Etxebarriarteun L, Alvarez L, Garcia Calabuig MA, Physicians Watcher Network of the Basque Country (2015) Epidemiology of accidents in a cohort of adults over 64 years old in the autonomous community of the

Basque Country. Rev Esp Geriatr Gerontol 50 (6):281–284
Dix MR, Hallpike CS (1952) The pathology, symptomatology and diagnosis of certain common disorders of the vestibular system. Proc R Soc Med 54:341–354
Epley JM (1992) The canalith repositioning procedure: for treatment of benign paroxysmal positional vertigo. Otolaryngol Head Neck Surg 107:399–404
Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, MA MB, Cardiovascular Health Study Collaborative Research Group (2001) Frailty in older adults: evidence for a phenotype. J Gerontol A Biol Sci Med Sci 56:M146–M157
Halmagyi GM, Curthoys IS (1988) A clinical sign of canal paresis. Arch Neurol 45:737–739
Herdman SJ (1994) Canalith repositioning maneuver. Otolaryngol Head Neck Surg 111:690–691
Herdman SJ, Tusa RJ, Blatt P, Suzuki A, Venuto PJ, Roberts D (1998) Computerized dynamic visual acuity test in the assessment of vestibular deficits. Am J Otolaryngol 19:790–796
Honrubia V, Bell TS, Harris MR, Baloh RW, Fisher LM (1996) Quantitative evaluation of dizziness characteristics and impact on quality of life. Am J Otolaryngol 17:595–602
Israel Central Bureau of Statistics (2015) http://www.cbs.gov.il/reader/cw_usr_view_Folder?ID=141; Accessed on 30 Oct 2016
Jyrkkä J, Enlund H, Lavikainen P, Sulkava R, Hartikainen S (2011) Association of polypharmacy with nutritional status, functional ability and cognitive capacity over a three-year period in an elderly population. Pharmacoepidemiol Drug Saf 20:51–522
Kamei T (1988) Two types of head shaking tests in vestibular examination. Acta Otolaryngol 458:108–112
Muir SW, Berg K, Chesworth B, Speechley M (2008) Use of the berg balance scale for predicting multiple falls in community-dwelling elderly people: a prospective study. Phys Ther 88:449–459
Powell LE, Myers AM (1995) The activities-specific balance confidence (ABC) scale. J Gerontol A Biol Sci Med Sci 50:M28–M34
Semont A, Freyss G, Vitte E (1988) Curing the BPPV with a liberatory maneuver. Adv Otorhinolaryngol 42:290–293
Sinoff G, Ore L, Zlotogorsky D, Tamir A (1999) Short anxiety screening test - a brief instrument for detecting anxiety in the elderly. Int J Geriatr Psychiatry 14:1062–1071
Yang XJ, Hill K, Moore K, Williams S, Dowson L, Borschmann K, Simpson JA, Dharmage SC (2012) Effectiveness of a targeted exercise intervention in reversing older people's mild balance dysfunction: a randomized controlled trial. Phys Ther 92(1):24–37
Zur O, Berner YN, Carmeli E (2006) Correlation between vestibular function and hip fracture following falls in the elderly: a case-controlled study. Physiotherapy 92:208–213
Zur O, Shaki T, Carmeli E (2016) Concurrent validity and reliability of a new balance scale used in older adults. Adv Exp Med Biol 910:63–70

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 73–81
DOI 10.1007/5584_2017_102

Published online: 14 September 2017

# Activities of Lysosomal Enzymes in Alloxan-Induced Diabetes in the Mouse

Bożena Witek, Danuta Rochon-Szmejchel, Iwona Stanisławska, Marek Łyp, Krzysztof Wróbel, Arkadiusz Zapała, Agnieszka Kamińska, and Adam Kołątaj

**Abstract**

The study investigated a panel of lysosomal enzymes in the liver and kidney tissues in alloxan-induced diabetes in the mouse. The mice were divided into six experimental groups receiving 10% alloxan at a dose of 50 and 75 mg/kg over a period of four, eight, and twelve days; each group was compared with controls receiving 0.9% NaCl. The findings were that diabetes induced by both doses of alloxan was accompanied by significant increases in the lysosomal activities of acid phosphatase and the glycosidases investigated: β-glucuronidase, β-galactosidase, β-glucosidase, and N-acetyl-hexosaminidase. The lysosomal enzyme activity in both liver and kidney cells peaked 12 days after onset of diabetes for most enzymes, at the time when hyperglycemia and hyperinsulinemia already started abating after their peak at 8 days into the course of diabetes. The enzyme activity was in most cases higher with the higher dose of alloxan and thus higher level of glycemia. Lysosomal enzymes degrade glycoconjugates, the molecules that are present in the basement membrane of endothelial cells where they contribute to capillary wall stability. Thus, enhanced activity of these enzymes could presage the progression of diabetic microangiopathy, atherosclerosis, and the development of microvascular complications.

**Keywords**

Alloxan • Diabetes • Lysosomal enzymes • Glucose • Insulin • Kidney • Liver

## 1 Introduction

Diabetes mellitus is a disease that has been extensively discussed in the scientific literature over decades (Gilinsky et al. 2015; Perkisas and Vandewoude 2016). WHO has recently classified

B. Witek
Department of Animal Physiology, Institute of Biology, The Jan Kochanowski University, Kielce, Poland

D. Rochon-Szmejchel
Dandiete Dietic Outpatient Clinic, Nowe Miasto Lubawskie, Poland

I. Stanisławska (✉), M. Łyp, and A. Kołątaj
Department of Dietetics, College of Rehabilitation, 49 Kasprzaka Street, 01-234 Warsaw, Poland
e-mail: iwonabiol@wp.pl

K. Wróbel
NZOZ Expertdent, Non-Public Health Care Center, Kielce, Poland

A. Zapała
Department of Urology, Regional Specialist Healthcare Center for Tuberculosis and Lung Diseases, Kielce, Poland

A. Kamińska
Faculty of Family Studies, The Cardinal Wyszyński University, Warsaw, Poland

diabetes as a disease of the twenty-first century (WHO 2012). Yet diabetes pathogenesis is not full clear, in particular concerning the biochemical aspects of its development (Kido 2013; Krischer et al. 2003). Growing attention in this respect has been devoted to lysosomal enzymes that play a key role in the cellular pathways of protein, lipid, carbohydrate, and xenobiotic degradation (Hamer et al. 2012; Heltianu et al. 2011; Kołątaj et al. 2001). The enzymatic activity is considered a significant factor in maintaining the cell in a state of dynamic homeostasis, notably concerning the synthesis and catabolism of energy-providing substances (Witek et al. 2001, 2014; Platt et al. 2012). The aim of the present study was to determine the direction and extend of changes in the activity of enzymes in the lysosomal fraction of liver and kidney cells in the mouse with alloxan (mesoxalylurea)-induced experimental diabetes.

## 2 Methods

The study protocol was approved by the Bioethics Committee of the Swiętokrzyska Chamber of Physicians in the city of Kielce, Poland (permit no. 50/2016), and was conducted in accord with the guidelines for the Care and Use of Experimental Animals of the Canadian Council on Animal Care. The experiments was performed in the Department of Animal Physiology of the Jan Kochanowski University in Kielce, Poland.

The experiments were conducted in 108 male Swiss mice aged 8–9 weeks and weighing of 25.0 ±1.4 grams. The animals were randomly selected from a mated population of 1,000 individuals bred in a standard mice farm at the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences in Jastrzębiec, Poland. The animals were kept in plastic cages, with free access to commercial chow (16% protein and energy value of 14.04 MJ/kg) and water, at temperature of 21–22 °C, relative humidity of 50–60%, and in a 12-h light/dark cycle.

There were nine groups, three control and six experimental, consisting of 12 animals each. Mice from the experimental groups (Group 2, 5 and 8) received alloxan (10% solution in 0.9% NaCl v/v) in a dose of 50 mg/kg daily (Sigma Chemical Company, St. Louis, MO) during 4, 8, and 12 days in respective groups, whereas mice from the Groups 3, 6, and 9 received the alloxan in a dose of 75 mg/kg in like manner. Mice from the control groups (Group 1, 4, and 7) received a 0.9% NaCl solution over the respective time periods. All injections were of 250 µl volume intraperitoneally and were made once a day between 8.00-9.00 a.m. Table 1 sums up the experimental paradigm.

Thirty minutes after the last injection, the mice were euthanized by intraperitoneal injection of thiopental (40 mg/kg). The blood was taken to determine the concentration of glucose, and fragments of kidney and liver were collected. The fragments were superfused with 0.9% NaCl solution at 4 °C, and were then suspended in 100 mM phosphate buffer solution, pH 7.0 at 4 °C, at a concentration of 500 mg tissue per 5 ml buffer. Liver and kidney tissue suspensions were homogenized at 200 rpm using a Potter-Elvehjem tissue grinder fitted with a Teflon pestle (Omni Inc; NW Kennesaw, GA) according to the method of Beaufay (1972). To obtain the lysosomal fractions from liver and kidney cells, homogenates were centrifuged for 8 min at 5,000 rpm (MPW-351R centrifuge; MPW Med. Instruments, Warsaw, Poland). Supernatants were collected and centrifuged for 20 min at 14,000 rpm (Sorvall RC-5C centrifuge; GMI

**Table 1** Experimental paradigm of substance administration; each group consisting of 12 mice

| Groups | Substance | Dose | Time after injection (*days*) |
|---|---|---|---|
| 1, 4, 7 | 0.9% NaCl in water | 250 µl | 4, 8, 12 |
| 2, 5, 8 | 10% alloxan in 0.9% NaCl | 50 mg/kg | 4, 8, 12 |
| 3, 6, 9 | 10% alloxan in 0.9% NaCl | 75 mg/kg | 4, 8, 12 |

Inc, Ramsey, MN). The pellets were dissolved in 4 ml of 0.1% Triton X-100 cooled to 4°C, frozen and thawed several times, and then kept frozen at -20 °C until further analysis. In the supernatants, lysosomal activities of acid phosphatase (ACP, EC 3.1.3.2), β-glucuronidase (β-GlcUr, EC 3.2.1.31), β-galactosidase (β-Gal, EC 3.2.1.23), β-glucosidase (β-Glu, EC 3.2.1.21), and β-N-acetyl-hexosaminidase (Hex, EC 3.2.1.52) were determined according to the method of Barrett and Heath (1977).

In the supernatants of liver and kidney homogenates, a total protein level was also determined using the Lowry method (Lowry et al. 1951) in modification of Kirschke and Wiederanders (1984). Glucose and insulin levels were determined in blood plasma using the Bio-La-Test diagnostic kit (Erba Lachema s.r.o., Brno, Czechia). The enzyme activity was expressed in nmol/mg protein/h, the level of glucose in mmol/l and insulin in μU/ml. All substrates were purchased from Serva Feinbiochemica GmbH (Heidelberg, Germany). The results were statistically elaborated using the multivariate analysis of variance (MANOVA). A $p$-value $<0.05$ defined statistically significant changes. The evaluation was performed with SAS/STAT (SAS Institute Inc; Cary, NC) and Origin v5.0 (Microcal Inc; Northampton, MA) commercial statistical packages.

## 3 Results and Discussion

Alloxan administration made all the mice diabetic, without exception. The result was hyperglycemia, with a nearly fivefold increase in glucose level, and hyperinsulinemia, with a sevenfold increase in insulin level over baseline (Table 2).

The mean absolute and percentage changes in the activity of the hydrolases investigated in the cells of mouse liver and kidneys in alloxan-induced diabetes are presented in sequential Tables 3, 4, 5, 6 and 7. There were significant increases in ACP activity in the liver after 4, 8, and 12 days and in the kidney after 12 days after injection of both doses of alloxan (Table 3). The increases might be caused by increased plasma glucose content. An increase in ACP has also been found in the rat model of alloxan-induced diabetes in a study of El-Demerdash et al. (2005). Those authors observed concomitant increases in ACP and glutathione transferase (GST); the most probably reflection of the cellular antioxidative protection against reactive oxygen species (ROS), generated in diabetes. Increased ACP in the liver, resulting from hyperglycemia-induced oxidative stress, has also been reported in streptozotocin-induced diabetes (McAnuff et al. 2003). Enhanced oxidative stress in diabetes is a known inducer of lipid peroxidation. The increased ACP activity observed in the present

**Table 2** Glucose and insulin content in mouse peripheral blood in the control Groups 1, 4, and 7, and the alloxan-injected Groups 2, 3, 5, 6, 8, and 9, with two doses of alloxan and each dose at three different day-intervals after injection

| Groups | Glucose (mmol/l) | Insulin (μU/ml) |
|---|---|---|
| Control | | |
| Groups 1, 4, and 7 | 6.12 ± 1.20 | 2.60 ± 0.87 |
| Alloxan † | | |
| Group 2–4 days – 50 mg/kg | 20.28 ± 4.26 | 15.51 ± 2.50 |
| Group 3–4 days – 75 mg/kg | 24.10 ± 5.64 | 17.09 ± 4.23 |
| Group 5–8 days – 50 mg/kg | 31.14 ± 7.88 | 20.67 ± 4.44 |
| Group 6–8 days – 75 mg/kg | 30.94 ± 6.15 | 18.82 ± 3.86 |
| Group 8–12 days – 50 mg/kg | 21.17 ± 5.10 | 16.43 ± 3.15 |
| Group 9–12 days – 75 mg/kg | 23.36 ± 4.71 | 17.33 ± 2.90 |

†$p < 0.001$; increases in glucose and insulin content were significant in all groups of alloxan-induced diabetes, compared with the control level

**Table 3** Acid phosphatase activity (ACP) (nmol/mg protein/h) in the liver and kidney in alloxan-induced diabetes in mice; each group consisting of 12 mice

| Groups | Liver | | Kidney | |
|---|---|---|---|---|
| | mean ±SD | % change | mean ±SD | % change |
| 1. NaCl – 4 days | 0.47 ±0.12 | 100 | 0.29 ±0.04 | 100 |
| 2. Alloxan 4 days – 50 mg | 0.59 ±0.16* | 124 | 0.27 ±0.03 | 96 |
| 3. Alloxan 4 days –75 mg | 0.62 ±0.15** | 131 | 0.29 ±0.04 | 102 |
| 4. NaCl – 8 days | 0.45 ±0.16 | 100 | 0.30 ±0.05 | 100 |
| 5. Alloxan 8 days – 50 mg | 0.74 ±0.12*** | 148 | 0.33 ±0.06 | 112 |
| 6. Alloxan 8 days – 75 mg | 0.73 ±0.01*** | 146 | 0.34 ±0.05 | 116 |
| 7. NaCl – 12 days | 0.48 ±0.08 | 100 | 0.29 ±0.05 | 100 |
| 8. Alloxan 12 days – 50 mg | 0.73 ±0.15*** | 152 | 0.35 ±0.10* | 121 |
| 9. Alloxan 12 days – 75 mg | 0.75 ±0.11*** | 155 | 0.36 ±0.05* | 125 |

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

**Table 4** β-glucuronidase activity (β-GlcUr) (nmol/mg protein/h) in the liver and kidney in alloxan-induced diabetes in mice; each group consisting of 12 mice

| Groups | Liver | | Kidney | |
|---|---|---|---|---|
| | mean ±SD | % change | mean ±SD | % change |
| 1. NaCl – 4 days | 0.43 ±0.11 | 100 | 0.32 ±0.03 | 100 |
| 2. Alloxan 4 days – 50 mg | 0.51 ±0.15 | 119 | 0.37 ±0.03 | 117 |
| 3. Alloxan 4 days –75 mg | 0.53 ±0.13* | 124 | 0.43 ±0.05** | 135 |
| 4. NaCl – 8 days | 0.39 ±0.13 | 100 | 0.35 ±0.05 | 100 |
| 5. Alloxan 8 days – 50 mg | 0.50 ±0.01* | 128 | 0.46 ±0.06** | 131 |
| 6. Alloxan 8 days – 75 mg | 0.55 ±0.13*** | 141 | 0.48 ±0.14** | 137 |
| 7. NaCl – 12 days | 0.41 ±0.08 | 100 | 0.24 ±0.03 | 100 |
| 8. Alloxan 12 days – 50 mg | 0.39 ±0.08 | 95 | 0.26 ±0.05 | 109 |
| 9. Alloxan 12 days – 75 mg | 0.40 ±0.12 | 86 | 0.26 ±0.08 | 108 |

*$p < 0.05$;**$p < 0.01$; ***$p < 0.001$

**Table 5** β-galactosidase activity (β-Gal) (nmol/mg protein/h) in the liver and kidney in alloxan-induced diabetes in mice; each group consisting of 12 mice

| Groups | Liver | | Kidney | |
|---|---|---|---|---|
| | mean ±SD | % change | mean ±SD | % change |
| 1. NaCl – 4 days | 0.15 ±0.04 | 100 | 0.14 ±0.03 | 100 |
| 2. Alloxan 4 days – 50 mg | 0.15 ±0.04 | 103 | 0.13 ±0.03 | 98 |
| 3. Alloxan 4 days –75 mg | 0.15 ±0.02 | 104 | 0.13 ±0.05 | 96 |
| 4. NaCl – 8 days | 0.13 ±0.03 | 100 | 0.12 ±0.05 | 100 |
| 5. Alloxan 8 days – 50 mg | 0.14 ±0.03 | 108 | 0.12 ±0.06 | 104 |
| 6. Alloxan 8 days – 75 mg | 0.14 ±0.03 | 107 | 0.12 ±0.14 | 106 |
| 7. NaCl – 12 days | 0.15 ±0.03 | 100 | 0.12 ±0.03 | 100 |
| 8. Alloxan 12 days – 50 mg | 0.23 ±0.04*** | 146 | 0.16 ±0.05 | 133 |
| 9. Alloxan 12 days – 75 mg | 0.22 ±0.03*** | 143 | 0.17 ±0.08 | 136 |

***$p < 0.001$

**Table 6** β-glucosidase activity (β-Glu) (nmol/mg protein/h) in the liver and kidney in alloxan-induced diabetes in mice; each group consisting of 12 mice

| Groups | Liver | | Kidney | |
|---|---|---|---|---|
| | mean ±SD | % change | mean ±SD | % change |
| 1. NaCl – 4 days | 0.13 ±0.02 | 100 | 0.11 ±0.01 | 100 |
| 2. Alloxan 4 days – 50 mg | 0.15 ±0.05 | 115 | 0.13 ±0.02 | 118 |
| 3. Alloxan 4 days – 75 mg | 0.16 ±0.03* | 121 | 0.13 ±0.02* | 120 |
| 4. NaCl – 8 days | 0.14 ±0.06 | 100 | 0.12 ±0.03 | 100 |
| 5. Alloxan 8 days – 50 mg | 0.15 ±0.03 | 112 | 0.14 ±0.03 | 115 |
| 6. Alloxan 8 days – 75 mg | 0.15 ±0.02 | 109 | 0.14 ±0.02 | 113 |
| 7. NaCl – 12 days | 0.13 ±0.02 | 100 | 0.13 ±0.02 | 100 |
| 8. Alloxan 12 days – 50 mg | 0.15 ±0.03 | 114 | 0.14 ±0.03 | 108 |
| 9. Alloxan 12 days – 75 mg | 0.15 ±0.02 | 115 | 0.14 ±0.04 | 109 |

*$p < 0.05$

**Table 7** N-acetyl-hexosaminidase activity (Hex) (nmol/mg protein/h) in the liver and kidney in alloxan-induced diabetes in mice; each group consisting of 12 mice

| Groups | Liver | | Kidney | |
|---|---|---|---|---|
| | mean ±SD | % change | mean ±SD | % change |
| 1. NaCl – 4 days | 0.54 ±0.13 | 100 | 0.41 ±0.04 | 100 |
| 2. Alloxan 4 days – 50 mg | 0.55 ±0.20 | 115 | 0.43 ±0.05 | 105 |
| 3. Alloxan 4 days –75 mg | 0.60 ±0.15 | 102 | 0.44 ±0.05 | 107 |
| 4. NaCl – 8 days | 0.59 ±0.24 | 111 | 0.48 ±0.12 | 100 |
| 5. Alloxan 8 days – 50 mg | 0.67 ±0.18 | 114 | 0.53 ±0.12 | 111 |
| 6. Alloxan 8 days – 75 mg | 0.72 ±0.01* | 122 | 0.58 ±0.09* | 123 |
| 7. NaCl – 12 days | 0.60 ±0.16 | 100 | 0.52 ±0.11 | 100 |
| 8. Alloxan 12 days – 50 mg | 0.82 ±0.09** | 136 | 0.67 ±0.07* | 129 |
| 9. Alloxan 12 days – 75 mg | 0.87 ±0.13*** | 145 | 0.71 ±0.07** | 137 |

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

study could also be connected with a heightened sensitivity of the hydrolase to changes in cellular redox potential. Another mechanism could be a greater requirement for organophosphates that actively participate in the utilization of carbohydrates (Al-Attas et al. 2011).

β-glucuronidase activity (β-GlcUr) did not reveal any significant changes 4 days after alloxan administration in a dose of 50 mg/kg, while it increased from 0.43 ±0.11 to 0.53 ±0.13 nmol/mg protein/h ($p < 0.05$) in the liver and from 0.32 ±0.03 to 0.43 ±0.05 nmol/mg protein/h ($p < 0.01$) in the kidney after the 75 mg/kg dose of alloxan. Percentagewise, the β-GlcUr increases clearly peaked 8 days after either dose of alloxan in both liver and kidney, abating thereafter (Table 4). The observations of Mohanam and Bose (1984) and Zhao et al. (2013) point to the rate of glycosaminoglycan metabolism in shaping the level of β-GlcUr activity in tissue. Thus, a distinct increase in β-GlcUr beginning 8 days after alloxan administration in the present experiment could be due to a considerable increase in the amount of plasma glycosaminoglycans within that time, parallel to the increase in glucose content (Oshima et al. 1994). Another factor leading to an increase in β-GlcUr could be a concurrent decrease in the level of heparin and heparan sulfate, resulting from accelerated hydrolysis of glucuronides, stimulated by the presence of alloxan in the liver (Faure et al. 2013; Hinohara et al. 1974).

β-galactosidase activity (β-Gal) did not significantly increase either in the liver or kidney until after 12 days from alloxan administration. In the liver, the increases were from 0.15 ±0.03 to 0.23 ±0.04 and to 0.22 ±0.03 nmol/mg protein/h ($p < 0.001$) at the doses of 50 mg and

75 mg/kg of alloxan, respectively. In the kidney, the increases were from 0.12 ±0.03 to 0.16 ±0.05 and 0.17 ±0.08 nmol/mg protein/h ($p < 0.01$) at the respective doses of alloxan (Table 5). The increase in β-Gal observed might be due to increased rate of glucose metabolism in diabetes. Stroev et al. (1993) have interpreted increased β-Gal activity the liver of diabetic rats as a 'catabolic response' of the organ to alloxan. Kutryk et al. (1987) have also reported increased β-Gal in the heart of rats 16 weeks after onset of streptozotocin-induced diabetes. In line with that report, Fushimi et al. (1980) have found a considerable increase in β-Gal in mice with streptozotocin-induced diabetes treated with insulin. In contrast, Chang et al. (1977), who examined the effects of streptozotocin-induced diabetes on the activity of β-Gal, β-Glu, and Hex in the liver, kidney, and pancreas of Chinese hamsters, failed to find any appreciable changes in β-Gal and Hex, while the β-Glu decreased. A study of Miralles et al. (1993) has revealed that in both diabetes mellitus type 1 and 2, production of glucose in the kidney is increased and amount to as much as 30% of the total gluconeogenesis.

β-glucosidase activity (β-Glu) significantly increased in both liver and kidney as early as 4 days of after alloxan in the higher dose of 75 mg/kg. The increases were from 0.13 ±0.02 to 0.16 ±0.03 nmol/mg protein/h and from 0.11 ±0.01 to 0.13 ±0.02 nmol/mg protein/h in the liver and kidney, respectively ($p < 0.05$) (Table 6). β-Glu is a glycosidase which catalyzes the breakdown of 1,4-glycosidic bonds, so that its increase already 4 days after alloxan administration might be indicative of the enzyme greater synthesis. A high content of glucose in diabetes inhibits the activity of glucose-6-phosphate dehydrogenase; a key enzyme of the pentose phosphate pathway, which contributes to protein glycosylation and, consequently, increases β-Glu activity (Kumari and Sahib 1993). An earlier study of Panin et al. (1982) has revealed increased β-Glu activity in the liver of diabetic rabbits which were additionally given hydrocortisone and adrenalin. Likewise, Grötsch et al. (1986) have also reported increased β-Glu in the rat liver in streptozotocin-induced diabetes. In the present study, interestingly, no differences in β-Glu were found after a longer time elapsing from alloxan administration, which was eight and twelve days.

N-acetyl-hexosaminidase activity (Hex) showed no changes four after days alloxan administration, but it increased significantly after 8 days with the higher 75 mg/kg alloxan; from 0.59 ±0.24 to 0.72 ±0.01 nmol/mg protein/h in the liver and from 0.48 ±0.12 to 0.58 ±0.09 in the kidney ($p < 0.05$). Hex remained significantly increased also 12 days after both doses of alloxan in both organs (Table 7). Here, the greatest increase was after the bigger 75 mg/kg dose of alloxan, changing from 0.60 ±0.16 to 0.87 ±0.13 nmol/mg protein/h ($p < 0.001$) in the liver and from 0.52 ±0.11 to 0.71 ±0.07 nmol/mg protein/h ($p = 0.01$) in the kidney.

The results on Hex activity obtained in the present study are consistent with the observations of other authors. Increases in plasma and lysosomal Hex in the liver and kidney have been found in a study of Takumi et al. (1985) in streptozotocin-induced diabetes in rats. Mohanam and Bose (1983) have reported that activity of some lysosomal hydrolases, including Hex, significantly increased over the baseline control level in alloxan- and streptozotocin-induced diabetes in rats. Alloxan contributed to a rapid increase in glucose content, increasing glutathione peroxidase activity and decreasing the aspartate aminotransferase/alanine aminotransferase ratio; the changes that might underlie an enhancement in Hex activity. Bhimji et al. (1985) have found a considerable increase in Hex activity in the heart of diabetic rabbits, which they interpreted as caused by diabetic cardiomyopathy. In line with the above outlined, Kutryk et al. (1987) have found a late increase in Hex activity in the streptozotocin-induced cardiomyopathy in rats, expressed 16 weeks after onset of diabetes. According to Mohanam and Bose (1984) and Gambaro et al. (1994), the processes of glycosaminoglycan degradation is essential for the development of diabetic nephropathy, also manifest in increased Hex activity. McAuliffe et al. (1996) have found an increased level of heparan sulfate in urine of

**Table 8** Analysis of variance for the lysosomal enzymes studied

| Source of variation | F |
|---|---|
| **ACP** | |
| condition (alloxan – time * dose) | 10.80 † |
| organ (liver or kidney) | 433.85 † |
| condition * organ | 4.19 |
| **β-GlcUr** | |
| condition (alloxan – time * dose) | 12.83 † |
| organ (liver or kidney) | 55.22 † |
| conditionr * organ | 1.19 |
| **β-Gal** | |
| condition (alloxan – time * dose) | 15.40 † |
| organ (liver or kidney) | 33.75 † |
| condition * organ | 1.82 |
| **β-Glu** | |
| condition (alloxan – time * dose) | 1.94 |
| organ (liver or kidney) | 10.49 |
| condition * organ | 0.22 |
| **Hex** | |
| condition (alloxan – time * dose) | 14.83 † |
| organ (liver or kidney) | 49.58 † |
| condition * organ | 0.20 |

†$p < 0.001$

individuals with type 2 diabetes as compared to that in healthy individuals. Mohanam and Bose (1983) pointed to the inductive effect of alloxan, manifest in increased Hex activity not only in the liver or kidney, but also in the skin, spleen and plasma of experimental rats.

Multivariate analysis of variance demonstrates significant effects on the dependent variables, i.e., the enzymes studied, of the experimental conditions, i.e., varying doses of alloxan and the time after alloxan administration, for all except the β-Glu enzyme. The analysis failed to reveal any interactions between the organ (liver or kidney) and the experimental conditions (Table 8).

## 4 Summary and Conclusions

The major findings of the present study were that the activities of a panel of five lysosomal enzymes in the liver and kidney tissue appreciably increased in the course of experimental alloxan-induced diabetes in the mouse. Of note, lysosomal enzyme activity peaked 12 days after onset of diabetes for most enzymes, at the time when hyperglycemia and hyperinsulinemia already started abating after their peak at 8 days into the course of diabetes. Although we did not attempt to follow the association of lysosomal enzyme activity and hyperglycemia/hyperinsulinemia in detail, the enzyme activity was in most cases higher with the higher dose of alloxan and thus higher level of glycemia.

The findings of this study confirm the increases in the lysosomal enzymes found in varies models of diabetes evoked in experimental animals and also in human diabetes mellitus as above discussed (Waters et al. 1992). Thus, increase in lysosomal enzyme activity has to do with hyperglycemia rather than with a direct toxic effect on beta cells of the pancreatic islets of an agent used to evoke experimental diabetes. Diabetes, irrespective of its pathogenesis and type, is underlain by distorted cellular metabolism; a primary consequence of hyperglycemia and hyperinsulinemia (Dell'aquila and Ellger 2013; Mandrup-Poulsen 2013). The role of lysosomal enzymes is to maintain cells in a state of dynamic biochemical balance (Witek et al. 2001, 2004). Therefore, diabetes is bound to affect the enzyme activity, which likely reflects cellular adaptation at the molecular level.

Lysosomal enzymes degrade membrane glycoconjugates, a class of carbohydrates linked to various other chemical species formed in the process of glycosylation. These molecules are present in the basement membrane of endothelial cells where they are supposed to be an integral part of the permeability properties of the capillary wall. A reduced membrane glycoconjugate content, caused by enhanced lysosomal enzyme activity, could constitute an essential trait in progression of diabetic microangiopathy and atherosclerosis, particularly developing in poorly controlled hyperglycemia (Waters et al. 1992; Rohrbach and Martin 1982). Although the exact molecular role of lysosomal enzymes remains to be fully elucidated in alternative study designs, we believe we have conclusively demonstrated that these enzymes' activity is enhanced in uncontrolled experimental diabetes. Since

lysosomal enzyme activity is highly conducive to the development of diabetic microangiopathy, the assessment of this activity could potentially be useful in the monitoring of diabetic therapy and microvascular complications.

## References


Al-Attas OS, Al-Daghri NM, Alfadda AA, Abd-Alrahman SH, Sabico S (2011) Blood thiamine and its phosphate esters as measured by high-performance liquid chromatography: levels and associations in diabetes mellitus patients with varying degrees of microalbuminuria. J Endocrinol Invest 35 (11):951–956

Barrett AJ, Heath MF (1977) Lysosomal enzymes. In: Dingle JT (ed) Lysosomes. A Laboratory Handbook. North-Holland Publishers, Amsterdam/New York/ Oxford, pp 19–145

Beaufay H (1972) Methods for isolation of lysosomes. In: Dingle JT (ed) Lysosomes. A Laboratory Handbook. North-Holland Publishers, Amsterdam/New York/ Oxford, pp 1–30

Bhimji S, Godin DV, McNeill JH (1985) Biochemical and functional changes in hearts from rabbits with diabetes. Diabetologia 28(7):452–457

Chang AY, Noble RE, Wyse BM (1977) Streptozotocin-induced diabetes in the Chinese hamster. Biochemical and Endocrine Disorders. Diabetologia 13(6):595–602

Dell'aquila AM, Ellger B (2013) Perioperative glycemic control: what is worth the effort? Curr Opin Anaesthesiol 26(4):438–443

El-Demerdash FM, Yousef MI, El-Naga NI (2005) Biochemical study on the hypoglycemic effects of onion and garlic in alloxan-induced diabetic rats. Food Chem Toxicol 43(1):57–63

Faure AM, Werder J, Nyström L (2013) Reactive oxygen species responsible for beta-glucan degradation. Food Chem 141(1):589–596

Fushimi H, Shibata M, Tarui S (1980) Glycosidase activities in the liver and kidney of hereditary diabetic mice. J Biochem 87(3):941–949

Gambaro G, Venturini AP, Noonan DM, Fries W, Reg G, Garbisa S, Milanesi C, Pesarini A, Borsatti A, Marchi E (1994) Treatment with a glycosaminoglycan formulation ameliorates experimental diabetic nephropathy. Kidney Int 46(3):797–806

Gilinsky AS, Kirk AF, Hughes AR, Lindsay RS (2015) Lifestyle interventions for type 2 diabetes prevention in women with prior gestational diabetes: a systematic review and meta-analysis of behavioural, anthropometric and metabolic outcomes. Prev Med Rep 24:448–461

Grötsch H, Hropot M, Kief H, Klaus E (1986) Enzymuria in streptozotocin-diabetes rats. J Clin Chem Clin Biochem 24(8):533–539

Hamer I, Van Beersel G, Arnould T, Jadot M (2012) Lipids and lysosomes. Curr Drug Metab 13 (10):1371–1387

Heltianu C, Robciuc A, Botez G, Musina C, Stancu C, Sima AV, Simionescu M (2011) Modified low density lipoproteins decrease the activity and expression of lysosomal acid lipase in human endothelial and smooth muscle cells. Cell Biochem Biophys 61 (1):209–216

Hinohara Y, Takanashi S, Nagashima R, Shioya A (1974) Glucuronic acid pathway in alloxan diabetic rabbits. (I). Urinary excretion of metabolites related to the glucuronic acid pathway. Jpn J Pharmacol 24 (6):869–878

Kido Y (2013) Progress in diabetes. Rinsho Byori 61 (10):941–947

Kirschke H, Wiederanders B (1984) Methoden zur Aktivitätsbestimmung von Proteinasen (Methods for determining proteinase activity). In: Kongress und Tagungsberichte der Martin-Luther-Universität, Halle-Wittenberg, Halle; pp. 11–17 (Article in German)

Kołątaj A, Śliwa-Jóźwik A, Jóźwik A (2001) The lysosomal cell complex as a stress response indicator. Anim Sci Pap Rep 19(3):177–192

Krischer JP, Cuthbertson DD, Yu L (2003) Screening strategies for the identification of multiple antibody-positive relatives of individuals with type 1 diabetes. J Clin Endocrinol Metab 88(1):103–108

Kumari K, Sahib MK (1993) Susceptibility of different rat tissue to non-enzymatic protein glycosylation in experimental diabetes. Indian J Exp Biol 31 (2):194–195

Kutryk MJ, Pierce GN, Dhalla NS (1987) Alterations in heart and serum lysosomal activities in streptozotocin-induced diabetes. Basic Res Cardiol 82(3):271–278

Lowry OH, Rosebrough NJ, Farr L, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265–266

Mandrup-Poulsen T (2013) Type 2 diabetes mellitus: a metabolic autoinflammatory disease. Dermatol Clin 31(3):495–506

McAnuff MA, Omoruyi FO, Morrison EY, Asemota HN (2003) Hepatic function enzymes and lipid peroxidation in streptozotocin-induced diabetic rats fed bitter Yam (Dioscorea Polygonoides) steroidal sapogenin extract. Diabetologia Croat 32(1):17–23

McAuliffe AV, Fisher EJ, McLennan SV, Yue DK, Turtle JR (1996) Urinary glycosaminoglycan excretion in NIDDM subjects: its relationship to albuminuria. Diabet Med 13(8):758–763

Miralles JM, Velasco J, Villabona V, Sanchez-Bernal C, Perez N, Corrales JJ, Garcia-Diez LC, Villar E (1993) Prospective study of the enzymatic activities in urine of N-acetyl-beta-D-glucosaminidase, alpha-D-mannosidase, alpha- and beta-D-glucosidases, alpha-L- and beta-D-fucosidases, and beta-D-galactosidase in type I diabetes mellitus with early nephropathy. J Diabetes Complications 7(3):99–203

Mohanam S, Bose SM (1983) Influence of streptozotocin- and alloxan-induced diabetes in the rat on collagenase and certain lysosomal enzymes in relation to the degradation of connective tissue proteins. Diabetologia 25 (1):66–70

Mohanam S, Bose SM (1984) Influence of streptozotocin- and alloxan-induced diabetes on the metabolism of glycosaminoglycans. Acta Diabetol Lat 21 (3):203–210

Oshima Y, Isogai S, Mogami K, Ohuchi H, Ohe K (1994) Protective effect of heparin on renal glomerular anionic sites of streptozotocin-injected rats. Diabetes Res Clin Pract 25(2):83–89

Panin LE, Kliment'eva TK, Maianskaia NN (1982) Effect of glucocorticoids and catecholamines on the state of the tissue lysosomal apparatus in rabbits with experimental diabetes. Probl Endokrinol (Mosk) 28 (1):70–73

Perkisas S, Vandewoude M (2016) Where frailty meets diabetes. Diabetes Metab Res Rev 32(Suppl 1):261–267

Platt FM, Boland B, van der Spoel AC (2012) The cell biology of disease: lysosomal storage disorders: the cellular impact of lysosomal dysfunction. J Cell Biol 199(5):723–734

Rohrbach DH, Martin GR (1982) Structure of basement membrane in normal and diabetic tissue. Ann N Y Acad Sci 401:203–211

Stroev EA, Chugunova LG, Dubinina II (1993) Activity of lysosomal enzymes in diabetes mellitus patients along with diffuse enlargement of the thyroid gland. Vopr Med Khim 39(2):35–36

Takumi T, Kodama S, Takahashi T, Matsuo T (1985) N-acetyl-beta D-glucosaminidase activity in serum, kidney and liver of streptozotocin-induced diabetic rat. Enzyme 34(3):166–473

Waters PJ, Flynn MD, Corrall RJM, Pennock CA (1992) Increases in plasma lysosomal enzymes in Type 1 - (insulin-dependent) diabetes mellitus: relationship to diabetic complications and glycaemic control. Diabetologia 35(10):991–995

WHO (2012) World Health Statistics 2012. WHO Press, Geneva

Witek B, Król T, Kołątaj A, Ochwanowska E, Stanisławska I, Slewa A (2001) The insulin, glucose and cholesterol level and activity of lysosomal enzymes in the course of the model alloxan diabetes. Neuro Endocrinol Lett 22(4):240–244

Witek B, Ochwanowska E, Kołątaj A, Baranowska D, Król T, Rafay J (2004) Insulin-induced changes of proteolytic activity of the lysosomal enzymes. Neuro Endocrinol Lett 25(1-2):83–86

Witek B, Fronczyk W, Jóźwik A, Walczak M, Kamińska A, Kołątaj A (2014) The effect of dietary protein level on selected degradative enzymes activity of hepatocytes cellular subfractions in experimental mice. Anim Sci Pap Rep 32(3):269–283

Zhao T, Lu X, Davies NM, Gong Y, Guo J, Zhang H, Li Z, Hong J, Fu G, Li P (2013) Diabetes results in structural alteration of chondroitin sulfate in the urine. J Pharm Pharm Sci 16(3):486–493

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 83–99
DOI 10.1007/5584_2017_104

Published online: 6 October 2017

# Proteomics in the Diagnosis of Inborn Encephalopathies of Unknown Origin: A Myth or Reality

Anna Kupniewska, Krystyna Szymanska, and Urszula Demkow

**Abstract**

Synaptopathy underlies a great variety of neurological or neurodevelopmental disorders, including neurodegenerative diseases and the highly complex neuropsychiatric syndromes. Standard diagnostic assays in the majority of synaptopathies are insufficient to make an appropriate and fast diagnosis, which has spurred a search for more accurate diagnostic methods using recent technological advances. As synaptopathy phenotypes strictly depend on genetics and environmental factors, the best way to approach these diseases is the investigation of entire sets of protein characteristics. Thus, proteomics has emerged as a mainstay in the studies on synaptopathies, with mass spectrometry as a technology of choice. This review is an update on the proteomic methods and achievements in the understanding, diagnostics, and novel biomarkers of synaptopathies. The article also provides a critical point of view and future perspectives on the application of neuroproteomics in clinical practice.

**Keywords**

Biomarkers • Diagnostics • Mass spectrometry • Phenotype • Proteomics • Synaptopathy • Synaptosome

## 1 Introduction

Around eighty-six billion of human brain neurons and about equal number of glial cells blend into a functioning unified whole, with many constrains (Azevedo et al. 2009). The constraints make the nervous system extremely sensitive to tiniest alterations, which usually result in neurological disorder. Point-to-point connections among brain neurons are provided by synaptic junctions. Each neuron may be linked to as many as 10,000 others. Numerous ion channels, located in the cell membrane of neurons, enable the conduction of signals along the nerve fibers. Voltage-gated ion channels and sodium-potassium pumps participate in maintaining neuronal resting and action

A. Kupniewska (✉) and U. Demkow
Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, 63A Zwirki and Wigury Street, 02-091 Warsaw, Poland
e-mail: aniakk22@gmail.com

K. Szymanska
Department of Clinical and Experimental Neuropathology, The Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawińskiego Street, 02-106 Warsaw, Poland

Department of Child Psychiatry, Warsaw Medical University, Warsaw, 24 Marszalkowska Street, 00-576 Warsaw, Poland

potentials. The action potential is conducted down to the presynaptic terminals where electrical signals are converted to chemical molecular signals due to exocytotic neurotransmitter release. A typical presynaptic nerve terminal in the brain has approximately 100 to 200 synaptic vesicles filled with a variety of neurotransmitters (Südhof 2013). Neurotransmitter receptors are located in both pre- and postsynaptic membranes. Binding of a neurotransmitter to a metabotropic receptor triggers a cascade of events with the activation of G proteins as a first step.

Synapses are made up of thousands of proteins, and each type of protein has a specific role to help neuronal signal transmission go smoothly (Coba et al. 2009). There are more than 2000 protein groups in highly purified post-synaptic densities, which are spatially organized and dynamically regulated macromolecular complexes essential for the stability of a postsynaptic architecture (Distler et al. 2014; Sheng and Kim 2011). Synapses are extremely dynamic structures are continuously formed, eliminated, and remodeled throughout the life cycle (Grutzendler et al. 2002; Trachtenberg et al. 2002). As every complicated biological process, neurotransmission is extremely fragile and any derangement in availability or activity of synaptic proteins may lead to a disease (Craft et al. 2013). Synaptic-related disorders, known as synaptopathies, are usually a consequence of an inherited genetic aberration. The term synaptopathy implies a disruption in synaptic structure or function; consequently also in neuronal signal transmission between neurons.

Since synapses are the fundamental information processing units in the brain, synaptic dysfunction underlies most disorders of CNS. There are at least 200 genes unraveled whose disruption leads to numerous different brain and neurodevelopmental disorders (Bayés et al. 2011, 2012). A number of genetic studies show that protein aberrations intimately involved in the synapse formation, synaptic signaling, and synaptic metabolism are responsible for complex neuropsychiatric disorders of synaptopathic origin, such as depression, schizophrenia, autism, and for neurodegenerative diseases such as Alzheimer and Parkinson's disease and others (Grant 2012; Grabrucker et al. 2011). The accurate diagnosis of synaptopathies requires an individual approach to each patient with an extensive clinical analysis consisting of neuroimaging, sophisticated biochemical testing, and genetic analysis (Shevell et al. 2003). Nonetheless, the diagnosis usually remains hard to be firmly established, so that there is a constant search for new, more accurate and efficient diagnostic methods taking advantage of recent technological developments.

Initially, synaptopathies were investigated by genotyping techniques, genome wide association studies, and the large-scale transcriptome analyses. However, genes profiling could provide only a static view of a diseased state due to a number of reasons such as: (i) differences in gene splicing; (ii) posttranslational modifications; (iii) influence by external stimuli at the protein level; and (iv) additive effects on the pathological phenotype of two or more protein mutations of smaller significance. Moreover, proteins almost never act individually, which is particularly true for synapses whose function relies on a vast array of compartmentalized protein-protein interactions. That ensures fidelity in the synaptic vesicle cycling at presynaptic site, neurotransmitter release and receptor localization, and postsynaptic signaling (Grant 2012). Thus, profiling of a whole set of proteins reflects much better the state of an organism in both health and disease, making proteomics a promising strategy in the investigation of synaptopathies of late.

The proteome was defined around 15 years ago as a total set of proteins expressed by the cell, tissue, or organism at a given time under the determined condition (Wilkins et al. 1996). This definition has been extended to the entire collection of proteins encoded by the genome of any organism, including protein isoforms and post-translational modifications (Taurines et al. 2011). Recently, protein-protein interactions were added to the proteomic studies. Proteomic measurements enable not only to obtain a snapshot of protein concentrations associated with different cell, tissue, body fluid, or whole living

organism's states but also protein identification, quantification, and to determine protein modifications and localizations (Yates et al. 2009; Wilkins et al. 1996). The snapshot of all synaptic proteins is called synaptosome. In an unsuitable manner, although growing evidence suggests that pathologic alterations of proteins and protein interactions involved in synaptic neurotransmission underlie a number of neurological and psychiatric disorders, the synaptosome is rather rarely included in the array of investigations (Duarte et al. 2011).

The best tissue to study synaptosome is of course the neuronal tissue, but it is only available from post-mortem cases. Body fluids may reveal protein signatures likely to provide "real-time" investigation of a disease for diagnosis and taking a therapeutic decision. The noninvasiveness issue is the best addressed by urine or blood. However these fluids are too distant from the tissues of interests in case of synaptopathies – neuronal tissue. Thus, the body fluid, which best fulfills the noninvasiveness and the proximity criteria, is CSF. It is known that CSF receives a wide variety of molecules released by different neuronal cell populations as a result of large contact with neuronal tissue (Thouvenot et al. 2008; Zougman et al. 2008). Even membrane CNS proteins could already be abundantly detected in CSF as theirs proteolytic fragments (Duarte et al. 2011; Yuan et al. 2002), full form or even as a part of small nanostructures ejected from neuronal cells (Harrington et al. 2009). It gives the possibility for indirect access to synaptosome composition and its alterations with the advantage of performing the studies *in vivo*.

## 2 Proteomic Studies – Methodology

Quantitative or also called comparative proteomics is essential for characterizing a disease proteome in relation to its normal counterpart. Proteomes can also be analyzed and compared in order to detect changes in response to the development, treatment, environment, and many other factors. The comparison usually includes control (normal) *versus* experimental (disease) samples. The challenge of comparative proteomics is to sift through entire proteomes and to identify a few proteins that differ between the samples being compared. Differences in the synaptosome makeup of patients with synaptopathies and their healthy counterparts are extremely informative for the pathology elucidation and diagnosis.

Proteomic studies are typically divided into two categories: (i) discovery and (ii) targeted proteomics. The quantitative discovery proteomics, called also differential proteomics, relies on the ability of mass spectrometry to detect small changes in protein and peptide abundance in response to an altered state (Ong and Mann 2005). While quantitative discovery proteomic experiments are compared to the search of a needle in a haystack, the targeted proteomics is designed to quantify a limited number of chosen proteins with very high precision, sensitivity, specificity, and throughput.

### 2.1 Quantitative Discovery Proteomic Tools

There are two complementary methods for proteomic discovery studies: (i) two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) which is able to analyze intact proteins and (ii) - high-performance mass spectrometry-based peptide analysis which have a greater sensitivity and reproducibility with less material consumption (Lottspeich et al. 2010).

#### 2.1.1 Two-Dimensional Polyacrylamide Gel Electrophoresis

2D-PAGE, a form of 2 dimensional gel electrophoresis, is a traditional proteomic approach. It consists of two main steps. Firstly, protein molecules are resolved according to their isoelectric point (pI), and secondly by molecular weight in polyacrylamide gels containing sodium dodecyl sulphate (SDS-PAGE). In the end, the proteins or rather protein spots are visualized by dyes, fluorophores, or radioactivity. The intensity of stained bands/spots can provide a rough idea

of a relative amount of each protein. 2D-PAGE can provide information about changes in proteomes upon certain conditions, e.g., a disease. In this case, the technique is called 2D–DIGE (two-dimensional differentiating gel electrophoresis) and includes a comparison of stained patterns of proteins from two samples (Anderson and Anderson 1998).

Nonetheless, this broadly used method has a certain number of drawbacks. It requires many replicates and intensive image analysis that could be user subjective. 2D-PAGE is also unsuitable for highly acidic or basic proteins as separation below pH 3 and above pH 11 is poor because of the lack of good ampholytes. Membrane proteins are often poorly separated due to the acidic character and poor solubility in the absence of detergents. Furthermore, the dynamic range of protein expression can vary by as much as 7–12 orders of magnitude within a biological sample, and 2D-PAGE can only visualize the most abundant proteins. As a result, 2D-PAGE gels have largely been superseded by the mass spectrometry (MS)-based proteomic methods (Corthals et al. 2000; Anderson and Anderson 1998).

### 2.1.2 Mass Spectrometry

Mass spectrometry (MS) is the most comprehensive and versatile tool in large-scale proteomics with a growing presence in laboratory medicine (Yates et al. 2009). The method is relatively simply and fast, which makes it more reproducible. In contrast to 2D-PAGE, MS addresses the issues of poor representation of low-abundance, low mas, and basic or acidic proteins.

Mass spectrometers are generally composed of three fundamental parts: (i) ionization source; (ii) mass analyzer; and (iii) detector. Sample pre-fractionation is an optional step in mass spectrometry, but necessary in most proteomics studies such as protein identification where target proteins need to be isolated from complex mixtures (e.g., serum, blood, or CSF) (Robards et al. 2004). High-performance liquid chromatography (HPLC), which in proteomics studies is often replaced by ultra-high-performance chromatography (UPLC), is currently one of the most popular fractionation methods coupled with MS. In the pre-fractionantion step, protein or peptide mixtures are separated by their hydrophobicity. This limits the quantity of analytes that are simultaneously introduced into a spectrometer, and thus increases the sensitivity of measurements (Robards et al. 2004).

The key issue to use MS for solutions is the ability to transfer the analytes into the electromagnetic field under a vacuum. As ions are actually analyzed in MS, the most important reaction is the conversion of analytes of interest into gas phase ions. Once in the ionization source, the molecules gain a charge. This process in proteomic studies is often handled by an electrospray ion source (ESI). Solutions introduced into ESI are sprayed, vaporized, and ionized. The proteins or theirs fragments are converted to a form $MH^+$ as they have numerous carboxylic group that readily accept a proton ($H^+$) (de Hoffmann and Stroobant 2007). The ESI has some very impressive attributes that allow the resolution of a wide variety of biological problems. There seems to be no inherent limit to the size of molecules that can be ionized. In addition, ESI is a very 'soft' technique that allows the analysis of whole biomolecules or even non-covalent biomacromolecular complexes.

Ions are extracted into the analyzer region of the mass spectrometer where they are separated according to their mass-to-charge ratios (m/z). All mass spectrometers, regardless of type, ionization mode, or performance characteristics, produce mass spectra (m/z spectra) being a plot of the mass-to-charge ratio of the ions detected (x-axis) *versus* detected ion abundance (y-axis). The separated ions are detected and the signal is stored in the form of m/z ratios, along with the ion relative abundance, for the display as the m/z spectrum (Domon and Aebersold 2006).

A dominant sample analysis workflow in proteomics by mass spectrometry utilizes site-specific enzymatic proteases such as trypsin to digest proteins to peptides. To identify the proteins in the sample mixture the protein peptides need to be sequenced by applying a tandem mass spectrometry mode (MS/MS mode). This mode requires two mass analyzers sequentially reassembled. The first analyzer is

used to select the user-specified sample ions arising from a particular component; usually the molecular-related ions. These chosen ions (parent ions) pass into the collision cell, where are bombarded by the neutral gas molecules. The high energy collisions with the gas cause their fragmentation into peptides of different lengths. Next, the created peptides are separated according to their mass to charge ratio by the second analyzer. The resulting spectra (MS/MS spectra) are used to retrieve the corresponding peptide sequence from the database. Protein identification is straightforward, as only two unique peptides are usually sufficient to recognize a protein (Domon and Aebersold 2006).

This approach is called the shotgun mass spectrometry and is recently the most popular in the proteomic toolbox methods of identification proteins in a proteome of interest. Advances in MS technology now enable the detection and identification of several thousand proteins in complex biological samples with a high level of confidence. This method can also provide quantitative data, as the signal intensity refers to the abundance of a peptide in a sample (Etzioni et al. 2003).

### 2.1.3 Differential Analysis by MS

Differential analysis is generated from LC-MS experiments and can be carried out using both label and label-free approaches. A label-free method uses the signal intensity to estimate the peptide ratios between analyses and typically requires complex data normalization (Old et al. 2005). For the label-based approaches, proteins from one sample are modified to include an isotopic signature that identifies the sample upon mixing and provides the basis for peptide comparison (Flory et al. 2002).

The label-free quantitative proteomic techniques are the methods of first choice from several reasons: (i) these methods are fast, simple and inexpensive (expensive chemical synthesis of labeled peptides is avoided); (ii) there is no principle limit to the number of experiments to compare with each other, which is especially required when a number of patients with different symptoms are going to be compared; (iii) MS measurements provide the mass spectra that are relatively of low complexity, and may thus be easier analyzed; (iv) MS measurements can be performed with a higher dynamic range of protein concentrations, thus the proteins with highly varied concentrations (8–10 fold) can be measured; and (v) virtually any soluble biological material can be used (Schulze and Usadel 2010; Bantscheff et al. 2007).

In the label-free technique, quantification is performed using the peak-intensity (Zhu et al. 2010) or spectral counting (Wang et al. 2008). The former is more popular since ion chromatograms for every peptide are extracted from the liquid chromatography–mass spectrometry (LC-MS) run, and their peak areas are integrated over the chromatographic time scale. These values can be compared to respective values in other experiments for relative quantitation. Only the same ion species can be compared between different samples. Differential analysis is highly dependent on the scan speed, sensitivity, and the ability to isolate precursor ions for selection to tandem MS/MS (Michalski et al. 2011). Thus, these techniques require the use of highly accurate and resolute mass spectrometers like, for instance, the Quadropule-OrbiTrap (Q-OrbiTrap).

Quantitative proteomic experiments in the discovery phase are likely to generate several hundred putative protein candidates which are significantly different between the healthy and ill individuals. Ideally, MS-based discovery phase experiments should generate no false positive identifications (i.e., all proteins detected and reported would be truly present in the sample); they should generate no false negative identifications either (i.e., every protein present in the sample will be detected by the MS); and every protein present should be able to be quantified with a high degree of accuracy. Unfortunately, proteomic technologies are poorly capable of globally analyzing a proteome of interest with the depth and reproducibility required. The quantitative information provided by proteomic profiling also is imperfect since a large number of hypotheses are tested in a small number of samples (Whiteaker et al. 2011;

Gramolini et al. 2008). Consequently, '-omics' based discovery experiments are fraught with false discoveries, and validation studies using independent methods ought to be performed to verify the clinical utility of candidate proteins.

## 3 Targeted Proteomics

Targeted proteomic methods are ideal to validate differences in proteomes in a highly selective manner. This technique is increasingly used in pharmaceutical and diagnostic applications to quantify proteins and metabolites in complex samples (Köhler and Seitz 2012; Domon and Aebersold 2006; Farr et al. 2004).

Currently, the most commonly applied approaches in targeted proteomics are immune-based sensitive methods such as the enzyme-linked immunosorbent assay (ELISA), immunoblotting (Western-blot), or chip immunoassay arrays (Köhler and Seitz 2012; Kingsmore 2006). Despite their high sensitivity, the immuno-based methods have disadvantages related to the performance of antibodies. The antibodies may not distinguish highly similar homologs and sequence variants arising from polymorphisms and mutations. That usually results in multiple bands on the immunoblotting membranes and significantly complicates the analysis. Finally, even if some antibodies can detect specific modification sites on proteins (e.g., phosphotyrosine), only few can reliably distinguish the differences between such modifications.

Consequently, the immune-based methods are replaced by a more reliable single or multiple reaction monitoring by mass spectrometry (SRM or MRM). This method was selected as the Nature Method of the Year 2012 and is recognized as the most sensitive and specific way to detect pre-selected components in a complex matrix (Colangelo et al. 2013; Picotti and Aebersold 2012; Pan et al. 2009; Domon and Aebersold 2006). The method is used for both verification/validation protein candidates from discovery proteomics as well as for a routine measurement in clinical investigations.

The underlying concept of SRM/MRM is that the proteins may be quantified by measuring their specific constituent peptides by mass spectrometry following proteolytic digestion. The acquisition of data exclusively for the selected peptides rather than sifting through enormous amounts of data enables the measurement with higher precision, sensitivity, and throughput. This technology enables a reliable quantitation of low abundance selected proteins in complex mixtures. It also enables a simultaneous quantitation and identity confirmation in a single LC-MS/MS run (Lange et al. 2008).

The SRM/MRM is performed on mass spectrometer with three mass analyzers sequentially connected that usually are of a quadrupole type called the triple-quadrupole (QqQ). A peptide mixture is introduced into the first quadrupole after ionization where the chosen precursor ion is precisely separated from other ions and uniquely passed to the second quadrupole. There the ion is subjected to a high energy fragmentation and its daughter ions pass to the third quadrupole where all of them or only the selected ones can be detected. A combined measurement of intact peptide ions (precursors) and resulting specific fragment ions (products) in SRM/MRM constitutes a transition that is specific for the monitored peptide sequences. The transition, with its maximum specificity, provides a high certainty of a determination of chosen macromolecules (Lange et al. 2008).

The SRM/MRM also enables the absolute quantitation of protein targets (AQUA). This is achieved by spiking complex samples with stable isotope-labeled synthetic peptides (AQUA-peptides). AQUA-peptides are usually labeled by incorporation of ($^{13}C_6{}^{15}N_2$)lysine or ($^{13}C_6{}^{15}N_4$)arginine. They act as internal standards for specific chosen peptides as they are designed to have the identical sequence with them and only slightly differ in mass. Thus, AQUA-peptides have the same chemical character as their original analogs. That ensures their exact co-elution in liquid chromatography and the same behavior in MS analysis as those of the peptides of interests. The quantitation values are subsequently obtained by comparing the intensities or

peak areas of signals from the peptides of interests and isotopically labeled AQUA-peptides (Gerber et al. 2003).

The absolute quantification of a native light peptide enables, by extrapolation, the quantity determination of its parent protein. The AQUA method provides the greatest protection against a system's drift and chromatographic instability that underlie measurement variations among inter-laboratory investigations. As the method provides the highest analytical precision, it is appropriate for the analyses conducted over an extended period of time or across multiple laboratories (Cham et al. 2010).

## 4 Neuroproteomics in Biomarker Discovery and Synaptopathy Deciphering

Targeted quantitative MS approaches with accurate and statistically reliable outcomes are essential for the development of biomarkers assays. The US National Institute of Health defines a biomarker as a "*characteristic that is objectively measured and evaluated as an indicator of a normal biological process, pathogenic process, or pharmacologic responses to therapeutic intervention*" (Biomarker Definition Working Group 2001). The biomarker can be any biological substance such as a protein, a metabolite, or a specific post-translational modification pattern. Since molecular changes underlying pathological conditions are usually complex and heterogeneous, a solution to improve the accuracy of biomarkers lies in the assembling of biomarker panels.

The ultimate aim of biomarker discovery is to develop a simple differential test as an easy and fast clinical evaluation tool. That requires a long process which involves candidate discovery, verification, validation, and translation to clinical laboratory use. The MS-based proteomic technologies are ideally suited for the workflow of biomarker discovery in the absence of any prior knowledge of quantitative changes in the protein content.

An initial discovery phase relies on untargeted MS-based approaches resulting in the identification of a vast number of potential biomarkers. As described above, the discovery phase results in many putative biomarkers that need to be verified and validated further on. A verification phase ensures that only the most promising putative candidates for biomarkers go on to the validation stage. To address this challenge, targeted proteomics workflows involving SRM/MRM usually are applied. However, an orthogonal verification by ELISA or Western-blot usually has to be also performed (Orton and Doucette 2013; Surinova et al. 2011; Rifai et al. 2006). Biomarker candidates are then validated across a large number of samples and a large cohort in a targeted population. Candidates are prioritized based on their functional/biological relevance. Here, also quantitative MS-based targeted approach such as SRM/MRM or AQUA is applied (Frantzi et al. 2014). Only biomarker candidates or theirs panels that passed through the validation phase can be translated into clinical laboratory tests (Latterich and Schnitzer 2011).

Due to recent technical advances in biomolecular mass spectrometry, a great deal of international effort has gone into the discovery of biomarkers. The development of new diagnostic biomarkers has a great potential and solutions are being tested in both pharmaceutical industry and academic medicine settings (Végvári et al. 2011; Végvári and Marko-Varga 2010; Anderson 2005; Zolg and Langen 2004). In the prevalent neuropsychiatric syndromes such as schizophrenia, major depressive syndrome (MDS), or autism spectrum disorder (ASD), and in other inborn encephalopathies, pathologic symptoms arise from the interplay between genetic and environment aberrations (Nascimento and Martins-de-Souza 2015; Diaz-Beltran et al. 2013; Filiou et al. 2011; Fernández et al. 2009). This interplay precludes drawing straight conclusions from genetic tests for the diagnosis and therapy. Thus, proteome investigations may shed a new light on the pathogenesis of those disorders. Even though CSF represents the most relevant specimen for neurological analyses (Zougman et al. 2008), there are a number of

studies pointing to protein biomarkers in other body fluids such as blood, serum, or urine, as well as post-mortem studies of brain tissue, which provide hints for the plausible biological pathway involved with a disorder. There are several lines of evidence showing that proteomics of body fluids is fruitful in the establishing of biomarker candidates of schizophrenia, MDS, ASD, and neurodegenerative diseases. Consequently, novel therapeutic targets can also be established.

## 5 Proteomics in Neurodegenerative Diseases

### 5.1 Alzheimer's Disease

In CSF of Alzheimer's disease (AD) patients most frequent biomarkers used to diagnose and monitor the disease, which correlate well with neuropathological lesions, are the following: decreased level of Ab42, and elevated levels of Tau, p-Tau, and their truncated forms (Bateman et al. 2012; Blennow et al. 2006). As Ab42 has a stronger tendency to aggregate, the ratio Ab42/Ab40 has a higher sensitivity for AD diagnosis, and it is less affected by variations in endogenous Ab levels and less biased by sample preparation (Perret-Liaudet et al. 2012). Also, the ratio of Ab42/Tau or p-Tau can be helpful for an early diagnosis of AD (Brunnström et al. 2013; Parnetti et al. 2006; Hulstaert et al. 1999). Proteomic studies have also shown that oligomers and different forms of peptide Ab of variable length well reflect dementia (Wang-Dietrich et al. 2013; Portelius et al. 2010).

In AD, neuroimaging cannot reflect direct clinical lesions nor the effect of treatment, which spurs a constant search for more precise methods. The proteomic MS investigations in AD have shown the utility of multiple reaction monitoring (MRM) in replacing the traditional ELISA test in the assessment of Tau content in CSF. An underrepresented Tau content in CSF can be overcome by a two-step CSF pre-fractionation technique with perchloric acid and micro-solid phase extraction instead of a traditional immunocapture. Studies reveal a potential of current MRM assays in the AD diagnostics and therapy monitoring (Bros et al. 2015).

Apart from Tau and Ab peptides, a prominent pathological feature of AD and of other neurological diseases is a severe loss of synapses. Previous reports have shown that presynaptic dysfunction occurs early in the AD disease process (Overk and Masliah 2014; Masliah et al. 2001; Blennow et al. 1996; Terry et al. 1991; Davies et al. 1987). Soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins, which mediate cytoplasmic vesicle fusion, are at the fore-front of research on the markers of synaptic dysfunction at the early stage of AD (Sollner et al. 1993). Using a combination of affinity purification and mass spectrometry (IP-MS) in brain samples from AD patients it has been found that synaptic pathology well reflects a decrease in SNARE proteins. The IP-MS enables a simultaneous quantitation of all four SNARE complex proteins. Moreover, a good association between the IP-MS- and sandwich ELISA-assessed SNAP-25 protein indicates that the two methods are comparably reproducible and sensitive.

The IP-MS well investigates the synaptic pathology in neuropsychiatric or neurodegenerative diseases and to evaluate drugs aimed at restoring synaptic function. The method can also be employed in studies in various animal models, human post-mortem tissue, and possibly in biological fluids (Brinkmalm et al. 2014).

### 5.2 Parkinson Disease

Parkinson disease (PD) is the second after AD most common neurodegenerative disorder. It is characterized by motor function impairment caused by a loss of dopamine (DA)-containing neurons in the brainstem and the presence of intracellular protein inclusions in the substantia nigra, known as Lewy bodies. The abnormal accumulation of proteins, particularly heavily ubiquitinated alpha-synuclein, may play a role in the pathogenesis of PD (Thomas and Beal 2007). Despite many efforts, reliable diagnostic assays for PD have not yet been developed (Tolosa et al. 2006), particularly for patients at

the early stage of the disease. Additionally, no objective measures of disease progression or treatment effects exist. Thus, specific, reliable, and reproducible biomarkers are clearly needed to aid the diagnosis of PD and to track or predict the disease progression.

A biomarker discovery in PD is quite challenging. There are only few CSF peptides/proteins reported as putative biomarkers. The most frequently studied are those which play a role in the pathogenesis of PD, such as α-synuclein and deglycase DJ-1 proteins (Mollenhauer et al. 2011; Hong et al. 2010). Unfortunately, neither shows sufficient and verifiable sensitivity/specificity to be of clinical use.

One especially thorough analysis seems to have established a panel of proteins in CSF that yield a good diagnostic sensitivity/specificity for PD and well correlates with PD severity. This biomarker panel includes such proteins as: neuroprotective osteopontin, receptors implicated in signaling pathways associated with neurodegeneration, in particular with inflammatory responses (low density lipoprotein receptor-related protein 1, colony-stimulating factor receptor 1, ephrin type-A receptor 4), tissue inhibitor of metalloproteinases-1, and amyloid-like protein. The two lastly-mentioned proteins perfectly correlate with PD severity (Shi et al. 2015). However, these protein candidates await validation in independent studies.

Other profound study using a 2DE MS/MS approach has provided a panel of potential PD plasma biomarkers, including haptoglobin, transthyretin, apolipoprotein A-1, serum amyloid P component, apolipoprotein E, complement factor H, fibrinogen γ, thrombin, and complement C3 (Alberio et al. 2013). Also, 2D–DIGE combined with LC-MS/MS or with iTRAQ labeling has provided another panel of 26 differentially expressed proteins, including *inter alia* up-regulated sero-transferrin and clusterin and down regulated complement component 4B, apolipoprotein A-I, α-2 antiplasmin, and coagulation factor V (Zhang et al. 2012).

## 6 Proteomics in Neuropsychiatric Disorders

Neuropsychiatric disorders are one of the most challenging synaptic diseases for medical research, with their complex and multifactorial characteristics. To-date, there are no firmly set and validated biomarkers for any such disorders, although there are many candidate proteins or protein panels consisting of a number of proteins. The reasons for that are the following: (1) multifactorial characteristics of disorders; (2) multigenic contribution, when each gene may exert a small effect; and (3) heavy environmental influence in these disorders. Nonetheless, proteomics is optimal for biomarker establishing in neuropsychiatric disorders as it is the closest linked to the phenotype. Of note, the most needed type of biomarkers for neuropsychiatric disorders, as for any other disorder, is the one that predicts the likelihood of treatment success. The 'one treatment fits all' notion is inapplicable in the management of psychiatric symptoms, since they vary significantly among patients according to their phenotype (Martins-de-Souza 2013).

### 6.1 Schizophrenia

Schizophrenia (SCZ), neuropsychiatric debilitating syndrome hereditary in 80–85% with polygenic contribution, encompasses an umbrella of disorders as in addition to genetic predisposition there are many other factors which contribute to the disease onset such as metabolic and developmental disturbances, with a substantial environmental influence (Rodriguez-Murillo et al. 2012).

Countless combinations of biochemical and environmental factors in SCZ lead to intricate webs of molecular interactions. Thus, understanding of schizophrenia requires the characterization of molecular interactions (Martins-de-Souza 2012) Although the main tissue studied in schizophrenia is post-mortem brain, recent

studies focus on peripheral tissues such as blood serum, plasma, CSF, liver, fibroblasts, skin, and saliva. Studying the other tissues seems to have been a better way to monitor the earliest phase of SCZ, develop treatments, avoid chronic medication-related influences, and to address the sample heterogeneity and age issues. Despite many efforts, until now there is no validated biomarker of SCZ. However, proteomic studies have been invaluable in the elucidation of SCZ pathophysiology (Levin et al. 2010). The proteins found differentially expressed in SCZ patients are mostly involved in the crucial neuronal processes of neurotransmission, synaptic plasticity, and neurite outgrowth. Notably, N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor proteins are downregulated in SCZ and since it targets oxidative phosporylation system it can contribute to alterations in mitochondria. Other proteins downregulated in SCZ are neurofilaments such as NEFL, which are involved in the neuronal morphogenesis and directly associated with the NMDA receptor, glutamate-ammonia ligase (GLUL) that removes glutamate from synapses, dihydropyrimidinase-related protein 2 (DPYSL2) that is involved in the axon guidance and neurosignaling, guanine nucleotide-binding proteins (G proteins) that are involved in glutamatergic signaling, and with many other proteins associated with NMDA signaling and synaptic plasticity (Nascimento and Martins-de-Souza 2015; Föcking et al. 2015).

It is a long time since it has been known that energy metabolism is hampered in SCZ. Frequently observed symptoms, especially in the early onset SCZ, such as hyperglycemia, impaired glucose tolerance, and insulin resistance are a case in point of hampered metabolism. Numerous proteomic analyses of brain and CSF samples reveal a distortion of glycolysis-gluconeogenesis pathways in SCZ. Most frequently detected imbalances are those in aldolase C, pyruvate kinase, and nicotinamide adenine dinucleotide phosphate (NADPH) content. A lower content of NADPH may compromise oxidative phosphorylation. Other glycolysis enzymes found differentially expressed in SCZ are enolase 1 and 2, lactate dehydrogenase B, phosphoglycerate mutase 1, phosphoglycerate kinase 1, glyceraldehyde-3 phosphate dehydrogenase, hexokinase, triose phosphate isomerase, and aconitase-2 and malate-dehydrogenase. The two last enzymes, along with pyruvate kinase, may compromise the Krebs cycle function in SCZ brain (Nascimento and Martins-de-Souza 2015; Martins-de-Souza 2011).

A direct consequence of impaired mitochondrial network of self-regulating enzymes is an overproduction of hazardous reactive oxygen species (ROS) and induction of oxidative stress; fundamental pathophysiologic features in the SCZ brain. Brain proteomics have sorted out the exact mitochondrial enzymes whose compromised activity leads to ROS production, such as superoxide dismutase that catalyzes the dismutation of superoxide anions ($O^{2-}$) into oxygen and hydrogen peroxide; peroxiredoxins that are responsible for hydrogen peroxide reduction, cellular detoxification, glutathione reduction and ROS neutralization; members of gluthatione S transferase family; and carbonyl reductase 1 and 3 and quinoid dihydropteridine reductase (QDPR) that are NADPH-dependent oxidoreductases potentially responsible for NADP/NADPH imbalance in the SCZ thalamus (Martins-de-Souza et al. 2011). In addition, imbalance in $Ca^{2+}$ regulatory proteins in SCZ contributes to the induction of oxidative stress. A disturbance in $Ca^{2+}$ regulation may perturb myelination and dopamine receptors, and may cause neurodegeneration; the processes in which $Ca^{2+}$ plays a key role. Proteomics have unraveled many proteins that are engaged in maintaining the calcium homeostasis, such as calcineurin that regulates dopaminergic and glutamatergic neurotransmissions, both frequently compromised in SCZ (Martins-de-Souza 2011). A number of other calcium-regulating proteins are downregulated in the SCZ brain; the exemplary may be calmodulin, calcium/calmodulin-dependent protein kinase II, voltage-dependent anion channels, or plasma membrane calcium-transporting ATPase 4 to name just a few. Proteomics has unraveled

changes in $Ca^{2+}$-regulating proteins, such as calmodulin-like proteins and the S100 family calcium-binding proteins also in secreted fluids such as sweat or saliva (Föcking et al. 2015). A low content of these proteins may interfere with phospholipase A2 activity and may lead to dopaminergic impairment.

Proteomic studies have confirmed that cytoskeleton imbalances may substantially factor in the SCZ pathogenesis. Cytoskeleton proteins take part in shaping cell polarity, neuritogenesis, and neurotransmission. Imbalances in the expression of these proteins disturb synaptic plasticity and cell metabolic processes. Frequently, imbalance in the expression of tubulin is detected in proteomic studies of SCZ brain tissue, which especially affects mitosis, cytokinesis, and vesicular transport. Proteomics has revealed both up- and down-regulation of some intermediate filaments such as glial fibrillary acidic protein (GFAP) that has an influence on glutamatergic transmission in the SCZ brain. Changes in dynamin 1 (DNM1), a microtubule-binding protein that acts in synaptic clefts as a mechano-chemical enzyme and takes part in dopaminergic and glutamatergic transmission, are consistently reported in the SCZ brains (Martins-de-Souza 2011).

Proteomic profiling has also confirmed the theory of oligodendrocyte dysfunction as another factor in the SCZ pathogenesis (Martins-de-Souza et al. 2009). The main oligodendrocytic pathway disturbed in SCZ is clathrin-mediated endocytosis and clathrin interactome may become a useful therapy target (Martins-de-Souza 2012). The main role of oligodendrocytes in the central nervous system consists of myelination of axons, along with growth factor synthesis, and support of neuronal survival, neurodevelopment, and neurotransmission. Several proteomic and transcriptomic studies of brain tissue have revealed that 2′,3′-cyclic nucleotide 3′ phosphodiesterase (CNP) expression is changed in SCZ. This tubulin binding protein is responsible for oligodendrocytic microtubule assembly and thus it is important in cell growth, axongenesis, RNA metabolic processes, and synaptic transmission. The association of CNP with SCZ has been confirmed by genetic analysis. Transferrin is another oligodendrocyte protein engaged in myelination whose expression also is changed in SCZ (Nascimento and Martins-de-Souza 2015). The impediment in expression of meylin constituents, such as myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) in SCZ has been confirmed using both proteomic and transcriptomic investigations.

Interleukins may also be included into a group of potential biomarkers of diagnosis and treatment in SCZ as their content increases in CSF and blood even at the earliest stage of the disease. In addition, the content of defensins – host defense peptides, migration inhibitory factor, several growth factors, and S100A12 protein changes in SCZ (Nascimento and Martins-de-Souza 2015).

## 6.2 Major Depression Syndrome

Major depression syndrome (MDS) is another complex neuropsychiatric disorder that involves a multitude of brain systems and environmental factors, which makes the establishment of a single marker of the diagnosis and therapy improbable at the present time (Ising et al. 2009). Nonetheless, comparison of CSF proteomes using 2D-PAGE- and LC-MS-based differential expression investigations has revealed a number of candidate biomarker proteins. Proteins that are differentially expressed in MDS are the following: neurofilament heavy polypeptide involved in the nervous system development and cell death (Petzold et al. 2009; Pijnenburg et al. 2007), neuronal cell adhesion molecule (NCAM) involved in the neuronal growth and repair (Gnanapavan et al. 2010), pigment epithelium-derived factor (PEDF) involved in neuroprotective effects (Yabe et al. 2010; Tombran-Tink and Barnstable 2003), prostaglandin-D synthase (PGDS) – an endogenous sleep regulating, nociceptive, and hormone releasing protein (Urade et al. 1996; Sri Kantha et al. 1994; Hayaishi 1991; Ito et al. 1989), ApoE involved in the lipoprotein metabolism and cystatin C – a cysteine proteinase inhibitor, both already implicated in some other CNS disease such as AD (Riedel et al. 2016),

dihydropyrimidinase like 2 (DPYSL2) protein involved in the nervous system development and cell differentiation, and many other proteins such as, for instance, transthyretin precursor, alpha-1B glycoprotein, beta-2-glycoprotein, or hemopexin. A proteomic differential analysis has been capable of unraveling an enhanced content of one alternative-spliced exon isoform of NCAM (140 kDa isoform) in CSF of depressed patients (Ditzen et al. 2012), which definitely confirmed which definitely confirmed earlier observations (Vawter 2000).

A set of the four proteins PEDF, ApoE, PGDS, and cystatin C, all of which have a key role in brain metabolism, has been chosen for the validation phase in one biomarker study (Ditzen et al. 2012). An increase in PEDF in depression could represent a compensation mechanism to counteract endogenous pro-apoptotic stress as PEDF is known to activate the cAMP-response element binding (CREB) protein in primary neuronal cultures (Yabe et al. 2005). In contrast, a decrease in cystatin C in CSF could indicate the presence of cognitive symptoms in depression and also could reflect a higher susceptibility to a neurodegenerative disease, such as AD. A decreased level of PGDS in CSF of depressed patients may explain disturbances in slow-wave sleep, especially that infusion of PGD2 into the rat brain induces this kind of sleep. Imbalance in ApoE pattern (predominance of E2 and E3 isoforms in MDS) could be one of the connecting links between MSD and metabolic syndrome that often accompanies MDS. The markers outlined above enable the disclosure of subgroups of depressed patients who share common mechanism.

Some reports have demonstrated that proteomics of CSF enable the monitoring of antidepressant treatment in selected patients. It is possible to detect fluctuation in CSF proteins, such as heat shock protein 9A, protein disulfide isomerase, creatine kinase, prohibitin, and others in the response to antidepressive therapy. In addition, the understanding of a role of brain plasticity in MDD has been substantially enhanced by the identification of glyoxalase-I and enolase phosphate isoform as markers of cellular plasticity (Huang et al. 2014; Ditzen et al. 2012).

### 6.3 Autism Spectrum Disorder

Genetics is unable to elucidate the etiology of autism spectrum disorder (ASD) neither. The biomarker candidates of ASD have been found in the serum and CSF of young patients, using the LC-MS/MS methodology. For instance, there is an increase in ApoE involved in the immune regulation, nerve regeneration, lipolytic enzyme activation, synaptogenesis, and which may be a ligand for various receptors. ApoE, along with cholesterol, is essential for maintaining both myelin and neuronal synapses (Vila-Rodriguez et al. 2011; Takeda et al. 2010). Other proteins found differentially expressed in ASD are ApoA1 that plays a role in cognitive and mental function and ApoA4, produced in the hypothalamus, which acts as a satiety signal (Ngounou Wetie et al. 2014). ApoA1 and ApoA4, along with ApoB, play a role in cholesterol metabolism and interact with each other.

CSF proteomic studies have also demonstrated an upregulation of the complement factor H-related protein, complement C1q, fibronectin 1, and apoB-100 (Corbett et al. 2007) These findings are consistent with the current theory that disturbances in the lipid system may underlie some forms of ASD (Woods et al. 2012).

## 7 Conclusions

A challenge facing any biomarker development is the sheer complexity and a range of concentrations within the human proteome. In addition, disease-specific proteins usually are very low in abundance and difficult to detect among a ‘sea’ of proteins. These issues have spurred a rapid development of proteomic technology. Likewise, mass spectrometry instruments continue to improve. As a result, there is a continual increase in the depth of coverage and accuracy that are required to discern small changes in peptide abundance and modifications that delineate a disease.

Nevertheless, a search for distinct and validated biomarkers of any psychiatric disorders

has been as yet futile. Since psychiatric syndromes are multifactorial, multigenic, and vulnerable to environmental influences, the best hitherto outcome is the identification of panels of biomarkers rather than one protein that would be common for all disease subgroups. It may be surmised that a targeted proteomic approach, which includes multiple reaction monitoring (MRM), could give better outcomes in the future, as this technology is capable of conducting simultaneous quantitative investigations of all enzymes of a given pathway of interest. The MRM can also be used as a diagnostic or treatment monitoring tool by the assessment of panels of biomarkers comprising specific pathways in a multivariate manner.

The detection of numerous biomarker candidates for complicated psychiatric syndromes has often confirmed a previously known or presumed pathogenesis. Thus proteomics has revealed itself as an interesting tool for a better comprehension of synaptopathic pathobiology. That makes proteomics a future mainstay of diagnosis and therapy design at least in less complicated than psychiatric syndromes neurodegenerative diseases and a few pathway synaptopathies. A rapid unraveling of proteins whose expression is imbalanced in the patient body fluids will provide an immediate hint for the accurate diagnosis and selection of appropriate therapy.

As vast efforts have been put in the biomarker discovery at the preclinical level and many proteins have been designated as potential biomarkers, there is a prevalent need of thorough and critical evaluation to exclude false positive results. Here, biological data from proteomics, genomics, and metabolomics need to be integrated with other scientific disciplines such as computational research and more importantly with clinical empiricism. For this purpose, a close dialog between researchers of different disciplines and clinicians is invaluable despite rapid technologic and bioinformatic developments.

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

## References


Alberio T, Bucci EM, Natale M, Bonino D, Di Giovanni M, Bottacchi E, Fasano M (2013) Parkinson's disease plasma biomarkers: an automated literature analysis followed by experimental validation. J Proteome 90:107–114

Anderson NL (2005) The roles of multiple proteomics platforms in a pipeline for new diagnostics. Mol Cell Proteomics 4:1441–1444

Anderson NL, Anderson NG (1998) Proteome and proteomics: new technologies, new concepts, and new words. Electrophoresis 19:1853–1861

Azevedo FA, Carvalho LR, Grinberg LT, Farfel JM, Ferretti RE, Leite RE, Jacob Filho W, Lent R, Herculano-Houzel S (2009) Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. J Comp Neurol 513:532–541

Bantscheff M, Schirle M, Sweetman G, Rick J, Kuster B (2007) Quantitative mass spectrometry in proteomics: a critical review. Anal Bioanal Chem 389 (4):1017–1031

Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC (2012) Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 367(9):795–804

Bayés A, van de Lagemaat LN, Collins MO, Croning MD, Whittle IR, Choudhary JS, Grant SG (2011) Characterization of the proteome, diseases and evolution of the human postsynaptic density. Nat Neurosci 14:19–21

Bayés A, Collins MO, Croning MDR, Van De Lagemaat LN, Choudhary JS, Grant SG (2012) Comparative study of human and mouse postsynaptic proteomes finds high compositional conservation and abundance differences for key synaptic proteins. PLoS One 7(10): e46683

Biomarker Definition Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69 (3):89–95

Blennow K, Bogdanovic N, Alafuzoff I, Ekman R, Davidsson P (1996) Synaptic pathology in Alzheimer's disease: relation to severity of dementia, but not to senile plaques, neurofibrillary tangles, or the ApoE4 allele. J Neural Transm 103:603–618

Blennow K, de Leon MJ, Zetterberg H (2006) Alzheimer's disease. Lancet 368(9533):387–403

Brinkmalm SA, Brinkmalm G, Honer WG, Moreno JA, Jakobsson J, Mallucci GR, Zetterberg H, Blennow K, O'Hrfelt A (2014) Targeting synaptic pathology with a novel affinity mass spectrometry approach. Mol Cell Proteomics 13(10):2584–2592

Bros P, Vialaret J, Barthelemy N, Delatour V, Gabelle A, Lehmann S, Hirtz C (2015) Antibody-free quantification of seven tau peptides in human CSF using targeted mass spectrometry. Front Neurosci 9:302

Brunnström H, Hansson O, Zetterberg H, Londos E, Englund E (2013) Correlations of CSF tau and amyloid levels with Alzheimer pathology in neuropathologically verified dementia with Lewy bodies. Int J Geriatr Psychiatry 28(7):738–744

Cham JA, Bianco L, Barton C, Bessant C (2010) MRMaid-DB: a repository of published SRM transitions. J Proteome Res 9(1):620–625

Coba MP, Pocklington AJ, Collins MO, Kopanitsa MV, Uren RT, Swamy S, Croning MD, Choudhary JS, Grant SG (2009) Neurotransmitters drive combinatorial multistate postsynaptic density networks. Sci Signal 2(68):ra19

Colangelo CM, Chung L, Bruce C, Cheung KH (2013) Review of software tools for design and analysis of large scale MRM proteomics datasets. Methods 61 (3):287–298

Corbett BA, Kantor AB, Schulman H, Walker WL, Lit L, Ashwood P, Rocke DM, Sharp FR (2007) A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. Mol Psychiatry 12(3):292–306

Corthals GL, Wasinger VC, Hochstrasser DF, Sanchez JC (2000) The dynamic range of protein expression: a challenge for proteomic research. Electrophoresis 21 (6):1104–1115

Craft GE, Chen A, Nairn AC (2013) Recent advances in quantitative neuroproteomics. Methods 61:186–218

Davies CA, Mann DM, Sumpter PQ, Yates PO (1987) A quantitative morphometric analysis of the neuronal and synaptic content of the frontal and temporal cortex in patients with Alzheimer's disease. J Neurol Sci 78 (2):151–164

de Hoffmann E, Stroobant V (2007) Mass spectrometry: principles and applications, 3rd edn. Wiley-Interscience, New York

Diaz-Beltran L, Cano C, Wall DP, Esteban FJ (2013) Systems biology as a comparative approach to understand complex gene expression in neurological diseases. Behav Sci 3:253–272

Distler U, Schmeisser MJ, Pelosi A, Reim D, Kuharev J, Weiczner R, Baumgart J, Boeckers TM, Nitsch R, Vogt J, Tenzer S (2014) In-depth protein profiling of the postsynaptic density from mouse hippocampus using data-independent acquisition proteomics. Proteomics 14(21–22):2607–2613

Ditzen C, Tang N, Jastorff AM, Teplytska L, Yassouridis A, Maccarrone G, Uhr M, Thomas Bronisch T, Miller CA, Holsboer F, Turck CW (2012) Cerebrospinal fluid biomarkers for major depression confirm relevance of associated pathophysiology. Neuropsychopharmacology 37:1013–1025

Domon B, Aebersold R (2006) Mass spectrometry and protein analysis. Science 312(5771):212–217

Duarte ST, Ortez C, Pérez A, Artuch R, García-Cazorla A (2011) Analysis of synaptic proteins in the cerebrospinal fluid as a new tool in the study of inborn errors of neurotransmission. J Inherit Metab Dis 34(2):523–528

Etzioni R, Urban N, Ramsey S, NcIntish M, Schwartz S, Reid B, Radich J, Anderson G, Hartwell L (2003) The case for early detection. Nat Rev Cancer 3(4):243–252

Farr CD, Gafken PR, Norbeck AD, Doneanu CE, Stapels MD, Barofsky DF, Minami M, Saugstad JA (2004) Proteomics analysis of native metabotropic glutamate receptor 5 protein complexes reveals novel molecular constituents. J Neurochem 91(2):438–450

Fernández E, Collins MO, Uren RT, Kopanitsa MV, Komiyama NH, Croning MD, Zografos L, Armstrong JD, Choudhary JS, Grant SG (2009) Targeted tandem affinity purification of PSD-95 recovers core postsynaptic complexes and schizophrenia susceptibility proteins. Mol Syst Biol 5:269

Filiou MD, Turck CW, Martins-de-Souza D (2011) Quantitative proteomics for investigating psychiatric disorders. Proteomics Clin Appl 5(1–2):38–49

Flory MR, Griffin TJ, Martin D, Aebersold R (2002) Advances in quantitative proteomics using stable isotope tags. Trends Biotechnol 20(12 Suppl):S23–S29

Föcking M, Lopez LM, English JA, Dicker P, Wolff A, Brindley E, Wynne K, Cagney G, Cotter DR (2015) Proteomic and genomic evidence implicates the postsynaptic density in schizophrenia. Mol Psychiatry 20 (4):424–432

Frantzi M, Bhat A, Latosinska A (2014) Clinical proteomic biomarkers: relevant issues on study design and technical considerations in biomarker development. Clin Transl Med 3(1):7

Gerber SA, Rush J, Stemman O, Kirschner MW, Gygi SP (2003) Absolute quantification of proteins and phosphoproteins from cell lysates by tandem MS. Proc Natl Acad Sci U S A 100(12):6940–6845

Gnanapavan S, Grant D, Illes-Toth E, Lakdawala N, Keir G, Giovannoni G (2010) Neural cell adhesion molecule–description of a CSF ELISA method and evidence of reduced levels in selected neurological disorders. J Neuroimmunol 225(1–2):118–122

Grabrucker AM, Schmeisser MJ, Schoen M, Boeckers TM (2011) Postsynaptic ProSAP/Shank scaffolds in the cross-hair of synaptopathies. Trends Cell Biol 21 (10):594–603

Gramolini AO, Peterman SM, Kislinger T (2008) Mass spectrometry-based proteomics: a useful tool for biomarker discovery? Clin Pharmacol Ther 83 (5):758–760

Grant SG (2012) Synaptopathies: diseases of the synaptome. Curr Opin Neurobiol 22(3):522–529

Grutzendler J, Kasthuri N, Gan WB (2002) Long-term dendritic spine stability in the adult cortex. Nature 420(6917):812–816

Harrington MG, Fonteh AN, Oborina E, Liao P, Cowan RP, McComb G, Chavez JN, Rush J, Biringer RG, Hühmer AF (2009) The morphology and biochemistry of nanostructures provide evidence for synthesis and

signaling functions in human cerebrospinal fluid. Cerebrospinal Fluid Res 6:10
Hayaishi O (1991) Molecular mechanisms of sleep-wake regulation: roles of prostaglandins D2 and E2. FASEB J 5(11):2575–2581
Hong Z, Shi M, Chung KA, Quinn JF, Peskind ER, Galasko D, Jankovic J, Zabetian CP, Leverenz JB, Baird G, Montine TJ, Hancock AM, Hwang H, Pan C, Bradner J, Kang UJ, Jensen PH, Zhang J (2010) DJ-1 and alpha-synuclein in human cerebrospinal fluid as biomarkers of Parkinson's disease. Brain 133:713–726
Huang TL, Sung ML, Chen TY (2014) 2D-DIGE proteome analysis on the platelet proteins of patients with major depression. Proteome Sci 12(1):1
Hulstaert F, Blennow K, Ivanoiu A, Schoonderwaldt HC, Riemenschneider M, De Deyn PP, Bancher C, Cras P, Wiltfang J, Mehta PD, Iqbal K, Pottel H, Vanmechelen E, Vanderstichele H (1999) Improved discrimination of AD patients using beta-amyloid (1-42) and tau levels in CSF. Neurology 52 (8):1555–1562
Ising M, Lucae S, Binder EB, Bettecken T, Uhr M, Ripke S, Kohli MA, Hennings JM, Horstmann S, Kloiber S, Menke A, Bondy B, Rupprecht R, Domschke K, Baune BT, Arolt V, Rush AJ, Holsboer F, Müller-Myhsok B (2009) A genomewide association study points to multiple loci that predict antidepressant drug treatment outcome in depression. Arch Gen Psychiatry 66(9):966–975
Ito S, Narumiya S, Hayaishi O (1989) Prostaglandin D2: a biochemical perspective. Prostaglandins Leukot Essent Fatty Acids 37(4):219–234
Kingsmore SF (2006) Multiplexed protein measurement: technologies and applications of protein and antibody arrays. Nat Rev Drug Discov 5(4):310–321
Köhler K, Seitz H (2012) Validation processes of protein biomarkers in serum – a cross platform comparison. Sensors 12(9):12710–12728
Lange V, Picotti P, Domon B, Aebersold R (2008) Selected reaction monitoring for quantitative proteomics: a tutorial. Mol Syst Biol 4:222
Latterich M, Schnitzer JE (2011) Streamlining biomarker discovery. Nat Biotechnol 29(7):600–6002
Levin Y, Wang L, Schwarz E, Koethe D, Leweke FM, Bahn S (2010) Global proteomic profiling reveals altered proteomic signature in schizophrenia serum. Mol Psychiatry 15(11):1088–1100
Lottspeich F, Kellermann J, Keidel EM (2010) Molecular biology tools: proteomics techniques in biomarker discovery. Scand J Clin Lab Invest Suppl 242:19–22
Martins-de-Souza D (2011) Proteomics as a tool for understanding schizophrenia. Clin Psychopharmacol Neurosci 9(3):95–101
Martins-de-Souza D (2012) Proteomics tackling schizophrenia as a pathway disorder. Schizophr Bull 38 (6):1107–1108
Martins-de-Souza D (2013) Biomarkers for psychiatric disorders: where are we standing? Dis Markers 35 (1):1–2
Martins-de-Souza D, Gattaz WF, Schmitt A, Rewerts C, Marangoni S, Novello JC, Maccarrone G, Turck CW, Dias-Neto EJ (2009) Alterations in oligodendrocyte proteins, calcium homeostasis and new potential markers in schizophrenia anterior temporal lobe are revealed by shotgun proteome analysis. Neural Transm (Vienna) 116(3):275–289
Martins-de-Souza D, Harris LW, Guest PC, Bahn S (2011) The role of energy metabolism dysfunction and oxidative stress in schizophrenia revealed by proteomics. Antioxid Redox Signal 15(7):2067–2079
Masliah E, Mallory M, Alford M, DeTeresa R, Hansen LA, McKeel DW, Jr, Morris JC (2001) Altered expression of synaptic proteins occurs early during progression of Alzheimer's disease. Neurology 56:127–129
Michalski A, Cox J, Mann M (2011) More than 100,000 detectable peptide species elute in single shotgun proteomics runs but the majority is inaccessible to data dependent LCMS/ MS. J Proteome Res 10 (4):1785–1793
Mollenhauer B, Locascio JJ, Schulz-Schaeffer W, Sixel-Döring F, Trenkwalder C, Schlossmacher MG (2011) Alpha-synuclein and tau concentrations in cerebrospinal fluid of patients presenting with parkinsonism: a cohort study. Lancet Neurol 10:230–240
Nascimento JM, Martins-de-Souza D (2015) The proteome of schizophrenia. NPJ Schizophr 1:14003
Ngounou Wetie AG, Wormwood K, Thome J, Dudley E, Taurines R, Gerlach M, Woods AG, Darie CC (2014) A pilot proteomic study of protein markers in autism spectrum disorder. Electrophoresis 35(14):2046–2054
Old WM, Meyer-Arendt K, Aveline-Wolf L, Pierce KG, Mendoza A, Sevinsky JR, Resing KA, Ahn NG (2005) Comparison of label-free methods for quantifying human proteins by shotgun proteomics. Mol Cell Proteomics 4(10):1487–1502
Ong SE, Mann M (2005) Mass spectrometry-based proteomics turns quantitative. Nat Chem Biol 1(5):252–262
Orton DJ, Doucette AA (2013) Proteomics workflows for biomarker identification using mass spectrometry – technical and statistical considerations during initial discovery. Proteomes 1:109–127
Overk CR, Masliah E (2014) Pathogenesis of synaptic degeneration in Alzheimer's disease and Lewy body disease. Biochem Pharmacol 88(4):508–516
Pan S, Aebersold R, Chen R, Rush J, Goodlett DR, McIntosh MW, Zhang J, Brentnall TA (2009) Mass spectrometry based targeted protein quantification: methods and applications. J Proteome Res 8 (2):787–797
Parnetti L, Lanari A, Silvestrelli G, Saggese E, Reboldi P (2006) Diagnosing prodromal Alzheimer's disease: role of CSF biochemical markers. Mech Ageing Dev 127(2):129–132

Perret-Liaudet A, Pelpel M, Tholance Y, Dumont B, Vanderstichele H, Zorzi W, ElMoualij B, Schraen S, Moreaud O, Gabelle A, Thouvenot E, Thomas-Anterion C, Touchon J, Krolak-Salmon P, Kovacs GG, Coudreuse A, Quadrio I, Lehmann S (2012) Cerebrospinal fluid collection tubes: a critical issue for Alzheimer disease diagnosis. Clin Chem 58 (4):787–789

Petzold A, Thompson EJ, Keir G, Quinn N, Holmberg B, Dizdar N, Wenning GK, Rascol O, Tolosa E, Rosengren L (2009) Longitudinal one-year study of levels and stoichiometry of neurofilament heavy and light chain concentrations in CSF in patients with multiple system atrophy. J Neurol Sci 279 (1–2):767–769

Picotti P, Aebersold R (2012) Selected reaction monitoring-based proteomics: workflows, potential, pitfalls and future directions. Nat Methods 9 (6):555–566

Pijnenburg YA, Janssen JC, Schoonenboom NS, Petzold A, Mulder C, Stigbrand T, Norgren N, Heijst H, Hack CE, Scheltens P, Teunissen CE (2007) CSF neurofilaments in frontotemporal dementia compared with early onset Alzheimer's disease and controls. Dement Geriatr Cogn Disord 23 (4):225–230

Portelius E, Bogdanovic N, Gustavsson MK, Volkmann I, Brinkmalm G, Zetterberg H, Winblad B, Blennow K (2010) Mass spectrometric characterization of brain amyloid beta isoform signatures in familial and sporadic Alzheimer's disease. Acta Neuropathol 120 (2):185–193

Riedel BC, Thompson PM, Brinton RD (2016) Age, APOE and sex: triad of risk of Alzheimer's disease. J Steroid Biochem Mol Biol 160:134–147

Rifai N, Gillette MA, Carr SA (2006) Protein biomarker discovery and validation: the long and uncertain path to clinical utility. Nat Biotechnol 24(8):971–983

Robards K, Haddad PR, Jackson PE (2004) Principles and practice of modern chromatographic methods. Academic Press, London

Rodriguez-Murillo L, Gogos JA, Karayiorgou M (2012) The genetic architecture of schizophrenia: new mutations and emerging paradigms. Annu Rev Med 63:63–68

Schulze WX, Usadel B (2010) Quantitation in mass spectrometry- based proteomics. Ann Rev Plant Biol 61:491–516

Sheng M, Kim E (2011) The postsynaptic organization of synapses. Cold Spring Harb Perspect Biol 3(12). https://doi.org/10.1101/cshperspect.a005678

Shevell M, Ashwal S, Donley D, Flint J, Gingold M, Hirtz D, Majnemer A, Noetzel M, Sheth RD, Quality Standards Subcommittee of the American Academy of Neurology; Practice Committee of the Child Neurology Society (2003) Practice parameter: evaluation of the child with global developmental delay: report of the quality standards Subcommittee of the American Academy of neurology and the practice committee of the child neurology society. Neurology 60(3):367–380

Shi M, Movius J, Dator R, Aro P, Zhao Y, Pan C, Lin X, Bammler TK, Stewart T, Zabetian CP, Peskind ER, S-C H, Quinn JF, Galasko DR, Zhang J (2015) Cerebrospinal fluid peptides as potential Parkinson disease biomarkers: a staged pipeline for discovery and validation. Mol Cell Proteomics 14(3):544–555

Sollner T, Whiteheart SW, Brunner M, Erdjument-Bromage H, Geromanos S, Tempst P, Rothman JE (1993) SNAP receptors implicated in vesicle targeting and fusion. Nature 362:318–324

Sri Kantha S, Matsumura H, Kubo E, Kawase K, Takahata R, Serhan CN, Hayaishi O (1994) Effects of prostaglandin D2, lipoxins and leukotrienes on sleep and brain temperature of rats. Prostaglandins Leukot Essent Fatty Acids 51(2):87–93

Südhof TC (2013) Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. Neuron 80 (3):675–690

Surinova S, Schiess R, Huttenhain R, Cerciello F, Wollscheid B, Aebersold R (2011) On the development of plasma protein biomarkers. J Proteome Res 10 (1):5–16

Takeda M, Martínez R, Kudo T, Tanaka T, Okochi M, Tagami S, Morihara T, Hashimoto R, Cacabelos R (2010) Apolipoprotein E and central nervous system disorders: reviews of clinical findings. Psychiatry Clin Neurosci 64(6):592–607

Taurines R, Dudley E, Grassl J, Warnke A, Gerlach M, Coogan AN, Thome J (2011) Proteomic research in psychiatry. J Psychopharmacol 25:151–196

Terry RD, Masliah E, Salmon DP, Butters N, De Teresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572–580

Thomas B, Beal MF (2007) Parkinson's disease. Hum Mol Genet 16(2):183–194

Thouvenot E, Urbach S, Dantec C, Poncet J, Séveno M, Demettre E, Jouin P, Touchon J, Bockaert J, Marin P (2008) Enhanced detection of CNS cell secretome in plasma protein-depleted cerebrospinal fluid. J Proteome Res 7(10):4409–4421

Tolosa E, Wenning G, Poewe W (2006) The diagnosis of Parkinson's disease. Lancet Neurol 5:75–86

Tombran-Tink J, Barnstable CJ (2003) PEDF: a multifaceted neurotrophic factor. Nat Rev Neurosci 4 (8):628–636

Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature 420(6917):788–794

Urade Y, Hayaishi O, Matsumura H, Watanabe K (1996) Molecular mechanism of sleep regulation by prostaglandin D2. J Lipid Mediat Cell Signal 14(1–3):71–82

Vawter MP (2000) Dysregulation of the neural cell adhesion molecule and neuropsychiatric disorders. Eur J Pharmacol 405(1–3):385–395

Végvári Á, Marko-Varga G (2010) Clinical protein science and bioanalytical mass spectrometry with an emphasis on lung cancer. Chem Rev 110:3278–3298

Végvári Á, Rezeli M, Döme B, Fehniger TE, Marko-Varga G (2011) Translation science for targeted personalized medicine treatments. In: Selected presentations from the 2011 Sino-American symposium on clinical and translational medicine. Science/AAAS, Washington, DC

Vila-Rodriguez F, Honer WG, Innis SM, Wellington CL, Beasley CL (2011) ApoE and cholesterol in schizophrenia and bipolar disorder: comparison of grey and white matter and relation with APOE genotype. J Psychiatry Neurosci 36(1):47-55

Wang M, You J, Bemis KG, Tegeler TJ, Brown DP (2008) Label-free mass spectrometry-based protein quantification technologies in proteomics analysis. Brief Funct Genomic Proteomic 7(5):329–339

Wang-Dietrich L, Funke SA, Kühbach K, Wang K, Besmehn A, Willbold S, Cinar Y, Bannach O, Birkmann E, Willbold D (2013) The amyloid-β oligomer count in cerebrospinal fluid is a biomarker for Alzheimer's disease. J Alzheimers Dis 34(4):985–994

Whiteaker JR, Lin C, Kennedy J, Hou L, Trute M, Sokal I, Yan P, Schoenherr RM, Zhao L, Voytovich UJ, Kelly-Spratt KS, Krasnoselsky A, Gafken PR, Hogan JM, Jones LA, Wang P, Amon L, Chodosh LA, Nelson PS, McIntosh MW, Kemp CJ, Paulovich AG (2011) A targeted proteomics-based pipeline for verification of biomarkers in plasma. Nat Biotechnol 29:625–634

Wilkins MR, Pasquali C, Appel RD, Ou K (1996) From proteins to proteomes: large scale protein identification by two-dimensional electrophoresis and amino acid analysis. Biotechnology (NY) 14:61–65

Woods AG, Sokolowska I, Taurines R, Gerlach M, Dudley E, Thome J, Darie CC (2012) Potential biomarkers in psychiatry: focus on the cholesterol system. J Cell Mol Med 16(6):1184–1195

Yabe T, Kanemitsu K, Sanagi T, Schwartz JP, Yamada H (2005) Pigment epithelium-derived factor induces pro-survival genes through cyclic AMP-responsive element binding protein and nuclear factor kappa B activation in rat cultured cerebellar granule cells: implication for its neuroprotective effect. Neuroscience 133(3):691–700

Yabe T, Sanagi T, Yamada H (2010) The neuroprotective role of PEDF: implication for the therapy of neurological disorders. Curr Mol Med 10(3):259–266

Yates JR, Ruse CI, Nakorchevsky A (2009) Proteomics by mass spectrometry: approaches, advances, and applications. Annu Rev Biomed Eng 11:49–79

Yuan X, Russell T, Wood G, Desiderio DM (2002) Analysis of the human lumbar cerebrospinal fluid proteome. Electrophoresis 23(7–8):1185–1196

Zhang X, Yin X, Yu H, Liu X, Yang F, Yao J, Jin H, Yang P (2012) Quantitative proteomic analysis of serum proteins in patients with Parkinson's disease using an isobaric tag for relative and absolute quantification labeling, two-dimensional liquid chromatography, and tandem mass spectrometry. Analyst 137 (2):490–495

Zhu W, Smith JW, Huang CM (2010) Mass spectrometry based label-free quantitative proteomics. J Biomed Biotechnol 2010:840518

Zolg JW, Langen H (2004) How industry is approaching the search for new diagnostic markers and biomarkers. Mol Cell Proteomics 3:345–354

Zougman A, Pilch B, Podtelejnikov A, Kiehntof M, Schnabel C, Kumar C (2008) Integrated analysis of the cerebrospinal fluid peptidome and proteome. J Proteome Res 7:386–339

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 101–112
DOI 10.1007/5584_2016_165

Published online: 14 October 2016

# Expression of Inflammatory Mediators in Induced Sputum: Comparative Study in Asthma and COPD

Magdalena Paplińska-Goryca, Patrycja Nejman-Gryz, Katarzyna Górska, Katarzyna Białek-Gosk, Joanna Hermanowicz-Salamon, and Rafał Krenke

**Abstract**

Asthma and COPD are the most common obstructive lung diseases characterized by inflammation in the lower airways which contribute to airflow limitation. Different inflammatory mediators are thought to play a key role in these diseases. This study was conducted in 13 patients with asthma, 12 patients with COPD, and 13 control subjects. The expression of mRNA of IL-6, IL-13, CXCL8, TSLP, IL-33, IL-25, IL-17, ECP, mast cell tryptase, CCL24, and CCL26 was assessed in induced sputum cells by real time PCR. We found that CXCL8 was strongly related to the neutrophil percentage but differed significantly in COPD and asthma patients. The expression of IL-17 was lower in patients with atopic asthma compared to non-atopic asthma. The percentage of macrophages correlated negatively with the expression of mast cell tryptase and ECP in COPD, and with CXCL8 in asthma. The expression of ECP correlated negatively with the severity of COPD symptoms measured by CAT. We conclude that asthma and COPD demonstrate a significant overlap in the airway cytokine profile. Thus, differentiation between the two diseases is difficult as based on a single cytokine, which suggests the coexistence of phenotypes sharing a common cytokine network in these obstructive lung diseases.

**Keywords**

Airway • Asthma • COPD • Eosinophils • CXCL8 • IL-17 • IL-6 • Inflammation • Mediators • Neutrophils • Respiration

## 1 Introduction

Asthma and COPD are complex airway diseases that share some common features including clinical presentation and pulmonary function impairment. Although both diseases are associated with chronic airway inflammation, they differ in terms of the inflammatory cells and mediators involved. The immunopathology of asthma is characterized by peribronchial infiltration of eosinophils, macrophages, and

The original version of this chapter has been revised. An erratum to this chapter can be found at http://dx.doi.org/10.1007/5584_2016_188

M. Paplińska-Goryca (✉), P. Nejman-Gryz, K. Górska, K. Białek-Gosk, J. Hermanowicz-Salamon, and R. Krenke
Department of Internal Medicine, Pneumology and Allergology, Warsaw Medical University, 1A Banacha Street, 02-097 Warsaw, Poland
e-mail: mpaplinska@wum.edu.pl

T lymphocytes. Eosinophils are thought the major inflammatory cells in asthma but the role of mast cells and T-helper cells is also well established (Bradding 2008; Moqbel and Odemuyiwa 2008). The influx of neutrophils into the airways observed in severe asthma may be a predictor of steroid resistance (Macedo et al. 2009). Neutrophils also are the most abundant inflammatory cells present in the bronchial wall and lumen of COPD patients. Increased airway neutrophilia correlates with lung function decline, and characterize acute exacerbations of COPD (Singh et al. 2010; Fujimoto et al. 2005).

As the spectrum of clinical presentations and other features of asthma and COPD is wide, different phenotypes have been defined in order to better characterize both diseases, their mechanisms, and the effect of different therapies. The type of airway inflammation defined by the predominant inflammatory cells is one of the most important factor defining asthma phenotypes. Even though the major COPD phenotypes have been defined by clinical and radiological presentations, features of airway inflammation and remodeling may also be used to cluster the disease (Górska et al. 2010; Han et al. 2010).

Identification of biological markers reflecting the underlying disease process also seems to be important. Parallel to the differences in the cellular composition of the inflammatory infiltrates in asthma and COPD, different mediators are thought to play the main role in both diseases. In patients with COPD, markers of systemic inflammation can be measured in peripheral blood but these markers do not seem to sufficiently reflect the inflammatory processes within the airways (Röpcke et al. 2012). Thus, a reliable assessment of local airway inflammation in asthma and COPD must be based on biological samples taken from the site of ongoing inflammation. Recently evaluated mediators of asthma include interleukin 25 (IL-25), IL-33, thymic stromal lymphopoietin (TSLP), and periostin (Bobolea et al. 2015; Byers 2014), while neutrophil associated proteins, including neutrophil elastase and matrix metalloproteinases in sputum have been reported as potential markers of airway remodeling in COPD (Paone et al. 2011; Vernooy et al. 2004). However, there are as yet no established and recommended biomarkers that would reliably reflect the type and severity of airway inflammation (Koutsokera et al. 2013).

Induced sputum (IS) has been extensively studied as a non-invasive sampling method useful in the evaluation of the cellular pattern and the concentration of different inflammatory mediators. However, protein levels of various inflammatory mediators measured by commercially available kits are often undetectable in IS supernatant, being below test sensitivity. The use of dithiothreitol for IS processing can further decrease the concentration of many proteins. The assessment of mRNA expression may be a more sensitive method for mediator evaluation in IS (Gelder et al. 1995). Some data show that mRNA expression may better reflect airway hyperresponsiveness and airflow limitation than sputum eosinophilia (Jung et al. 2014). IL-4, IL-5, and IL-13 mRNA expression in IS cells can be used to classify asthma into $Th_2$-high and $Th_2$-low endotypes (Peters et al. 2014).

Based on the data presented above we undertook a study aimed to: 1/compare the mRNA expression of different inflammatory proteins IL-6, IL-13, chemokine (C-X-C motif) ligand 8 (CXCL8), thymic stromal lymphopoietin, IL-33, IL-25, IL-17, eosinophil cationic protein (ECP), mast cell tryptase, eotaxin-2 (CCL24), eotaxin-3 (CCL26) in cells obtained from IS of asthma and COPD patients and 2/characterize the relationship between the expression of mRNA of different mediators and sputum cellular phenotypes of asthma and COPD.

## 2 Methods

### 2.1 Characteristics of Patients

The study protocol was approved by the Ethics Committee of the Medical University of Warsaw (KB/275/2012) and informed written consent was obtained from all the participants. The study involved 13 patients with asthma, 12 patients with COPD, and 13 control subjects. All asthma and COPD patients were stable and free from exacerbations for at least 8 weeks

before the study onset. None of patients or control subjects have respiratory infections for at least one month preceding the enrollment.

Asthma was diagnosed according to the GINA recommendations (GINA 2015). Atopic status was assessed by skin prick testing with a panel of fifteen aeroallergens. Asthma control test (ACT) was performed in all asthma patients. Short-acting β2-agonists (SABA) on demand was the only treatment in eight patients, one patient was treated only with long acting β2-agonists (LABA). Two patients received low doses of inhaled corticosteroids (ICS) and LABA, while two others received moderate doses of ICS and LABA.

COPD was diagnosed according to the GOLD recommendations (GOLD 2015). COPD assessment test (CAT) and modified Medical Research Council (mMRC) Questionnaire were used to measure severity of symptoms. Two COPD patients were treated with a low dose of ICS plus LABA, five patients with LABA and long-acting muscarinic antagonists (LAMA), four patients received LABA and short-acting muscarinic antagonists (SAMA) one used LABA only.

The control group consisted of healthy non-smoking subjects, with normal results of spirometry.

## 2.2 Sputum Induction

Sputum induction was preceded by premedication with 400 μg of inhaled salbutamol. After postbronchodilator spirometry the patients inhaled sterile hypertonic saline (NaCl) at increasing concentrations (3 %, 4 %, and 5 % solutions, 10 min per inhalation) *via* an ultrasonic nebulizer (ULTRA-NEB TM 2000, DeVilbiss, USA). After each inhalation, spirometry was performed to detect a potential forced expiratory volume in 1 s ($FEV_1$) decrease. The procedure was stopped when a significant ($\geq$20 %) $FEV_1$ decline as compared to postbronchodilator baseline value was noted (Chmielowicz et al. 2008).

Induced sputum was processed immediately on receipt as described by other authors (Hargreave et al. 1998). The volume of sputum was measured and the plugs were separated and weighed. A freshly prepared 0.1 % solution of dithiothreitol (Sigma Aldrich; St. Louis, USA) was added in a volume equal to four the weight of the sputum, and the mixture was shaken for 15 min. Subsequently, a double volume of phosphate-buffered saline (PBS) was added and the mixture was vortexed briefly. After filtration through two layers of a sterile gauze, sputum was centrifuged for 10 min at 1800 *g*. The cells were counted, and the percentage of dead and epithelial cells was assessed. The pellet of cells was suspended in RNAlater solution (Qiagen; Valencia, USA) and stored at −80 ° C for further investigation. The smears were stained with MG-Giemsa staining. The criteria for appropriate IS quality were as follows: a minimum of 2 ml of expectoration; less than 50 % squamous epithelial cells, and more than 300 non-epithelial cells on one slide.

## 2.3 RNA Isolation and cDNA Synthesis

Total RNA was isolated using Nucleo Spin RNA II Columns Kit (Machery & Nagel; Düren Germany). The concentration and purity of isolated RNA was measured on a DU650 spectrophotometer (Beckman Coulter, Brea, USA). Eight microliters of total RNA was used for reverse transcription using SuperScript III First-Strand Synthesis Super Mix for qRT-PCR (Invitrogen, Carlsbad, USA).

## 2.4 Real-Time Quantitative PCR

For real-time PCR, 0.8 μl of cDNA was amplified in 16 μl PCR volume, containing a Power SYBR Green PCR mastermix (Applied Biosystems, Foster City, CA) with 150 nM of specific primers. Sequences of the applied primers are shown in Table 1. PCR protocol consisted of one cycle at 95 °C for 10 min followed by 40 cycles at 95 °C for 15 s, and 60 °C for 1 min. Each sample was measured in duplicate. 18S rRNA was applied for each sample as an internal control in order to normalize gene expression levels. The results were expressed as relative quantification (RQ) units (fold change). The PCR evaluation was performed with an ABI-Prism 7500 Sequence Detector System (Applied Biosystems, Foster City, CA).

**Table 1** Sequence of primers used in PCR

| | Forward primer | Reverse primer | Product size |
|---|---|---|---|
| 18 s rRNA | GGATGAGGTGGAACGTGTGAT | AGGTCTTCACGGAGCTTGTTG | 148 |
| IL-6 | CCGGGAACGAAAGAGAAGCT | GCGCTTGTGGAGAAGGAGTT | 67 |
| IL-13 | TCAACATCACCCAGAACCAGAA | AGCTGTCAGGTTGATGCTCCATA | 70 |
| IL-8 | GAGCACTCCATAAGGCACAAACT | ATCAGGAAGGCTGCCAAGAG | 149 |
| ECP | TTTGCCATCCAGCACATCA | TTAATTGCCCGCATTGCA | 61 |
| Mast cell tryptase | CACCGCCATTTCCTCTGAAG | GCGTCACAAATGTGGTTTTCC | 60 |
| IL-17 | AGGAATCACAATCCCACGAAAT | GGTGAGGTGGATCGGTTGTAGT | 149 |
| IL-25 | TGGTCCCTTTTTGGGAAACC | TGTGCAGAAGTGCAGGCTTT | 153 |
| IL-33 | GCCTAGATGAGACACCGAATTAACA | CCAGGGTCAGAAGGGATGGT | 85 |
| TSLP | TCTTGAATTCCCGCTGCAA | CCACTGGTGTTTATAGGGTTCTGA | 78 |
| CCL24 | CACATCATCCCTACGGGCTCT | GGTTGCCAGGATATCTCTGGACAGGG | 88 |
| CCL26 | GGAACTGCCACACGTGGGAGTGAC | CTCTGGGAGGAAACACCCTCTCC | 354 |

*IL* interleukin, *ECP* eosinophil cationic protein, *CCL24* eotaxin-2, *CCL26* eotaxin-3, *TSLP* thymic stromal lymphopoietin

Relative quantification values were calculated by the $2^{-\Delta\Delta CT}$ method. The cycle threshold (CT) for the target amplicon and CT for endogenous control (18S rRNA) were determined for each sample. Differences were calculated between these two CTs and called ΔCT, in order to account for the difference in the amount of total nucleic acid added to each reaction. The values of ΔCT for cDNA from sputum cells of healthy subjects (calibrator) were subtracted from the ΔCT of each sample and termed ΔΔCT. The target normalized to endogenous control ratio, relative to the calibrator, was then calculated by the formula $2^{\Delta\Delta CT}$. The calculation was made, CT of the cDNA of sputum cells from patients with asthma or COPD was calibrated with the average CT of sputum cells from healthy volunteers.

## 2.5 Statistical Analysis

Results are given as medians and range of values. Differences between continuous variables were tested using the nonparametric Mann-Whitney U or Kruskal-Wallis test. Correlations between variables were analyzed with Spearman's rank test. Differences were considered statistically significant at $p < 0.05$. Statistical analysis was performed using Statistica 9.0 software (StatSoft Inc., Tulsa, OK).

# 3 Results

Baseline patients' characteristics are presented in Table 2. In the asthma group, 8, 3, and 2 patients had mild, moderate, and severe disease, respectively. The degree of disease control was as follows: well-controlled asthma – 7 patients, partly controlled asthma – 4 patients, and uncontrolled asthma – 2 patients. The median value of ACT was 20 (range 11–25) points.

The number of COPD patients classified as GOLD A, GOLD B, and GOLD D category was 4, 6, and 2 respectively. The use of spirometric classification of COPD severity based on post-bronchodilator $FEV_1$ value revealed 11 patients with moderate (GOLD 2) and one patient with very severe (GOLD 4) disease (Rabe et al. 2007). The median value of CAT was 15.5 (range 4–23) points.

Patients with COPD were significantly older and had a significantly greater tobacco smoke exposure than healthy volunteers and asthma patients. Both asthma and COPD patients had impaired lung function (lower $FEV_1$/FVC and $FEV_1$% predicted) as compared to controls. Significantly greater impairment of respiratory function (lower $FEV_1$% predicted) was demonstrated in COPD than in asthma patients.

The cellular composition of IS in asthmatics, controls, and COPD patients is shown in Table 3. The percentage of lymphocytes and epithelial

**Table 2** Demographic and clinical data of patients with asthma, COPD, and healthy controls

| | Asthma | COPD | Controls | p |
|---|---|---|---|---|
| Gender (male/female) | 1/12 | 7/5 | 7/6 | NA |
| Atopy (n) | 8 | 3 | 0 | NA |
| Age (yr) | 53 (22–75) | 70 (59–82) | 42 (31–64) | $0.0030^{\#\diamond}$ |
| Smoking (pack-years) | 0 (0–10) | 55 (14–100) | 0 (0–20) | $0.0001^{\#\diamond}$ |
| $FEV_1$ (L/s) | 2.9 (1.1–3.1) | 1.5 (0.5–1.8) | 3.9 (2.0–5.1) | 0.0001* |
| $FEV_1$ %pred. | 88 (45–121) | 51 (21–78) | 106 (89–125) | $0.0001^{*\#}$ |
| FVC (L) | 4.1 (2.4–4.5) | 2.7 (1.5–3.8) | 4.6 (2.8–7.0) | $0.0001^{*\diamond}$ |
| FVC %pred. | 105 (83–139) | 93 (45–124) | 113 (84–132) | $0.0082^{\diamond}$ |
| $FEV_1$/FVC (%) | 69 (46–84) | 45 (36–61) | 81 (70–96) | $0.0001^{*\#\diamond}$ |

*COPD* chronic obstructive pulmonary disease, *$FEV_1$* forced expiratory volume in one second, *FVC* forced vital capacity, *NA* non-applicable

Data are medians (range); $p < 0.05$, significant differences for paired comparisons (Kruskal-Wallis test): $^{\#}$asthma *vs.* COPD, *asthma *vs.* control, $^{\diamond}$COPD *vs.* control

**Table 3** Percentage of epithelial and inflammatory cells in induced sputum (IS) of asthmatics, COPD patients, and controls

| | Asthma | COPD | Controls | p |
|---|---|---|---|---|
| Epithelial cells | 22 (8–48) | 14 (4–37) | 13 (7–45) | 0.45 |
| Macrophages | 27.5 (14–65) | 12 (0–34) | 33 (7–61) | $0.07^{\diamond}$ |
| Lymphocytes | 5.5 (1–13) | 8 (1–16) | 6 (1–15) | 0.22 |
| Neutrophils | 52.5 (17–78) | 74 (54–91) | 55 (30–90) | $0.03^{\#\diamond}$ |
| Eosinophils | 3.5 (1–28) | 3 (0–10) | 1 (0–1) | $0.001^{*\diamond}$ |

The sum of all non-epithelial cells was taken as 100 %

Data are presented as medians (range); $p < 0.05$, significant differences for paired comparisons (Kruskal-Wallis test): *asthma *vs.* control, $^{\#}$asthma *vs.* COPD, $^{\diamond}$COPD *vs.* control

cells was similar in all groups. However, the percentage of macrophages and neutrophils was significantly higher in COPD patients than those in controls and asthmatics. The highest and lowest percentages of eosinophils were found in the asthma patients and controls, respectively.

Of all the measured gene expressions only CXCL8 differed significantly between COPD and asthma patients: 3.9 (range 0.2–54.5) *vs.* 0.8 (range 0.2–162.2) (RQ units), respectively (Fig. 1). The analysis of asthma and COPD patients subclassified according to the IS eosinophil and neutrophil percentage showed a significantly elevated CXCL8 expression in asthmatics with neutrophilia (≥60 %) as compared with the remaining asthma patients [1.3 (range 0.8–7.9) *vs.* 0.4 (range 0.2–162.2) (RQ units), $p < 0.045$]. We observed increased $FEV_1$/FVC [74 (range 68–84) *vs.* 67 (range 46–88) (%), $p < 0.045$] in neutrophilic as compared to non-neutrophilic asthmatics (Table 4). No other significant differences in pulmonary function parameters in the asthma and COPD groups classified according the eosinophil/neutrophil phenotype of sputum were found. CXCL8 expression in IS was insignificantly lower in COPD patients with high percentage of eosinophils (≥3 %) than in COPD patients with a lower eosinophil percentage (<3 %) [2.2 (range 0.2–13.5) *vs.* 9.4 (range 3.0–54.5) (RQ units), $p = 0.07$].

The analyses within asthma and COPD groups showed some relationships between mRNA expression and patients' characteristics. In the non-atopic asthmatics, IL-17 expression was significantly higher [1.4 (range 0.6–13.0) (RQ units)] than in the atopic ones [0.5 (range 0.2–1.4) (RQ units), $p = 0.05$] (Fig. 2). In COPD, CAT score correlated significantly with ECP expression ($r = -0.69$, $p = 0.01$) (Fig. 3). None of the evaluated mediators correlated with age either in asthma or COPD.

The percentage of macrophages correlated negatively with the expression of almost all evaluated mediators, except for IL-33 and CCL26 in asthma, IL-6 and IL-13 in COPD, and CCL24 in both diseases. This correlation

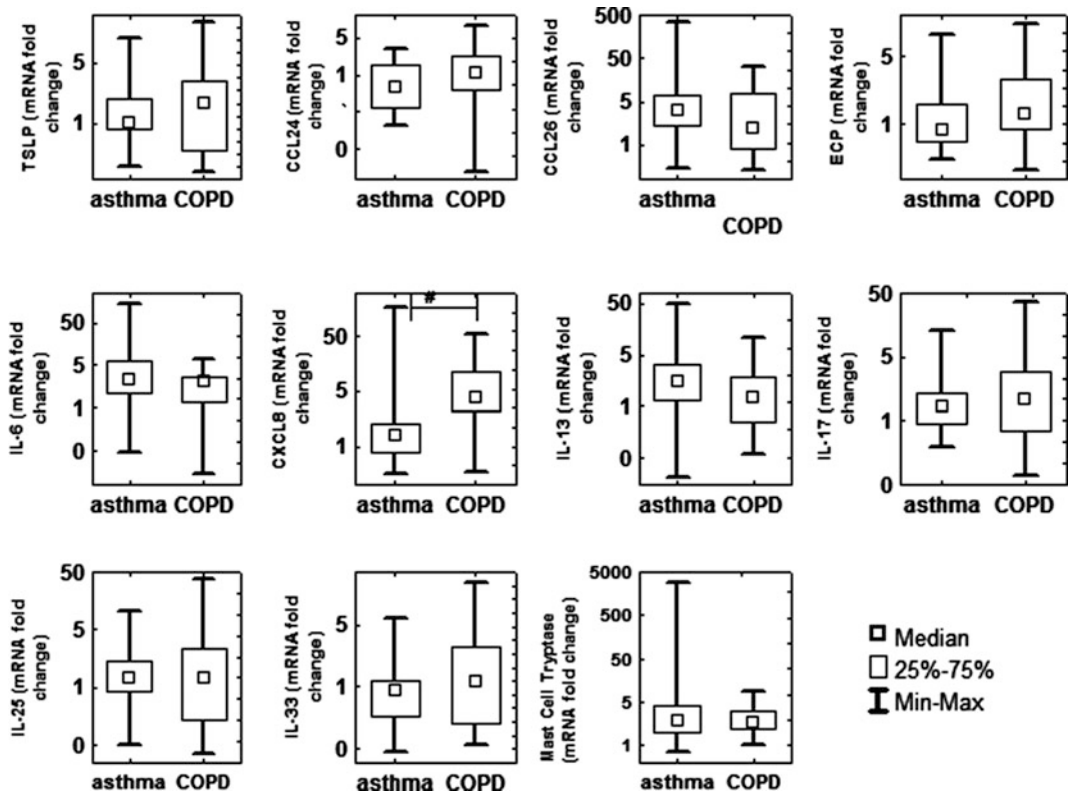



**Fig. 1** Box plots of expression of CCL24, CCL26, ECP, IL-13, IL-25, IL-33, TSLP, mast cell tryptase, IL-17, IL-6, and CXCL8 in induced sputum of asthma and COPD patients (logarithmic scale). $^{\#}p < 0.05$ (Mann-Whitney *U* test)

**Table 4** Expression of chemokine (C-X-C motif) ligand 8 (CXCL8) in induced sputum (IS) and spirometry in asthma and COPD patients stratified according to eosinophil or neutrophil predominance

| Asthma | Non-eosinophilia *vs.* eosinophilia | | | Non-neutrophilia *vs.* neutrophilia | | |
|---|---|---|---|---|---|---|
| | <3 % eosinophils n = 6 | ≥3 % eosinophils n = 7 | p | <60 % neutrophils n = 5 | ≥60 % neutrophils n = 8 | p |
| CXCL8 (RQ) | 1.0 (0.4–4.2) | 0.4 (0.2–162.2) | 0.14 | 0.4 (0.2–162.2) | 1.3 (0.8–7.9) | 0.045* |
| $FEV_1$ % pred. | 82 (45–121) | 83 (65–93) | 0.95 | 77 (45–93) | 86 (69–121) | 0.280 |
| $FEV_1$/FVC (%) | 71 (46–84) | 69 (52–75) | 0.53 | 67 (46–75) | 74 (68–84) | 0.045* |
| COPD | Non-eosinophilia *vs.* eosinophilia | | | Non-neutrophilia *vs.* neutrophilia | | |
| | <3 % eosinophils n = 7 | ≥3 % eosinophils n = 5 | p | <60 % neutrophils n = 3 | ≥60 % neutrophils n = 9 | p |
| CXCL8 (RQ) | 9.4 (3.0–54.5) | 2.2 (0.2–13.5) | 0.07 | 2.2 (0.9–13.5) | 4.7 (0.2–54.5) | 0.73 |
| $FEV_1$ % pred. | 47 (21–78) | 54 (48–74) | 0.80 | 65 (58–74) | 50 (21–78) | 0.06 |
| $FEV_1$/FVC (%) | 42 (36–6) | 50 (41–55) | 0.58 | 51 (50–55) | 44 (36–61) | 0.21 |

Data are medians (range); *COPD* chronic obstructive pulmonary disease, *RQ* relative quantification units, *$FEV_1$* forced expiratory volume in one second, *FVC* forced vital capacity; *$p < 0.05$, significant differences between neutrophilia *vs.* non-neutrophilia in asthmatics (Mann–Whitney *U* test)

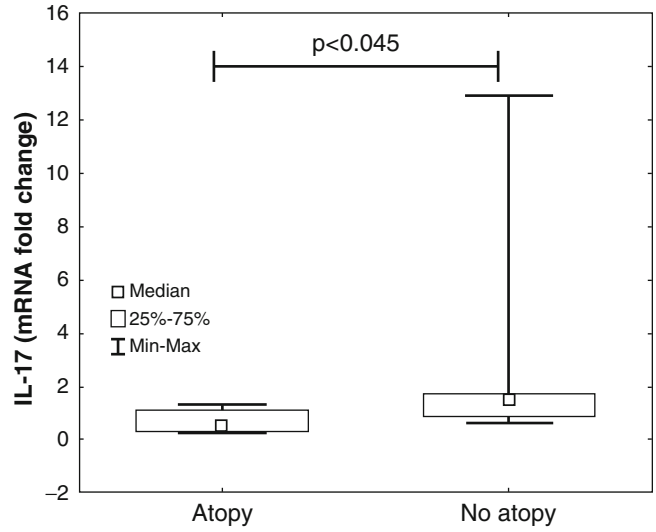



**Fig. 2** Interleukin (IL)-17 expression in induced sputum of asthma patients with and without atopy (Mann-Whitney *U* test)

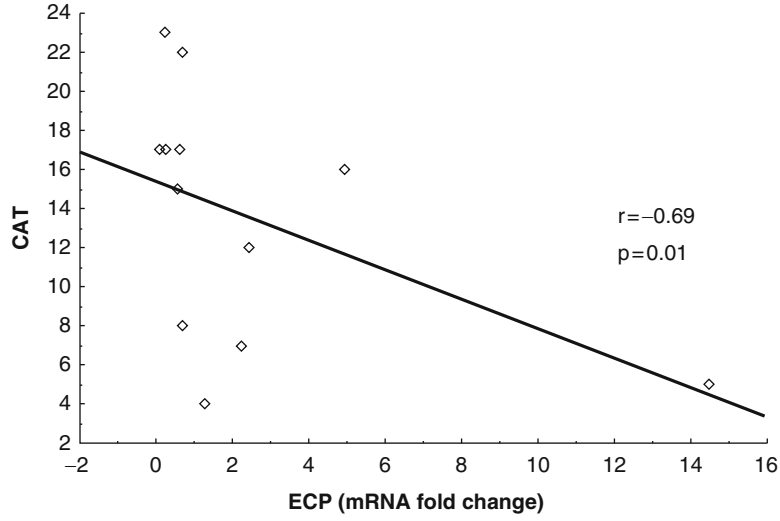



**Fig. 3** Correlations between eosinophil cationic protein (ECP) expression in induced sputum and disease assessment test (CAT) score in COPD patients (Spearman's rank correlation)

was significant in three cases only: mast cell tryptase in COPD ($r = -0.70$, $p = 0.01$), CXCL8 in asthma ($r = -0.72$, $p < 0.01$), and ECP in COPD ($r = -0.60$, $p = 0.04$) (Table 5).

## 4 Discussion

This study evaluated the expression of eleven different mediators in induced sputum cells from asthma and COPD patients. We found that CXCL8 was the only cytokine with a significantly different expression in asthma and COPD. There were no appreciable differences in terms of cytokine expression in IS cells in patients with different asthma or COPD phenotypes based on clinical characteristics. The exception was IL-17 whose level was significantly lower in atopic asthmatics compared with non-atopic ones. We also found that the neutrophilic phenotype was characterized by a higher CXCL8 mRNA level compared with the eosinophilic phenotype, irrespective of the underlying disease. An interesting observation was a

**Table 5** Correlations between expressions of CXCL8, IL-6, IL-8, IL-33, IL-25, TSLP, IL-17, mast cell tryptase, IL-13, ECP, CCL24, and CCL26 and macrophage percentage in induced sputum (IS) of asthma and COPD patients

| Macrophages (%) | Asthma | COPD |
|---|---|---|
| CXCL8 | $r = -0.72$, $p = 0.01$ | $r = -0.06$, $p = 0.86$ |
| IL-6 | $r = -0.17$, $p = 0.55$ | $r = 0.41$, $p = 0.19$ |
| IL-33 | $r = 0.13$, $p = 0.96$ | $r = -0.30$, $p = 0.34$ |
| IL-25 | $r = -0.36$, $p = 0.25$ | $r = -0.45$, $p = 0.14$ |
| TSLP | $r = -0.35$, $p = 0.26$ | $r = -0.48$, $p = 0.11$ |
| IL-17 | $r = -0.31$, $p = 0.28$ | $r = -0.40$, $p = 0.19$ |
| Mast cell tryptase | $r = -0.27$, $p = 0.35$ | $r = -0.70$, $p = 0.01$ |
| IL-13 | $r = -0.13$ $p = 0.66$ | $r = 0.04$, $p = 0.90$ |
| ECP | $r = -0.49$, $p = 0.09$ | $r = -0.60$, $p = 0.04$ |
| CCL24 | $r = 0.08$, $p = 0.80$ | $r = 0.54$, $p = 0.07$ |
| CCL26 | $r = 0.01$, $p = 0.96$ | $r = -0.05$, $p = 0.87$ |

*COPD* chronic obstructive pulmonary disease, *CXCL8* chemokine (C-X-C motif) ligand 8, *IL* interleukin, *TSLP* thymic stromal lymphopoietin, *ECP* eosinophil cationic protein, *CCL24* eotaxin-2, *CCL26* eotaxin-3. Statistical analysis was performed using Spearman's rank test

negative correlation between ECP expression in IS and the severity of COPD symptoms measured by CAT.

Elevated CXCL8 expression in COPD is consistent with the results of earlier studies which have reported higher CXCL8 concentrations in sputum and bronchoalveolar lavage fluid of COPD patients compared with asthmatics (Hollander et al. 2007; Yamamoto et al. 1997). CXCL8, a strong neutrophil chemoattractant, is produced by macrophages, dendritic cells, bronchial epithelial cells and airway smooth muscle cells particularly after bacterial or pro-inflammatory stimulation (e.g., TNF-α, LPS, or cigarette smoke) (Paplinska et al. 2011; Sarir et al. 2010; Gosens et al. 2009). In the present study, CXCL8 expression in IS positively correlated with neutrophil and inversely with eosinophil percentage. There is evidence that sputum neutrophilia is associated with more severe asthma phenotype and disease exacerbations (Moore et al. 2014; Paone et al. 2011), and with impaired lung function in COPD and asthma (Shaw et al. 2007; Boulet et al. 2003). We failed to find significant differences between the degree of airflow limitation in patients with eosinophilic *vs.* non-eosinophilic and neutrophilic *vs.* non-neutrophilic phenotypes of asthma or COPD. The only exception was a higher $FEV_1$/FVC in asthmatics with IS neutrophilia compared with asthma patients without IS neutrophilia. However, albeit $FEV_1$/FVC was slightly higher in asthmatics with IS neutrophil percentage $\geq$60 %, there were no significant differences in terms of $FEV_1$% of predicted value. We believe that the lack of significant differences between pulmonary function parameters in patients with neutrophilic *vs.* non-neutrophilic disease in the present study might be, in part, explained by a rather mild nature of asthma in 8 (62 %) out of the 13 patients; there were only 2 (15 %) patients with severe asthma. In earlier studies, the relationship between IS neutrophils and impaired lung function was reported mainly in patients with severe asthma. For instance, Little et al. (2002) have observed that sputum neutrophil count and activation was negatively associated with the maximal $FEV_1$ value obtained after treatment with oral steroids or maximal dose of fluticasone in combination with salbutamol.

Asthma and COPD are heterogeneous diseases with various inflammatory phenotypes. That may explain the lack of significant differences in the expression of the majority of the cytokines investigated. Although we carefully selected patients for this study and enrolled only patients with unequivocal clinical signs and symptoms of asthma or COPD, the cellular composition of IS in these two groups was variable, with some COPD patients presenting an increased percentage of eosinophils. The role of

these cells in COPD is a matter of discussion. Some studies have shown that COPD might be associated with eosinophil predominance in the airways (10–40 % of COPD subjects), which is particularly pronounced during COPD exacerbations (Siva et al. 2007). Induced sputum eosinophilia in COPD has also been reported in our previous work (Gorska et al. 2008). The results of the present study suggest that the degree of eosinophilic inflammation in stable COPD, measured by ECP expression in IS, may inversely correlate with the severity of symptoms assessed by CAT. That is consistent with our previous study that has indicated that expression of the eosinophil chemoattractant CCL26 in nasal mucosa could be a negative regulator for neutrophil airway infiltration and was strongly positively correlated with $FEV_1$/FVC in COPD patients (Paplińska et al. 2012). The assumption is that a higher eosinophil percentage would be related to a lower number of neutrophils in IS and, in consequence, to the lack of a negative influence of neutrophil airway infiltration on lung function. This assumption is strengthened by the observation that neutrophils rather than eosinophils are associated with lung function decline and irreversible airway obstruction (Boulet et al. 2003).

The only significant relation between the expression of pro-inflammatory cytokines in IS and clinical phenotypes of asthma and COPD was low IL-17 expression in patients with atopic asthma. As the role of Th17 cells, main source of IL-17, in atopy is not fully understood, the significance of this finding is unclear. IL-17 promotes neutrophilic influx into the airways in the allergic mode of asthma (Sergejeva et al. 2005). At the same time, IL-17 strongly promotes IgE production (Milovanovic et al. 2010). Some authors have shown a decreased number of circulating Th17 cells in patients with atopic dermatitis (Hayashida et al. 2011), while others have shown elevated IL-17 expression in IS from patients with allergic asthma (Manise et al. 2013). The present results suggest that Th2 and Th17 pathways can directly antagonize each other and that atopy status is associated with lower IL-17 expression in IS in asthma.

The present study suggests that macrophage function is dysregulated in obstructive lung diseases. Given the fact that macrophages are important effector cells which influence the course of asthma and COPD, we assume that dysfunction of these cells could play a role in the pathogenesis of obstructive lung diseases. Although our study demonstrates a weak negative correlation between the percentage of macrophages in IS and the expression of mediators, except for IL-13 in asthma, CCL26 in COPD, and CCL24 in both, we believe these results should be highlighted. According to the literature, macrophage function is impaired in asthma and COPD. Hodge et al. (2003) have shown that alveolar macrophages from COPD patients have a reduced ability to phagocytose apoptotic bronchial epithelial cells. It has also been reported that macrophage phagocytosis is reduced in non-eosinophilic asthma (Simpson et al. 2013). In yet another study, eosinophilic inflammation in asthma has been associated with decreased CD16 expression in bronchial macrophages (Moniuszko et al. 2007). The relationship between airway hyperresponsiveness and dysfunction of bronchial macrophages has been confirmed in mild asthma (Alexis et al. 2001). The plausibility arises that neutrophilia in severe asthma and COPD might be a consequence of reduced and inefficient phagocytotic and efferocytotic function (clearance of apoptotic cells) of macrophages. The prolonged state of ineffective airway clearance enhances bacterial colonization (Matkovic and Miravitlles 2013). In the present study, expression of ECP in COPD patients correlated negatively with the total percentage of macrophages in IS. ECP is produced by monocytes, not macrophages (Byström et al. 2001) and is thought to attract macrophages (Liu et al. 2012b). We suppose that this correlation could be associated with M1 phenotype of macrophages, which plays a role in the response to bacterial colonization, often present in the airways of patients with COPD (Patel et al. 2002).

The present investigation has several limitations. Firstly, the study groups were relatively small. Secondly, some patients with

asthma and COPD were treated with low or moderate doses of ICS, which may affect both the cellular composition of IS and the expression of different cytokines mRNA. Inhaled ICS reduce eosinophil count (van Rensen et al. 1999), airway hyperresponsiveness (Liu et al. 2012a), improve lung function (Jatakanon et al. 1998) and decrease the level of inflammatory mediators in asthmatic airways (Basyigit et al. 2004). On the other hand, ICS have no or meager anti-inflammatory action in stable COPD and neutrophilic asthma (Green et al. 2002; Culpitt et al. 1999). As our intention was to evaluate patients with stable disease (relatively well balanced pro-inflammatory and anti-inflammatory activity), treatment with ICS was not the exclusion criterion. In fact, patients treated with ICS are usually not excluded from studies that evaluate various aspects of airway inflammation (Peters et al. 2014; Sanchez-Cuellar et al. 2012). We indeed observed significantly lower ECP and IL-25 expression in the whole group of patients compared with those without ICS treatment, but a small number of ICS-treated patients made a further interpretation of this observation not feasible. Thirdly, the same control group was used for comparisons with asthma and COPD. As there were some differences between patients with asthma and COPD (e.g., age), the control group could not be well-matched to the patients of both diseases. As a result, there were significant differences between some features of COPD and control subjects (e.g., age or tobacco exposure). We accepted this drawback because the major aim of our study was to compare the data from asthma and COPD patients. We believe that a comparison of results from COPD patients with those from non-smoking control subjects, representing normal airway environment, might provide data of interest.

## 5 Conclusions

The present study demonstrates CXCL8 as the only cytokine in induced sputum showing significantly different expression between asthma and COPD. There is a significant overlap in expression of cytokines in patients with asthma and COPD. CXCL8 expression was related to the percentage of neutrophils in both COPD and asthma, which makes differentiation between the two diseases hard on the basis of a single cytokine expression. The corollary is that many common phenotypes share similar cytokine network in these obstructive lung diseases. Interestingly, higher expression of eosinophil mediators in induced sputum correlated with better COPD control as assessed by CAT. Macrophage phenotypes and macrophage-derived cytokines involved in the pathogenesis of asthma and COPD as well as the influence of eosinophils on COPD severity should be further explored in detail using alternative study designs.

**Acknowledgements** The authors thank Dr. Marta Maskey-Warzęchowska for a help in the preparation of this manuscript.

**Conflicts of Interest** The authors declare no conflict of interest related to the content of this article.

## References

Alexis NE, Soukup J, Nierkens S, Becker S (2001) Association between airway hyperreactivity and bronchial macrophage dysfunction in individuals with mild asthma. Am J Physiol Lung Cell Mol Physiol 280: L369–L375

Basyigit I, Yildiz F, Ozkara SK, Boyaci H, Ilgazli A (2004) Inhaled corticosteroid effects both eosinophilic and non-eosinophilic inflammation in asthmatic patients. Mediators Inflamm 13:285–291

Bobolea I, Barranco P, Del Pozo V, Romero D, Sanz V, López-Carrasco V, Canabal J, Villasante C, Quirce S (2015) Sputum periostin in patients with different severe asthma phenotypes. Allergy 70:540–546

Boulet LP, Turcotte H, Turcot O, Chakir J (2003) Airway inflammation in asthma with incomplete reversibility of airflow obstruction. Respir Med 97:739–744

Bradding P (2008) Asthma: eosinophil disease, mast cell disease, or both? Allergy, Asthma Clin Immunol 4:84–90

Byers DE (2014) Defining the roles of IL-33, thymic stromal lymphopoietin, and IL-25 in human asthma. Am J Respir Crit Care Med 190:715–716

Byström J, Tenno T, Håkansson L, Amin K, Trulson A, Högbom E, Venge P (2001) Monocytes, but not macrophages, produce the eosinophil cationic protein. APMIS 109:507–516

Chmielowicz B, Obojski A, Barczyk A, Sozańska E, Kycler Z, Demkow U, Domagała-Kulawik J, Polskie Towarzystwo Chorób Płuc (2008) Methodology of induced sputum induction and processing: recommendation of Polish Respiratory Society. Pneumonol Alergol Pol 76:378–394 (Article in Polish)

Culpitt SV, Maziak W, Loukidis S, Nightingale JA, Matthews JL, Barnes PJ (1999) Effect of high dose inhaled steroid on cells, cytokines, and proteases in induced sputum in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 160:1635–1639

Fujimoto K, Yasuo M, Urushibata K, Hanaoka M, Koizumi T, Kubo K (2005) Airway inflammation during stable and acutely exacerbated chronic obstructive pulmonary disease. Eur Respir J 25:640–646

Gelder CM, Thomas PS, Yates DH, Adcock IM, Morrison JF, Barnes PJ (1995) Cytokine expression in normal, atopic, and asthmatic subjects using the combination of sputum induction and the polymerase chain reaction. Thorax 50:1033–1037

GINA (2015) Global strategy for asthma management and prevention. http://www.ginasthma.org. Accessed on 11 Aug 2016

GOLD (2015) Global initiative for chronic obstructive lung disease http://www.goldcopd.it/materiale/2015/GOLD_Report_2015.pdf. Accessed on 11 Aug 2016

Gorska K, Krenke R, Domagala-Kulawik J, Korczynski P, nejman-Gryz P, Kosciuch J, Hildebrand K, Chazan R (2008) Comparison of cellular and biochemical markers of airway inflammation in patients with mild-to-moderate asthma and chronic obstructive pulmonary disease: an induced sputum and bronchoalveolar lavage fluid study. J Physiol Pharmacol 59(Suppl 6):271–283

Górska K, Maskey-Warzechowska M, Krenke R (2010) Airway inflammation in chronic obstructive pulmonary disease. Curr Opin Pulm Med

Gosens R, Rieks D, Meurs H, Ninaber DK, Rabe KF, Nanninga J, Kolahian S, Halayko AJ, Hiemstra PS, Zuyderduyn S (2009) Muscarinic M3 receptor stimulation increases cigarette smoke-induced IL-8 secretion by human airway smooth muscle cells. Eur Respir J 34:1436–1443

Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID (2002) Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax 57:875–879

Han MK, Agusti A, Calverley PM et al (2010) Chronic obstructive pulmonary disease phenotypes: the future of COPD. Am J Respir Crit Care Med 182:598–604

Hargreave FE, Pizzichini E, Pizzichini M (1998) Induced sputum examination. J Allergy Clin Immunol 101:569–570

Hayashida S, Uchi H, Moroi Y, Furue M (2011) Decrease in circulating Th17 cells correlates with increased levels of CCL17, IgE and eosinophils in atopic dermatitis. J Dermatol Sci 61:180–186

Hodge S, Hodge G, Scicchitano R, Reynolds PN, Holmes M (2003) Alveolar macrophages from subjects with chronic obstructive pulmonary disease are deficient in their ability to phagocytose apoptotic airway epithelial cells. Immunol Cell Biol 81:289–296

Hollander C, Sitkauskiene B, Sakalauskas R et al (2007) Serum and bronchial lavage fluid concentrations of IL-8, SLPI, sCD14 and sICAM-1 in patients with COPD and asthma. Respir Med 101:1947–1953

Jatakanon A, Lim S, Chung KF, Barnes PJ (1998) An inhaled steroid improves markers of airway inflammation in patients with mild asthma. Eur Respir J 12:1084–1088

Jung JW, Kang HR, Lee HS, Park HW, Cho SH, Min KU, Sohn SW (2014) Expression levels of eosinophil granule protein mRNAs in induced sputum reflect airway hyperresponsiveness and airflow limitation. Tohoku J Exp Med 233:49–56

Koutsokera A, Kostikas K, Nicod LP, Fitting JW (2013) Pulmonary biomarkers in COPD exacerbations: a systematic review. Respir Res 14:111. doi:10.1186/1465-9921-14-111

Little SA, MacLeod KJ, Chalmers GW, Love JG, McSharry C, Thomson NC (2002) Association of forced expiratory volume with disease duration and sputum neutrophils in chronic asthma. Am J Med 112:446–452

Liu M, Liu K, Zhu N, Xia J, Chen X (2012a) Inflammatory mediators in induced sputum and airway hyperresponsiveness in cough variant asthma during long-term inhaled corticosteroid treatment. Mediators Inflamm 2012:403868. doi:10.1155/2012/403868

Liu YS, Tsai PW, Wang Y, Fan TC, Hsieh CH, Chang MD, Pai TW, Huang CF, Lan CY, Chang HT (2012b) Chemoattraction of macrophages by secretory molecules derived from cells expressing the signal peptide of eosinophil cationic protein. BMC Syst Biol 6:105

Macedo P, Hew M, Torrego A, Jouneau S, Oates T, Durham A, Chung KF (2009) Inflammatory biomarkers in airways of patients with severe asthma compared with non-severe asthma. Clin Exp Allergy 39:1668–1676

Manise M, Holtappels G, Van Crombruggen K, Schleich F, Bachert C, Louis R (2013) Sputum IgE and cytokines in asthma: relationship with sputum cellular profile. PLoS One 8:e58388

Matkovic Z, Miravitlles M (2013) Chronic bronchial infection in COPD. Is there an infective phenotype? Respir Med 107:10–22

Milovanovic M, Drozdenko G, Weise C, Babina M, Worm M (2010) Interleukin-17A promotes IgE production in human B cells. J Invest Dermatol 130:2621–2628

Moniuszko M, Bodzenta-Lukaszyk A, Kowal K, Dabrowska M (2007) Bronchial macrophages in asthmatics reveal decreased CD16 expression and substantial levels of receptors for IL-10, but not IL-4 and IL-7. Folia Histochem Cytobiol 45:181–189

Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, Wenzel SE, Peters SP, Meyers DA, Bleecker ER, National Heart, Lung, and Blood Institute's Severe Asthma Research Program (2014) Sputum neutrophil counts are associated with more severe asthma phenotypes using cluster analysis. J Allergy Clin Immunol 133:1557–1563

Moqbel R, Odemuyiwa SO (2008) Allergy, asthma, and inflammation: which inflammatory cell type is more important? Allergy, Asthma Clin Immunol 4:150–156

Paone G, Conti V, Vestri A, Leone A, Puglisi G, Benassi F, Brunetti G, Schmid G, Cammarella I, Terzano C (2011) Analysis of sputum markers in the evaluation of lung inflammation and functional impairment in symptomatic smokers and COPD patients. Dis Markers 31:91–100

Paplinska M, Chazan R, Grubek-Jaworska H (2011) Effect of phoshpodiesterase 4 (PDE4) inhibibtors on eotaxin expression in humen bronchial epithelial cells. J Physiol Pharmacol 62:303–311

Paplińska M, Hermanowicz-Salamon J, Nejman-Gryz P, Białek-Gosk K, Rubinsztajn R, Arcimowicz M, Placha G, Góra J, Chazan R, Grubek-Jaworska H (2012) Expression of eotaxins in the material from nasal brushing in asthma, allergic rhinitis and COPD patients. Cytokine 60:393–399

Patel IS, Seemungal TA, Wilks M, Lloyd-Owen SJ, Donaldson GC, Wedzicha JA (2002) Relationship between bacterial colonisation and the frequency, character, and severity of COPD exacerbations. Thorax 57:759–764

Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV (2014) Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. J Allergy Clin Immunol 133:388–394

Rabe KF, Hurd S, Anzueto A et al (2007) Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 176:532–555

Röpcke S, Holz O, Lauer G, Müller M, Rittinghausen S, Ernst P, Lahu G, Elmlinger M, Krug N, Hohlfeld JM (2012) Repeatability of and relationship between potential COPD biomarkers in bronchoalveolar lavage, bronchial biopsies, serum, and induced sputum. PLoS One 7:e46207

Sanchez-Cuellar S, de la Fuente H, Cruz-Adalia A, Lamana A, Cibrian D, Giron RM, Vara A, Sanchez-Madrid F, Ancochea J (2012) Reduced expression of galectin-1 and galectin-9 by leucocytes in asthma patients. Clin Exp Immunol 170:365–374

Sarir H, Mortaz E, Janse WT, Givi ME, Nijkamp FP, Folkerts G (2010) IL-8 production by macrophages is synergistically enhanced when cigarette smoke is combined with TNF-alpha. Biochem Pharmacol 79:698–705

Sergejeva S, Ivanov S, Lötvall J, Lindén A (2005) Interleukin-17 as a recruitment and survival factor for airway macrophages in allergic airway inflammation. Am J Respir Cell Mol Biol 33:248–253

Shaw DE, Berry MA, Hargadon B, McKenna S, Shelley MJ, Green RH, Brightling CE, Wardlaw AJ, Pavord ID (2007) Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. Chest 132:1871–1875

Simpson JL, Gibson PG, Yang IA, Upham J, James A, Reynolds PN, Hodge S, AMAZES Study Research Group (2013) Impaired macrophage phagocytosis in non-eosinophilic asthma. Clin Exp Allergy 43:29–35

Singh D, Edwards L, Tal-Singer R, Rennard S (2010) Sputum neutrophils as a biomarker in COPD: findings from the ECLIPSE study. Respir Res 11:77. doi:10.1186/1465-9921-11-77

Siva R, Green RH, Brightling CE, Shelley M, Hargadon B, McKenna S, Monteiro W, Berry M, Parker D, Wardlaw AJ, Pavord ID (2007) Eosinophilic airway inflammation and exacerbations of COPD: a randomised controlled trial. Eur Respir J 29:906–913

van Rensen EL, Straathof KC, Veselic-Charvat MA, Zwinderman AH, Bel EH, Sterk PJ (1999) Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. Thorax 54:403–408

Vernooy JHJ, Lindeman JHN, Jacobs JA, Hanemaaijer R, Wouters EF (2004) Increased activity of matrix metalloproteinase-8 and matrix metalloproteinase-9 in induced sputum from patients with COPD. Chest 126:1802–1810

Yamamoto C, Yoneda T, Yoshikawa M, Fu A, Tokuyama T, Tsukaguchi K, Narita N (1997) Airway inflammation in COPD assessed by sputum levels of interleukin-8. Chest 112:505–510

# Erratum to

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 113–114
DOI 10.1007/5584_2016_188

Published online: 16 July 2017

## Expression of Inflammatory Mediators in Induced Sputum: Comparative Study in Asthma and COPD

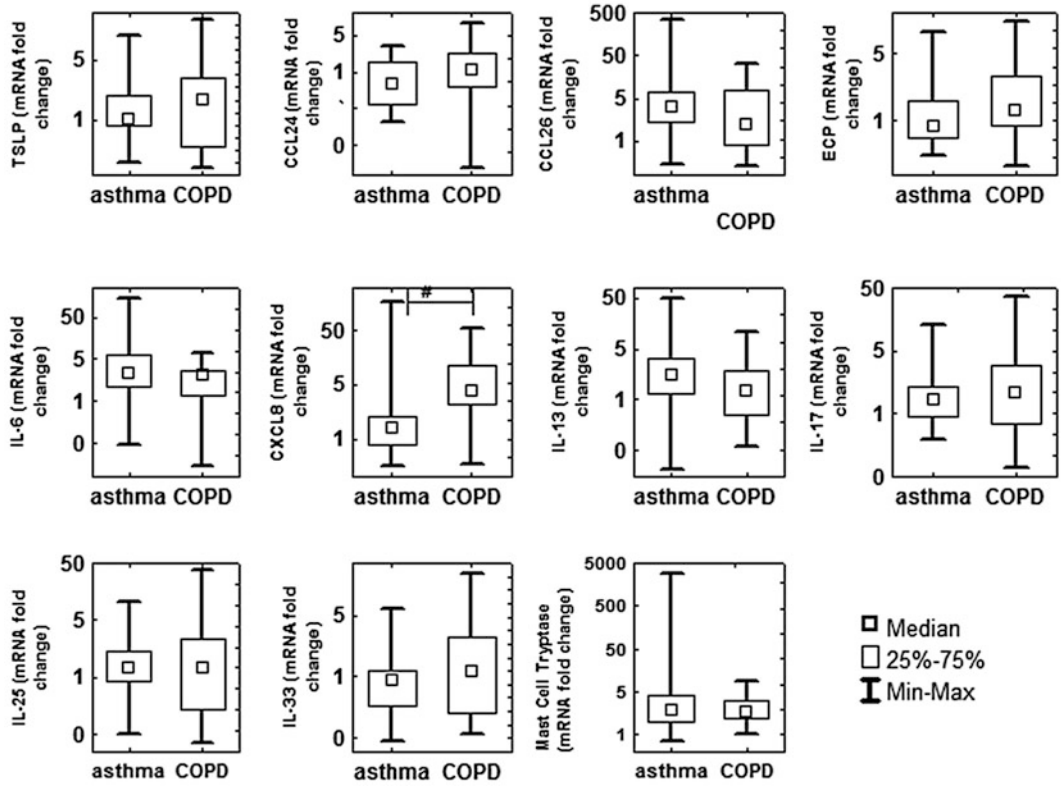
Magdalena Paplińska-Goryca, Patrycja Nejman-Gryz, Katarzyna Górska, Katarzyna Białek-Gosk, Joanna Hermanowicz-Salamon, and Rafał Krenke

Advs Exp. Medicine, Biology - Neuroscience and Respiration
DOI 10.1007/5584_2016_165


**DOI 10.1007/5584_2016_188**

Figure 1 of this chapter was inadvertently displayed incorrect. The correct form of the figure should be displayed as follows:

The updated online version of the original chapter can be found under
DOI 10.1007/5584_2016_165


TSLP (mRNA fold change)
CCL24 (mRNA fold change)
CCL26 (mRNA fold change)
ECP (mRNA fold change)
IL-6 (mRNA fold change)
CXCL8 (mRNA fold change)
#
IL-13 (mRNA fold change)
IL-17 (mRNA fold change)
IL-25 (mRNA fold change)
IL-33 (mRNA fold change)
Mast Cell Tryptase (mRNA fold change)
asthma
COPD
Median
25%-75%
Min-Max

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2018) 37: 115–116
https://doi.org/10.1007/978-3-319-74092-8


# Index