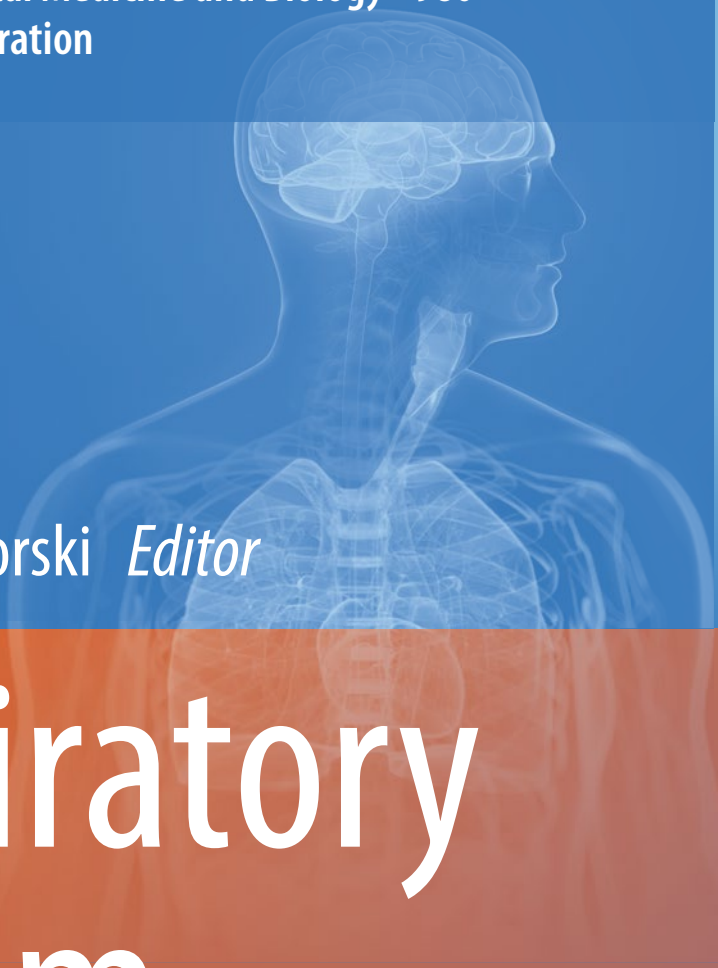
Advances in Experimental Medicine and Biology 980
Neuroscience and Respiration



Mieczyslaw Pokorski *Editor*

Respiratory System Diseases

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Volume 980

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Mieczyslaw Pokorski
Editor

Respiratory System Diseases

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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 foused on pulmonary disorders as the respiratory tract is upfront at the first line of defense of the organism 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, or the mind-to-body connection. The authors focus on the modern diagnostic techniques and the leading-edge therapeutic concepts, methodologies, and innovative treatments. The action and pharmacology of existing drugs and the development and evaluation of new agents are the heady area of research. Practical, data-driven options to manage patients will be considered. New research is presented regarding older drugs, performed from a modern perspective or from a different pharmacotherapeutic angle. The introduction of new drugs and treatment approaches in both adults and children also is discussed.

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, by decreasing stress, can play a major role in disease therapy.

Neuromolecular and carcinogenetic aspects relating to gene polymorphism and epigenesis, involving both heritable changes in the nucleotide sequence and functionally relevant changes to the genome that do not involve a change in the nucleotide sequence, leading to disorders will also be tackled. Clinical advances stemming from molecular and biochemical research are but possible if the research findings are translated into diagnostic tools,

therapeutic procedures, and education, effectively reaching physicians and patients. All that cannot be achieved without a multidisciplinary, collaborative, bench-to-bedside approach involving both researchers and clinicians. The role of science in shaping medical knowledge and transforming it into practical care is undeniable.

Concerning the respiratory disorders, their societal and economic burden has been on the rise worldwide leading to disabilities and shortening of life span. COPD alone causes more than three million deaths globally each year. Concerted efforts are required to improve this situation, and part of those efforts are gaining insights into the underlying mechanisms of disease and staying abreast with the latest developments in diagnosis and treatment regimens. It is hoped that the 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 Mr. Paul Roos, Ms. Tanja Koppejan, and Ms. Cynthia Kroonen of Springer SBM 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

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Impaired Vascular Function in Sarcoidosis Patients

I. Tuleta, D. Skowasch, L. Biener, C. Pizarro, R. Schueler, G. Nickenig, N. Schahab, C. Schaefer, and S. Pingel

Abstract

A common feature of sarcoidosis and atherosclerosis is a chronic systemic inflammatory reaction. Our hypothesis was that sarcoidosis may negatively influence the vessel status. We addressed the issue by examining preatherosclerotic vascular alternations using an ultrasound-based speckle-tracking method in 72 sarcoidosis patients and 15 matched controls. To find potential factors which may have a deleterious influence on arterial performance, different subgroups of sarcoidosis, such as sarcoidosis with or without cortisone therapy, pulmonary sarcoidosis in early and advanced stages, pulmonary sarcoidosis alone or combined with extrapulmonary sarcoidosis, and sarcoidosis with or without elevated blood levels of angiotensin converting enzyme (ACE)/soluble interleukin 2 receptor (sIL-2R) were investigated. We found in the general collective of sarcoidosis patients that circumferential strain (2.68 ± 0.19%), circumferential strain rate (0.21 ± 0.01 1/s), and radial displacement (0.10 ± 0.01 mm) were significantly decreased compared to controls (3.77 ± 0.35%, 0.28 ± 0.02 1/s, and 0.14 ± 0.02 mm, respectively). Vascular strains were more impaired in patients with cortisone therapy, pulmonary sarcoidosis in stages III-IV, and in pulmonary sarcoidosis accompanied by extrapulmonary involvement. The level of ACE/sIL-2R had no relevant influence on the angiological parameters. In conclusion, sarcoidosis is associated with increased vascular stiffness. Cortisone therapy and advanced stages of pulmonary sarcoidosis with extrapulmonary manifestations may account for the impaired vascular function in this patient collective.

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Keywords
Arterial Elastic Properties • Atherosclerosis • Granulomatous Disease • Inflammation • Sarcoidosis Vasculopathy • Vascular Ultrasound

1 Introduction

Sarcoidosis (Newman et al. 1997) and atherosclerosis (Ross 1999) are both chronic inflammatory diseases. To-date there is a limited knowledge about a potential influence of sarcoidosis on the atherosclerotic vessel changes. Our hypothesis was that sarcoidosis, a granulomatous disorder with a systemic inflammatory reaction, could predispose to the development of pathological vascular alternations. The aim of the present study was to assess whether sarcoidosis patients are at higher risk for preatherosclerosis compared to control subjects. In order to verify this hypothesis we used an established and sensitive method of an ultrasound-based speckle-tracking analysis of arteries. Additionally, subgroups of sarcoidosis patients were created to examine what kind of factors may influence vessel status of these patients.

2 Methods

2.1 Patients

The research was conducted according to the principles of the Declaration of Helsinki and was aproved by a local Ethics Committee. Written informed consent was obtained from all subjects. We enrolled 72 sarcoidosis patients and 15 matched control subjects into our study between May–December/2015. There were no significant differences in terms of demographics or the presence of classical cardiovascular risk factors. Blood level of angiotensin converting enzyme (ACE), but not that of soluble interleukin 2 receptor (sIL-2R), was significantly increased in sarcoidosis patients compared with controls. More than half of sarcoidosis patients were on the cortisone therapy. No person in the control group received cortisone medication (Table 1). 93.5% of sarcoidosis patients suffered from pulmonary sarcoidosis in stages I or II (68.7% of patients with pulmonary sarcoidosis) and III or IV (31.3% of patients with pulmonary sarcoidosis). In 43.1% of patients, pulmonary sarcoidosis was accompanied by extrapulmonary sarcoidosis. Only 5 out of the 72 patients (6.9%) presented solely extrapulmonary manifestations of the disease. Cortisone therapy was significantly more frequent in patients with pulmonary sarcoidosis in stages III-IV compared with pulmonary sarcoidosis in stages I-II. There were no statistically significant differences in cortisone therapy between pulmonary sarcoidosis and pulmonary sarcoidosis accompanied by extrapulmonary sarcoidosis as well as sarcoidosis with normal blood ACE/sIL-2R and sarcoidosis with elevated ACE/sIL-2R. Detailed characteristics of sarcoidosis patients are shown in Table 2.

2.2 Angiological Examinations

Vascular stiffness was that assessed by ultrasound examination of the right common carotid artery, recording axial loops of at least 3 consecutive heart beats, as previously described (Zhang et al. 2014). Afterward, the vessel wall motion was analyzed by an off-line speckle tracking based software (Image arena, Tomtec Imaging Systems, Unterschleissheim, Germany), calculating global circumferential and radial strain, global circumferential and radial strain rate, and global radial displacement.

Table 1 Baseline characteristics of sarcoidosis patients and control subjects. There were no differences in age, sex, BMI, or the presence of cardiovascular risk factors between the groups. More than half of sarcoidosis patients were treated with cortisone. ACE and sIL-2R in the blood were increased in sarcoidosis vs. control group

	Sarcoidosis (n=72)	Control (n=15)
Age (years)	54.7 ± 1.4	52.7 ± 2.2
Gender (male)	30 (41.7%)	6 (40.0%)
BMI (kg/m^2)	25.8 ± 0.5	24.3 ± 1.0
Arterial hypertension	28 (38.9%)	6 (40.0%)
Hypercholesterolemia	31 (43.1%)	4 (26.7%)
Diabetes mellitus	13 (18.1%)	0 (0%)
Nicotine abuse – never	47 (65.3%)	7 (46.7%)
Nicotine abuse – former	19 (26.4%)	5 (33.3%)
Nicotine abuse – current	6 (8.3%)	3 (20.0%)
Cortisone therapy	41 (56.9%)	0 (0%)*
ACE (U/l)	39.7 ± 3.3	23.5 ± 4.3*
sIL-2R (U/ml)	730.1 ± 58.9	513.9 ± 65.9

The results are presented as means ± SEM or n (%) of study participants in respective groups
BMI body mass index, *ACE* angiotensin converting enzyme, *sIL-2R* soluble interleukin 2 receptor
*$p<0.05$

Table 2 Prevalence of cortisone therapy in different sarcoidosis subgroups

	Cortisone therapy			
	Lack n (%)	Low n (%)	High n (%)	p-value
Sarcoidosis patients with cortisone therapy (n=41)	0 (0)	36 (87.8)	5 (12.2)	<0.05
Sarcoidosis patients without cortisone therapy (n=31)	0 (0)	0 (0)	0 (0)	
Pulmonary sarcoidosis in stage I-II (n=46)	27 (58.7)	15 (32.6)	4 (8.7)	<0.05
Pulmonary sarcoidosis in stage III-IV (n=21)	2 (9.5)	18 (85.7)	1 (4.8)	
Pulmonary sarcoidosis (n=41)	19 (46.3)	20 (48.8)	2 (4.9)	ns
Pulmonary and extrapulmonary sarcoidosis (n=31)	12 (38.7)	16 (51.6)	3 (9.7)	
Sarcoidosis with normal ACE/sIL-2R (n=43)	22 (51.2)	20 (46.5)	1 (2.3)	ns
Sarcoidosis with higher ACE/sIL-2R (n=29)	9 (31.0)	16 (55.2)	4 (13.8)	

The results are presented as n (%) of study participants in respective groups. Cortisone therapy: low = ≤15 mg prednisolone/day; high = > 15 mg prednisolone/day
ACE angiotensin converting enzyme, *sIL-2R* soluble interleukin 2 receptor, *ns* non-significant

2.3 Blood Tests

ACE and sIL-2R were measured in the blood of sarcoidosis patients and in control individuals using standard laboratory tests at the time of recruitment into the study.

2.4 Statistical Analysis

Angiological measures were presented as means ± SEM. Differences between the mean values in sarcoidosis and control groups were calculated by means of a *t*-test. A p-value of less than 0.05 defined statistically significant differences.

3 Results

The main finding was that circumferential strain and circumferential strain rate were significantly reduced in sarcoidosis (2.68 ± 0.19% and 0.21 ± 0.01 1/s, respectively) compared to the control subjects (3.77 ± 0.35%, 0.28 ± 0.02 1/s, respectively) (Fig. 1a, c). Likewise, radial displacement was also relevantely decreased in sarcoidosis compared to the control subjects

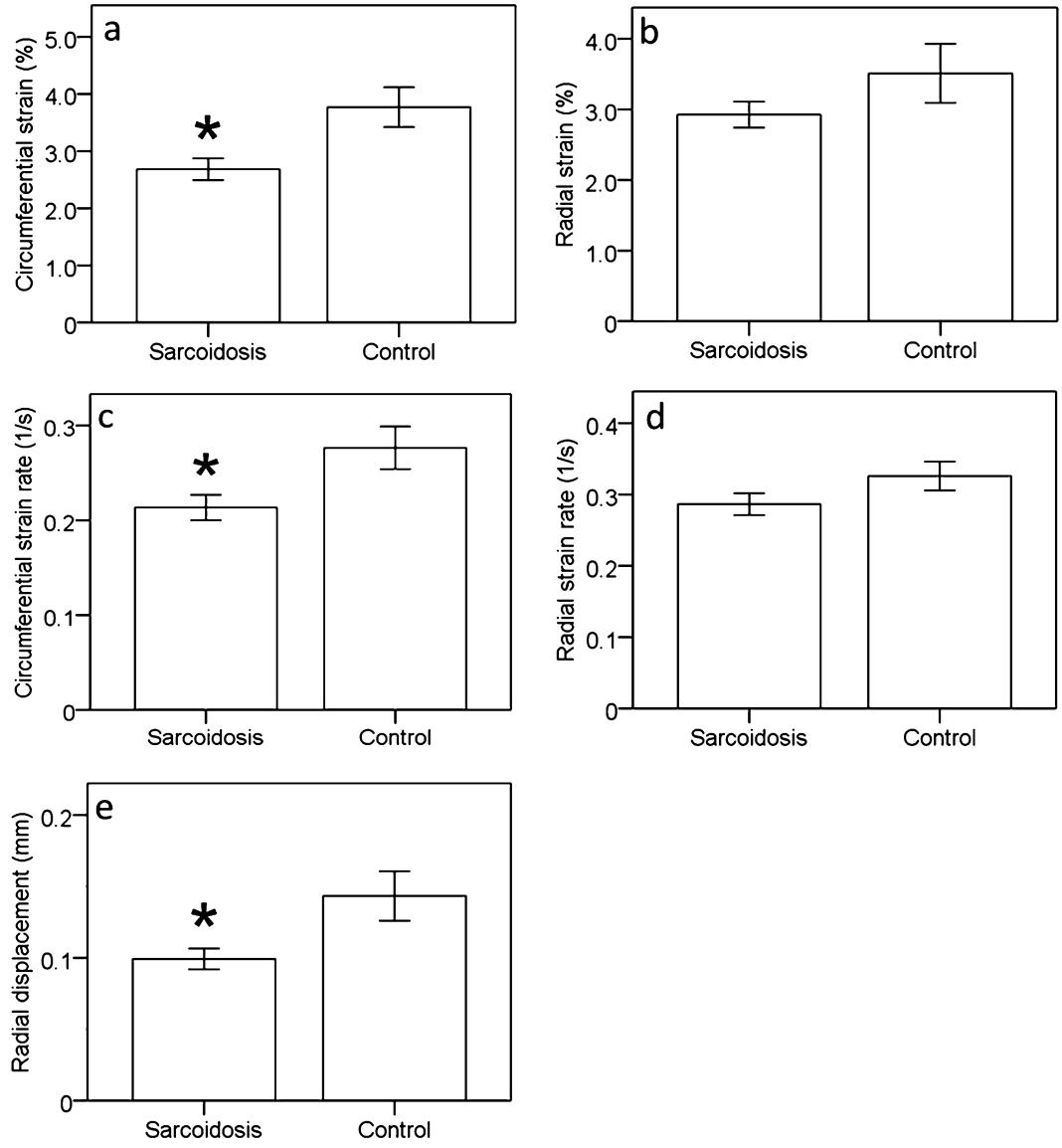

Fig. 1 Blood vessel wall motion and deformation parameters: (**a**) circumferential strain, (**b**) radial strain, (**c**) circumferential strain rate, (**d**) radial strain rate, and (**e**) radial displacement. All these parameters were reduced in sarcoidosis compared to control subjects; *$p<0.05$

(0.10 ± 0.01 mm vs. 0.14 ± 0.02 mm, respectively) (Fig. 1e). Radial strain and radial strain rate showed an insignificant trend toward lower values compared to the control subjects (2.93 ± 0.18% and 0.29 ± 0.02 1/s vs. 3.51 ± 0.42% and 0.33 ± 0.02 1/s, respectively) (Fig. 1b, d).

An elucidation of different sarcoidosis subgroups provided further insight into the vessel status depending on various factors. In detail, sarcoidosis patients on cortisone therapy had a significantly reduced circumferential strain (2.25 ± 0.23%) (Fig. 2a), circumferential strain rate (0.18 ± 0.02 1/s), and radial displacement values (0.08 ±0.01 mm) (Fig. 2b) vs. control

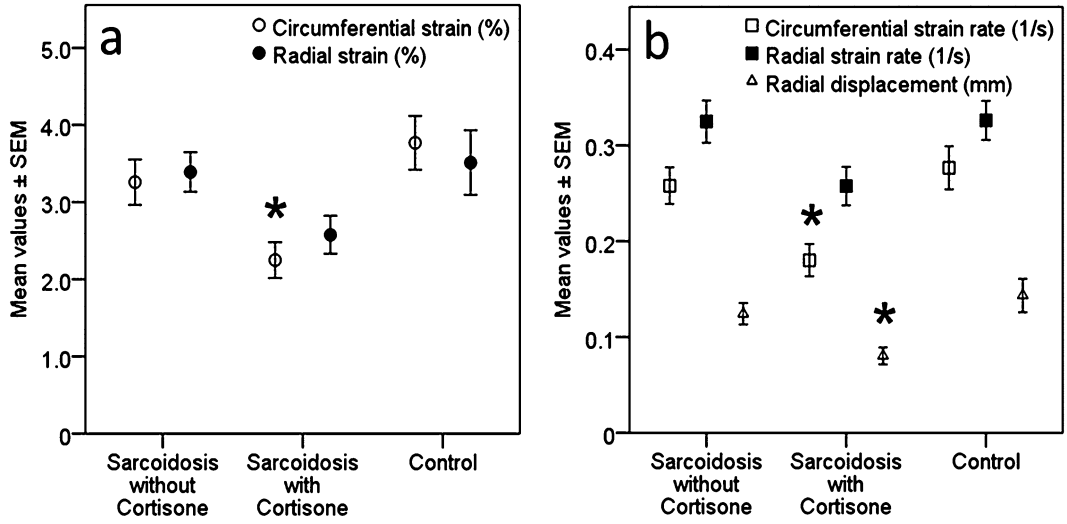

Fig. 2 Blood vessel wall motion and deformation parameters in sarcoidosis patients with and without cortisone therapy. Circumferential strain (**a**), and circumferential strain rate and radial displacement (**b**) were significantly reduced in patients on cortisone therapy compared to those in control subjects; *$p<0.05$

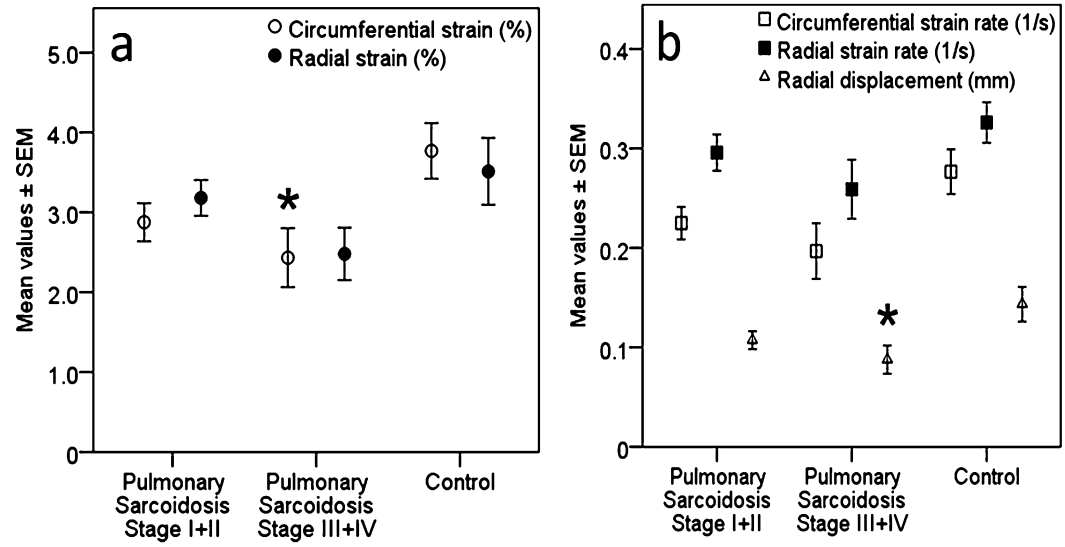

Fig. 3 Blood vessel wall motion and deformation parameters in patients with pulmonary sarcoidosis in stages I-II and III-IV. Circumferential strain (**a**) and radial displacement (**b**) were significantly reduced in pulmonary sarcoidosis in stages III-IV. compared to those in control subjects; *$p<0.05$

subjects. The patients without cortisone therapy did not show any relevant differences in the same angiological parameters (3.26 ± 0.29%, 0.26 ± 0.02 1/s, and 0.12 ± 0.01 mm, respectively) (Fig. 2).

Analogically, only did patients with pulmonary sarcoidosis in stages III-IV demonstrate significantly lower circumferential strain (2.43 ± 0.37%) and radial displacement (0.09 ± 0.01 mm) vs. control subjects (Fig. 3a, b). In contrast, patients with pulmonary

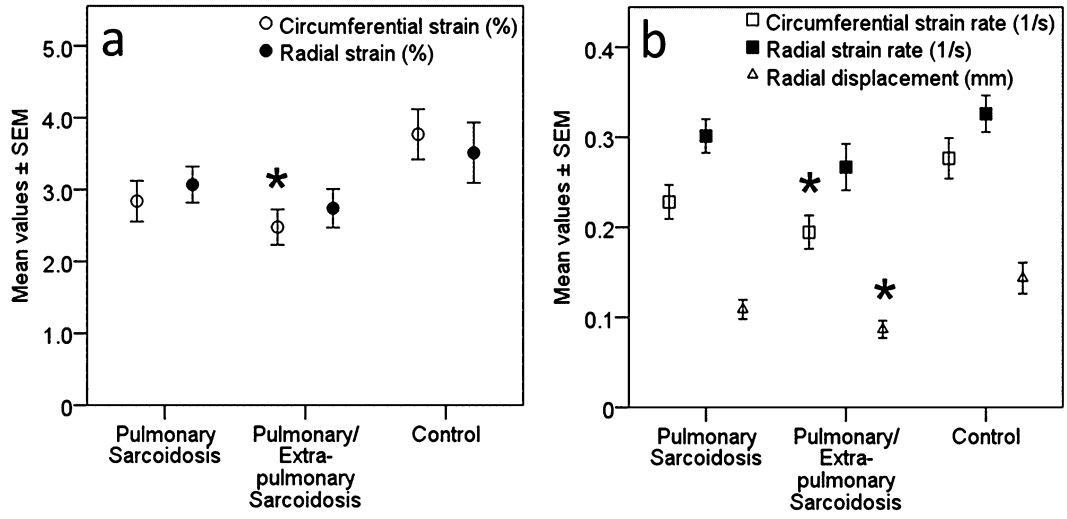

Fig. 4 Blood vessel wall motion and deformation parameters in patients with pulmonary sarcoidosis combined with extrapulmonary manifestations compared to control subjects. Circumferential strain (**a**), and circumferential strain rate and radial displacement (**b**) were significantly reduced in pulmonary sarcoidosis with extrapulmonary manifestations compared to those in control subjects; *$p<0.05$

sarcoidosis in stages I-II demonstrated the circumferential strain (2.87 ± 0.24%) and radial displacement (0.11 ± 0.01 mm) similar to those present in the control subjects (Fig. 3a, b). Interestingly, patients with pulmonary sarcoidosis in stages III-IV obtained relevantly more often cortisone treatment compared with the patients in stages I-II of sarcoidosis (Table 2).

In a group of pulmonary and extrapulmonary sarcoidosis, circumferential strain, circumferential strain rate, and radial displacement were all significantly decreased vs. control subjects, which was not observed in a group with isolated pulmonary sarcoidosis (Fig. 4a, b). Of note, there were no significant differences in the frequency of cortisone therapy between these both groups (Table 2). Increased sarcoidosis markers, such as ACE and sIL-2R, had no relevant influence on the angiological parameters (Fig. 5a, b). The number of sarcoidosis patients with the elevated ACE and/or sIL-2R markers, who were treated with cortisone, was not significantly different from that with the markers being in the normal range (Table 2).

4 Discussion

The present study shows an impaired vessel wall motion and deformation measured by means of an ultrasound-based speckle-tracking in sarcoidosis patients compared to control subjects. We have previously reported on a significant increase in pulse wave index in sarcoidosis patients, which may indicate early preatherosclerotic artery changes, albeit we have not found any differences in the atherosclerotic plaque burden between sarcoidosis and control subjects (Tuleta et al. 2016). Other works have demonstrated an augmented vessel stiffness assessed by classical angiological parameters, such as pulse wave velocity (Yildiz 2010). Pathological vessel alternations are also observed in other chronic inflammatory disorders, including systemic sclerosis (Au et al. 2011), systemic lupus erythematosus (Tyrrell et al. 2010), and in granulomatosis with polyangiitis (de Leeuw et al. 2005). Precise mechanisms promoting atherosclerosis in these diseases are unknown. Chronic systemic inflammation (Yildiz 2010)

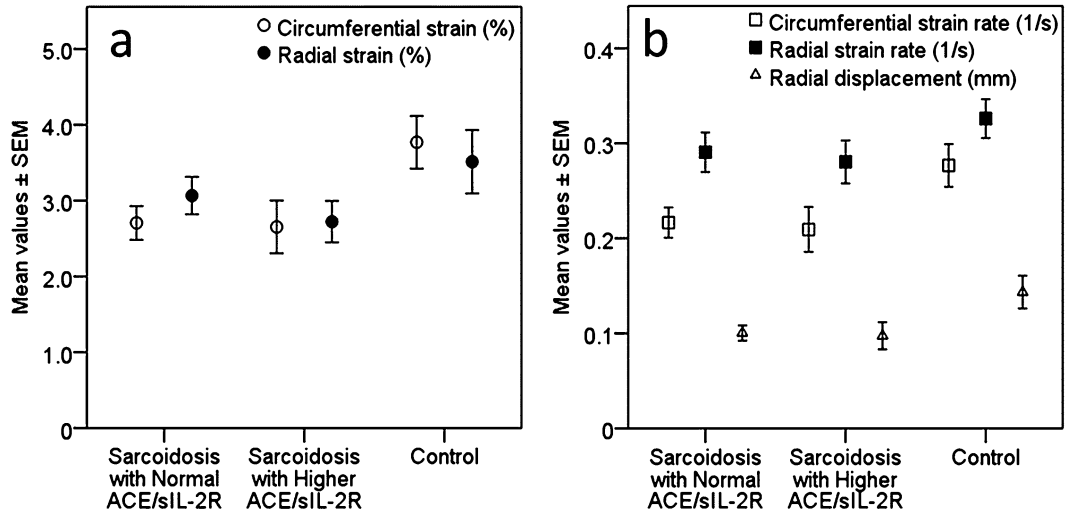

Fig. 5 Relation of blood vessel wall motion and deformation parameters to the blood levels of sarcoidosis markers: angiotensin converting enzyme (ACE) and soluble interleukin 2 receptor (sIL-2R). There was no significant influence of the elevated markers on the vascular strain in sarcoidosis patients compared to control subjects

and oxidative stress (Ivanišević et al. 2012) seem to play the most important role. As systemic inflammation is a known cardiovascular risk factor (Ross 1999), one could assume that the cortisone therapy might exert positive effects not only on the course of the disease, but also on the vascular changes. However, our present data do not support this notion. On the contrary, cortisone treatment was associated with an impaired vessel function. Sarcoidosis patients without cortisone therapy presented similar vascular strain results as that present in control subjects. One explanation for this finding could be a proatherogenic action of cortisone (Hermanowski-Vosatka et al. 2005). This action, however, would be mediated by mechanisms other that the cortisone-induced development of metabolic syndrome (Parker et al. 2013), as there were no significant differences in the cardiovascular risk markers between sarcoidosis and control subjects in the present study. Cortisone could worsen the vessel status in sarcoidosis patients via an induction of other potentially proatherogenic pathways (Hermanowski-Vosatka et al. 2005). Generally, the exact impact of cortisone on the progression of atherosclerosis is very complex and still not entirely clear. In previous studies, cortisone has been described as a proatherogenic agent. However, there are also works showing no or even a positive influence of cortisone on the vasculature under certain conditions (Gordon et al. 1954). In sarcoidosis patients without pulmonary fibrosis, cortisone therapy may improve vasculopathy that underlies pulmonary hypertension (Nunes et al. 2006). In systemic sclerosis, cortisone therapy is linked to the augmented atherosclerotic plaque load (Vettori et al. 2010). Analogically, intima-media-thickness (IMT) is higher in granulomatosis with polyangiitis (Nienhuis et al. 2007) and in lupus erythematosus patients (Tyrrell et al. 2010). However, other authors have reported on a missing effect of cortisone on IMT, endothelial function, and on the prevalence of atherosclerotic plaques in rheumatoid arthritis (Hafström et al. 2007) or a mitigation of the development of premature atherosclerosis in granulomatosis with polyangitis (Souza et al. 2014).

In the present study, patients with pulmonary sarcoidosis in stages III-IV were characterized by a significant reduction of vascular strain compared with control subjects, whereas such a relation was not detected in stages I-II of sarcoidosis.

At this point it should be mentioned that patients with pulmonary sarcoidosis in stages III-IV received cortisone therapy significantly more often than those in earlier stages of pulmonary disease. It is hard to judge whether the advanced stage of sarcoidosis or the cortisone therapy had a predominant deleterious effect on the arteries. Probably both factors contributed to the poor angiological results since both the disease duration along with resulting tissue damage and the cortisone therapy may act proatherogenic, as it has been shown for systemic lupus erythematosus (Frerix et al. 2014). Additionally, we demonstrate that a combined pulmonary and extrapulmonary sarcoidosis was related to the decreased vascular performance compared with the solely pulmonary sarcoidosis. The frequency of cortisone therapy did not differ between both groups. Interestingly, it has been shown that ocular sarcoidosis impairs the flow-mediated dilation, and increases the augmentation index and pulse wave velocity compared with sarcoidosis without the ocular involvement (Siasos et al. 2015). The reason for this is unknown. One may surmise that the manifestation of sarcoidosis involving more than one organ reflects the disease activity, which may have a more pronounced influence on the arterial disorders. Surprisingly, we noticed no relevant impact of the sarcoidosis blood markers, such as ACE and sIL-2R, on the angiological results. The lack of relation of markers of illness activity to vessel changes have also been obtained in systemic sclerosis (Vettori et al. 2010) and lupus erythematosus (Frerix et al. 2014). Since it is widely accepted that augmented systemic inflammation is negatively associated with vessel status (Hingorani et al. 2000), we hypothesize that ACE and sIL-2R may not mirror the complexity of the immunological response in sarcoidosis and may serve as an indicator for enhanced risk for pathological vessel alternations only together with other markers, clinical status, and the therapy administrated.

In conclusion, sarcoidosis is associated with reduced functional vessel status. Advanced stages of pulmonary sarcoidosis, involvement of extrapulmonary organs, and cortisone therapy seem to predispose to impaired vascular function in sarcoidosis patients.

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

References

Au K, Singh MK, Bodukam V, Bae S, Maranian P, Ogawa R, Spiegel B, McMahon M, Hahn B, Khanna D (2011) Atherosclerosis in systemic sclerosis: a systematic review and meta-analysis. Arthritis Rheum 63:2078–2090

de Leeuw K, Sanders JS, Stegeman C, Smit A, Kallenberg CG, Bijl M (2005) Accelerated atherosclerosis in patients with Wegener's granulomatosis. Ann Rheum Dis 64:753–759

Frerix M, Stegbauer J, Kreuter A, Weiner SM (2014) Atherosclerotic plaques occur in absence of intima-media thickening in both systemic sclerosis and systemic lupus erythematosus: a duplexsonography study of carotid and femoral arteries and follow-up for cardiovascular events. Arthritis Res Ther 16:R54

Gordon D, Kobernick SD, McMillan GC, Duff GL (1954) The effect of cortisone on the serum lipids and on the development of experimental cholesterol atherosclerosis in the rabbit. J Exp Med 99:371–386

Hafström I, Rohani M, Deneberg S, Wörnert M, Jogestrand T, Frostegård J (2007) Effects of low-dose prednisolone on endothelial function, atherosclerosis, and traditional risk factors for atherosclerosis in patients with rheumatoid arthritis--a randomized study. J Rheumatol 34:1810–1816

Hermanowski-Vosatka A, Balkovec JM, Cheng K, Chen HY, Hernandez M, Koo GC, Le Grand CB, Li Z, Metzger JM, Mundt SS, Noonan H, Nunes CN, Olson SH, Pikounis B, Ren N, Robertson N, Schaeffer JM, Shah K, Springer MS, Strack AM, Strowski M, Wu K, Wu T, Xiao J, Zhang BB, Wright SD, Thieringer R (2005) 11beta-HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice. J Exp Med 202:517–527

Hingorani AD, Cross J, Kharbanda RK, Mullen MJ, Bhagat K, Taylor M, Donald AE, Palacios M, Griffin GE, Deanfield JE, MacAllister RJ, Vallance P (2000) Acute systemic inflammation impairs endothelium-dependent dilatation in humans. Circulation 102:994–999

Ivanišević J, Kotur-Stevuljević J, Stefanović A, Jelić-Ivanović Z, Spasić S, Videnović-Ivanov J, Vučinić-Mihailović V, Ilić J (2012) Dyslipidemia and oxidative stress in sarcoidosis patients. Clin Biochem 45:677–682

Newman LS, Rose CS, Maier LA (1997) Sarcoidosis. N Engl J Med 336:1224–1234

Nienhuis HL, de Leeuw K, Smit AJ, Bijzet J, Stegeman CA, Kallenberg CG, Bijl M (2007) Enhanced endothelium-dependent microvascular responses in patients with Wegener's granulomatosis. J Rheumatol 34:1875–1881

Nunes H, Humbert M, Capron F, Brauner M, Sitbon O, Battesti JP, Simonneau G, Valeyre D (2006) Pulmonary hypertension associated with sarcoidosis: mechanisms, haemodynamics and prognosis. Thorax 61:68–74

Parker B, Urowitz MB, Gladman DD, Lunt M, Bae SC, Sanchez-Guerrero J, Romero-Diaz J, Gordon C, Wallace DJ, Clarke AE, Bernatsky S, Ginzler EM, Isenberg DA, Rahman A, Merrill JT, Alarcón GS, Fessler BJ, Fortin PR, Hanly JG, Petri M, Steinsson K, Dooley MA, Manzi S, Khamashta MA, Ramsey-Goldman R, Zoma AA, Sturfelt GK, Nived O, Aranow C, Mackay M, Ramos-Casals M, van Vollenhoven RF, Kalunian KC, Ruiz-Irastorza G, Lim S, Kamen DL, Peschken CA, Inanc M, Bruce IN (2013) Clinical associations of the metabolic syndrome in systemic lupus erythematosus: data from an international inception cohort. Ann Rheum Dis 72:1308–1314

Ross R (1999) Atherosclerosis is an inflammatory disease. Review. Am Heart J 138:S419–S420

Siasos G, Paraskevopoulos T, Gialafos E, Rapti A, Oikonomou E, Zaromitidou M, Mourouzis K, Siasou G, Gouliopoulos N, Tsalamandris S, Vlasis K, Stefanadis C, Papavassiliou AG, Tousoulis D (2015) Vascular function and ocular involvement in sarcoidosis. Microvasc Res 100:54–58

Souza AW, de Leeuw K, van Timmeren MM, Limburg PC, Stegeman CA, Bijl M, Westra J, Kallenberg CG (2014) Impact of serum high mobility group box 1 and soluble receptor for advanced glycation end-products on subclinical atherosclerosis in patients with granulomatosis with polyangiitis. PLoS One 9:e96067

Tuleta I, Pingel S, Biener L, Pizarro C, Hammerstingl C, Öztürk C, Schahab N, Grohé C, Nickenig G, Schaefer C, Skowasch D (2016) Atherosclerotic vessel changes in Sarcoidosis. Adv Exp Med Biol 910:23–30

Tyrrell PN, Beyene J, Feldman BM, McCrindle BW, Silverman ED, Bradley TJ (2010) Rheumatic disease and carotid intima-media thickness: a systematic review and meta-analysis. Arterioscler Thromb Vasc Biol 30:1014–1026

Vettori S, Maresca L, Cuomo G, Abbadessa S, Leonardo G, Valentini G (2010) Clinical and subclinical atherosclerosis in systemic sclerosis: consequences of previous corticosteroid treatment. Scand J Rheumatol 39:485–489

Yildiz M (2010) Arterial distensibility in chronic inflammatory rheumatic disorders. Open Cardiovasc Med J 4:83–88

Zhang L, Yin JK, Duan YY, Liu X, Xu L, Wang J, Yang YL, Yuan LJ, Cao TS (2014) Evaluation of carotid artery elasticity changes in patients with type 2 diabetes. Cardiovasc Diabetol 13:39

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Effectiveness of Bronchofiberoscopy in Diagnosis of Lung Lesions

Artur Nahorecki, Katarzyna Postrzech-Adamczyk, Ewelina Straszak, Andrzej Szuba, Dariusz Janczak, and Mariusz Chabowski

Abstract

Lung cancer is the most common cause of cancer-related deaths. A short survival rate often results from belated diagnosis made in advanced stages. Therapy individualization requires the collection of a viable material for histopathological examination, which often brings difficulties. This study was performed in a group of 110 patients suspected of malignancy in chest computed tomography. All subjects underwent bronchofiberoscopy. Bronchoalveolar lavage (BAL) and endobronchial brushing were performed in all cases, whereas forceps tissue biopsy was taken if mucous membrane abnormalities were observed. In case of a negative result of bronchofiberoscopy invasive methods were implemented. A malignant neoplasm was diagnosed in 106 cases. Overall, cancer cells (positive result) were found in 45 patients (42.0%) subjected to bronchofiberoscopy. Cytology was positive in 38 (35.8%) and histopathological examination in 30 (28.3%) specimens. Eleven samples of BAL (10.3%) were positive. Endobronchial brushing was more effective, with 27 positive samples (25.5%). Forceps tissue biopsy was performed in 33 cases with 90% sensitivity. The most frequent cancer subtype found was squamous cell carcinoma. No severe complications of bronchofiberoscopy were observed. We conclude that bronchofiberosocpy is a safe diagnostic procedure for lung lesions, but its sensitivity and specificity are low. Only

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when there are mucous macroscopic changes observed, a precise diagnosis is possible.

Keywords
Bronchoalveolar lavage • Bronchofiberoscopy • Cancer cells • Endobronchial brushing • Lung cancer • Squamous cell carcinoma • Tissue biopsy

1 Introduction

A diagnosis of lung cancer is associated with poor prognosis. The overall 5-year survival rate in this group of patients does not exceed 15% (Siegel et al. 2016; Dela Cruz et al. 2011). In most patients, diagnosis is delayed due to the lack of alarming symptoms that would point to the disease. About 90% of patients with early stage lung cancer are asymptomatic. Only a small percentage of them presents with cough, hemoptysis, dyspnea, or fatigue, all of which are not characteristic for a malignant disease (Beckles et al. 2003). The chest computed tomography is performed as an initial test for screening and evaluation of suspected cancer. However, additional invasive examinations are usually performed afterward.

Advances in radiologic technology have led to the development of new computed tomography (CT) devices that allow reducing the radiation dose and increase sensitivity. The National Lung Screening Trial has shown superiority of a low-dose helical CT over chest radiograph in early detection of lung cancer in the elderly current and former heavy smokers in terms of mortality from lung cancer (NLSTRT 2011). A contrast-enhanced chest CT examination is routinely performed in patients with known or suspected lung cancer to enable the diagnosis and disease staging and may be followed by fiberoptic bronchoscopy or another invasive procedure, which are chosen based on the location of lesions and the spread of tumor (NICE 2011). In case of centrally located tumors, bronchoscopy allows for sampling pathologic material by means of forceps tissue biopsy, bronchial brushing or bronchoalveolar lavage (BAL). Peripheral lesions are often difficult to access. In cases, in which clinical and imaging characteristics suggest malignancy, video-assisted thoracoscopy biopsy, trucut biopsy under CT guidance or open thoracotomy may be used (Eberhardt et al. 2015; Vansteenkiste et al. 2013).

The aim of this study was to elucidate the diagnostic process in patients suspected of lung cancer. Additionally, we assessed the usefulness of bronchofiberoscopy in diagnosing lung cancer in comparison with other methods.

2 Methods

This study was approved by the Ethics Committee of Wroclaw Medical University in Poland. All patients provided written informed consent to have their data used in the study. This is an observational study performed in a group of 110 patients (61 males, 49 females, and mean age of 67.7 $\pm$ 10.9 years) who were suspected for malignancy based on the chest CT. The study included patients diagnosed between January 1/2013 and June 15/2016. CT scans were obtained using a 128-slice Somatom Definition Scanner (Siemens AG; Munich, Germany). All patients underwent bronchofiberoscopy. The procedure was performed by three specialists with at least 5-year experience. Both BAL and endobronchial brushing (ENB) were performed in all cases, whereas forceps tissue biopsy (FTB) was taken when there were macroscopic mucous membrane abnormalities observed. In case of a negative bronchofiberoscopy result, invasive

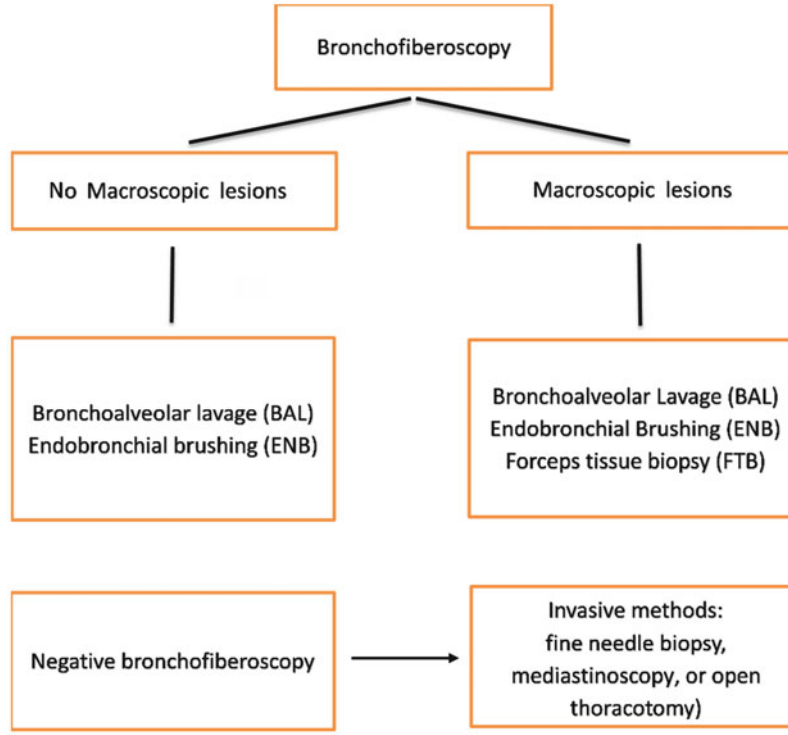

Fig. 1 Diagnostic algorithm of lung cancer employed in the study. *BAL*, bronchoalveolar lavage; *ENB*, endobronchial brushing; *FTB*, forceps tissue biopsy

methods were implemented, including fine needle biopsy, mediastinoscopy, or open thoracotomy. The diagnostic algorithm used in shown in Fig. 1. Histopathological material was then delivered to a pathomorphologist with over 15-year experience. BAL and ENB enable to define the presence of malignant cells, whereas forceps tissue biopsy provides the information about histopathological tumor structure. All data were collected in Excel sheets.

3 Results

Malignant neoplasm was diagnosed in 106 cases. A positive result (cancer cells) in bronchofiberscopy specimens was described in 45 subjects (42.0%). No severe complications of this procedure were observed. Mild complications including elevated body temperature and hemoptysis, were rare.

Cytology (BAL, ENB) was positive in 38 samples (35.8%) and histopathological examination (FTB) in 30 samples (28.3%). Based on BAL, 11 samples (10.3%) turned out positive, yielding nine non-small cell lung cancer (NSCLC), and one small cell (SCLC) and undifferentiated lung cancer each. Brushing was more effective than BAL yielding 27 positive samples (25.5%): twenty NSCLC, six SCLC, and one undifferentiated cancer. The FTB was performed in 33 patients (31.0%) providing the following diagnostic yield: twelve squamous cell carcinoma, nine SCLC, six adenocarcinomas, one lung carcinoid tumor, and three negative results; with the overall effectiveness of 90% (Fig. 2).

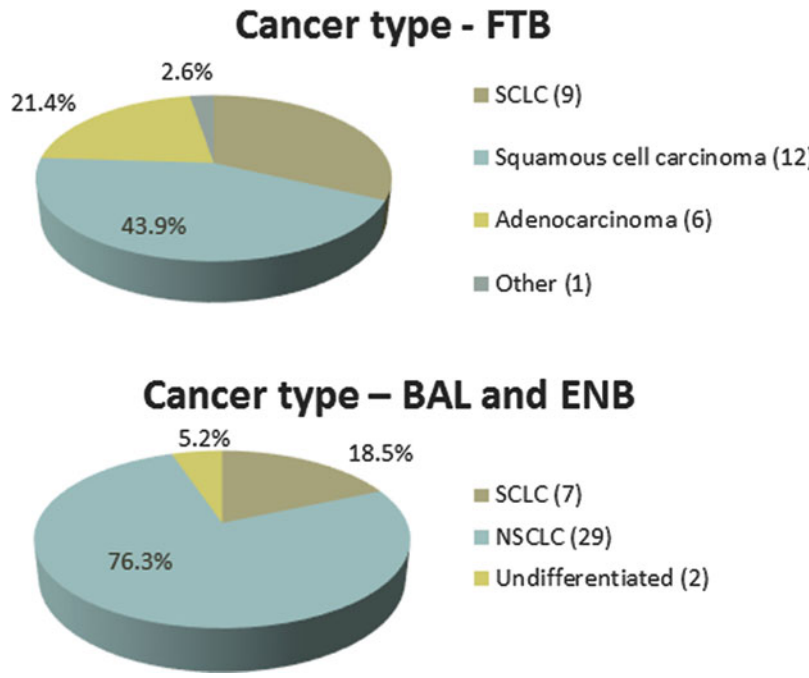

Fig. 2 Cancer types. *FTB*, forceps tissue biopsy; *BAL*, bronchoalveolar lavage; *ENB*, endobronchial brushing; *SCLC*, small cell lung cancer; *NSCLC*, non-small cell lung cancer

4 Discussion

The present study encompassed patients suspected for malignant tumors in the lungs based on the chest CT alone or in combination with symptoms suggestive of lung cancer. Further tests confirmed the presence lung cancer in 106 patients, which yields a true positive rate of 96.4%. This diagnostic result is comparable to that obtained for forceps tissue biopsy with a true positive rate of 90%, and much higher compared with the BAL and ENB results, which yielded a true positive rate 10.3% and 25.5%, respectively.

A high sensitivity of CT examination is due likely to the characteristics of the patients studied and the CT criteria for positive screening. Up-to-date, there is no consensus on the definition of a positive result which affects sensitivity of CT examination. In the present study, all patients referred to our institution had been suspected of lung cancer and presented symptoms of advanced stages of the disease. Oliveira and Saraiva (2010) have examined a cohort of 70 patients suspected of lung cancer who underwent chest CT and bronchoscopy. They confirmed cancer in 37 (52.9%) patients, which gives relatively low sensitivity. For low-dose CT screening tests, Toyoda et al. (2008) have shown sensitivity of 88.9% and specificity of 92.6%. Lung cancer was diagnosed when ground-glass opacity nodules were over 10 mm in diameter. Smaller lesions were observed but were not subjected to invasive treatment. In the NELSON study, scans with nodules of a diameter over 9.8 mm were regarded positive. Additionally, nodules between 5 and 9.8 mm were considered as suspected and observed. Such nodules were regarded positive in case of 25% growth in volume after 3 month (Horeweg et al. 2013). In the National Lung Screening Trial, non-calcified nodules or masses with diameter of at least 4 mm were considered a positive screening result (NLSTRT 2011).

Bronchofiberoscopy is generally regarded as a safe and effective way to diagnose and stage patients with lung cancer. BAL and ENB are often performed routinely in every patient suspected of lung cancer during bronchofiberoscopy. CT examination performed prior to

bronchofiberoscopy helps obtain samples of lesions located beneath the mucosa or pathologically changed lymph nodes. BAL is mainly used for cytological evaluation. Additionally, measurement of potential biomarkers including hepatocyte growth factor, interleukins, high mobility group proteins, and chemokine ligands in the bronchoalveolar lavage fluid and serum help diagnose different types of lung cancer (Jakubowska et al. 2015; Naumnik et al. 2016; Pastor et al. 2016). A diagnostic value of BAL differs for cancers depending on the location of lesions and their characteristics. The BAL test is based on the evaluation of cells exfoliated from malignant tumor. Lower sensitivity of BAL may be rooted in the fact that some of the lung tumors do not exfoliate cells useful for the diagnostic process. Bezel et al. (2016) have examined patients with suspected peripheral lung cancer. They showed that cytologic examination was truly positive in 18% and truly negative in 34% of cases. It had a sensitivity of 29% and a negative predictive value of 44% for the diagnosis of malignancy, while other bronchoscopic techniques, such as transbronchial forceps biopsy (sensitivity of 41%), bronchial washing (sensitivity of 36%), and bronchial brush biopsy (sensitivity of 38%) had a better diagnostic performance. Labbe et al. (2015) have reported the overall sensitivity of BAL at a level of 11.6% and ENB of 16.5%. They also revealed a higher diagnostic yield in patients with larger lesions, central/intermediate distance from the hilum and in case of the presence of a bronchus sign. The results of the present study are generally in line with the findings of other researchers as we found a lower overall sensitivity of BAL and ENB than that of forceps tissue biopsy.

Bronchial forceps biopsy can be performed either in case of a visible bronchial lesion or more distal, endobronchially invisible lesions. In the present study, forceps tissue biopsy was carried out when macroscopic mucous membrane abnormalities were seen during bronchofiberoscopy. This type of biopsy has a lower complication rate than open biopsy has. However, peripheral lung lesions often remain inaccessible. Binesh et al. (2015) have compared a diagnostic value of transbronchial lung biopsy with that of BAL in patients with pulmonary lesions suggestive of lung cancer. They confirmed a low specificity of BAL for detecting lung cancer, along with its usefulness in case of peripheral lesions. Thus, a combination of BAL with forceps biopsy may increase a positive diagnostic rate.

Introduction of advanced modalities such as endobronchial ultrasound transbronchial aspiration (EBUS-TBNA), video-assisted thoracoscopic lung biopsy, and virtual bronchoscopy enables to further improve the diagnostics. Those techniques supplement conventional examinations, especially in the assessment of lymph nodes involvement, staging of lung cancer, or in difficult cases in which the diagnosis could not be made based on other methods (Fukusumi et al. 2016; Khan et al. 2016; Adamczyk et al. 2013; Szlubowski et al. 2009; Silvestri et al. 1996). Despite technological advances, patients still benefit from the use of conventional bronchoscopic techniques such as BAL, brush and forceps biopsy. Combining conventional bronchoscopic techniques with new diagnostic modalities reduces the length of time between the first abnormal CT finding and the diagnosis of lung cancer, and also helps limit the number of consecutive procedures, which hastens the commencement of curative treatment (Binesh et al. 2015; Verma et al. 2015; Salomaa et al. 2005).

5 Conclusions

Bronchofiberoscopy is a safe diagnostic method for lung nodules, but its sensitivity and specificity are rather low, including both cytological and histopathological results. The efficacy of endobronchial brushing is better than that of BAL, even with the absence of macroscopic lesions. Only a third of performed endoscopies enables to visualize lesions in the bronchial tree. When mucous macroscopic changes are observed, a precise histopathologic diagnosis may be made based on forceps tissue biopsy whose effectiveness is very high, reaching 90%.

The most common cancer type in this study was NSCLC; squamous cell carcinoma subtype. In almost 80% of cases, alternative methods of sampling were required for qualification to oncological treatment.

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

References

Adamczyk M, Tomaszewski G, Naumczyk P, Kluczewska E, Walecki J (2013) Usefulness of computed tomography virtual bronchoscopy in the evaluation of bronchi divisions. Pol J Radiol 78 (1):30–41

Beckles MA, Spiro SG, Colice GL, Rudd RM (2003) Initial evaluation of the patient with lung cancer: symptoms, signs, laboratory tests, and paraneoplastic syndromes. Chest 123(1 Suppl):97S–104S

Bezel P, Tischler V, Robinson C, Baumueller S, Bode-Lesniewska B, Kohler M, Freitag L, Franzen D (2016) Diagnostic value of bronchoalveolar lavage for diagnosis of suspected peripheral lung cancer. Clin Lung Cancer 17(5):e151–e156

Binesh F, Pirdehghan A, Mirjalili MR, Samet M, Majomerd ZA, Akhavan A (2015) Comparative assessment of the diagnostic value of transbronchial lung biopsy and bronchoalveolar lavage fluid cytology in lung cancer. Asian Pac J Cancer Prev 16 (1):201–204

Dela Cruz CS, Tanoue LT, Matthay RA (2011) Lung cancer: epidemiology, etiology, and prevention. Clin Chest Med 32(4):605–644

Eberhardt WE, De Ruysscher D, Weder W, Le Pechoux C, De Leyn P, Hoffmann H, Westeel V, Stahel R, Felip E, Peters S, Panel Members (2015) 2nd ESMO consensus conference on lung cancer: locally advanced stage III non-small-cell lung cancer. Ann Oncol 26(8):1573–1588

Fukusumi M, Ichinose Y, Arimoto Y, Takeoka S, Homma C, Matsuoka H, Mouri A, Hamamoto Y, Matsumoto J, Kamimura M (2016) Bronchoscopy for pulmonary peripheral lesions with virtual fluoroscopic preprocedural planning combined with EBUS-GS: a pilot study. J Bronchol Interv Pulmonol 23(2):92–97

Horeweg N, van der Aalst CM, Vliegenthart R, Zhao Y, Xie X, Scholten ET, Mali W, Thunnissen E, Weenink C, Groen HJ, Lammers JW, Nackaerts K, van Rosmalen J, Oudkerk M, de Koning HJ (2013) Volumetric computed tomography screening for lung cancer: three rounds of the NELSON trial. Eur Respir J 42(6):1659–1667

Jakubowska K, Naumnik W, Niklinska W, Chyczewska E (2015) Clinical significance of HMGB-1 and TGF-beta level in serum and balf of advanced non-small cell lung cancer. Adv Exp Med Biol 852:49–58

Khan KA, Nardelli P, Jaeger A, O'Shea C, Cantillon-Murphy P, Kennedy MP (2016) Navigational bronchoscopy for early lung cancer: a road to therapy. Adv Ther 33(4):580–596

Labbe C, Beaudoin S, Martel S, Delage A, Joubert P, Drapeau C, Provencher S (2015) Diagnostic yield of non-guided flexible bronchoscopy for peripheral pulmonary neoplasia. Thorac Cancer 6(4):517–523

Naumnik W, Naumnik B, Niklinska W, Ossolinska M, Chyczewska E (2016) Clinical implications of hepatocyte growth factor, interleukin-20, and interleukin-22 in serum and bronchoalveolar fluid of patients with non-small cell lung cancer. Adv Exp Med Biol 952:41–49

NICE (National Institute for Health and Care Excellence) (2011) Lung cancer: diagnosis and management. Clinical guideline (CG121). Available from http://www.nice.org.uk/guidance/CG121. Accessed 1 Sept 2016

NLSTRT (National Lung Screening Trial Research Team), Aberle DR, Berg CD, Black WC, Church TR, Fagerstrom RM, Galen B, Gareen IF, Gatsonis C, Goldin J, Gohagan JK, Hillman B, Jaffe C, Kramer BS, Lynch D, Marcus PM, Schnall M, Sullivan DC, Zylak CJ (2011) The National Lung Screening Trial: overview and study design. Radiology 258(1):243–253

Oliveira C, Saraiva A (2010) Comparative study between computed tomography and bronchoscopy in the diagnosis of lung cancer. Radiol Bras 43(4):229–235

Pastor MD, Nogal A, Molina-Pinelo S, Quintanal-Villalonga A, Melendez R, Ferrer I, Romero-Romero-B, De-Miquel MJ, Lopez-Campos JL, Corral J, Garcia-Carbonero R, Carnero A, Paz-Ares L (2016) IL-11 and CCL-1: novel protein diagnostic biomarkers of lung adenocarcinoma in bronchoalveolar lavage fluid (BALF). J Thorac Oncol. doi:10.1016/j.jtho.2016.07.026

Salomaa ER, Sallinen S, Hiekkanen H, Liippo K (2005) Delays in the diagnosis and treatment of lung cancer. Chest 128(4):2282–2288

Siegel RL, Miller KD, Jemal A (2016) Cancer statistics. CA Cancer J Clin 66(1):7–30

Silvestri GA, Hoffman BJ, Bhutani MS, Hawer RH, Coppage L, Sanders-Ciette A, Reed CE (1996) Endoscopic ultrasound with fine-needle aspiration in the diagnosis and staging of lung cancer. Ann Thorac Surg 61(5):1441–1445

Szlubowski A, Kuzdzal J, Kolodziej M, Soja J, Pankowski J, Obrochta A, Kopinski P, Zielinski M (2009) Endobronchial ultrasound-guided needle aspiration in the non-small cell lung cancer staging. Eur J Cardiothorac Surg 35(2):332–335

Toyoda Y, Nakayama T, Kusunoki Y, Iso H, Suzuki T (2008) Sensitivity and specificity of lung cancer screening using chest low-dose computed tomography. Br J Cancer 98(10):1602–1607

Vansteenkiste J, De Ruysscher D, Eberhardt WE, Lim E, Senan S, Felip E, Peters S, ESMO Guidelines Working Group (2013) Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 24(Suppl 6):vi89–vi98

Verma A, Lim AY, Tai DY, Goh SK, Kor AC, Dokeu Basheer AA, Chopra A, Abisheganaden J (2015) Timeliness of diagnosing lung cancer: number of procedures and time needed to establish diagnosis: being right the first time. Medicine (Baltimore) 94 (29):e1216

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Hospital Morbidity Database for Epidemiological Studies on Churg-Strauss Syndrome

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Abstract

Churg-Strauss syndrome or more accurately eosinophilic granulomatosis with polyangiitis (EGPA) is a small-vessel necrotizing vasculitis with a characteristic late-onset allergic rhinitis and asthma. The use of hospital morbidity database is an important element of the epidemiological analysis of this rare disease. The present study was undertaken to assess the incidence of EGPA and factors related to its epidemiology in Poland; the first analysis of the kind in Poland, enabling a comparison in the European context. This is a retrospective, population-based study using hospital discharge records with EGPA diagnosis, collected for a National Institute of Public Health survey covering the period from 2008 to 2013. The group consisted of 344 patients (206 females and 138 males) with the first-time hospitalization for EGPA. The major findings are that the annual incidence of EGPA in Poland was 1.5 per million (95% confidence intervals: 1.2–1.8), with the point prevalence of 8.8 per million at the end of 2013. A greater incidence of EGPA was observed in the regions with urban predominance. We conclude that discharge records may be a useful element of epidemiological studies on EGPA.

Keywords

ANCA-associated • Disease prevalence • Eosinophilic granulomatosis • Epidemiology • Morbidity • Polyangiitis • Vasculitis

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1 Introduction

The Churg-Strauss syndrome, recently renamed to eosinophilic granulomatosis with polyangiitis (EGPA) (Jennette et al. 2013), is a systemic necrotizing vasculitis which affects small-to-medium-sized vessels and is related to anti-neutrophil cytoplasmic antibodies (ANCA). The disease is often associated with late-onset severe allergic rhinitis or asthma, with eosinophilia in blood and tissue (Groh et al. 2015). Clinical picture and histologic examination of the organ involved, whenever available, are helpful in establishing diagnosis that should be best shaped on the basis of the appropriateness criteria of the American College of Rheumatology (Bloch et al. 1990; Masi et al. 1990) or Lanham's criteria (Lanham et al. 1984). Recently, recommendations have been made to provide physicians with tools for effective and individually tailored management of EGPA patients. Tissue biopsies from patients with suspected EGPA are also encouraged to enhance diagnostic yield and to help develop further targeted research (Groh et al. 2015).

The main EGPA manifestations include peripheral neuropathy (51.4%), ear, nose, and throat symptoms (48.0%), skin lesions (39.7%), lung infiltrates (38.6%), and cardiomyopathy (16.4%) (Comarmond et al. 2013). It has been reported that ANCA-positive patients at the time of diagnosis have a significantly higher prevalence of renal (53%) and skin involvement (93.3%), and of peripheral neuropathy in the form of mononeuritis multiplex (60%) compared to ANCA-negative with 7.7%, 57.1%, and 25.7%, respectively. On the better side, however, ANCA-positive patients experience cardiac manifestations less frequently as reported for the entire period of follow-up (33.3% vs. 68.6%; $p = 0.021$) (Sokolowska et al. 2014).

1.1 Incidence of EGPA

EGPA is a rare disease. The annual incidence is 0.8/1 million in Turkey (Pamuk et al. 2016), 0.6/million in southern Spain as of 2010 (Romero-Gomez et al. 2015), or 0.9 (95% CI: 0.0–1.7)/million in southern Sweden (Mohammad et al. 2009). In another study, the point prevalence of EGPA per million inhabitants within a defined population in southern Sweden was estimated at 14 (95% CI: 0.3–27.0) on January 1, 2003 (Mohammad et al. 2007). According to a medical register of vasculitis cases in a large region in northern Germany, annual incidence of EGPA was 2/million adults inhabitants (95% CI: 0.0–4.0) in 2002 (Reinhold-Keller et al. 2005), whereas in the UK it was 1.4/million (95% CI: 0.3–3.9) (Watts et al. 2008). In a retrospective study in the Burgundian population in France, annual EGPA incidence amounted to 1.2 new cases/million, with the average number of EGPA patients in the entire population of 11.3/million (Vinit et al. 2011). In yet another study, incidence of EGPA was estimated at 10.7/million (95% CI 5.0–17.0) in a French urban multiethnic population in 2000 (Mahr et al. 2004). The EGPA incidence in the Australian Capital Territory and southeastern New South Wales has been reported at a level of 2.2–2.3/million per year (Ormerod and Cook 2008). In Japan, 1866 patients were found suffering from EGPA in 2014, which gives the incidence of 17.8/million (Sada et al. 2014).

1.2 Hospital Morbidity Database for Epidemiological Studies

There is a growing interest in the development of tools and methods for the surveillance of chronic rheumatic diseases with the use of existing resources, such as hospital morbidity databases (Bernatsky et al. 2011). For instance, the prevalence of polymyositis and dermatomyositis has been ascertained on the basis of population-based administrative data obtained from the Quebec physician billing and hospitalization databases (Bernatsky et al. 2009). Data acquired from the 2010 national database of hospitalized patients of Thai Health Coding Center has been used to clarify the admission rate, disease determination, hospital mortality rate, length of stay, and

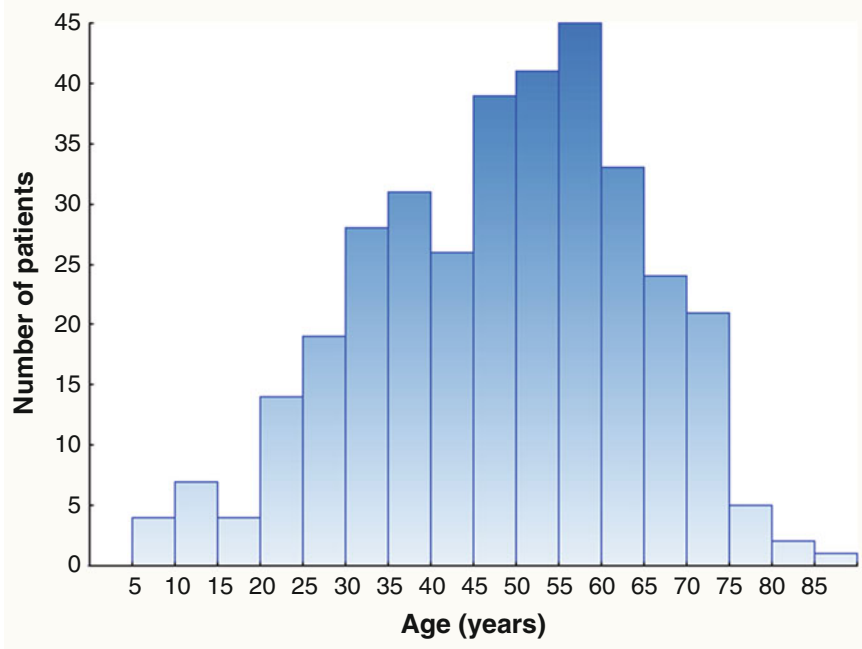

Fig. 1 Age distribution of patients with eosinophilic granulomatosis with polyangiitis (EGPA) during 2008–2013

hospital charges among patients diagnosed with systemic connective tissue disorders (Foocharoen et al. 2013). In another study, data on the patients hospitalized with rheumatic disease in California has been abstracted from a state hospitalization database (Ward 2004).

1.3 Study Aims

The present study seeks to determine the incidence rate and epidemiology of EGPA in Poland. The disease is one of the rarest, life-threatening systemic necrotizing vasculitides, predominantly affecting small vessels. There is little information regarding the epidemiology of EGPA in Poland. The use of hospital morbidity database is an important element of epidemiological analysis of this rare disease. This study is the first such evaluation in Poland and provides an opportunity to compare the incidence rate of EGPA in the European context.

2 Methods

The study was approved by an institutional Ethics Committee and it was conducted in accordance with the Declaration of Helsinki for Human Research. This is a retrospective, population-based study using the hospital discharge records with EGPA diagnosis, derived from the Polish National Institute of Public Health survey covering the period from 2008 to 2013. The survey was part of the European Hospital Morbidity Database that evaluates morbidity and hospital activity patterns in European counties (HMDB 2016). Data on all first-time hospitalized patients with EGPA diagnosis from all hospitals, except psychiatric and military ones, were evaluated in the study. These anonymous data included information on hospitalization with ICD10-coded diagnoses, date of admittance and discharge, birth date, sex, and the place of residence. The incidence rate was calculated using the number of EGPA patients and the corresponding census data. In addition,

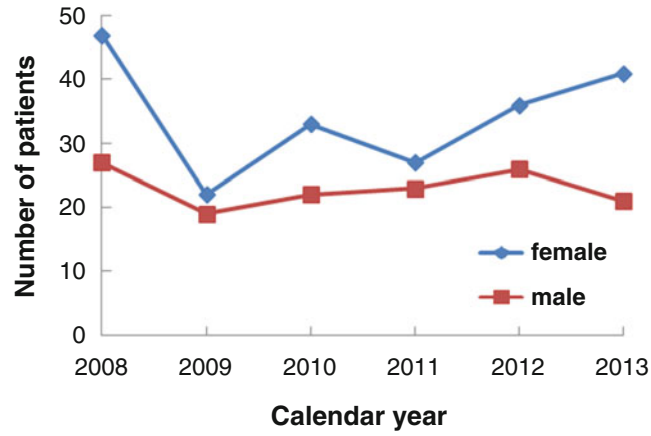

Fig. 2 Incidence of eosinophilic granulomatosis with polyangiitis (EGPA) by gender during 2008–2013

demographic data for the general Polish population were obtained from the Central Statistical Office in Poland (Central Statistical Office of Poland 2016). The EGPA, as a life-threatening systemic necrotizing vasculitis, often times requires hospitalization; thus, evaluation of hospitalized patients provides a good incidence estimate.

2.1 Statistical Analysis

Categorical data were expressed as percentages. Assuming the Poisson distribution of the cases analyzed, 95% confidence intervals (CI) were calculated. Non-parametric tests were used when the normality assumptions were unmet. A p-value of <0.05 defined statistically significant differences. The incidence rate was calculated using the number of disease cases divided as the population figure. All analyses were performed using StatSoft ver. 2012 and WINPEPI software (Abramson 2011).

3 Results

There were 953 cases of hospitalization with the EGPA diagnosis in the period of 2008–2013. Three hundred and forty four cases of those represented the patients with the first-time EGPA diagnosis. This group encompassed 138 male and 206 female patients, with the median age of 51 years. We failed to find any significant difference in patients' age in relation to gender. The majority of patients were in the sixth decade of life (Fig. 1). The number of EGPA cases by gender per year is shown in Fig. 2. The disease was significantly more common in females than males (60% vs. 40%, respectively; $p < 0.001$). There also were significant differences in the EGPA prevalence between urban and rural regions, with more cases in the former (237 patients) than latter region (107 patients); 69% vs. 31%, respectively, $p < 0.001$ (Fig. 3). The average annual incidence of EGPA in Poland was estimated at 1.5 per million (95% CI: 1.2–1.8) and the point prevalence at the end of 2013 was 8.8 per million. In the entire group of 953 hospitalized patients with EGPA, 10 deaths occurred in adult patients during the study period (six females and four males; mean age 65, min-max: 52–77 years). Six out of the 10 occurred in patients hospitalized with the first-time diagnosis of EGPA (four females and two males).

4 Discussion

Although some epidemiological studies on the EGPA incidence have been reported in

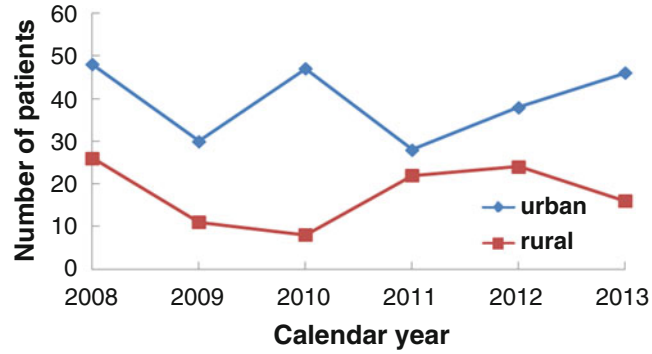

Fig. 3 Incidence of eosinophilic granulomatosis with polyangiitis (EGPA) by the place of living during 2008–2013

European countries, no such study has yet been performed in Poland. In the present study, we noted several interesting points concerning the EPGA epidemiology based on the inpatient discharge records. The estimation of the average annual incidence of EGPA in Poland amounted to 1.5 cases per million people. This finding is in line with those coming from other countries (Pamuk et al. 2016; Romero-Gomez et al. 2015; Sada et al. 2014; Vinit et al. 2011; Mohammad et al. 2009; Watts et al. 2008; Ormerod and Cook 2008). A population-based vasculitis register covering 2.78 million inhabitants of northern Germany over a period of 5 years has revealed a stable incidence of EGPA (Reinhold-Keller et al. 2005). Any fluctuations would be due to unknown factors and would not be related to environmental causes. The mean age of our patients with the first EGPA hospitalization was 49 years, which also was comparable to the 50.3 years in a study by Comarmond et al. (2013) or 55 years in that by Sada et al. (2014). We found that more patients lived in urban than rural areas, which could suggest the presence of risk factors associated with the environment or lifestyle. Such factors, however, could not be substantiated. By contrast, Herlyn et al. (2014) have found a significantly higher prevalence of EGPA in rural than urban regions in northern Germany.

The present study shows the male-to-female ratio of EGPA patients of 0.67. This ratio was 0.50 in a Japanese study (Sada et al. 2014). In another study, no gender predominance was noted, but female patients were significantly younger at the time of EGPA diagnosis compared with males (Comarmond et al. 2013). In yet another study performed in a large group of 2195 patients, a predominance of females has been found, with the participation of only 42.1% male patients (Hasegawa et al. 2015). In that study it has also been reported that in-hospital deaths occurred in 97 out of the 2195 patients (4.4%); a lower mortality rate was associated with female gender, and a higher rate concerned unscheduled admissions. In the present study, 10 deaths of EGPA patients (3%) took place. All deaths occurred in adults, six of whom were hospitalized with the first-time diagnosis of EGPA. A study by Phillip and Luqmani (2008) has reported a 68–100% survival rate for EGPA patients within 5 years. Comarmond et al. (2013) have reported a 5-year relapse-free survival rate of 58.1% (95% CI: 45.6–68.6) for ANCA-positive and 67.8% (95% CI: 59.8–74.5) for ANCA-negative patients; the difference between the two was statistically insignificant. That is in line with a report from a Polish tertiary referral center, which shows that the presence of ANCA does not appreciably affect the frequency of relapses, although ANCA status may cause differences in clinical presentation of EGPA (Sokolowska et al. 2014).

The present study has several limitations. The database used provided general epidemiological information regarding hospitalization of EGPA patients. Detailed risk factors and other correlates of the disease could not be assessed

in individual patients. Another limitation has to do with relying on the hospital discharge records, which may have excluded outpatients. The appearance of EGPA diagnosis in the discharge records is not necessarily the date of the first diagnosis. This inaccuracy may lead to an overestimation of the count of incident cases. However, a long observation time in this study may have mitigated any possible overestimation. The disease is a systemic necrotizing vasculitis with multi-organ involvement which usually requires advanced diagnostic procedures that can be done only in inpatient settings.

5 Conclusions

The average annual incidence of EGPA in Poland was estimated at 1.5 per million people (95% CI: 1.2–1.8), and the point incidence was 8.8 per million at the end of 2013. These figures are comparable to those reported for other European countries. Hospital discharge records may be a useful tool for epidemiological studies on EGPA, a life-threatening rare systemic vasculitis.

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

References

Abramson JH (2011) WINPEPI updated: computer programs for epidemiologists, and their teaching potential. Epidemiol Perspect Innov 8(1):1

Bernatsky S, Joseph L, Pineau CA, Belisle P, Boivin JF, Banerjee D, Clarke AE (2009) Estimating the prevalence of polymyositis and dermatomyositis from administrative data: age, sex and regional differences. Ann Rheum Dis 68:1192–1196

Bernatsky S, Lix L, Hanly JG, Hudson M, Badley E, Peschken C, Pineau CA, Clarke AE, Fortin PR, Smith M et al (2011) Surveillance of systemic autoimmune rheumatic diseases using administrative data. Rheumatol Int 31:549–554

Bloch DA, Michel BA, Hunder GG, McShane DJ, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Fries JF, Leavitt RY et al (1990) The American College of Rheumatology 1990 criteria for the classification of vasculitis. Patients and methods. Arthritis Rheum 33(8):1068–1073

Central Statistical Office of Poland. http://stat.gov.pl. Accessed 13 Oct 2016

Comarmond C, Pagnoux C, Khellaf M, Cordier JF, Hamidou M, Viallard JF, Maurier F, Jouneau S, Bienvenu B, Puéchal X et al (2013) Eosinophilic granulomatosis with polyangiitis (Churg-Strauss): clinical characteristics and long-term followup of the 383 patients enrolled in the French Study Group cohort. Arthritis Rheum 65:270–281

Foocharoen C, Thavornpitak Y, Mahakkanukrauh A, Suwannaroj S, Nanagara R (2013) Admission rate and characteristics of hospitalized systemic connective tissue disorders: analysis from a nationwide Thailand healthcare database. Int J Rheum Dis 16:41–46

Groh M, Pagnoux C, Baldini C, Bel E, Bottero P, Cottin V, Dalhoff K, Dunogué B, Gross W, Holle J et al (2015) Eosinophilic granulomatosis with polyangiitis (Churg-Strauss) (EGPA) Consensus Task Force recommendations for evaluation and management. Eur J Intern Med 26:545–553

Hasegawa W, Yamauchi Y, Yasunaga H, Sunohara M, Jo T, Matsui H, Fushimi K, Takami K, Nagase T (2015) Factors that predict in-hospital mortality in eosinophilic granulomatosis with polyangiitis. Allergy 70:585–590

Herlyn K, Buckert F, Gross WL, Reinhold-Keller E (2014) Doubled prevalence rates of ANCA-associated vasculitides and giant cell arteritis between 1994 and 2006 in northern Germany. Rheumatology (Oxford) 53:882–889

HMDB (2016) European Hospital Morbidity Database. Copenhagen, WHO Regional Office for Europe. http://www.euro.who.int/en/data-and-evidence/databases/european-hospital-morbidity-database-hmdb2. Accessed on 16 Oct 2016

Jennette JC, Falk RJ, Bacon PA, Basu N, Cid MC, Ferrario F, Flores-Suarez LF, Gross WL, Guillevin L, Hagen EC et al (2013) 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides. Arthritis Rheum 65:1–11

Lanham JG, Elkon KB, Pusey CD, Hughes GR (1984) Systemic vasculitis with asthma and eosinophilia: a clinical approach to the Churg-Strauss syndrome. Medicine 63:65–81

Mahr A, Guillevin L, Poissonnet M, Aymé S (2004) Prevalences of polyarteritis nodosa, microscopic polyangiitis, Wegener's granulomatosis, and Churg-Strauss syndrome in a French urban multiethnic population in 2000: a capture-recapture estimate. Arthritis Rheum 51:92–99

Masi AT, Hunder GG, Lie JT, Michel BA, Bloch DA, Arend WP, Calabrese LH, Edworthy SM, Fauci AS, Leavitt RY (1990) The American College of Rheumatology 1990 criteria for the classification of Churg-Strauss syndrome (allergic granulomatosis and angiitis). Arthritis Rheum 33:1094–1100

Mohammad AJ, Jacobsson LT, Mahr AD, Sturfelt G, Segelmark M (2007) Prevalence of Wegener's granulomatosis, microscopic polyangiitis, polyarteritis nodosa and Churg-Strauss syndrome within a defined population in southern Sweden. Rheumatology (Oxford) 46:1329–1337

Mohammad AJ, Jacobsson LT, Westman KW, Sturfelt G, Segelmark M (2009) Incidence and survival rates in Wegener's granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and polyarteritis nodosa. Rheumatology (Oxford) 48:1560–1565

Ormerod AS, Cook MC (2008) Epidemiology of primary systemic vasculitis in the Australian Capital Territory and south-eastern New South Wales. Intern Med J 38:816–823

Pamuk ON, Donmez S, Calayir GB, Pamuk GE (2016) The epidemiology of antineutrophil cytoplasmic antibody-associated vasculitis in northwestern Turkey. Clin Rheumatol 35:2063–2071

Phillip R, Luqmani R (2008) Mortality in systemic vasculitis: a systematic review. Clin Exp Rheumatol 26 (5 Suppl 51):94–104

Reinhold-Keller E, Herlyn K, Wagner-Bastmeyer R, Gross WL (2005) Stable incidence of primary systemic vasculitides over five years: results from the German vasculitis register. Arthritis Rheum 53:93–99

Romero-Gomez C, Aguilar-Garcia JA, Garcia-de-Lucas MD, Cotos-Canca R, Olalla-Sierra J, Garcia-Alegria JJ, Hernández-Rodríguez J (2015) Epidemiological study of primary systemic vasculitides among adults in southern Spain and review of the main epidemiological studies. Clin Exp Rheumatol 33 (2 Suppl 89):11–18

Sada KE, Amano K, Uehara R, Yamamura M, Arimura Y, Nakamura Y, Makino H, Research Committee on Intractable Vasculitides, the Ministry of Health, Labour, Welfare of Japan (2014) A nationwide survey on the epidemiology and clinical features of eosinophilic granulomatosis with polyangiitis (Churg-Strauss) in Japan. Mod Rheumatol 24:640–644

Sokolowska BM, Szczeklik WK, Wludarczyk AA, Kuczia PP, Jakiela BA, Gasior JA, Bartyzel SR, Rewerski PA, Musial J (2014) ANCA-positive and ANCA-negative phenotypes of eosinophilic granulomatosis with polyangiitis (EGPA): outcome and long-term follow-up of 50 patients from a single Polish center. Clin Exp Rheumatol 32(3 Suppl 82):41–47

Vinit J, Muller G, Bielefeld P, Pfitzenmeyer P, Bonniaud P, Lorcerie B, Besancenot JF (2011) Churg-Strauss syndrome: retrospective study in Burgundian population in France in past 10 years. Rheumat Int 31:587–593

Ward MM (2004) Decreases in rates of hospitalizations for manifestations of severe rheumatoid arthritis, 1983–2001. Arthritis Rheum 50:1122–1131

Watts RA, Scott DG, Jayne DR, Ito-Ihara T, Muso E, Fujimoto S, Harabuchi Y, Kobayashi S, Suzuki K, Hashimoto H (2008) Renal vasculitis in Japan and the UK--are there differences in epidemiology and clinical phenotype? Nephrol Dial Transplant 23:3928–3931

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Public Perception of the Risks Associated with Infectious Diseases in Poland: Ebola and Influenza and Their Impact on the Attitude to Vaccination

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Abstract

While the Ebola outbreak in 2014 was strongly highlighted in mainstream media and perceived as a threat to public health in Poland, influenza was regarded as a triviality and the vaccination coverage was low. In the present study, by analyzing feedback from an on-line questionnaire (from November 2014 to January 2015) we assessed the knowledge concerning Ebola and influenza together with attitudes to immunization of 544 respondents (45% medical staff). The findings were that 92.6% of respondents declared readiness to vaccination before traveling to endemic regions if a vaccine against Ebola would have existed, but adverse reactions, high costs, and low effectiveness would adversely affect that decision. While 84.2% of respondents declared awareness of influenza attributing significantly to the cause of death, only 65.4% considered influenza as an actual danger for people in Poland and 46.7% thought that Poland was not an endemic region for influenza. Nearly 23% declared that they were already vaccinated against influenza. The majority of respondents (67.5%) were not going to be vaccinated. We conclude that awareness of risk related to infectious diseases is an important determinant when deciding whether to vaccinate. However, negative information about the vaccine has some bearing on the decision to get vaccinated.

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Decision-making • Healthcare workers • Immunization • Prevention • Sociocultural epidemiology

1 Introduction

The outbreak of Ebola in 2014 was strongly highlighted in the mainstream media and caused a high level of fear in the European and American societies. While Ebola was perceived as a threat to public health in Poland, influenza was regarded as a triviality and the vaccination coverage rate was low in the 2014/2015 season, even lower than in the preceding season. We assumed that the most important reason for this situation was a false perception of a mild threat of influenza infection and media misinformation. Using an online questionnaire during the epidemic period of both Ebola and influenza infections (November 2014–January 2015) we compared the knowledge about the two and the attitude to prophylaxis by immunization, depending on the age, gender, and the level of general and medical education.

Ebola virus – a member of *Filoviridae* family – causes a severe hemorrhagic fever, further referred to as Ebola virus disease (EVD), which is characterized by suppression of the innate immune system. In general, outbreaks of filovirus hemorrhagic fevers occur with 20–90% lethality, depending on the viral species; an average EVD fatality rate is around 50%. The virus is transmitted to people from poorly defined wild animals and then spreads through human-to-human transmission *via* direct contact with bodily fluids. Today, there is no proven causative treatment available for EVD. Nor are there any licensed vaccines available, although potential vaccines have been undergoing human safety tests (Kanapathipillai 2014)

A dramatic course with gruesome death that frequently accompanies EVD has caused that the disease is etched deeply in the public imagination. All of Ebola outbreaks have originated in Africa. Because of a rapid fatal ending, outbreaks tended to be self-limiting. A recent epidemic in West Africa, which had begun in March 2014 and ended in March 2016, was the largest Ebola outbreak since the virus discovery in 1976. According to the WHO, there were 28,646 cases of EVD and 11,323 people died worldwide. The vast majority of cases took place in the endemic regions in West Africa, with only a few cases spread by air transportation to the US (4 cases, 1 patient died) and Europe (3 cases, no deaths) (WHO 2014b, 2015a)

There was a negative influence of excessive fear connected with EVD. Social panic resulted in irrational acts, exacerbating the outbreak and disease spreading, as well as stigma in population during the 2014 Ebola outbreak. The fear was enhanced by the mainstream media all over the world. As the epidemic was rising during the autumn months, Ebola was the main health-connected topic in press, TV and Internet portals also in Poland, a country with very low risk of EVD epidemic, where there were no cases of Ebola. In October 2015, EVD dominated public health concern, while another important infectious disease – influenza, was trivialized and forced out of the public debate.

Seasonal influenza (viruses A, B, and C) circulate and mutate causing respiratory infections all over the world with the annual prevalence estimated at 5–10% in adults and 20–30% in children. They spread readily with air droplets from person to person. In Poland, as in other temperate climate countries, disease tends to occur seasonally in winter months causing annual epidemics. Classical symptoms include acute fever, malaise, cough, muscle and joint aches, coryza, and runny nose. Children up to 2 years of age, elderly people, pregnant women, the morbidly obese and people with chronic conditions, such as diabetes or immunodeficiency, are at risk of complications and death. It

is estimated that annual epidemics result in about 3–5 million cases of severe disease and about 250,000–500,000 deaths worldwide (WHO 2014a; Fiore et al. 2008) There were 3,164,405 in 2013 and 3,137,056 in 2014 cases of influenza and influenza-like disease reported in Poland. Hospitalization rate was 0.45% and 0.32%, respectively (Czarkowski et al. 2014, 2015). An updated vaccine against seasonal influenza is available every year. Except children below the age of 6 months, vaccination is universally recommended. It is especially important for people at high risk of serious influenza complications, and for people who live with, or care for, high-risk individuals, including healthcare workers. Even though it has been demonstrated that vaccination is the most effective prophylaxis, its coverage is low in most Eastern European countries (Fiore et al. 2008). Likewise, despite many educational campaigns in Poland, vaccination rate is far below the WHO indicated threshold of 75% of high-risk populations. In 2010 alone, 5.2% of general population and only 16% of elderly population was vaccinated against influenza. Vaccination rate of medical staff was as much disappointingly low amounting to about 6%.

Studies demonstrate that numerous factors bear on the decision to get vaccinated. One of them is the knowledge on the preventability of a disease and its source (Nyhan et al. 2014; Dubé et al. 2013; Scullard et al. 2010). Therefore, in this study we seek to determine the level of knowledge on influenza and on EVD, and the perception of risk of contracting these diseases in the general public and specifically in healthcare workers.

2 Methods

2.1 Study Population

The study was approved by the Ethics Committee of Wroclaw Medical University in Poland. The cohort investigated consisted of 552 subjects, aged 18–50 odd years, 73% of women, who were surveyed with an online questionnaire. Participation in the study was on a voluntary basis. The majority of respondents (74%) declared having a master's degree and 45% of them were medical staff. The questionnaire was hosted by MySurveyLab.com and was available online. Of the 552 participants who responded to the questionnaire, eight provided the returns after the collection deadline, which was January of 2015; those data were discarded. Therefore, the final population sample consisted of 544 subjects. Basic demographics and characteristics are presented in Table 1.

2.2 Measures

The questionnaire was distributed to a varied group of respondents by social media services and *via* e-mailing. It consisted of the following parts:

- Items requesting socio-demographic information, including gender, age and education;
- Questions about Ebola and approach to Ebola vaccination if it existed, and about the attitude to participation in research on Ebola vaccination;
- Questions about influenza and the attitude to immunization against it.

Table 1 Characteristics of study subjects

	n = 544	%
Gender		
Male	147	73.0
Female	397	27.0
Age-groups (year)		
≤20	28	5.2
21–30	287	52.8
31–40	133	24.5
41–50	50	9.2
>50	46	8.5
Educational level		
Primary	5	0.9
Secondary	136	25.9
University	403	74.1
Medical staff	240	44.1

2.3 Data Analysis

The analyzed variables were qualitative in nature (nominal). Chi-squared tests were used to determine how selected categorical variables, including demographical factors, were related to the knowledge about Ebola and influenza and the attitude to vaccination. Chi-squared tests with Yates' correction and Fisher's exact test were also used. The odds ratio (OR) were calculated to show quantitatively how strong the association between the presence/absence of any two factors in the population was the correspondence analysis was used to determine the relationship between variables in multi-way tables. The level of statistical significance was set $\alpha = 0.05$. Statistica 10.0 software (StatSoft; Tulsa, OK) was used for data elaboration.

3 Results

3.1 Ebola Assessment

Ebola was perceived by 97.1% of respondents as a life-threatening condition and almost all of them (94.6% of healthcare professionals and 89.9% of others) considered traveling to the endemic regions of Ebola outbreak as dangerous because of infection risk. Medical education factored significantly in this perception ($Chi^2 = 3.95$; $p = 0.047$). In the general group, women answered more often than men that traveling to the endemic regions of Ebola is dangerous (94.2% *vs.* 86.4%, respectively; $Chi^2 = 8.99$; $p < 0.003$). A vast majority (88.1%) of respondents did not regard the Ebola virus as a real health threat to people living in Poland. Persons with a higher educational level disagreed more often about Ebola virus as a threat than those with lower education (primary or secondary school) (89.8% *vs.* 83.0%; $Chi^2 = 4.66$; $p < 0.031$).

A second question tested the attitude to Ebola vaccination in a hypothetical situation of its existence. As many as 92.7% of respondents declared that they would get vaccinated before traveling to the endemic region, while 15.0% declared that they would also get vaccinated while staying in Poland. The individual's age was connected with that decision as the youngest respondents were more inclined to get vaccinated while staying in the country than the older ones; 35.7% of persons aged <20 *vs.* no more than 16% of those in all the other age-groups ($Chi^2 = 11.20$; $p = 0.024$). Well-educated persons more often disagreed about getting vaccinated while staying in the country than those with lower education (87.8% *vs.* 77.3% respectively, $Chi^2 = 9.15$; $p = 0.0025$).

A third questions tested the attitude to vaccination (regardless of traveling or not to endemic regions) with regards to negative information about vaccination, including its adverse effects, were available. When, it was belived that, Ebola vaccine would cause intense pain in the arms and fever, only would 59.2% of all respondents (69.6% of healthcare workers/medical students and 50.0% of others) would get vaccinated. Medical education made a significant difference in this judgment ($Chi^2 = 21.35$; $p < 0.0001$; $OR = 2.3$). When the vaccine cost were rather high (*ca.* 100 $), 60.9% of all respondents (68.8% of health care workers/students and 54.6% of others) would still get vaccinated. Medical education made again a significant difference in this judgment ($Chi^2 = 11.25$; $p = 0.001$; $OR = 1.83$). When the effectiveness of vaccination was stated as being only 50%, only 48.2% of all respondents would get vaccinated (57.1% of health care workers/students and 40.4% of others; $Chi^2 = 14.80$; $p = 0.0001$; $OR = 1.96$).

Further questions tested the attitude to participation in clinical trials on Ebola vaccination. Only 28.5% of respondents declare that they would take part in such trial. Age influenced that decision and persons from the extreme age-groups (<20 and >50 years of age) declared their consent for participation in such research more often (42.9% and 45.7%, respectively) than the others. Among the persons who would participate in clinical trials, 42.6% would take part regardless of the nature of organizer, 38.1% would participate only in the non-profit international trials, and 19.4% would take part in trials

organized by a pharmaceutical company. This choice also was also influenced by age, as the trial was of no interest for people >50 years of age (23.9%). A majority of younger respondents, especially those <20 years of age, participated in the trials organized by the non-profit international organizations. Medical education influenced that decision as well. Healthcare workers/medical students more often declared a willingness to participate in trials organized by the non-profit international organizations – 13.8% *vs.* 8.8% of the others ($Chi^2 = 9.99$; $p = 0.019$). The majority (72.4%) who answered that they did not consider Ebola as a real threat to persons living in Poland declared that they would not take part in a clinical trial on Ebola vaccination.

3.2 Influenza Assessment

The majority of respondents (81.1%, including 88.8% of healthcare workers and 74.8% of the others) considered influenza as dangerous for general health. Medical education had a significant bearing on the recognition of influenza danger ($Chi^2 = 16.91$; $p < 0.0001$; OR = 2.66). Also, well-educated respondents agreed with that statement more often than the less educated (83.1% *vs.* 75.2% respectively; $Chi^2 = 4.30$; $p = 0.038$). Likewise, the majority (84.2%, including 92.9% of healthcare workers/medical students and 76.8% of the others) were aware of the potential fatality of influenza. Medical education was an obvious differentiating factor in this regard ($Chi^2 = 25.74$; $p < 0.00001$; OR = 2.66). The socio-demographic factors also influenced the knowledge on influenza fatality. Male *vs.* female gender (90.5% *vs.* 81.0% persons, respectively; $Chi^2 = 5.98$; $p = 0.014$), older *vs.* younger persons (96% aged 41–50 *vs.* 93.5% aged > 50; $Chi^2 = 10.75$; $p = 0.029$), and higher *vs.* lower education (86.6% *vs.* 77.3% of persons, respectively; $Chi^2 = 6.78$; $p = 0.01$) were all predictors for the knowledge on influenza fatality.

The next question tested the perception of influenza as a real threat to people living in Poland. The majority of respondents (65.4%, including 73.3% healthcare workers/medical students and 58.3% of the others) regarded influenza as a threat. Medical education was a significant factor enhancing this knowledge ($Chi^2 = 13.27$; $p < 0.0003$; OR = 1.97). Age was also a determining factor in providing a positive answer: 86.0% of persons aged 41–50 compared with 43.6% of those aged < 20, and 61.7% of those aged 21–30 ($Chi^2 = 13.41$; $p = 0.01$). Likewise, a higher level of education was another factor in determining a positive answer (68.2% *vs.* 57.5% of those with low education; $Chi^2 = 5.38$; $p = 0.02$).

A slight majority (53.3%) of respondents knew that Poland was an endemic region for influenza, but only 47.1% of healthcare workers/medical students were aware of this compared with the 57.6% of the others ($Chi^2 = 5.86$; $p = 0.015$; OR=0.66). The most striking result was that only 23.0% of respondents (37.1% of health care workers/medical students and 10.4% of the others) declared that they were vaccinated against influenza in the 2014/2015 season. The medical education greatly influenced respondent answers ($Chi^2 = 54.31$; $p < 0.00001$; OR = 5.06). Persons aged 41–50 and >50 were vaccinated more often (52.0% and 52.2%, respectively), while younger persons declared they were vaccinated less often ($Chi^2 = 56.25$, $p < 0.00001$). Likewise, better educated persons were vaccinated more often than those with less education (26.1% *vs.* 14.2%, respectively; $Chi^2 = 8.31$; $p = 0.004$).

The majority (67.5%) of respondents declared that they did not intend to be vaccinated in the current season. Age and medical education were the important factors. People aged < 40 intended to get vaccinated less often than the older ones ($Chi^2 = 64.87$; $p < 0.00001$), while healthcare workers/medical students intended to receive vaccination more often than the others (16.3% *vs.* 7.7%, respectively; $Chi^2 = 73.28$; $p < 0.00001$; OR = 2.66). Persons who considered influenza as a potentially deadly disease also were going to receive vaccination more often than the others ($Chi^2 = 11.34$; $p = 0.003$).

Nearly all respondents (97.5%) who declared that they would not get vaccinated against Ebola before traveling to endemic regions, even if such vaccination existed, also declared they were not vaccinated against influenza ($Chi^2 = 10.23$, $p = 0.001$). The vast majority of them (92.5%) also declared they were not going to get vaccinated against influenza ($Chi^2 = 11.96$; $p < 0.003$).

4 Discussion

Because of the rapid spread of an EVD and its significant negative life consequences, the WHO declared in August of 2014 the EVD outbreak a Public Health Emergency of International Concern (PHEIC) (IHR 2014). People in the endemic regions needed international support. There were also concerns about a possible wide spread of the epidemic, although the epidemiologic projections failed to directly envisage such a possibility, barring the cases in the epidemic regions (Bogoch et al. 2015). Indeed, only a few cases of EVD were brought to the US and Europe (four cases in the US, one in Italy, one in Spain, and one in the UK) *via* a commercial air travel in 2014. Even though the EVD was not a public threat in Europe or the US, it was considered in public debates as the most important health-related topic of the time (Gidado et al. 2015; Iliyasu et al. 2015; Lancet Editorial 2014). Social media, such as Twitter, played a huge role in the rapid worldwide spreading of information. During the initial 8 days of the Ebola epidemic in 2014 (from July 24 to August 1) an enormous number of 42,236 of English-language tweets (16,499 unique and 25,737 retweets) mentioning a word 'Ebola' were shared (Odlum and Yoon 2015). However, most Twitter-shared information was misleading and myth (Oyeyemi et al. 2014). Undoubtedly, mainstream and social media participated in creating a terrifying picture of Ebola in the public eye, enhancing fear.

To date, there are not many publications assessing the knowledge about EVD. Most of them have been conducted with healthcare workers and medical students and revealed that the knowledge about EVD is not conclusive especially outside the epidemiologic regions (Alfaki et al. 2015; Fazekas et al. 2015; Iliyasu et al. 2015; Lisk et al. 2015; Patiño-Barbosa et al. 2015) During the outbreak of EVD in 2014, the knowledge about the symptoms and ways of transmission was low in the general public, the level of fear was high, and in some situations labelled as a mass hysteria (Goldstone and Brown 2015; Kobayashi et al. 2015). In countries outside the epidemic regions a high level of fear and misperception of risk was conspicuous (Rübsamen et al. 2015). In the present survey, one in eight persons considered that EVD might be dangerous for people living in Poland. Similar observations have been made in the US where, despite health officials' assurance that it was unlikely to happen, four in ten Americans were afraid that there might be a large outbreak of EVD in their country (McCarthy 2014). In another study, which compared the attitude to EVD and influenza in the US, similar results were obtained (Whiteside et al. 2016). The authors have reported that nearly one in five persons thought there was some likelihood they could come down with EVD, although there was no tangible risk to support this assumption. Concerning influenza, nearly two thirds of participants had a risk factor for complications, and only 48% of participants admitted they were vaccinated. The conclusion was that the perception of viral illness risk is incongruent with the risk of illness or the use of a preventive vaccination. In Germany, the general level of fear has not been shown as high, but 7% of people changed their behavior in response to the EVD outbreak. Among those, 68.8% avoided contact with African people in public places and 26.6% avoided using public transportation (Rübsamen et al. 2015).

The lack of knowledge and fear factor were sited as often a source of problems and stigma for people coming to the US or Europe from Africa, even from places that are far from the exposed regions (Chang 2014; Mangan 2014; Schnirring 2014). The WHO has emphasized the need to improve the EVD-related health information to dispel misconceptions. At the same time, efforts have been made to establish treatment and

effective ways of prevention. To date, there is about 15 vaccines under development in North America, Europe, Russia, and China, with possibly four effective candidates. The two leading potential vaccines have begun human clinical trials in September of 2014 and are now in phase II/III trials in the three affected countries (Kanapathipillai 2014; WHO 2015b).

During the EVD outbreak in Nigeria in 2014, the acceptance of a hypothetical vaccination was assessed. The study has revealed an impressive acceptance of the vaccine. The willingness to pay for it was conspicuous (Ughasoro et al. 2015). Likewise, the present study revealed a similar willingness for vaccination before traveling to the endemic regions, but the likelihood of a high cost of vaccine decreased that willingness by about 40%. We also checked the attitude to the hypothetical vaccination if adverse reactions would appear and if the effectiveness would only be 50%. All these appeared important factors in refusal to vaccination. The fear of side effects is one of the most important factors influencing hesitancy to vaccinations (Dubé et al. 2013). The present study also revealed a lack of the ability to properly estimate the risk-benefit ratio in a population.

Concerning influenza, the present study revealed an insufficient level of knowledge in the general public on its gravely damaging or lethal potential. In the past, Poland had not been considered a region for influenza epidemics. A number of unvaccinated people and those not willing to be vaccinated exceeded those with an opposite, positive attitude toward vaccination. Even if someone is aware of influenza risk, he often avoids vaccination for irrational reasons. The same observation has been made by other authors in a study on pregnant women (Chamberlain et al. 2015). Despite many educational campaigns carried out, the knowledge on influenza is, generally, not satisfying. The vaccine coverage is far too low and it is hard to reach the 75% population threshold of vaccination (Lu et al. 2016; Kardas et al. 2011).

While evaluating the reasons for reluctance in getting vaccinated, the following main factors should be considered: knowledge, perceived importance of vaccination, risk perception, health professionals' recommendations, safety evaluation, and media broadcast content (Dubé et al. 2013). Physician's recommendation and attitude to vaccination are the strongest factors influencing the decision to get vaccinated. As Nessler et al. (2014) have revealed, patients of physicians who were themselves vaccinated, get vaccinated more often. The present study demonstrates a lack of sufficient knowledge about influenza and a low vaccination rate among the healthcare workers/medical students. This may be one reason underlying a low vaccination coverage in the general public as well. Another possible reason is a rising influence of mainstream media on the public opinion in Poland. During recent years, there apparently arise anti-vaccination movements observed in societies worldwide. The information on vaccination, lacking positive qualities, on the one side, and underlining the adverse effects and low effectiveness, on the other side, has been disseminated. A study by Scullard et al. (2010) simulating a patient's search for advice on the potential link between the measles-mumps-rubella (MMR) vaccine and autism, with the use the Google search engine, has shown that only 51% of websites provides correct information that the association between the MMR vaccine and autism has never been conclusively demonstrated. In Italy, three cases of death were blamed on the Fluad vaccine during the 2014/2015 influenza epidemic season (Odone et al. 2015). The information was rapidly disseminated in prime time of international TV broadcasting and in newspapers. When any connection between the deaths and the vaccine was disapproved, much less time was devoted to straightening up the previous misleading information. Such conduct is not neutral and obviously facilitates an apprehensive attitude toward vaccination. Another example of uneven media reporting on influenza is the H1N1 pandemic in Germany between April of 2009 and August of 2010. The approach to the subject was systematically examined. The media reaction dynamically changed and corresponded to the rising number of infections. Even though there

were thousands of articles on influenza, information avaliable on vaccinations appeared in just about 33% of articles (Husemann and Fischer 2015).

The public is overwhelmed by the amount of information on vaccines and is confused with discrepancies. In effect, difficulty arises in risk assessment and the decision to get vaccinated is postponed, which is often tantamount to not getting vaccinated at all. Unfortunately, healthcare workers, who are an important link in the decision-making chain on vaccination (Dubé et al. 2013), are not themselves the best sources of knowledge. Only 42% of Polish physicians do recommend vaccination against influenza to their patients. Most physicians are unaware of the influence they have on the patient's decision on influenza vaccination and it is evident that their recommendation does matter and is expected by patients. In the present study, the knowledge on influenza and vaccination among the healthcare professionals was better than that of laymen, but still highly unsatisfying bearing in mind their medical education. Similar observations have been previously made in other countries (Antón-Ladislao et al. 2015; Hollmeyer et al. 2009; Pearson et al. 2006). Therefore, raising the level of education regarding influenza and vaccination should be of prime importance in medical curriculum.

Social media can be a useful tool in fast communication and epidemiologic supervision. It is also a means of sharing medical knowledge with the general public. However, media should not be a source of misinformation, misinterpretation of facts, or bias. This, in particular concerns, widely available internet portals, which represent the major sources of health-related topics in modern times. Concerning both Ebola disease and influenza, mass media has had a significant influence in shaping the knowledge and attitude toward vaccination in the lay public, which unfortunately, at times, appeared skewed (Del Rio and Guarner 2015). Social media could actually help integrate disease surveillance, medical practice and outbreak management. Dissemination of factual health-related facts and risks linked to vaccine-preventable diseases is essential for shaping a positive attitude toward vaccinations, (Charles-Smith et al. 2015; Horne et al. 2015), although it is not always tantamount to acquiring a positive attitude to vaccination (Nyhan et al. 2014). Unfortunately for the time being, the role played by media is insufficient. The American Academies emphasize the need to improve the kind of public understanding on what science and research are all about, how clinical trials are conducted, and how to interpret scientific information in an impartial way (National Academies of Sciences, Engineering, and Medicine 2015).

5 Conclusions

- Mainstream and social media greatly influence public opinion and awareness of emerging diseases.
- Influenza seems a concealed threat as it is usually regarded as nonfatal and devoid of major complications, despite the evidence to the contrary that it is in fact a prevalent endemic infection.
- The lay public is hardly able to properly estimate the risk-benefit ratio related to vaccination. Excessive fear of adverse effects and economic costs deter many from being vaccinated, causing up to two-fold effect in the number of people willing to get immunized against deadly diseases.
- Although education is a key factor influencing the decision to get vaccinated, this knowledge rarely translates into action. Unfortunately in the case of doctors being vaccinated against influenza, the number still remains at a disappointingly low level. By far the best way to attract new vaccinees would be of course for doctors to take the lead in they themselves getting vaccinated.

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

References

Alfaki MM, Salih AM, Elhuda DA, Egail MS (2015) Knowledge, attitude and practice of health care providers toward Ebola virus disease in hotspots in Khartoum and White Nile states, Sudan, 2014. Am J Infect Control 44:20–23

Antón-Ladislao A, García-Gutiérrez S, Soldevila N et al (2015) Visualizing knowledge and attitude factors related to influenza vaccination of physicians. Vaccine 33:885–891

Bogoch II, Creatore MI, Cetron MS et al (2015) Assessment of the potential for international dissemination of Ebola virus via commercial air travel during the 2014 West African outbreak. Lancet 385:29–35

Chamberlain AT, Seib K, Ault KA et al (2015) Factors associated with intention to receive influenza and tetanus, diphtheria, and acellular pertussis (TDAP) vaccines during pregnancy: a focus on vaccine hesitancy and perceptions of disease severity and vaccine safety. PLoS Curr 7. doi:10.1371/currents.outbreaks.d37b61bceebae5a7a06d40a301cfa819

Chang D (2014) African students scheduled to start at NJ school will stay home past waiting period amid Ebola concerns. http://www.nbcphiladelphia.com/news/local/Ebola-Fears-and-Arrival-of-2-African-Students-Prompt-Parents-to-Ke. Accessed10 May 2016

Charles-Smith LE, Reynolds TL, Cameron MA, Conway M, Lau EH, Olsen JM, Pavlin JA, Shigematsu M, Streichert LC, Suda KJ, Corley CD (2015) Using social media for actionable disease surveillance and outbreak management: a systematic literature review. PLoS One 10(10):e0e139701. doi:10.1371/journal.pone.0139701

Czarkowski M, Cieleba E, Kondej B, Staszewska E (2014) Infectious diseases and poisonings in Poland in 2013. National Institute of Public Health – National Institute of Hygiene – Department of Epidemiology, Warsaw

Czarkowski M, Cieleba E, Kondej B, Staszewska E (2015) Infectious diseases and poisonings in Poland in 2014. National Institute of Public Health – National Institute of Hygiene – Department of Epidemiology, Warsaw

Del Rio C, Guarner J (2015) Ebola: implications and perspectives. Trans Am Clin Climatol Assoc 126:93–112

Dubé E, Laberge C, Guay M, Bramadat P, Roy R, Bettinger JA (2013) Vaccine hesitancy: an overview. Hum Vaccin Immunother 9:1763–1773

Editorial L (2014) The medium and the message of Ebola. Lancet 384(9955):1641. doi:10.1016/S0140-6736(14)62016-X

Fazekas B, Moledina M, Fazekas J, Karolyhazy B (2015) Ebola virus disease: awareness among junior doctors in England. J Hosp Infect 90:260–262

Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, Bresee JS, Cox NJ (2008) Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 57(RR-7):1–60

Gidado S, Oladimeji AM, Roberts AA et al (2015) Public knowledge, perception and source of information on Ebola virus disease – Lagos, Nigeria; September, 2014. PLoS Curr 7. doi:10.1371/currents.outbreaks.0b805cac244d700a47d6a3713ef2d6db

Goldstone BJ, Brown B (2015) The role of public knowledge, resources, and innovation in responding to the Ebola outbreak. Disaster Med Public Health Prep 9:595–597

Hollmeyer HG, Hayden F, Poland G, Buchholz U (2009) Influenza vaccination of health care workers in hospitals – a review of studies on attitudes and predictors. Vaccine 27:3935–3944

Horne Z, Powell D, Hummel JE, Holyoak KJ (2015) Countering antivaccination attitudes. Proc Natl Acad Sci U S A 112:10321–10324

Husemann S, Fischer F (2015) Content analysis of press coverage during the H1N1 influenza pandemic in Germany 2009-2010. BMC Public Health 15:386

IHR 2014 Emergency Committee Members and Advisers. Statement on the 1st meeting of the IHR Emergency Committee on the 2014 Ebola outbreak in West Africa. WHO. http://www.who.int/mediacentre/news/statements/2014/ebola-20140808/en/. Accessed 24 June 2015

Iliyasu G, Ogoina D, Otu AA, Dayyab FM, Ebenso B, Otokpa D, Rotifa S, Olomo WT, Habib AG (2015) A multi-site knowledge attitude and practice survey of Ebola virus disease in Nigeria. PLoS One 10(8):e0135955. doi:10.1371/journal.pone.0135955.s001

Kanapathipillai R (2014) Ebola virus disease – current knowledge. N Engl J Med 371(13):e18. doi:10.1056/NEJMp1410741

Kardas P, Zasowska A, Dec J, Stachurska M (2011) Reasons for low influenza vaccination coverage – a cross-sectional survey in Poland. Croat Med J 52:126–133

Kobayashi M, Beer KD, Bjork A et al (2015) community knowledge, attitudes, and practices regarding Ebola virus disease – five counties, Liberia, September-October, 2014. MMWR Morb Mortal Wkly Rep 64:714–718

Lisk C, Snell L, Haji-Coll M, Ellis J, Sufi S, Raj R, Sharma A, Smith C (2015) Doctors' knowledge of Viral haemorrhagic fevers. Acupunct Med 14(2):47–52

Lu PJ, O'Halloran A, Ding H, Srivastav A, Williams WW (2016) Uptake of influenza vaccination and missed opportunities among adults with high-risk conditions, United States, 2013. Am J Med 129(6):636.e1–636.e11. doi:10.1016/j.amjmed.2015.10.031

Mangan D (2014) Texas college rejects Nigerian applicants, cites Ebola cases. http://www.cnbc.com/id/102087542#. Accessed 24 May 2015

McCarthy M (2014) Four in 10 US people fear large outbreak of Ebola. BMJ 349:g5321. doi:10.1136/bmj.g5321

National Academies of Sciences, Engineering, and Medicine (2015) Strategies to link knowledge with action. In: Trust and Confidence at the Interfaces of the Life Sciences and Society. Does the Public Trust Science? National Academies Press (US), Washington DC

Nessler K, Krztoń-Królewiecka A, Chmielowiec T, Jarczewska D, Windak A (2014) Determinants of influenza vaccination coverage rates among primary care patients in Krakow, Poland and the surrounding region. Vaccine 32:7122–7127

Nyhan B, Reifler J, Richey S, Freed GL (2014) Effective messages in vaccine promotion: a randomized trial. Pediatrics 133(4):e835–e842. doi:10.1542/peds.2013-2365

Odlum M, Yoon S (2015) What can we learn about the Ebola outbreak from tweets? Am J Infect Control 43:563–571

Odone A, Chiesa V, Ciorba V, Cella P, Pasquarella C, Signorelli C (2015) Influenza and immunization: a quantitative study of media coverage in the season of the Fluad case. Epidemiol Prev 39(4 Suppl 1):139–145

Oyeyemi SO, Gabarron E, Wynn R (2014) Ebola, Twitter, and misinformation: a dangerous combination? BMJ 349:g6178. doi:10.1136/bmj.g6178

Patiño-Barbosa A-V, García-Ramírez V-AE, Arciniegas-Pantoja M, Rodriguez-Morales AJ, Paniz-Mondolfi AE (2015) Healthcare students' and workers' knowledge about epidemiology and symptoms of Ebola in one city of Colombia. J Hosp Infect 90:356–358

Pearson M, Bridges CB, Harper SA (2006) Healthcare Infection Control Practices Advisory Committee (HICPAC), Advisory Committee on Immunization Practices (ACIP). Influenza Vaccination of Health-Care Personnel. Recommendations of the Healthcare Infection Control Practices Advisory Committee (HICPAC) and the Advisory Committee on Immunization Practices (ACIP) MMWR Recomm Rep 24 (RR-2):1–16

Rübsamen N, Castell S, Horn J, Karch A, Ott JJ, Raupach-Rosin H, Zoch B, Krause G, Mikolajczyk RT (2015) Ebola risk perception in Germany, 2014. Emerg Infect Dis 21:1012–1018

Schnirring L (2014) WHO pushes back against Ebola-related flight bans. http://www.cidrap.umn.edu/news-perspective/2014/08/who-pushes-back-against-ebola-related-flight-bans. Accessed 30 May 2015

Scullard P, Peacock C, Davies P (2010) Googling children's health: reliability of medical advice on the internet. Arch Dis Child 95:580–582

Ughasoro MD, Esangbedo DO, Tagbo BN, Mejeha IC (2015) Acceptability and willingness-to-pay for a hypothetical Ebola virus vaccine in Nigeria. PLoS Negl Trop Dis 9(6):e0003838. doi:10.1371/journal.pntd.0003838

Whiteside LK, Fernandez R, Bammer J, Nichol G (2016) Perception of the risks of Ebola, Enterovirus-E68 and influenza among emergency department patients. Western J Emerg Med 17:391–395

WHO (2014a) Influenza (seasonal). http://www.who.int/mediacentre/factsheets/fs211/en/. Accessed 27 Oct 2015

WHO (2014b) Guinea: life after Ebola has new meaning for 2 survivors now helping others. http://www.who.int/features/2014/life-after-ebola/en/. Accessed 29 Oct 2015

WHO (2015a) Ebola situation report – 4 November 2015. http://apps.who.int/ebola/current-situation/ebola-situation-report-4-november-2015. Accessed 29 Oct 2015

WHO (2015b) Ebola vaccines. http://apps.who.int/medicines/ebola-treatment/emp_ebola_vaccines/en/index.html. Accessed 29 Oct 2015

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The Sentinel System as the Main Influenza Surveillance Tool

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Abstract

Poland has implemented the influenza surveillance system called Sentinel as of 2004. The system consists of both epidemiological and virological surveillance. It is an important tool for monitoring the situation in the entire country, coordinated by the National Influenza Center situated at the National Institute of Public Health-National Institute of Hygiene (NIPH-NIH) in Warsaw, Poland. In the 2015/2016 epidemic season, more than 1600 samples were tested in the Sentinel System, of which 38.6% were positive. The samples were evaluated in seven age-groups: 0–4, 5–9, 10–14, 15–25, 26–44, 45–64, and over 65 years of age. Significant differences were reported in the number of confirmed cases of infection caused by influenza and influenza-like viruses, depending on the age-group. The highest number of confirmed cases of infections was reported for the age range of 26–44 years, accounting for 30% of the total. In each age-group, the presence of infection caused by influenza-like viruses, collectively accounting for only 3.8% of all positive tests, was also confirmed. Weekly reports generated by the Sentinel System enable to determine and control a current influenza activity in the country, which is of essential importance in case of the emergence of a new strain with a pandemic potential.

Keywords

Surveillance • Sentinel • Influenza infection • Virology • Virus • Epidemic season

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1 Introduction

Over decades, the world has been attacked by many pandemics. The greatest havoc in the twentieth century was brought about by Spanish influenza, caused by the virus subtype A/H1N1/, which took place in the years 1918/1919. According to the latest data, it caused about 50 million deaths and large economic losses (WHO 2004). For this reason, the WHO launched an international influenza surveillance system in 1947. It served to observe changes in the structure of the influenza virus and to rapidly detect newly formed virus variants (Życińska and Brydak 2007). In 2011, the network of surveillance has evolved to be coordinated by the WHO Global Influenza Surveillance and Response System (GISRS) (Bednarska et al. 2016a, b).

The best proof of the importance of the preparation of virological and epidemiological reports are those drawn up in 1997 in the National Influenza Center in Hong Kong after the emergence of the avian influenza virus A/H5N1/ (Highly Pathogenic Avian Influenza – HPAI) (Brydak 2008). Thanks to the reports it was possible to react quickly and focus on preventing the spread of the virus. A quick reaction was essential because according to the latest WHO estimates, 60% of infections caused by this subtype are fatal (Brydak 2014). In 2003, the National Center of Influenza in Rotterdam identified a different dangerous subtype of the HPAI determined as A/H7N7/ (Elbers et al. 2004), which reaffirms the notion of the influenza virus constantly undergoing modifications. In view of this unpredictability and the virus spread in the world, National Influenza Centers have been created in many countries. By 2008, 122 such centers were in operation throughout the world (Brydak 2008). Currently, there are 142 National Influenza Centers that act as reference centers in different countries. In Poland, this center is placed at the National Institute of Public Health-National Institute of Hygiene (NIPH-NIH). Apart from the GISRS, surveillance of influenza activity in Europe also is conducted by the European Influenza Surveillance Network (EISN), which is overseen by the European Center for Disease Control and Prevention (ECDC). The EISN conducts both epidemiological and virological surveillance. Data on the situation in European countries are gathered in the platform of the European Surveillance System (TESS) that issues weekly reports assessing the current status of influenza spread in Europe (Bednarska et al. 2016a, b).

The Sentinel System of epidemiological and virological surveillance is in place in Poland. The initiation of the system was made possible after fulfilling the international requirements imposed by the European Influenza Surveillance Scheme (EISS) (Brydak 2008). Sentinel works in cooperation with general practitioners and 16 Voivodeship (province) Sanitary Epidemiological Stations (VSES) present. This enables a continuous observation of virological and epidemiological situation in the country (Romanowska and Brydak 2007). Family doctors, who make up at least 1% of all doctors, are obliged to collect clinical samples from patients and to report on the epidemiological situation in a given area (Woźniak-Kosek and Brydak 2013). The samples are then sent to the VSES where diagnostic tests are performed using biomolecular methods to confirm the presence of influenza and influenza-like viruses.

As of the 2013/2014 epidemic season, influenza surveillance has been modified by expanding the age-group stratification for recording infections with influenza and influenza-like viruses, which improves the clarity of results (Bednarska et al. 2016a, b). The results are sent weekly by representatives of VSES, enabling to report by the National Influenza Center to the GISRS and EISN networks. From the data collected, epidemiological reports are generated, posted every week on the website of the National Institute of Public Health-National Institute of Hygiene. The National Influenza Center, acting as a reference place, is responsible for controlling the performance of VSES by random checks to confirm the positive results of samples obtained from the VSES.

The goal of the present study was to investigate the efficacy of epidemiological data

elaboration in the Sentinel System with regard to the 2015/2016 influenza epidemic season in Poland.

2 Methods

2.1 Material

The study was approved by an institutional Ethics Committee and it was conducted in accordance with the Declaration of Helsinki for Human Research. In the 2015/2016 epidemic season, 1625 samples acquired by the country 16 VSES were tested in the Sentinel System. The clinical material was derived from swabs taken from the nose and throat. The results were evaluated in the following age-groups: 0–4, 5–9, 10–14, 15–25, 26–44, 45–64, and >65 years of age.

2.2 Isolation of RNA

RNA isolation was carried out using the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Corporation; Madison, WI) according to the manufacturer's instructions. The samples were suspended in physiological saline. The final volume of the sample after elution was 50 µl.

2.3 Real-Time Reverse Transcription Polymerase Chain Reaction (rRT-PCR)

The real-time reverse transcription (rRT) polymerase chain reaction (PCR) was performed using a Light Thermocycler 2.0 System (Roche Diagnostics; Rotkreuz, Switzerland). The final reaction volume in the capillaries was 20 µl; 5 µl of which was the previously isolated RNA. Primers and probes necessary to carry out the reaction were obtained through the Influenza Reagent Resource (IRR) program from the US Center for Disease Control (CDC). The reaction mixture consisted of bovine serum albumin (BSA) buffer, $MgSO_4$, RNase free water, and a SuperScript III/Platinum Taq Mix (Invitrogen Life Technologies-Thermo Fisher Scientific; Carlsbad, CA). Viruses included in the vaccine for the 2015/2016 epidemic season were the following: A/California/7/2009(H1N1)pdm09 and A/Switzerland/9715293/2013, and B/Phuket/3073/2013 functioned as a positive control. The negative control was RNase free water provided in the kit. Before the amplification process, in order to obtain cDNA, the RNA was subjected to reverse transcription (30 min 50 °C). The resulting DNA was subjected to a process of initiation (1 cycle 95 °C for 2 min), followed by 45 cycles of amplification: denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 20 s.

2.4 Conventional Multiplex RT-PCR

To confirm the genetic material of influenza-like viruses, RT-PCR reaction using an RV12 ACE Detection Kit (Seegene; Seul, South Korea) was performed. Influenza A and B viruses, human adenovirus, human respiratory syncytial A and B viruses, human metapneumovirus, human OC43 and 229E/NL63 coronaviruses, human parainfluenza 1, 2, and 3 viruses, and human rhinovirus A/B could be detected. Random hexamer-primed cDNA synthesis products were generated using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific; Carlsbad, CA) according to the manufacturer's instructions. Each cDNA preparation was subjected to the RV12 PCR procedure. Finally, amplicons were detected by gel electrophoresis.

3 Results

In the 2015/2016 epidemic season, 1625 tests were run in the Sentinel System, out of which 627 (38.6%) samples confirmed the presence of influenza and influenza-like viruses (Table 1). Infections caused only by influenza virus were present in 603 cases (37.1%). Influenza virus type A and B were the equally predominant contagions, accounting for 50.6% and 49.4% of

Table 1 Confirmations of influenza and influenza-like illness (ILI) in different age-groups

Age-group (years)	Influenza detections; n (%)	ILI detections; n (%)
0–4	75 (12.4)	6 (25.0)
5–9	145 (24.1)	1 (4.2)
10–14	33 (5.5)	0 (0)
15–25	53 (8.8)	4 (16.7)
26–44	181 (30.0)	7 (29.2)
45–64	92 (15.3)	6 (25.0)
>65	24 (4.0)	0 (0)

infections, respectively. Concerning the influenza A virus, the predominant subtype was A/H1N1/pdm09, which accounted for 44.1% of all infections. There was only one case (0.2%) of the presence of subtype A/H3N2/, and 6.3% of influenza A cases remained unsubtyped.

Positive samples were evaluated in the seven age-group division. The majority of confirmed influenza viruses were observed in the group of persons aged 26–44 – 181 (30.0%) cases and in children aged 5–9 – 145 (24.1%) cases, and the least in those aged over 65 (4.0%) (Table 1). Influenza virus type B predominated in the youngest groups of 0–4, 5–9, and 10–14 years of age, as opposed to subtype A/H1N1/pdm09 that predominated in the other age-groups (Table 2).

Most samples were tested in the province of Western Pomerania (n = 390) and the fewest in Malopolska (n = 12). Most confirmations of infection with influenza virus type A and subtype A/H1N1/pdm09 were recorded in the province of Podkarpackie, while the influenza virus type B was found most frequently in Western Pomerania (Table 3).

Influenza-like virus infections were confirmed only in 24 samples, which accounted for 1.5% of all those tested. The dominant influenza-like virus was RSV (41.7%). Moreover, the presence of the genetic material of viruses such as PIV3 (29.2%), adenovirus (12.5%) PIV1 (8.3%), PIV2 (4.2%), and human coronavirus (4.2%) was confirmed. The largest percentage of influenza-like viruses was reported in the group of persons aged 26–44 (29.2% of cases), while in those aged 10–14 and over 65 no influenza-like virus infections were reported (Table 2).

There were three co-infections detected in individuals of 7 (influenza type A and B), 12 (influenza A/H1N1/pdm09 and B), and 67 years of age (influenza A/H1N1/pdm09 and B).

4 Discussion

The Sentinel System is an important part of virological and epidemiological surveillance. Thanks to the data reported from VSES to the National Influenza Center of the National Institute of Public Health-National Institute of Hygiene (NIPH-NIH 2016), it is possible to determine the type of influenza and influenza-like viruses currently circulating in the population. That is essential for the proper selection of vaccine strains for the epidemic season and also provides information on the emergence of new viral subtypes and on antigenic changes in the circulating strains; all of which helps prevent the development of a pandemic.

Primary care doctors are the foremost participants of epidemiological surveillance. They are obliged to record cases of infections. The number of participating doctors ranges from 1 to 5% of all country physicians each season. The 2015/2016 season considered in the present study involved 524 primary care physicians; the number was comparable with those in previous seasons (Table 4). It is worth emphasizing that doctors work *pro publico bono* as they do not receive compensation for participation in surveillance.

The Sentinel System confirmed 627 cases of infections caused by influenza viruses in the 2015/2016 epidemic season. This figure is almost 2.7-fold greater than the 2014/2015 result and 3.5-fold greater than 2013/2014 result

Table 2 The number of confirmed cases of influenza and influenza-like viruses in seven age-groups in the 2015/2016 epidemic season in Poland

Age-group (years)	A[a]	A/H1N1/ pdm09	A/ H1N1	A/ H3N2	A total	B	A+B Total	ADV	RSV	PIV 1	PIV 2	PIV 3	hCoV	RhV	hMPV	Total ILI
0–4	1	32	0	0	33	42	75	0	4	0	1	1	0	0	0	6
5–9	6	32	0	1	39	106	145	0	0	0	0	1	0	0	0	1
10–14	0	6	0	0	6	27	33	0	0	0	0	0	0	0	0	0
15–25	10	19	0	0	29	24	53	0	2	0	0	2	0	0	0	4
26–44	9	96	0	0	105	76	181	2	2	0	0	2	1	0	0	7
45–64	9	70	0	0	79	13	92	1	2	2	0	1	0	0	0	6
>65	3	11	0	0	14	10	24	0	0	0	0	0	0	0	0	0
Total no of detections	38	266	0	1	305	298	603	3	10	2	1	7	1	0	0	24
Percent of total (%)	2.3	16.4	0.0	0.1	18.8	18.3	37.1	0.2	0.6	0.1	0.1	0.4	0.1	0.0	0.0	1.5
Percent of influenza viruses and of influenza-like viruses (%)	6.3	44.1	0.0	0.2	50.6	49.4	100	12.5	41.7	8.3	4.2	29.2	4.2	0.0	0.0	100

[a]*A* unsubtyped

Table 3 Samples tested in the Sentinel System stratified by Poland's provinces

Province	Samples (n)	Influenza virus confirmations				
		A[a]	A/H1N1	A/H1N1/pdm09	A(H3N2)	B
Podlasie	75	1	0	14	0	3
Kujawy-Pomerania	230	3	0	30	1	36
Pomerania	14	0	0	0	0	3
Lubuskie	85	0	1	13	0	11
Silesia	131	0	0	32	0	55
Swietokrzyskie	13	0	0	0	0	3
Małopolska	12	2	0	3	0	2
Lublin	31	2	0	9	0	4
Lodz	60	4	0	15	0	5
Warmia-Masuria	81	2	0	16	0	11
Opole	37	0	0	0	0	0
Greater Poland	177	2	0	28	0	31
Podkarpacie	162	19	0	63	0	11
West Pomerania	390	5	0	14	0	103
Mazovia	51	1	1	19	0	14
Lower Silesia	77	2	0	7	0	23
TOTAL	1625	43	2	263	1	314

[a]*A* unsubtyped.

Table 4 Comparison of Sentinel System data from the recent four epidemic seasons

Epidemic season	Samples tested; (n)	Confirmations; n (%)	Primary care physicians; (n)
2012/2013	1525	487 (31.9)	521
2013/2014	466	178 (38.2)	572
2014/2015	653	235 (36.0)	561
2015/2016	**1625**	**627 (38.6)**	**524**

(Table 4). That speaks for an extremely good and continuously increasing efficiency of the surveillance system in Poland, with passing years.

In the 2015/2016 epidemic season, same as in the preceding one, an equal predomination of influenza type A and B viruses was observed. However, subtypes of the influenza A virus differed, with A/H1N1/pdm09 predominating in 2015/2016 (16.4%) and A/H3N2/ (22.6%) in 2014/2015 (Bednarska et al. 2016a, b; WHO 2015). The results noted in Poland correspond well with those in other European countries, for instance, in Austria or Finland where the A/H1N1/pdm09 subtype also predominated (FluNews Europe 2016).

The present findings also underscore the possibility of regional variability in the influenza virus subtype. Type B virus predominated in western Poland whereas type A/H1N1/pdm09 did in eastern Poland (Table 3). This situation also was reflected in the results obtained in neighboring countries. On the western side, in Germany, type B virus predominated, whereas on the eastern side, in Ukraine, subtype A/H1N1/pdm09 predominated (FluNews Europe 2016), which is explicable by a readily spread of the virus across borders.

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

References

Bednarska K, Hallmann-Szelińska E, Kondratiuk K, Rabczenko D, Brydak LB (2016a) Novelties in influenza surveillance in Poland. Probl Hig Epidemiol (2):101–105 (Article in Polish)

Bednarska K, Hallmann-Szelińska E, Kondratiuk K, Brydak LB (2016b) Influenza surveillance. Postępy Hig Med Dosw 70:313–318 (Article in Polish)

Brydak LB (2008) Influenza, pandemic flu, myth or real threat? Rythm, Warsaw, 1–492 (Article in Polish)

Brydak LB (2014) Influenza – the greatest master of metamorphosis – constant puzzle. JHPOR 2:4–11

Elbers ARW, Fabri THF, de Vries TS, de Wit JJ, Pijpers A (2004) Koch G (2004) The highly pathogenic avian influenza A (H7N7) virus epidemic in the Netherlands in 2003 – lessons learned from the first five outbreaks. Avian Diseases: September 48(3):691–705

FluNews Europe (2016) Available from: https://flunewseurope.org/. Accessed 22 Nov 2016

NIPH-NIH (2016) Influenza and influenza-like illness in Poland Available from: http://wwwold.pzh.gov.pl/oldpage/epimeld/grypa/index.htm. Accessed 22 Nov 2016

Romanowska M, Brydak LB (2007) The role of surveillance in the fight against influenza including pandemic. Fam Med Prim Care Rev 9(3): 823–829 (Article in Polish)

WHO (2004) Wkly Epidemiol Rec 79:25–40

WHO (2015) Review of the 2014–2015 influenza season in the northern hemisphere. Available from: http://www.who.int/wer/2015/wer9023/en/. Accessed 22 Nov 2016

Woźniak-Kosek A, Brydak LB (2013) The epidemiological and virological surveillance of influenza in the Polish population – Sentinel. Fam Med Prim Care Rev 15(3):483–485

Życińska K, Brydak LB (2007) Influenza and flu prophylaxis – a current medical issue. Pol Arch Med Wewn 117(10): 464–469 (Article in Polish)

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The Activity of Influenza and Influenza-like Viruses in Individuals Aged over 14 in the 2015/2016 Influenza Season in Poland

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Abstract

Infections in every epidemic season induced by respiratory viruses, especially by the influenza virus, are the cause of many illnesses and complications which often end in death. The aim of this study was to determine the activity of influenza and influenza-like viruses in individuals aged over of 14 in Poland during the 2015/2016 epidemic season. A total of 5070 specimens taken from patients were analyzed. The presence of the influenza virus was confirmed in 40.2% of cases, among which the subtype A/H1N1/pdm09 (62.6% positive samples) predominated. The analysis of confirmed influenza and influenza-like viruses in individuals divided into four age-groups demonstrate that the highest morbidity was reported for the age ranges: 45–64 (13.1%) and 26–44 (12.6%) years. An increase in the number of influenza type B cases (23.7% positive samples), which was the main cause of morbidity in the age group 15–25 years, was noticeable. Given the epidemiological and virological data, the 2015/2016 season in Poland was characterized by increased activity of the influenza virus compared to the previous season. In the 2015/2016 season, there were more than 3.8 million cases and suspected cases of influenza and influenza-like illness, more than 15,000 hospitalizations, and up to 140 deaths.

Keywords

Adults • Epidemic Season • Influenza • Respiratory Infection • Respiratory Viruses • Upper Respiratory Tract

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1 Introduction

Influenza is an infectious viral disease of the respiratory system, caused by influenza viruses belonging to *Orthomyxoviridae*. There are three types of the influenza virus: A, B and C, among which infections caused by type C are asymptomatic. In every epidemic season, infections are induced by influenza virus type A and B, with usually a different course of illness for either type of virus, which also depends on the patient's age and the immune system's efficiency (Brydak 2008). Influenza A virus is divided into subtypes, depending on the combination of the surface antigens hemagglutinin (HA) and neuraminidase (NA). There are 18 types of HA and 11 types of NA described (Wu et al. 2014). Improperly treated infections caused by influenza viruses can lead to complications and consequently to death. Therefore, it is important to confirm the presence of influenza virus in a short period of time, using molecular biology methods (Bednarska et al. 2016b), which enables to apply antiviral drugs, i.e., neuraminidase inhibitors, soon after onset of symptoms (Fiore et al. 2011; Brydak 2008).

The 2015/2016 influenza season was characterized by an increase in the number of cases and suspected cases of the influenza and influenza-like viruses, compared to the 2014/2015 season. There has also been a significant increase in deaths due to complications of influenza, the number of which was up to 135 in adult patients, compared with the mere 11 fatal cases in the 2014/2015 season.

It is meaningful that in adult patients qualified to high-risk groups, e.g., those over 65 years of age, influenza may lead to exacerbations of chronic diseases and may be characterized by an acute fulminant course (Mastalerz-Migas et al. 2015; Brydak et al. 2009). However, the percentage of vaccinated population was only 2.39% in this age range in Poland in the epidemic season in question, despite the fact that the majority of local governments make the vaccination available free of charge for people over 50 years of age (Brydak et al. 2012).

The aim of the present study was to analyze the activity influenza and influenza-like viruses in people over the age of 14 in the 2015/2016 influenza season in Poland, according to the new reporting system (Bednarska et al. 2016a).

2 Methods

2.1 Collection of Specimens

The study was approved by an institutional Ethics Committee and it was conducted in accordance with the principles for biomedical human research set by the Declaration of Helsinki.

In the 2015/2016 influenza season, lasting from week 40 (01 Oct 2015) to week 34 (28 Aug 2016), a total of 8542 clinical specimens were tested. Of those specimens, 5070 were collected from people aged over 14, taken from patients in four age-groups: 15–25, 26–44, 45–64, and over 65 years of age, collected by the Sentinel and Non-Sentinel systems. Swabs from the nose and throat, and bronchial lavage fluid were analyzed in the Department Of Influezna Research, National Influenza Center (NIC) at the National Institute of Public Health – National Institute of Hygiene (NIPH-NIH) and in 16 Voivodeship (province) Sanitary Epidemiological Stations (VSES) in Poland. Specimens typed in VSES were sent to the NIC for the confirmation of the presence of influenza virus and determination of a viral subtype using real-time PCR (qRT-PCR).

2.2 Isolation of RNA

Viral RNA was isolated from a 200 μL sample suspended in phosphate buffered saline using Maxwell 16 Total Viral Nucleic Acid Purification Kit (Promega Corporation; Madison, WI) according to the manufacturer's instructions. The isolate was eluted in 50 μL of RNase-free water.

2.3 Reverse Transcription Polymerase Chain Reaction (RT-PCR)

The isolated material was analyzed using the RT-PCR to confirm the presence of influenza A and B viruses. A Transcriptor One-Step RT-PCR Kit (Qiagen; Venlo, The Netherlands) was used for the RT-PCR reaction. A 20 μL reaction mix, consisting of RNase free water, buffer, primer F (5 mM), primer R (5 mM), and the transcriptor enzyme mix, was incubated with 5 μL RNA isolated from a sample. For each tested sample, an internal control amplification was performed. Positive control consisted of RNA isolated from the reference viruses A/California/7/2009 (H1N1) and B/Phuket/3073/2013, recommended by the WHO for the 2015/2016 influenza season as the vaccine components. Negative control consisted of RNase-free water. Before amplification, isolated RNAs were reverse transcribed into cDNA at 50 °C for 15 min. cDNA was analyzed as follows: denaturation (1 cycle of 94 °C for 7 min), 45 cycles of amplification: denaturation at 94 °C for 10 s, annealing at 55 °C for 30 s, and elongation at 68 °C for 55 s. The reaction products were detected by gel electrophoresis.

2.4 Real-Time PCR (qRT-PCR)

qRT-PCR reactions were carried out in capillary tubes, using a Roche Light Cycler 2.0 System (Roche Diagnostics; Rotkreuz, Switzerland). The 15 μL reaction mix consisted of RNase free water, buffer, $MgSO_4$, 0.5 mM primers F and R, 0.2 μM probes and the SuperScript III/Platinum Taq Mix (Invitrogen Life Technologies – Thermo Fisher Scientific; Carlsband, CA), incubated with 5 μL sample of RNA per capillary tube. Positive control consisted of RNA isolated from the reference A/California/7/2009(H1N1), A/Switzerland/971593/2013(H3N2), and B/Phuket/3073/2013 viruses, and RNase-free water was used for negative control. Before the amplification step, isolated RNA were reverse transcribed into cDNA at 45 °C for 15 min. The cDNA was analyzed as follows: initialization step of 1 cycle at 95 °C for 2 min, followed by 45 cycles of amplification consisting of denaturation at 95 °C for 15 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 20 s.

2.5 Conventional Multiplex RT-PCR

The presence of influenza-like viruses was confirmed in two steps by RT-PCR using RV12 ACE Detection Kit (Seegene; Seul, South Korea). The material was tested for the following respiratory viruses: influenza virus A and B, human respiratory syncytial virus A (RSV A), human respiratory syncytial virus B (RSV B), human adenovirus (AdV), human metapneumovirus (hMPV), human coronavirus 229E/NL63, human coronavirus OC43, human parainfluenza virus 1, 2, and 3 (PIV-1, PIV-2, PIV-3), and human rhinovirus A/B (RhV A/B). Random hexamer-primed cDNA synthesis products were generated using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific; Carlsband, CA), according to the manufacturer's instructions. Each cDNA preparation was subjected to the RV12 PCR procedure according to the manufacturer's instructions (Seegene, Seoul, South Korea). Afterward, amplicons were detected by gel electrophoresis.

3 Results

The 2015/2016 epidemic influenza season in Poland was characterized by a high number of 3,864,731 cases and suspected cases of influenza and influenza-like viruses (ILI). There were 15,312 hospitalizations and 140 deaths due to complications of influenza, including 135 deaths among people over 14 years of age. The peak incidence of ILI occurred in week 8 of 2016. During the season more than 8500 samples were tested and more than 5000 of them were collected from people aged over 14. The distribution of confirmed cases of influenza viruses by

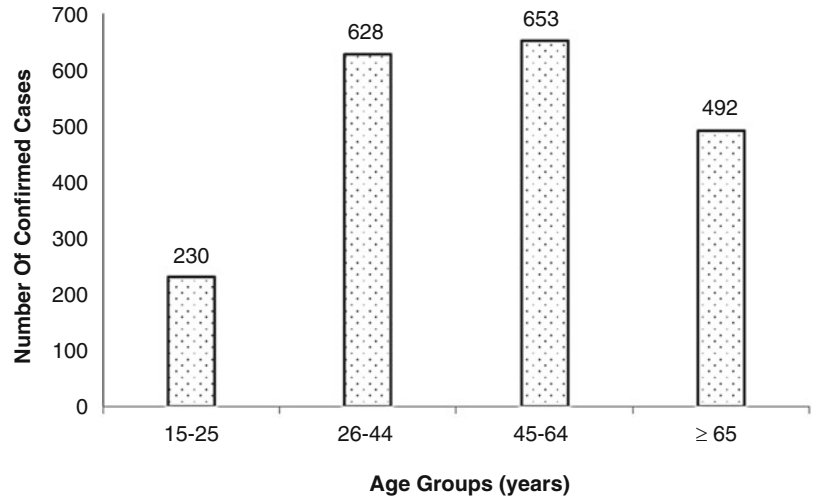

Fig. 1 Confirmed cases of influenza viruses in persons over 14 years of age in the 2015/2016 influenza epidemic season in Poland

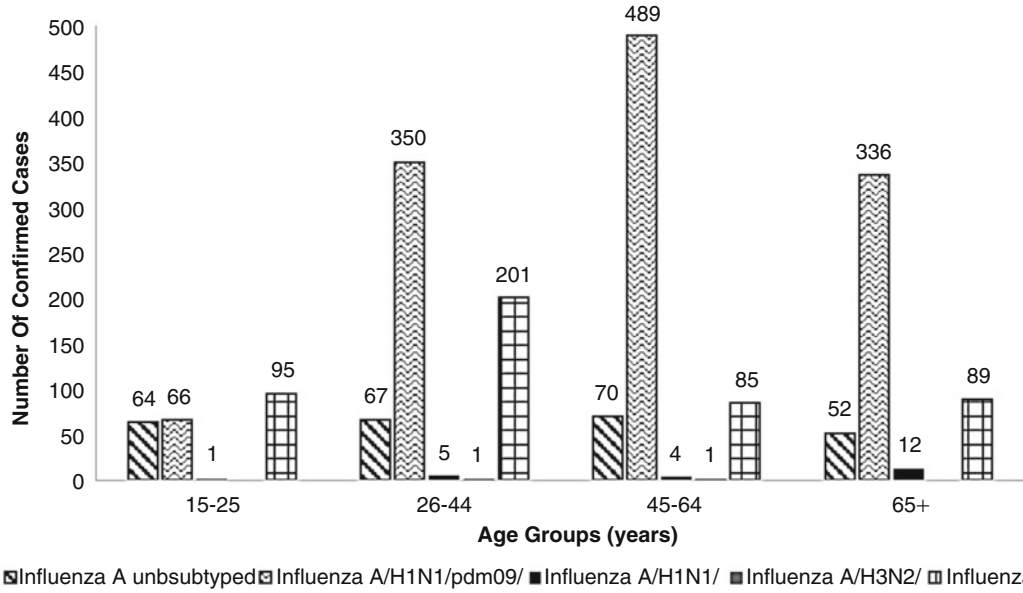

Fig. 2 Individual types and subtypes of influenza virus in persons over 14 years of age in the 2015/2016 influenza epidemic season in Poland

age and the viral type are shown in Figs. 1 and 2. The highest percentage of confirmations was observed in the persons aged 45–64 (13.1% of cases), less in those aged 26–44 (12.6%) and over 65 (9.9% of cases), while in the group of 15–25 years of age viruses were confirmed in 4.6% of cases. The dominant type was influenza A virus, subtype A/H1N1/pdm09. The highest number of confirmed cases of this subtype was reported in the persons aged 45–64 (n = 489), while in those aged 26–44 and over 65 the numbers of confirmed cases were comparable: 350 and 336, respectively. In the persons aged 15–25, subtype A/H1N1/pdm09 was confirmed in 66 cases. The subtype A/H1N1/ was detected in individual cases in all age-groups, with more than 10 cases present only in the persons aged over 65 (Fig. 1). Concerning the unsubtyped

Table 1 Epidemiological indicators of influenza and influenza-like infections (ILI) in the 2014/2015 and 2015/2016 epidemic seasons in Poland according to NIPH-NIH (2016)

Epidemic season	ILI cases (n)	Morbidity	Hospitalizations (n)	Deaths (n)	Specimens (n)
2014/2015	3,776,518	9,814,59	12,273	11	2416
2015/2016	3,864,731	10,054,65	15,312	140	8542

influenza virus type A, the number of confirmed cases was similar in all age-groups investigated, amounting to 64 cases in the persons aged 15–25, 67 cases in those aged 26–44, 70 cases in those aged 45–64, and 52 cases in those aged over 65. Concerning the subtype A/H3N2/, single cases were observed in persons aged 26–44 and 45–64 (Fig. 1).

There also were confirmed cases of influenza B in individuals over 14 years of age. This was actually the dominant type of influenza virus in persons aged 15–25. However, the highest number of cases of influenza virus type B was found in the persons aged 26–44 (n = 201), far fewer in those aged 15–25 years (n = 95), 45–64 years (n = 85), and over 65 years (n = 89) (Fig. 2).

In the 2015/2016 influenza season, there were just 16 cases of influenza-like viruses reported, with RSV (n = 6), PIV-3 (n = 4), and ADV (n = 3) reported most frequently and with individual cases of PIV 1, hCoV and RhV A/B virues.

4 Discussion

The number of 3,864,731 cases and suspected cases of influenza and influenza-like viruses in the 2015/2016 influenza epidemic season in Poland was comparable to that present in the preceding season (NIPH-NIH 2015/2016). However, the number of hospitalizations and deaths due to complications increased dramatically. The number of deaths increased from 11 in 2014/2015 to 140 in 2015/2016 (Table 1).

Despite a comparable number of cases and suspected cases of influenza and influenza-like viruses, the number of confirmed cases increased to 40.2% in 2015/2016 up from 21.2% in 2014/2015 (Bednarska et al. 2016). There also was a difference in the most frequent contagion between the two seasons, with subtype A/H1N1/pdm09 (62.6%) predominating in 2015/2016 as opposed to A/H3N2/ predominating the season before in persons over 14 years of age (Hallmann-Szelińska et al. 2016). The present study demonstrates that subtype A/H1N1/pdm09 predominated in all age groups, except for 15–24 years old persons in whom virus type B was dominant (Fig. 2). In both compared seasons, the highest incidence of influenza was reported in the age-groups of 45–64 and 26–44 years and the lowest one in the 15–24 years old persons (Fig. 1) (Bednarska et al. 2016b). Interestingly, the number of confirmed cases of influenza virus was the highest in the same age-groups of 45–64 and 26–44 years in the past 2013/2014 season (Bednarska et al. 2015). We also found that the activity of RSV and PIV-3 was increased in 2015/2016 compared with that in the season before (NIPH-NIH 2015/2016).

The following strains were included into the influenza vaccine in the 2015/2016 season: A/California/7/2009(H1N1)pdm09, A/Switzerland/9715293/2013(H3N2) and B/Phuket/3073/2013. A particular attention should be paid to the A/California/7/2009(H1N1)pdm09 strain which was a vaccine component since the 2010/2011 season, and also caused most influenza cases in persons over 14 years of age in the currently evaluated season. Given that the percentage of vaccinated population in this age range in Poland ranks at a dismally low level and has a decreasing trend from 1.95% in 2011 to 1.37% in 2015 (Epimeld 2016), it can be judged that that the recorded number of influenza cases reflects the immunodeficiency to this viral strain (Brydak 2015).

An increased number of confirmed cases of infection in the currently evaluated season was mainly caused by the influenza virus and to a lesser extent by influenza-like viruses, which

may lead to a severer disease course, complications, and in consequence to death. The death as a sequelae of influenza was due usually to complications arising from the underlying chronic comorbidities, especially cardiovascular and respiratory disorders that ended up in fulminant pneumonia.

The present findings of 40.2% of confirmed cases of influenza and influenza-like viruses in the 2015/2016 influenza season show a near doubling of laboratory confirmations compared with past seasons (Bednarska et al. 2016b; Bednarska et al. 2015). That result points to substantial improvements in infection surveillance and imbued nuances in the virological and epidemiological procedures despite a drastically low and inexplicably decreasing percentage of adult, mostly middle-aged, population getting vaccinated against influenza each epidemic season in Poland. The virological data presented in this article seek to call repeat attention to the need to vaccinate against influenza as the most effective method of preventing the infection and its severe complications, death included; a need advocated by the WHO, major medical societies, and 142 National Influenza Centers worldwide.

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References

Bednarska K, Hallmann-Szelińska E, Kondratiuk K, Brydak LB (2015) Evaluation of the activity of influenza and influenza-like viruses in the epidemic season. Adv Exp Med Biol 12:1–7

Bednarska K, Hallmann-Szelińska E, Kondratiuk K, Rabczenko D, Brydak LB (2016a) Novelties in influenza surveillence in Poland. Probl Hig Epidemiol 97(2):101–105

Bednarska K, Hallmann-Szelińska E, Kondratiuk K, Brydak LB (2016b) Antigenic drift of A/H3N2/virus and circulation of influenza-like viruses during the 2014/2015 influenza season in Poland. Adv Exp Med Biol 905:33–38

Brydak LB (2008) Influenza, pandemic flu, myth or real threat? Rythm, Warsaw, pp. 1–492 (in Polish)

Brydak LB (2015) Prophylaxis of influenza in general practice. Top Med Trends Guide Physician 1:9–11 (in Polish)

Brydak LB, Romanowska M, Nowak I, Ciszewski A, Bilińska ZT (2009) Antibody response to influenza vaccine in coronary artery disease: a substudy of the FLUCAD study. Med Sci Monit 15(7):PH85–PH91

Brydak LB, Roiz J, Faivre P, Reygrobellet C (2012) Implementating an influenza vaccination programme for adults aged ≥65 years in Poland. Clin Drug Investig 32:73–85

Epimeld (2016) Vaccinations in Poland in 2015. Available from: http://wwwold.pzh.gov.pl/oldpage/epimeld/index_p.html#05 (in Polish)

Fiore AE, Fry A, Shay D et al (2011) Antiviral agents for the treatment and chemoprophylaxis of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 60(RR01):1–24

Hallmann-Szelińska E, Bednarska K, Korczyńska M, Paradowska-Stankiewicz I, Brydak LB (2016) Virological characteristic of the 2014/2015 influezna season based on molecular analysis of biological material derived from I-MOVE study. Adv Exp Med Biol 857:45–40

Mastalerz-Migas A, Bujnowska-Fedak M, Brydak LB (2015) Immune efficacy of first and repeat trivalent influenza vaccine in healthy subjects and hemodialysis patients. Adv Exp Med Biol 836:47–54

NIPH-NIH (2015/2016) http://wwwold.pzh.gov.pl/oldpage/epimeld/grypa/index.htm. Accessed 22 Nov 2016

Wu Y, Wu Y, Tefsen B, Shi Y, Gao GF (2014) Bat-derived influenza-like viruses H17N10 and H18N11. Trends Microbiol 22(4):183–191

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Clinical Utility of Berlin Questionnaire in Comparison to Polysomnography in Patients with Obstructive Sleep Apnea

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Abstract

The aim of this study was to assess the utility of the Berlin questionnaire (BQ) in adult patients at high risk of obstructive sleep apnea (OSA). The study consisted of 64 patients recruited for the polysomnography diagnostics of sleep respiratory disturbances. The anthropometric assessment included body weight, height, and body mass index (BMI), all related to the risk of OSA. The BQ consisted of the following three categories: 1 – snoring, 2 – daytime somnolence, and 3 – hypertension. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were evaluated. Likelihood ratio was used to assess the diagnostic accuracy. We found that patients were, on average obese; the mean BMI amounted to 31.9 $\pm$ 6.0 kg/m^2. Polysomnography identified OSA in 73.4% of patients (AHI >5), where the BQ categorized 87.5% of patients at high risk of OSA. Sensitivity of the BQ was 87.2%, specificity 11.8%, PPV 73.2%, and NPV 25.0%. Diagnostic accuracy assessed by the likelihood ratio had a value of 1.00. The BQ had a false discovery rate of 31.2% and misclassification rate of 32.8%. We conclude that the BQ is a sensitive tool that should be used in clinical settings in which the benefit of high sensitivity outweighs the disadvantage of low specificity.

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Keywords
Apnea-hypopnea index • Diagnostic tool • Hypertension • Obesity • Obstructive sleep apnea • Polysomnography • Questionnaire • Sleep disordered breathing

1 Introduction

Obstructive sleep apnea (OSA) is a condition which typically requires lengthy and complex polysomnographic diagnostics (AASM 1999). The apnea-hypopnea index (AHI) forms the basis for the diagnosis and stratifies patients into mild (5.0–14.9), moderate (15.0–29.9), or severe (≥30.0) apnea-hypopnea events taking longer than 10 s per hour of sleep (Foster et al. 2009). The Berlin questionnaire (BQ) is a screening tool that captures OSA symptoms to identify patients at high risk of OSA (Enciso and Clark 2011) before going through a polysomnographic examination. It consists of 11 items that include such factors as snoring, wake-time sleepiness, fatigue, obesity, and elevated blood pressure (Ahmadi et al. 2008). The BQ is thought of as a suitable substitute for polysomnography that is uncomfortable for patients and pricey for healthcare services. Screening approaches, like rapid tests and questionnaires, are often used to facilitate clinicians' decisions of whether to subject a patient to more complex or invasive diagnostic procedures.

Clinical utility of the BQ has been recently questioned (Cowan et al. 2014; Iber et al. 2007). Therefore, in the present study we set out to assess the utility of this questionnaire in adult patients at high risk of OSA.

2 Methods

2.1 Study Population

The experimental protocol was approved by the Bioethics Committee of Poznan University of Medical Sciences. The study was performed in accordance with the Helsinki Declaration for Human Research. Written informed consent was obtained from all the subjects who agreed to participate in the study.

One hundred fifty three patients were screened due to a suspicion of OSA symptoms. Of that group, we qualified 64 individuals for admission to the Department of Pulmonology, Allergology and Respiratory Oncology at the Poznan University of Medical Sciences in Poland for the diagnostics of sleep-related respiratory disturbances. Patients were included in the study if they were over 18 years of age, had symptoms of OSA**,** were willing to participate in the study, and were on a habitual diet during the period of examination. The exclusion criteria included: pregnancy or lactation, cancer (excluding curatively treated with no evidence of disease for 5 years), severe liver and kidney diseases, and the diagnosed cardiovascular diseases such as myocardial infarct, stroke, or angina pectoris. Any active drug abuse, legal incompetence, and limited legal incompetence were additional exclusion criteria. Medical history, comorbidities, concomitant medications were recorded in the electronic database.

2.2 Assessment of Obstructive Sleep Apnea

Overnight polysomnography was used as a standard method for OSA diagnosis (Embla 4500; Beth Israel Deaconess Medical, Boston, MA). The recommendations of the American Academy of Sleep Medicine (AASM) (Oku and Okada 2008) regarding filters, sample signal rates, and configuration were followed. A respiratory flow trace was provided via a nasal cannula equipped with a thermistor. The thoracoabdominal motion was recorded with

piezoelectric bands. Oxygen saturation was measured with a pulse oximeter. Apnea was defined as a cessation of airflow for at least 10 s and hypopnea as an at least 80% reduction in airflow amplitude for at least 10 s (Sert Kuniyoshi et al. 2011). The AHI was defined as the total apneas plus hypopneas during total time asleep, divided by the number of hours asleep.

The BQ consists of three categories (category 1 – snoring, category 2 – daytime somnolence, category 3 – hypertension and body mass index (BMI)); all related to the risk of having sleep apnea. The BQ score was assessed based on the responses to ten questions from the three categories. Scores from the first and second category were assessed positively if the patient indicated frequent symptoms (>3–4 times/week). The score from the third category was positively evaluated when a history of hypertension or BMI >30 kg/m^2 was reported. If patients had a positive score in two or more categories, they were classified as being at high-risk for OSA. If only one or none category was scored positively, patients were classified being at low-risk (Cole 1990). Polysomnography and BQ were administered at the patient's first visit in the clinic.

Blood pressure (BP) was measured with a digital electronic tensiometer (Omron, Kyoto, Japan) after a resting period of 10 min. The mean of three consecutive measurements performed in the non-dominant arm at 3-min intervals was taken as the end-result. Regular or large adult cuffs were used, depending on the arm circumference of patients. BP measurements were performed in accordance with the guidelines of the European Society of Hypertension (Mancia et al. 2014).

2.3 Assessment of Covariates

Data on age, sex, smoking, educational status, and anthropometry were collected at the time of enrollment in the study using study-specific data collection tools. Anthropometric assessments included the measurement of weight and height. Weight was measured in light clothes without shoes and recorded to the nearest 100 g. Height was assessed in a similar manner to the nearest 1 cm. BMI was calculated to determine the degree of obesity (Netzer et al. 1999).

2.4 Statistical Analysis

Continuous variables were described as means ±SD, while categorical variables were described as percentages. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were evaluated. The likelihood ratio was used to assess the diagnostic accuracy. A p-value of less than 0.05 defined statistically significant changes All analysis were performed using SAS(R) 9.4 (Enterprise guide 6.1).

3 Results

The mean age of study participants was 56.6 ± 10.6 years and the mean BMI of 31.9 ± 6.1 kg/m^2 pointed to the prevailing obesity. Over 60% of subjects were smokers, and more than 50% had high school education (Table 1).

Polysomnography identified 73.4% of the patients as having OSA (AHI >5), while the

Table 1 Basic characteristics of studied patients (n = 64)

Parameter	Value
Age (years)	56.6 ± 10.6
BMI (kg/m^2)	31.9 ± 6.1
Smokers (%)	64.9
Education (%)	
Primary school	15.6
High school	54.7
University degree	29.7
AHI (events/h)	25.2 ± 22.7
SBP (mmHg)	126.9 ± 14.9
DBP (mmHg)	78.9 ± 8.4

BMI body mass index, *AHI* apnea/hypopnea index: normal (<5.0), mild (5.0–14.9), moderate (15.0–29.9), and severe (≥30.0 events per hour), *SBP* systolic blood pressure, *DBP* diastolic blood pressure

BQ categorized 87.5% of the patients as of high risk for OSA. There was no significant difference in the mean BMI value between OSA and non-OSA subjects identified according to the AHI value. However, BMI of subjects identified by the BQ as OSA was significantly higher than that of non-OSA subjects (Table 2). Moreover, a higher number of patients with OSA were identified by the BQ as hypertensive in comparison to the standard blood pressure measurement (Fig. 1).

Sensitivity of the BQ was 87.2%, specificity was 11.8%, positive predictive value (PPV) was 73.2%, and a negative predictive value (NPV) was 25.0%. Diagnostic accuracy assessed by the likelihood ratio had a value of 1.0. The BQ provided a false discovery rate of 31.2% and a misclassification rate of 32.8% (Table 3).

Table 2 Body mass index in patients with obstructive sleep apnea (OSA) vs. non-OSA identified by apnea-hypopnea index (AHI) and Berlin questionnaire (BQ)

	BMI (kg/m^2)	
Assessment Tool	OSA	Non-OSA
AHI	31.8 ± 6.2	30.3 ± 6.4
BQ	32.1 ± 6.3*	26.3 ± 2.1

*p < 0.05 for th difference between OSA – non-OSA

4 Discussion

This study demonstrates that the BQ has a high sensitivity but low specificity and low positive predictive value. Moreover, BQ has a high misclassification rate and its diagnostic accuracy is no different than a random chance. Our findings corroborate the results of Netzer et al. (1999) concerning the sensitivity of BQ, but not specificity, positive predictive value, and the likelihood ratio, all of which were greater in

Table 3 Performance of Berlin questionnaire against the gold standard polysomnography in the identification of patients at risk of obstructive sleep apnea (OSA)

	OSA (n)		
Berlin questionnaire	Positive	Negative	Total
Positive	41 (TP)	15 (FP)	56
Negative	6 (FN)	2 (TN)	8
Total	47	17	64
Sensitivity (%)	87.2 (74.5–95.2)		
Specificity (%)	11.8 (1.5–36.4)		
PPV (%)	73.2 (59.7–84.2)		
NPV (%)	25.0 (3.2–65.1)		
LR	1.0		

TP true positive, *FP* false positive, *FN* false negative, *TN* true negative, *PPV* positive predictive value, *NPV* negative predictive value, *LR* likelihood ratio, Percentage values are medians with 95% lower and upper confidence intervals

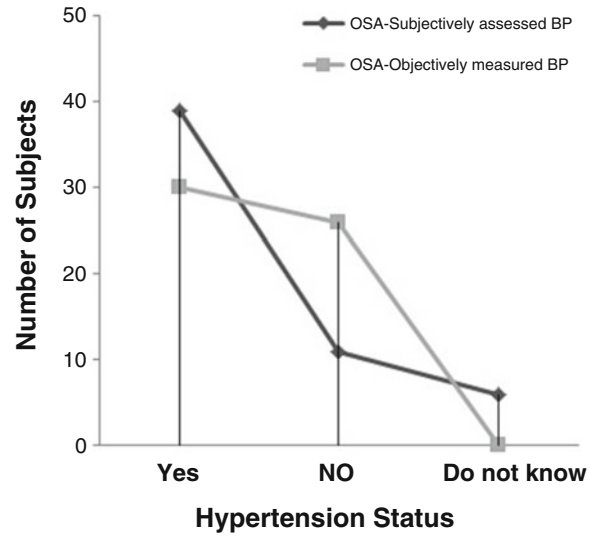

Fig. 1 Objectively and subjectively assessed hypertension status in obstructive sleep apnea (OSA) patients; *BP* blood pressure

high risk patients in the study of those authors, amounting to 77%, 87%, and 3.2, respectively.

In general, BQ has expectedly high sensitivity, as this tool has been developed for identifying high risk patients at the primary care level. However, low specificity and high misclassification rate suggest that BQ has a low discriminatory power and its utility is no different than the judgement of clinicians (Cowan et al. 2014; Sert Kuniyoshi et al. 2011). The present findings also support earlier studies showing that the BQ is of limited utility in specialized clinics (Ahmadi et al. 2008). Currently, clinicians look for a simple questionnaire that may be used as a tool to determine the risk of OSA syndrome and to predict the possible perioperative respiratory complications. The latter may improve clinical outcome when anesthesia and surgery are required (Gokay et al. 2016). The data from recently published systematic review suggest that the BQ is a questionnaire that enables to risk stratify patients for peri- and postoperative complications. However, testing of BQ is still required with a focus on specific surgery types, adjusted for potentially confounding factors (Dimitrov and Macavei 2016). A higher score of BQ in specific groups of patients after stroke or transient ischemic attack indicates that this tool is but moderately predictive for OSA exclusion (Boulos et al. 2016). It patients suffering from type 2 diabetes, BQ fails to identify 31% of patients with moderate-to-severe OSA, preventing such patients from receiving correct diagnosis and treatment. However, BQ may be suboptimal when OSA screening is done with home sleep monitoring devices (Westlake et al. 2016). An evaluation of BQ in Iranian patients with AHI >5 shows its sensitivity and specificity for OSA diagnosis as 77.3% and 23.1%, respectively, PPV of 68.0%, and NPV of 22.0% (Khaledi-Paveh et al. 2016), which is akin to present findings in the Polish population. The BQ has also been tested in Portuguese patients in whom it shows an acceptable reliability, but after excluding the following two questions: 'Has anyone noticed that you stop breathing during your sleep?' and 'Have you ever dozed off or fallen asleep while driving?' (Silva et al. 2016). Arunsurat et al. (2016) have assumed that the BQ may be useful as an OSA screening tool for the Thai or Asian populations after some adjustments. In addition, there is an apparent paucity of BQ testing in population samples comprising women and individuals of a low educational level (Silva et al. 2016). Interestingly, Gupta et al. (2016), in view of the unavailability of any screening tool for OSA in Hindi, have undertaken an attempt to explore the validity of a Hindi version of BQ, irrespective of the literacy status of subjects. The results have demonstrated sensitivity of 89%, specificity of 58%, PPV of 87%, and NPV of 63%, which supports the role of BQ as a valid tool for OSA screening OSA in that population. There is also a need to use simple tools for OSA screening in Africa, where the awareness of OSA is poor and its incidence is underreported, despite a high prevalence of symptoms (Desalu et al. 2016).

The current practice model of screening and assessment for OSA in primary care seems fragmented and ineffective (Miller and Berger 2016). Primary care providers encounter patients with OSA symptoms but do not routinely screen, assess, or refer to a sleep specialist. The present study contributes to the existing evidence that the BQ is not a reliable instrument to discriminate between high and low risk patients. Therefore, professionals should be exercised in the knowledge of using the BQ for mass screening since this tool is neither a perfect substitute to polysomnography in diagnosing OSA and predicting its course nor is it of perfect research utility as its application should be addressed to specific populations. In addition, since one-third of the score is assigned to hypertension or obesity, the misclassification of OSA cases among hypertensive and obese patients might be high. A low accuracy of BQ in identifying OSA patients has been confirmed by Margallo et al. (2014) in a large cohort of resistant hypertensive patients. Thurtell et al. (2011) have validated the BQ as a screening tool for OSA in idiopathic intracranial hypertension patients and found that only a low-risk

BQ score identifies such hypertensive patients who are unlikely to have OSA. Therefore, other protocols need to be developed to improve diagnostic accuracy in such patients.

In conclusion, the BQ is a sensitive tool that should be used in the setting in which the benefits of high sensitivity outweigh the disadvantage of low specificity. Further research is needed to improve specificity and accuracy of screening tools for OSA.

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

References

AASM (1999) American Academy of Sleep Medicine European Respiratory Society Australasian Sleep Association American Thoracic Society Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research: the report of an American Academy of Sleep Medicine Task Force. Sleep 22:667–689

Ahmadi N, Chung SA, Gibbs A, Shapiro CM (2008) The Berlin questionnaire for sleep apnea in a sleep clinic population: relationship to polysomnographic measurement of respiratory disturbance. Sleep Breath 12 (1):39–45

Arunsurat I, Luengyosluechakul S, Prateephoungrat K, Siripaupradist P, Khemtong S, Jamcharoensup K, Thanapatkaiporn N, Limpawattana P, Laohasiriwong S, Pinitsoontorn S, Boonjaraspinyo S, Sawanyawisuth K (2016) Simplified Berlin questionnaire for screening of high risk for obstructive sleep apnea among Thai male healthcare workers. J UOEH 38(3):199–206

Boulos MI, Wan A, Im J, Elias S, Frankul F, Atalla M, Black SE, Basile VS, Sundaram A, Hopyan JJ, Boyle K, Gladstone DJ, Murray BJ, Swartz RH (2016) Identifying obstructive sleep apnea after stroke/TIA: evaluating four simple screening tools. Sleep Med 21:133–139

Cole TJ (1990) The LMS method for constructing normalized growth standards. Eur J Clin Nutr 44:45–60

Cowan DC, Allardice G, Macfarlane D, Ramsay D, Ambler H, Banham S, Livingston E, Carlin C (2014) Predicting sleep disordered breathing in outpatients with suspected OSA. BMJ 4(4):e004519

Desalu O, Onyedum C, Sanya E, Fadare J, Adeoti A, Salawu F, Oluyombo R, Olamoyegun M, Fawale M, Gbadegesin B, Bello H (2016) Prevalence, awareness and reporting of symptoms of obstructive sleep apnoea among hospitalized adult patients in Nigeria: a multicenter study. Ethiop J Health Sci 26(4):321–330

Dimitrov L, Macavei V (2016) Can screening tools for obstructive sleep apnea predict postoperative complications? A systematic review of the literature. J Clin Sleep Med 12(9):1293–1300

Enciso R, Clark GT (2011) Comparing the Berlin and the ARES questionnaire to identify patients with obstructive sleep apnea in a dental setting. Sleep Breath 15 (1):83–89

Foster GD, Borradaile KE, Sanders MH, Millman R, Zammit G, Newman AB, Wadden TA, Kelley D, Wing RR, Pi-Sunyer FX, Reboussin D, Kuna ST, Sleep AHEAD Research Group of Look AHEAD Research Group (2009) A randomized study on the effect of weight loss on obstructive sleep apnea among obese patients with type 2 diabetes: the Sleep AHEAD study. Arch Intern Med 169(17):1619–1626

Gupta R, Ali R, Dhyani M, Das S, Pundir A (2016) Hindi translation of Berlin questionnaire and its validation as a screening instrument for obstructive sleep apnea. J Neurosci Rural Pract 7(2):244–249

Gokay P, Tastan S, Orhan ME (2016) Is there a difference between the STOP-BANG and the Berlin obstructive sleep apnoea syndrome questionnaires for determining respiratory complications during the perioperative period? J Clin Nurs 25(9–10):1238–1252

Iber C, Ancoli-Israel S, Chesson A, Quan SF (2007) The AASM manual for the scoring of sleep and associated events: rules, terminology and technical specifications, 1st edn. American Academy of Sleep Medicine, Westchester

Khaledi-Paveh B, Khazaie H, Nasouri M, Ghadami MR, Tahmasian M (2016) Evaluation of Berlin questionnaire validity for sleep apnea risk in sleep clinic populations. Basic Clin Neurosci 7(1):43–48

Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M et al (2014) Task Force for the Management of Arterial Hypertension of the European Society of Hypertension and the European Society of Cardiology. 2013 ESH/ESC Practice Guidelines for the Management of Arterial Hypertension. Blood Press 23 (1):3–16

Margallo VS, Muxfeldt ES, Guimarães GM, Salles GF (2014) Diagnostic accuracy of the Berlin questionnaire in detecting obstructive sleep apnea in patients with resistant hypertension. J Hypertens 32 (10):2030–2036

Miller JN, Berger AM (2016) Screening and assessment for obstructive sleep apnea in primary care. Sleep Med Rev 29:41–51

Netzer NC, Stoohs SA, Netzer CM, Clark K, Strohl KP (1999) Using the Berlin questionnaire to identify patients at risk for the sleep apnea syndrome. Ann Intern Med 131(7):485–491

Oku Y, Okada M (2008) Periodic breathing and dysphagia associated with a localized lateral medullary infarction. Respirology 13(4):608–610

Sert Kuniyoshi FH, Zellmer MR, Calvin AD, Lopez-Jimenez F, Albuquerque FN, van der Walt C, Trombetta IC, Caples SM, Shamsuzzaman AS, Bukartyk J, Konecny T, Gami AS, Kara T, Somers VK (2011) Diagnostic accuracy of the Berlin questionnaire in detecting sleep-disordered breathing in patients with a recent myocardial infarction. Chest 140(5):1192–1197

Silva KV, Rosa ML, Jorge AJ, Leite AR, Correia DM, Silva Dde S, Cetto DB, Brum Ada P, Silveira Netto P, Rodrigues GD (2016) Prevalence of risk for obstructive sleep apnea syndrome and association with risk factors in primary care. Arq Bras Cardiol 106(6):474–480

Thurtell MJ, Bruce BB, Rye DB, Newman NJ, Biousse V (2011) The Berlin questionnaire screens for obstructive sleep apnea in idiopathic intracranial hypertension. J Neuroophthalmol 31(4):316–319

Westlake K, Plihalova A, Pretl M, Lattova Z, Polak J (2016) Screening for obstructive sleep apnea syndrome in patients with type 2 diabetes mellitus: a prospective study on sensitivity of Berlin and STOP-Bang questionnaires. Sleep Med. doi:10.1016/j.sleep.2016.07.009

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Isolated Hearing Impairment Caused by *SPATA5* Mutations in a Family with Variable Phenotypic Expression

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Abstract

Biallelic mutations in the *SPATA5* gene, encoding ATPase family protein, are an important cause of newly recognized epileptic encephalopathy classified as epilepsy, hearing loss, and mental retardation syndrome (EHLMRS, OMIM: 616577). Herein we describe a family in which two *SPATA5* mutations with established pathogenicity (p.Thr330del and c.1714+1G>A) were found in the proband and her younger sister. The proband had a similar clinical picture to the previous descriptions of EHLMRS. In the sister, the only manifestation was an isolated sensorineural hearing loss. Our findings extend the phenotypic spectrum of *SPATA5*-associated diseases and indicate that *SPATA5* defects may account for a fraction of isolated sensorineural hearing impairment cases.

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Keywords
ATPase family protein • Epileptic encephalopathy • Exome sequencing • Hearing loss • Mental retardation • Mutations • Sensorineural deficit • *SPATA5*

1 Introduction

A significant proportion of all seizures with onset within the first years of life are part of epileptic encephalopathies (Myers and Mefford 2016). In such cases, recurrent seizures are accompanied by severe intellectual disability, motor disorders, and other signs contributing to the complex clinical picture (Drislane 2013; Noh et al. 2012; Engel 2001). Across all the known epileptic encephalopathy presentations, genetic causes are attributable to about one half (Esmaeeli Nieh and Sherr 2014). The majority of genetic epileptic encephalopathies are due presumably to autosomal dominant *de novo* pathogenic variants. However, a significant subgroup may exist where familial cases of variable expressivity and penetrance are found (e.g., autosomal recessive or X-linked recessive) (Depienne et al. 2012).

SPATA5-related early-onset encephalopathy, recently classified as epilepsy, hearing loss, and mental retardation syndrome (EHLMRS, OMIM: 616577) has been clinically characterized by microcephaly, variable degree of intellectual disability, early-onset seizures, hypo- or hypertonia, hearing loss, brain imaging changes, and gastrointestinal problems (Buchert et al. 2016; Kurata et al. 2016; Tanaka et al. 2015). In all the observed familial cases of the condition, the phenotype has been consistent between the affected siblings.

Herein, we present a familial case of two female siblings with biallelic *SPATA5* mutations whose pathogenicity has been proven by previous reports. The proband presented with full clinical picture of early-onset epileptic encephalopathy while her sibling had sensorineural hearing loss as the only finding. Our observations suggest a variable expression of *SPATA5*-associated disease, indicating that *SPATA5* pathogenic variants may account for a fraction of isolated congenital bilateral sensorineural hearing loss cases.

2 Cinical Report

The proband, one of the dizygotic twins, was born to a non-consanguineous 30-year-old mother and a 33-year-old father. No family history of note or exposure to teratogens was reported. The other twin was normal at birth and remains clinically healthy at the age of 4 years. The proband was born by cesarean section at 38 weeks gestation. Apgar scores were 10 after both 1 and 5 min. Birthweight was 2620 g (<25th centile), length 50 cm (75th centile) and occipito-frontal circumference 32 cm (50th centile). No congenital anomalies or dysmorphic features were noted at birth. Perinatal screening detected bilateral sensorineural hearing loss of medium size.

Although there were no feeding difficulties or slowing of physical growth within the first year of life, the achievement of major milestones was severely delayed when assessed for both gross and fine motor, speech, and social skills development (Table 1). The eye contact was very poor. Between 6 and 8 months of age short-time episodes of eye blinking and upward gaze were observed which receded without intervention. At the age of 14 months, she began to be very hypotonic, lost the ability to roll over on her stomach, the antigravity movements became weak and started to include choreiform elements. The pattern of movements became stereotypic. At this time, paroxysmal episodes returned (convergent strabismus with eyeballs tremor). She was treated with valproic acid 30 mg/kg/day

Table 1 Clinical characteristics and acquisition of major developmental milestones in the proband and her now 10-month-old sister. Months of the first two years of age are given at which the skills were acquired

Developmental milestone	Proband	Sister
Gross motor skills		
Head stabilization	3–4	2
Turning around body axis	9	6
Can sit when set down	Never acquired	7
Sits unsupported	Never acquired	Beginning at 9 months
Crawling on all fours	Never acquired	Can crawl backward
Minor motor skills		
Purposeful grip	8	3–4
Elbow grip	14	4
Palm grip	15	5
Scissor grasp	16	7
Pincer grasp	18	9
Speech		
Cooing	6	2–3
Babbling	Never acquired	5–6
Social skills		
Facial preference	4	2
Social smile	4	2
Emotions toward relatives	7	4
Reacts to own name	9 (on hearing aids)	8
Fear of strangers	11	9

without satisfactory effect; levetiracetam 30 mg/kg/day was added. This resulted in an increased frequency of paroxysmal episodes and after some weeks epileptic spasm appeared. Levetiracetam was discontinued. Subsequently, adrenocorticotropic hormone (ACTH) was administered and all types of seizures subsided.

Currently, at 4 years of age, the proband is on valproic acid 23 mg/kg/day and experiences no epileptic seizures. Her weight is 17 kg (50th centile), height 110 cm (97th centile), and occipito-frontal circumference 48 cm (<10th centile) She is severely developmentally delayed, although no formal psychological assessment has been performed yet. She cannot sit, there is short-duration eye-contact, response to commands is questionable. She does not speak a single word, presents with severe hypotonia with low muscle strength and a choreiform pattern of movements with stereotypies that do not wear off and do not respond to medication, although they often subside during the night. Episodes of upward eye gaze lasting up to 1 min without any epileptiform changes in EEG are observed (probably oculogyric crises). The proband wears hearing aids for bilateral congenital sensorineural deafness and has strabismus. She receives forlax daily for chronic constipation.

Head MRI performed at the age of 15 months was normal. Spinal MRI reveals occult spina bifida. Copper and ceruloplasmin levels were within normal limits. In EEG, epileptic graphoelements, burst of sharp and slow wave complexes, spikes, and polyspike-wave complexes were present. Acylcarnitines analysis in a dried blood spot by tandem mass spectrometry (MS/MS) and urine organic acids analysis by gas chromatography – mass spectroscopy (GC/MS) were normal. Amino acids in plasma and cerebrospinal fluid (CSF) showed no significant abnormalities. The CSF sample was taken according the standard protocol and the level of biogenic amine metabolites at the age of 21 months was normal.

A younger, now 10-month-old, sister of the proband was born by cesarean section at 39 weeks gestation. Birth weight was 3120 g

Table 2 Variants found by whole exome sequencing in the proband, which were considered potentially disease-causing

Gene	Position	ID	Effect	Disease (inheritance, other info)
Potentially autosomal recessive (potentially biallelic variants, freq. < 0.01 in available databases)				
SPATA5	chr4:123868644-G>A	rs149688478	NM_145207.2: c.1714+1G>A	Epilepsy, hearing loss, and mental retardation syndrome;
				OMIM 613940 (AR)
	chr4:123855728-TCAA>T	rs748291365	NM_145207.2: p.Thr330del	
GMPPA	chr2:220366610-G>T	rs753112469	NM_013335.3: p.Gly94Cys	Alacrimia, achalasia, and mental retardation syndrome;
				OMIM 615510 (AR)
	chr2:220366580-C>A	rs767718283	NM_013335.3: p.Gln84Lys	
Potentially autosomal dominant (potential loss of function, freq.= 0 in available databases)				
IARS2	chr1:220267799-C>CTT	–	NM_018060.3: p.Pro81_Asp82fs	Cataracts, growth hormone deficiency, sensory neuropathy, sensorineural hearing loss, and skeletal dysplasia; OMIM 612801 (AR)
PNKD	chr2:219209177-G>C	–	NM_022572.4: c.797-1G>C	Paroxysmal nonkinesigenic dyskinesia; OMIM 609023 (AD), *pLi = 0.001;
				All previously associated diseases variants were missense

*pLI – probability that mutation on a single chromosome, resulting in a loss of function, is pathogenic (ExAC Browser Beta (2016)

(<50th centile), length 51 cm (>75th centile), and occipito-frontal circumference 33 cm (50th centile). Apgar scores were 10 at both 1 and 5 min. She had no major malformations or dysmorphic features at birth. At 9 months her weight was 8 kg (25th centile), height 68 cm (10th centile), and occipito-frontal circumference 44.5 cm (50th centile). At 10 months, she is hypotonic but with normal pattern of movements. The gross motor development is slightly delayed, which is most likely due to hypotonia. She started to sit with support at 9 months. She is a very social girl, she has a very good eye contact and executes plays that are nearly adequate for age (Table 1). Bilateral sensorineural deafness of moderate degree was diagnosed using auditory brainstem response testing, and hearing aids were appropriately fitted. No episodes of seizures have occurred since birth. No other typical features of *SPATA5* encephalopathy have been observed. The EEG was normal and MRI of the head was not performed. Copper and ceruloplasmin levels were normal.

DNA extracted from the peripheral blood of the proband was analyzed by whole exome sequencing. The library was prepared with an Nextera DNA Library Prep Kit (Illumina; San Diego, CA) and sequencing was performed on HiSeq 1500 to the mean depth of 50x; 82% of target bases were covered at a minimum of 20x and 95% were covered at a minimum of 10x. Raw data were analyzed as previously described by Ploski et al. (2014), with Hg19 genomic build used for alignments. We found 563,432 variants passing a default quality filter. These variants were further filtered to include those affecting the coding sequence (by changing the aminoacid or splice site within 10 bp from exon/intron boundary) and having <1% of minor allele frequency in the following databases: ExAC Browser Beta (2016), 1000 Genomes (http://www.1000genomes.org/), ESP6500 (http://evs.gs.washington.edu/EVS), and an in-house database of ~500 Polish exomes. The final yield of 705 variants were screened against known mutations listed in the HGMD database (http://www.hgmd.cf.ac.uk) as pathogenic (Table 2). In parallel, the 705 variants were searched for

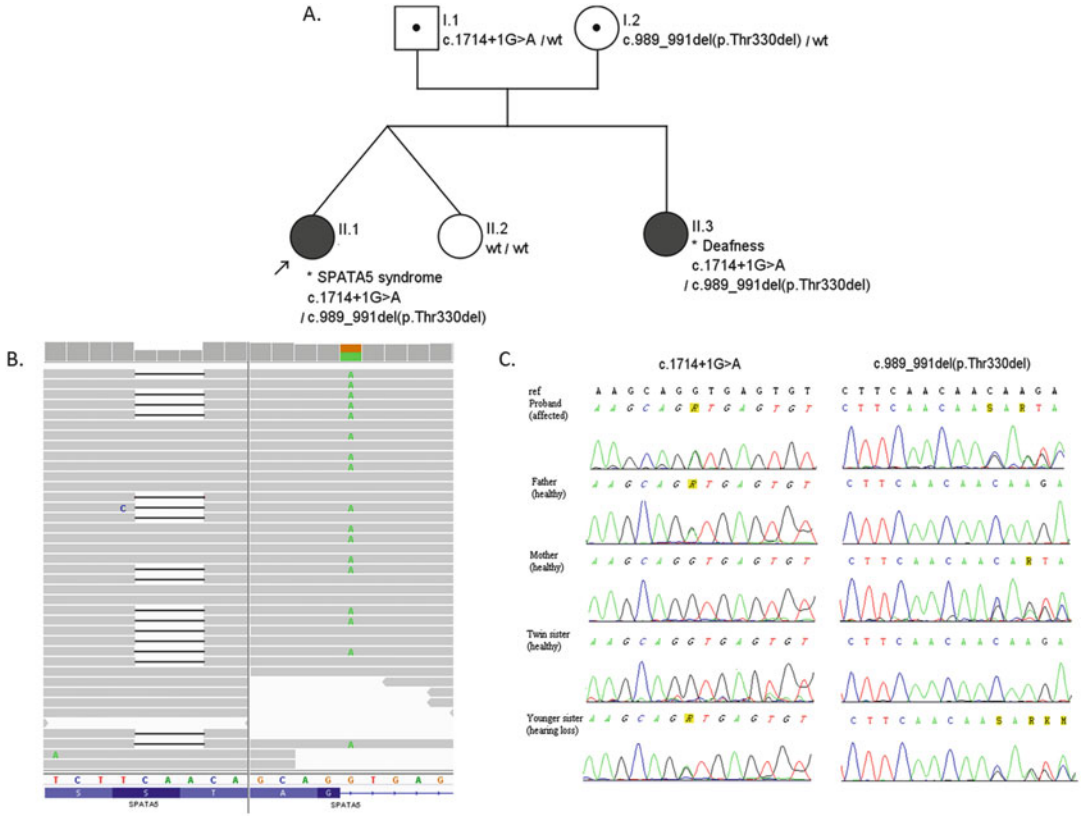

Fig. 1 **Sequencing results:** (**a**) pedigree of the studied family; (**b**) IGV screenshot showing the *SPATA5* mutations p.Thr330del (*left*) and c.1714+1G>A (*right*) detected by NGS in the proband, and (**c**) Sanger sequencing results showing the same mutations in the whole family

biallelic mutations consistent with autosomal recessive inheritance (Table 2) as well as for monoallelic variants potentially causative of an autosomal dominant or sex-linked condition. Here we considered variants not found in the available databases and predicted by SNP effect software to have a 'high' effect, i.e., to introduce a premature stop codon or affect a splice site. The variants finally considered are shown in Table 2.

Considering the phenotype and characteristics of the variants, we prioritized two mutations in *SPATA5* exon 5 p.Thr330del and exon 9 c.1714 +1G>A, both of which have been previously reported as causative of EHLMRS (OMIM: 616577) (Buchert et al. 2016; Kurata et al. 2016; Tanaka et al. 2015). Using Sanger sequencing, the mutations were confirmed in the proband's DNA and *trans* position was demonstrated by sequencing DNA samples from the parental DNA (Fig. 1).

3 Discussion

This study gives account of a family in which two mutations p.Thr330del and c.1714+1G>A in the *SPATA5* gene were found in the proband and her younger sister. The proband had a disease consistent with the description in previous reports on *SPATA5*-associated EHLMRS. In the proband's sister, the only manifestation was isolated sensorineural hearing loss and mild hypotonia.

Both *SPATA5* mutations observed in the family have been previously found in patients with typical *SPATA5* encephalopathy, which strongly implicates the presence of mutations'

Table 3 Comparison of clinical features of the present familial case with those observed by Tanaka et al. (2015), Kurata et al. (2016), and Buchert et al. (2016)

Clinical feature	Tanaka et al. (2015) (n = 14)	Kurata et al. (2016) (n = 3)	Buchert et al. (2016) (n = 8)	Proband/Younger sibling
Intellectual disability/developmental delay	14	3	8	+/−
Microcephaly	12	2	8	+/−
Seizures	13	3	some	+/−
Abnormal EEG	14	2	8	+/−
Hypotonia	13	1?	8	−/−
Extrapyramidal features, including hypertonia	9	1	1	+/−
Brain MRI abnormality	7	3	some	+/NA
Hearing impairment	14	3	some	+/+
Vision impairment	13	0	some	−/−
Gastrointestinal problems	13	0	7	+/−
Dysmorphic features	NA	3	0	−/−

NA not assessed

pathogenicity. Tanaka et al. (2015) have described p.Thr330del in a homozygous female patient and c.1714+1G>A in two male patients who were also compound heterozygotes for p.Trp626Cys. The female and one of the male patients exhibited microcephaly, hypotonia and spasticity, developmental delay, seizures, vision defect, hearing loss, and gastrointestinal problems. The second male had a similar phenotype but without spasticity. Kurata et al. (2016) have reported two siblings, a male and a female with p.Thr330del together with p.Glu711Profs*21. The phenotype of the male patient included intellectual disability, seizures, and hearing loss, whereas the female patient additionally had hypotonia. Finally, Buchert et al. (2016) have reported a patient with p.Thr330del and p.Gly694Glu who had intellectual disability, seizures, hypotonia, hearing loss, extrapyramidal symptoms, and vision defects.

The phenotype of the proband in the present study shares similarities with previously described patients totaling 25 affected patients from 14 families as shown in Table 3. Intellectual disability and developmental delay, microcephaly, seizures and abnormal EEG, hypertonicity with other extrapyramidal features, and hearing loss have consistently been observed in all those patients. A significant proportion of them also had variable head MRI abnormalities, vision and gastrointestinal problems, and some had facial dysmorphism (Buchert et al. 2016; Kurata et al. 2016; Tanaka et al. 2015).

The proband's sister had frequently been assessed neurologically since birth and no abnormalities other than mild hypotonia were found up to age 10 months. She had a normal EEG and her social functioning was undisturbed. Therefore, congenital bilateral hearing impairment was a single feature of the *SPATA5* syndrome. Other genetic or extragenetic causes of the isolated hearing impairment, which may have a heterogenous nature (Koffler et al. 2015), were in this case rather excluded. The audiological assessment in the parents revealed normal hearing in both of them. This observation is unusual since in individuals with *SPATA5* defects, neurological symptoms, microcephaly, and encephalopathy are congenital or occur within the first months of life. Seizures of various type usually start prior to or at around 7 months of age in at least 12 of the 19 individuals reported in all the studies on the subject. Further, epilepsy always commence on the background of other serious neurodevelopmental problems such as developmental delay, hyper- or hypotonia, extrapyramidal symptoms, and particularly an abnormal EEG pattern.

SPATA5 encodes a protein that is part of the superfamily of ATPases associated with diverse cellular activities (AAA). Proteins of this family are characterized by a highly conserved ATPase domain. Members of the AAA protein family are implicated in DNA replication, cellular membrane fusion, protein degradation, and in the microtubule organization (Bar-Nun and Glickman 2012). *SPATA5* is expressed in neural, muscular, secretory, and reproductive tissues as well as in internal organs. That also includes the core cerebral structures that play a central role in generating and transforming hearing signals.

Beside the *SPATA5* mutations above outlined, we found additional variants listed in Table 2, of which none are likely to contribute to the phenotype. Concerning the *GMPPA* phenotype found in the proband, it does not match that described in the literature. In particular, the characteristic achalasia and alacrimia were not observed in our proband (Koehler et al. 2013). Diseases caused by mutations in the *IARS2* gene have so far been linked to recessive inheritance, which makes their contribution to the disease in our family unlikely (Schwartzentruber et al. 2014). Mutations in the *PNKD* gene cause paroxysmal nonkinesigenic dyskinesia (PNKD) with dominant inheritance (Szczałuba et al. 2009). However, *PNKD* mutation found in our proband was unlikely to be pathogenic since it is predicted to cause aberrant splicing, which leads to a loss of protein function (c.797-1G>C affects canonical splice site whose constant feature is the presence of G or C but no other nucleotides (Pagani and Baralle 2004)). The loss of function with *PNKD* mutations are apparently benign, being present at expected frequency in ExAC population (12.1 *vs.* 12.0, for expected and observed, respectively, pLI = 0.00) (ExAC Browser Beta 2016). Further, according to the HGMD, all previously described disease causing *PNKD* mutations have been missense.

In conclusion, our report broadens the *SPATA5* disease spectrum by including an isolated hearing impairment. The phenotypic differences observed between siblings with the same mutations indicate a considerable variation in the *SPATA5* disease expression.

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References

Bar-Nun S, Glickman MH (2012) Proteasomal AAA-ATPases: structure and function. Biochim Biophys Acta 1823:67–82

Buchert R, Nesbitt AI, Tawamie H, Krantz ID, Medne L, Helbig I, Matalon DR, Reis A, Santani A, Sticht H, Abou Jamra R (2016) *SPATA5* mutations cause a distinct autosomal recessive phenotype of intellectual disability, hypotonia and hearing loss. Orphanet J Rare Dis 11(1):130

Depienne C, Gourfinkel-An I, Baulac S, et al (2012) Genes in infantile epileptic encephalopathies. In: Noebels JL, Avoli M, Rogawski MA, et al (eds) Jasper's basic mechanisms of the epilepsies (Internet), 4th edn. Bethesda: National Center for Biotechnology Information (US). Available from: http://www.ncbi.nlm.nih.gov/books/NBK98182/. Accessed 27 Nov 2016

Drislane FW (2013) Overlap of encephalopathies and epileptic seizures. J Clin Neurophysiol 30(5):468–476

Engel J Jr (2001) A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology. Epilepsia 42(6):796–803

Esmaeeli Nieh S, Sherr EH (2014) Epileptic encephalopathies: new genes and new pathways. Neurotherapeutics 11(4):796–806

ExAC Browser Beta (2016) Exome Aggregation Consortium. http://exac.broadinstitute.org/. Accessed 28 Nov 2016

Koehler K, Malik M, Mahmood S et al (2013) Mutations in *GMPPA* cause a glycosylation disorder characterized by intellectual disability and autonomic dysfunction. Am J Hum Genet 93(4):727–734

Koffler T, Ushakov K, Avraham KB (2015) Genetics of hearing loss: syndromic. Otolaryngol Clin N Am 48 (6):1041–1061

Kurata H, Terashima H, Nakashima M et al (2016) Characterization of SPATA5-related encephalopathy in early childhood. Clin Genet 90:437–444

Myers CT, Mefford HC (2016) Genetic investigations of the epileptic encephalopathies: recent advances. Prog Brain Res 226:35–60

Noh GJ, Asher YJT, Graham JM (2012) Clinical review of genetic epileptic encephalopathies. Eur J Med Genet 55(5):281–298

Pagani F, Baralle FE (2004) Genomic variants in exons and introns: identifying the splicing spoilers. Nat Rev Genet 5:389–396

Ploski R, Pollak A, Müller S, Franaszczyk M, Michalak E, Kosinska J, Stawinski P, Spiewak M, Seggewiss H, Bilinska Z (2014) Does p.Q247X in *TRIM63* cause human hypertrophic cardiomyopathy? Circ Res 114: e2–e5

Schwartzentruber J, Buhas D, Majewski J et al (2014) Mutation in the nuclear-encoded mitochondrial isoleucyl-tRNA synthetase *IARS2* in patients with cataracts, growth hormone deficiency with short stature, partial sensorineural deafness, and peripheral neuropathy or with Leigh syndrome. Hum Mutat 35 (11):1285–1289

Szczałuba K, Jurek M, Szczepanik E et al (2009) A family with paroxysmal nonkinesigenic dyskinesia: genetic and treatment issues. Pediatr Neurol 41(2):135–138

Tanaka AJ, Cho MT, Millan F et al (2015) Mutations in SPATA5 are associated with microcephaly, intellectual disability, seizures, and hearing loss. Am J Hum Genet 97(3):457–464

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Stress Response, Brain Noradrenergic System and Cognition

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Abstract

Locus coeruleus is a critical component of the brain noradrenergic system. The brain noradrenergic system provides the neural substrate for the architecture supporting the interaction with, and navigation through, an external world complexity. Changes in locus coeruleus tonic and phasic activity and the interplay between norepinephrine and α_1- and α_2-adrenoceptors in the prefrontal cortex are the key elements of this sophisticated architecture. In this narrative review we discuss how the brain noradrenergic system is affected by increased exposure to corticotropin-releasing hormone triggered by stress response. In particular, we present the mechanisms responsible for thinking inflexibility often observed under highly stressful conditions. Finally, the main directions for future research are highlighted.

Keywords

Brain Noradrenergic System • Corticotropin-Releasing Hormone • Locus Coeruleus • Mental Function • Stress Response

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1 Noradrenergic Circuitry

The locus coeruleus (LC) in the brainstem contains noradrenergic circuitry, using norepinephrine (NE) as a neurotransmitter, is increasingly recognized as a critical component of the neural architecture supporting interaction with, and navigation through, an external world complexity. In particular, LC seems to play a central role in governing behavioral processes, like switching between attention on task and novelty-seeking or sensory information processing and decision making (Berridge and Waterhouse 2003; Aston-Jones et al. 1997, 1994; Rajkowski et al. 1994). The switching is achieved through modulation of neural activity in response to stimuli in the form of enhancement or suppression depending on the perceived relevance, but not affective valence (Aston-Jones and Bloom 1981a, b; Foote et al. 1980). NE influences neuronal firing rates and stimulus-evoked cellular responses, thus optimizing the signal-to-noise ratio (Fig. 1) and the gain in target neurons (Woodward et al. 1991; Servan-Schreiber et al. 1990; Foote et al. 1975). Such a system enables the adaptive flexibly, i.e., disengaging from a task and switching to another one depending on the altered context or motivational state (Aston-Jones and Cohen 2005).

The LC is the largest group of noradrenergic neurons that extensively project to widespread areas of the brain and spinal cord. When the pathways involving the LC are delineated, it becomes evident that LC projections are selective and the afferents vary extensively (Aston-Jones and Waterhouse 2016; Samuels and Szabadi 2008). NE receptors on cells receiving input from the LC are classified as α_1, α_2, or β-adrenoceptors. Activation of α_1 and β -adrenoceptors by NE generally causes excitation, while activation of α_2-adrenoceptors causes inhibition of neurons (Jones 2005; Berridge and Waterhouse 2003). However, activation of α_2-adrenoceptors increases the prefrontal cortex (PFC) activity. It has been proposed that postsynaptic α_2-adrenoceptors are present on inhibitory γ-aminobutyric acid (GABA) interneurones in the PFC. Consequently, NE activates postsynaptic α_2-adrenoceptors located on GABA interneurons, hyperpolarizes them, which in turn disinhibits the PFC pyramidal cells (Andrews and Lavin 2006).

α_1- and α_2-adrenoceptor modulation in the PFC provides a physiological substrate for the neural mechanisms underlying the cognition-enhancing and therapeutic effects of psychostimulants such as methylphenidate or amphetamine (Berridge et al. 2012). More specifically, a variable reaction to psychostimulants across the PFC-dependent tasks is explained by differential actions of noradrenergic α_2- and α_1-adrenoceptors. For instance, α_1-adrenoreceptor activation within the PFC impairs, while activation of postsynaptic α_2-receptors improves, working memory (Arnsten and Dudley 2005). NE exerts an inverted-U shaped modulation of working memory within the PFC. A moderate NE concentration is associated with optimal performance, while both inadequate and excessive NE stimulation impair working memory (Robbins and Arnsten 2009). Consequently, working memory is impaired under low arousal conditions associated with low rates of NE release. During moderate arousal, and thus moderate rates of NE release, α_2-adrenoreceptor stimulation ensures the optimal working memory (Arnsten 2000). In contrast, under stressful and high arousal conditions, and thus high rates of NE release, stimulation of PFC α_1-adrenoceptors results in a stress-like impairment in working memory (Berridge and Waterhouse 2003).

In this review we seek to summarize recent achievements in this fascinating field of science. In particular, we discuss how stress influences the LC function and NE release in the PFC, with some cognitive consequences involved.

2 Stress Response

Stress can be broadly defined as any situation associated with augmented energy consumption that is beyond expected or physiological range. Alternatively, stress can be explained through the

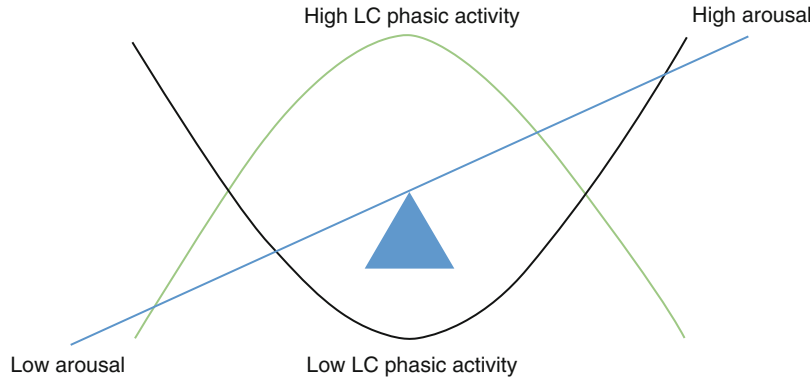

Fig. 1 Relationship between arousal and locus coeruleus (LC) tonic and phasic activity. Moderate arousal is associated with high phasic and low tonic LC activity, resulting in optimal signal-to-noise ratio and high cognitive performance

central concept of homeostasis. Physiological and biochemical processes in the organism maintain equilibrium; an ideal steady state that is hardly achievable. Environmental factors, internal or external stimuli, continually disrupt this equilibrium. Therefore, an ideal harmony or a homeostatic point can be considered as an attractor and the organism's current state as dynamic fluctuations around this attractor. Factors pushing the organism's functions to diverge too far from homeostasis are defined as stressors (Robert and Labat-Robert 2015; Chrousos 2009; Chrousos and Gold 1992).

To maintain homeostasis, organisms have developed an integrated response managed by the stress system. The system is equipped with central and peripheral neuroendocrine mechanisms to cope with challenges that threaten or are perceived as threatening for homeostasis (Selye 1936). Importantly, not all stressful conditions had adverse effects on human health. On the contrary, 'eustress' represents those states of stress that are associated with pleasant feelings, enhanced human growth and development at the emotional and intellectual levels. On the other hand, distress consists of stressful conditions that trigger pathologic alterations. Stress response depends not only on catecholamines originating from the adrenal medulla and the systemic sympathetic nervous system, but also on the adrenal cortex-derived glucocorticoids that exert a strong anti-inflammatory effect (Nicolaides et al. 2015; Szabo et al. 2012).

Nowadays, it is recognized that activation of the hypothalamic–pituitary–adrenal (HPA) axis in response to stress is highly dependent on the characteristics of individual stressors (Herman et al. 2016). Information about pathological stressors representing a straightforward threat to homeostasis, e.g., inflammation, hypoxia, hypoglycemia, or blood loss, is transmitted directly to the paraventricular hypothalamic nucleus (PVN) whose neurons release corticotropin-releasing hormone (CRH) via monosynaptic fibres originating in sensory organs (Herman et al. 2003). Therefore, blood volume or oxygenation changes are communicated via baro- and chemoreceptors to the solitary tract nucleus (Accorsi-Mendonca and Machado 2013) that sends noradrenergic projections to the PVN, triggering an immediate HPA axis response (Ulrich-Lai and Herman 2009).

In contrast, psychological stimuli are typically transmitted to the PVN via more complex circuitry including one or more limbic structures. These structures process polymodal sensory information in the context of available mnemonic information regarding a potential threat, and generate an anticipatory response to manage the real or perceived threat to health or well-being (Herman et al. 2003). Inputs from multiple limbic regions converge to provide direct projections to the PVN (Radley and Sawchenko 2011), so that the information from stress-excitatory and stress-inhibitory parts of limbic structures is reconfigured and streamlined aiming at the optimization of a net stress response

(Ulrich-Lai and Herman 2009). Different types of psychological stimuli may affect different limbic stress-regulatory pathways to an extent. Therefore, stressors has been classified by numerous investigators as physical, psychological or emotional (Jacobson 2005; Dayas et al. 2001), interoceptive vs. exteroceptive (Sawchenko et al. 2000), systemic vs. processive (Herman and Cullinan 1997), and more recently as reactive vs. anticipatory (Herman et al. 2003).

Components of the central stress system consist of the following: 1/ parvocellular neurons of the PVN, which secrete CRH; 2/ PVN neurons that secrete arginine vasopressin (AVP); 3/ CRH neurons that form the paragigantocellular and parabrachial nuclei of the medulla and the LC; and 4/ other neuronal groups in the medulla and pons forming the LC/NE system mostly secreting NE (Nicolaides et al. 2015). Stimulation of CRH neurons activates the LC/NE system and vice versa. In addition, the stress system communicates with the mesocortical and mesolimbic dopaminergic reward systems, receiving inhibitory afferent input from either system (Charmandari et al. 2003; Chrousos and Gold 1992). The stress system is linked to the central nucleus of the amygdala, a structure of the limbic system involved in the generation of fear and anger; both forming a positive regulatory feedback loop upon activation. CRH actions also modulate the transmission of sensory information by the thalamic and cortical neurons. In particular, during stress of high intensity, CRH acts at the LC to suppress neuronal processing of weak afferent stimuli within sensory systems (Devilbiss et al. 2012).

In the awake state, as long as homeostasis is maintained, LC exhibits low tonic discharge of 1–2 Hz (Aston-Jones and Bloom 1981a, b). Stressful events shift LC activity toward a high tonic mode of firing (3–8 Hz). The CRH is believed to drive the high tonic state while simultaneously decreasing phasic firing events, thus decreasing the signal-to-noise ratio (Page and Abercrombie 1999; Valentino and Foote 1988; Curtis et al. 1997). In normal conditions, PFC exerts an inhibitory control over the amygdala, HPA axis, and the LC-NE system. After activation by a highly stressful event, amygdala inhibits the PFC, which relieves both HPA axis and LC-NE system from PFC-controlled inhibition. The HPA and LC-NE activate the amygdala, which further mitigates the PFC. Taking together, PFC is inhibited under highly stressful stimulation, while the HPA axis, LC-NE, and amygdala create a positive feedback loop amplifying the effect and potentially leading to pathologic conditions (Gold and Chrousos 2002). It has been recently shown that release of amygdalar CRH into the LC promotes anxiety and aversive behavior; the processes in which LC and its afferent circuitry are critical for encoding and producing stress-induced anxiety (McCall et al. 2015). These findings are strengthened by the experiments in which the loss of hypothalamic CRH markedly reduces anxiety behavior (Zhang et al. 2016).

3 Prefrontal Cortex

The current knowledge on the LC-PFC axis is related to the research focused on the psychostimulant-enhanced PFC cognitive performance. Methylphenidate and amphetamine, the psychostimulants used in treatment of attention deficit hyperactivity disorder (ADHD), potently block NE and dopamine (DA) reuptake in the brain and consequently increase the extracellular level of these neurotransmitters (Arnsten et al. 1996; Kuczenski et al. 1995; Kuczenski and Segal 1992). DA and NE exert an inverted-U action on the PFC dependent working memory, PFC neuronal signaling, and in the case of DA on D1 receptors (Vijayraghavan et al. 2007). At low, clinically-relevant doses of these psychostimulants, elevated concentration of NE and DA improves the PFC cognitive function in both ADHD and healthy subjects. In contrast, systemically administered four to eight-fold higher doses impair working memory and cognition (Spencer et al. 2012). Importantly, low-doses of these psychostimulants exert very

modest effect on extracellular catecholamines in the subcortical regions associated with motor activation (e.g., nucleus accumbens) and arousal (e.g., medial septum). Therefore, the prototypical behavior-activating and arousal-enhancing effects of high-doses of psychostimulants are absent (Spencer et al. 2015).

Postsynaptic α_2-adrenoceptors have high-affinity to NE and are engaged at lower rates in NE release (Arnsten 2000). Consistently, a beneficial working memory effect of low-dose methylphenidate and atomoxetine is blocked with systemic treatment with α_2 and D_1 antagonists (Gamo et al. 2010; Arnsten and Dudley 2005). Likewise, the cognition-enhancing effects of intra-PFC infusion of methylphenidate are abolished by co-infusion of α_2 and D_1 antagonists (Berridge and Spencer 2016). Interestingly, methylphenidate injected intra-PFC does not impair cognitive performance, even at concentrations 16-fold and 32-fold higher than a clinically relevant dose (Spencer et al. 2012). It seems that PFC pathways remain operational at values close to zero of the descending limb of the inverted U-shaped curve. The mechanisms of cognition-impairing actions of high doses of systemically administered psychostimulants should thus be located outside of PFC, with HPA axis being the most likely candidate (Barsegyan et al. 2010).

Lower affinity α_1-adrenoceptors, responsible for impairment of working memory and PFC neuronal signaling, become involved at higher rates of NE release (Arnsten 2000). Consequently, α_1-adrenoceptors are activated by acute restraint stress (Alves et al. 2014), predatory stress (Rajbhandari et al. 2015), and maternal separation (Coccurello et al. 2014). Stimulation of α_1-adrenoceptors reinforces the stress response, while their blockade diminishes the HPA activation (Yang et al. 2012). Likewise, behavior-activating high doses of psychostimulants activate the HPA axis, and impair spatial working memory through activation of glucocorticoid receptors present in the PFC (Barsegyan et al. 2010). In line with these findings, exposure to prolonged stress, resulting in PFC hyperactivity and a loss of function in the nucleus accumbens, leads to behavioral inflexibility. An impairment in set-shifting may be responsible for the disengagement of attention in individuals who are exposed to traumatic events (Piao et al. 2016). Furthermore, high intensity stress profoundly suppresses the activity of neurons that are strongly tuned to key task events and, at the same time, it activates neurons displaying relatively weak task-related tuning. The end result is a profound collapse in the fidelity of goal-related coding across the PFC output neurons (Devilbiss et al. 2016).

4 Conclusions

Modulation of the LC-NE and HPA axes plays an essential role in cognitive function and task performance modifications. Nonetheless, a number of issues remain that need to be resolved. In particular, the interference of the LC-NE and HPA axes with other systems responsible for attention and alertness, such as serotoninergic, histaminergic, or cholinergic pathways needs to be further clarified. Likewise, the role of microglia and interactions among inflammatory agents, NE, and CRH merit detailed attention and research.

This review focused almost exclusively on rodent and macaque research. Recent studies have revealed that nonluminance-mediated changes in pupil diameter might provide a moment-to-moment index that reflects the LC-mediated coordination of noradrenergic neuronal activity. The exact mechanisms underlying the relation between pupil changes and NE-driven neural and behavioral phenomena still remain conjectural. Nonetheless, an appealing possibility arises of future non-invasive investigations in humans based on the pupil distention.

We briefly summarized the current knowledge on the neuroendocrine mechanisms that may account for the relation between stress and cognition. This knowledge is in line with the notion that moderate stress might be quite stimulating,

while the effect of exposure to intense, and prolonged hostile psychological environment is devastating regarding cognitive abilities and human general well-being. It is worth mentioning that this widely accepted popular notion is increasingly justified in light of recent research discoveries.

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

References

Accorsi-Mendonca D, Machado BH (2013) Synaptic transmission of baro- and chemoreceptors afferents in the NTS second order neurons. Auton Neurosci 175:3–8

Alves FH, Crestani CC, Resstel LB, Corrêa FM (2014) Both α1- and α2-adrenoceptors in the insular cortex are involved in the cardiovascular responses to acute restraint stress in rats. PLoS One 9(1):e83900

Andrews GD, Lavin A (2006) Methylphenidate increases cortical excitability via activation of alpha-2 noradrenergic receptors. Neuropsychopharmacology 31:594–601

Arnsten AF (2000) Through the looking glass: differential noradenergic modulation of prefrontal cortical function. Neural Plast 7:133–146

Arnsten AF, Dudley AG (2005) Methylphenidate improves prefrontal cortical cognitive function through alpha$_2$ adrenoceptor and dopamine D1 receptor actions: relevance to therapeutic effects in attention deficit hyperactivity disorder. Behav Brain Funct 1(1):2

Arnsten AF, Steere JC, Hunt RD (1996) The contribution of alpha 2-noradrenergic mechanisms of prefrontal cortical cognitive function. Potential significance for attention-deficit hyperactivity disorder. Arch Gen Psychiatry 53:448–455

Aston-Jones G, Bloom FE (1981a) Norepinephrine-containing locus coeruleus neurons in behaving rats exhibit pronounced responses to non-noxious environmental stimuli. J Neurosci 1:887–900

Aston-Jones G, Bloom FE (1981b) Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. J Neurosci 1:876–886

Aston-Jones G, Cohen JD (2005) Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. J Comp Neurol 493:99–110

Aston-Jones G, Waterhouse B (2016) Locus coeruleus: from global projection system to adaptive regulation of behavior. Brain Res 1645:75–78

Aston-Jones G, Rajkowski J, Kubiak P, Alexinsky T (1994) Locus coeruleus neurons in monkey are selectively activated by attended cues in a vigilance task. J Neurosci 14:4467–4480

Aston-Jones G, Rajkowski J, Kubiak P (1997) Conditioned responses of monkey locus coeruleus neurons anticipate acquisition of discriminative behavior in a vigilance task. Neuroscience 80:697–715

Barsegyan A, Mackenzie SM, Kurose BD, McGaugh JL, Roozendaal B (2010) Glucocorticoids in the prefrontal cortex enhance memory consolidation and impair working memory by a common neural mechanism. Proc Natl Acad Sci 107:16655–16660

Berridge CW, Spencer RC (2016) Differential cognitive actions of norepinephrine a2 and a1 receptor signaling in the prefrontal cortex. Brain Res 1641:189–196

Berridge CW, Waterhouse BD (2003) The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. Brain Res Brain Res Rev 42:33–84

Berridge CW, Shumsky JS, Andrzejewski ME, McGaughy JA, Spencer RC, Devilbiss DM, Waterhouse BD (2012) Differential sensitivity to psychostimulants across prefrontal cognitive tasks: differential involvement of noradrenergic α_1- and α_2-receptors. Biol Psychiatry 71:467–473

Charmandari E, Kino T, Souvatzoglou E, Chrousos GP (2003) Pediatric stress: hormonal mediators and human development. Horm Res 59:161–179

Chrousos GP (2009) Stress and disorders of the stress system. Nat Rev Endocrinol 5:374–381

Chrousos GP, Gold PW (1992) The concepts of stress and stress system disorders: overview of physical and behavioral homeostasis. JAMA 267:1244–1252

Coccurello R, Bielawski A, Zelek-Molik A, Vetulani J, Kowalska M, D'Amato FR, Nalepa I (2014) Brief maternal separation affects brain α1-adrenoceptors and apoptotic signaling in adult mice. Prog Neuro-Psychopharmacol Biol Psychiatry 48:161–169

Curtis AL, Lechner SM, Pavcovich LA, Valentino RJ (1997) Activation of the locus coeruleus noradrenergic system by intracoerulear microinfusion of corticotropin-releasing factor: effects on discharge rate, cortical norepinephrine levels and cortical electroencephalographic activity. J Pharmacol Exp Ther 281:163–172

Dayas CV, Buller KM, Crane JW, Xu Y, Day TA (2001) Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. Eur J Neurosci 14:1143–1152

Devilbiss DM, Waterhouse BD, Berridge CW, Valentino R (2012) Corticotropin-releasing factor acting at the locus coeruleus disrupts thalamic and cortical sensory-evoked responses. Neuropsychopharmacology 37:2020–2030

Devilbiss DM, Spencer RC, Berridge CW (2016) Stress degrades prefrontal cortex neuronal coding of goal-directed behavior. Cereb Cortex pii:bhw140

Foote SL, Freedman R, Oliver AP (1975) Effects of putative neurotransmitters on neuronal activity in monkey auditory cortex. Brain Res 86:229–242

Foote SL, Aston-Jones G, Bloom FE (1980) Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. Proc Natl Acad Sci U S A 77:3033–3037

Gamo NJ, Wang M, Arnsten AF (2010) Methylphenidate and atomoxetine enhance prefrontal function through alpha2-adrenergic and dopamine D1 receptors. J Am Acad Child Adolesc Psychiatry 49:1011–1023

Gold PW, Chrousos GP (2002) Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. Mol Psychiatry 7:254–275

Herman JP, Cullinan WE (1997) Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. TINS 20:78–83

Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, Cullinan WE (2003) Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Front Neuroendocrinol 24:151–180

Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, Scheimann J, Myers B (2016) regulation of the hypothalamic-pituitary-adrenocortical stress response. Commun Physiol 6:603–621

Jacobson L (2005) Hypothalamic-pituitary-adrenocortical axis regulation. Endocrinol Metab Clin N Am 34:271–292

Jones BE (2005) From waking to sleeping: neuronal and chemical substrates. Trends Pharmacol Sci 26:578–586

Kuczenski R, Segal DS (1992) Regional norepinephrine response to amphetamine using dialysis: comparison with caudate dopamine. Synapse 11:164–169

Kuczenski R, Segal DS, Cho AK, Melega W (1995) Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. J Neurosci 15:1308–1317

McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, Bruchas MR (2015) CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety. Neuron 87:605–620

Nicolaides NC, Kyratzi E, Lamprokostopoulou A, Chrousos GP, Charmandari E (2015) Stress, the stress system and the role of glucocorticoids. Neuroimmunomodulation 22:6–19

Page ME, Abercrombie ED (1999) Discrete local application of corticotropin-releasing factor increases locus coeruleus discharge and extracellular norepinephrine in rat hippocampus. Synapse 33:304–313

Piao C, Deng X, Wang X, Yuan Y, Liu Z, Liang J (2016) Altered function in medial prefrontal cortex and nucleus accumbens links to stress-induced behavioral inflexibility. Behav Brain Res 317:16–26

Radley JJ, Sawchenko PE (2011) A common substrate for prefrontal and hippocampal inhibition of the neuroendocrine stress response. J Neurosci 31:9683–9695

Rajbhandari AK, Baldo BA, Bakshi VP (2015) Predator stress-induced CRF release causes enduring sensitization of basolateral amygdala norepinephrine systems that promote PTSD-like startle abnormalities. J Neurosci 35:14270–14285

Rajkowski J, Kubiak P, Aston-Jones G (1994) Locus coeruleus activity in monkey: phasic and tonic changes are associated with altered vigilance. Brain Res Bull 35:607–616

Robbins TW, Arnsten AF (2009) The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annu Rev Neurosci 32:267–287

Robert L, Labat-Robert J (2015) Stress in biology and medicine, role in aging. Pathol Biol 63:230–234

Samuels ER, Szabadi E (2008) Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part I: principles of functional organisation. Curr Neuropharmacol 6:235–253

Sawchenko PE, Li HY, Ericsson A (2000) Circuits and mechanisms governing hypothalamic responses to stress: a tale of two paradigms. Prog Brain Res 122:61–78

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138:132

Servan-Schreiber D, Printz H, Cohen JD (1990) A network model of catecholamine effects: gain, signal-to-noise ratio, and behavior. Science 249:892–895

Spencer RC, Klein RM, Berridge CW (2012) Psychostimulants act within the prefrontal cortex to improve cognitive function. Biol Psychiatry 72:221–227

Spencer RC, Devilbiss DM, Berridge CW (2015) The cognition-enhancing effects of psychostimulants involve direct action in the prefrontal cortex. Biol Psychiatry 77:940–950

Szabo S, Tache Y, Somogyi A (2012) The legacy of Hans Selye and the origins of stress research: a retrospective 75 years after his landmark brief 'letter' to the editor of nature. Stress 15(5):472–478

Ulrich-Lai YM, Herman JP (2009) Neural regulation of endocrine and autonomic stress responses. Nat Rev Neurosci 10:397–409

Valentino RJ, Foote SL (1988) Corticotropin-releasing hormone increases tonic but not sensory-evoked activity of noradrenergic locus coeruleus neurons in unanesthetized rats. J Neurosci 8:1016–1025

Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AF (2007) Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat Neurosci 10:376–384

Woodward DJ, Moises HC, Waterhouse BD, Yeh HH, Cheun JE (1991) Modulatory actions of norepinephrine on neural circuits. Adv Exp Med Biol 287:193–208

Yang LJ, Liu X, Liu DX, Jiang H, Mao XQ, Wang C, Pan F (2012) Effects of different adrenergic blockades on the stress resistance of Wistar rats. Neurosci Lett 511:95–100

Zhang R, Asai M, Mahoney CE, Joachim M, Shen Y, Gunner G, Majzoub JA (2016) Loss of hypothalamic corticotropin-releasing hormone markedly reduces anxiety behaviors in mice. Mol Psychiatry. doi:10.1038/mp.2016.136

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Small Airway Obstruction in Chronic Obstructive Pulmonary Disease: Potential Parameters for Early Detection

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Abstract

The impulse oscillometry (IOS) is recognized as a complementary method to spirometry in the diagnostics of obstructive pulmonary disorders. The IOS enables to measure total respiratory resistance (R5) and proximal respiratory resistance (R20), with the R5-R20 difference reflecting small airway resistance. This study seeks to evaluate the usefulness of R5-R20, maximal mid-expiratory flow (MMEF), and forced expiratory volume in 3 s/forced vital capacity ratio (FEV_3/FVC), in the assessment of small airway obstruction in chronic obstructive pulmonary disease (COPD). One hundred and six COPD patients and 43 control subjects, aged over 55, were investigated. Spirometry and IOS were used to assess pulmonary function. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) were evaluated. The findings demonstrate significant reductions in FEV_3/FVC and MMEF, and an increase in R5-R20 difference in COPD patients; the changes that depended on the severity of airway obstruction. The sensitivity of R5-R20 in reflecting the MMEF was 84%, specificity 44.2%, PPV 72.4%, and NPV 61.3%. We conclude that the R5-R20 difference is superior to spirometry in the assessment of

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small bronchi obstruction. A high sensitivity of R5-R20 in reflecting the MMEF makes the IOS method particularly useful for detection of mild lung injury, while a high specificity of the spirometric FEV_3/FVC ratio makes it useful to exclude obstruction of small airways. Both methods are thus complimentary.

Keywords
Airflow Limitation • Airways Obstruction • Chronic Lung Disease • Lung Function • Respiratory Resistance

1 Introduction

Spirometry is a standard, objective, and commonly used lung function test for diagnosing and staging the airflow limitation in chronic obstructive pulmonary disease (COPD). However, the assessment of small airways (less than 2 mm of internal diameter) obstruction, particularly important in COPD patients, still poses a problem. The variables available so far for measuring the airflow in peripheral airways have limitations due to their volatility, and the results do not specifically reflect a small airway disease in individual patients. Changes associated with small airway obstruction are expressed in a concave shape of the flow-volume curve, reduction of the maximal expiratory flow (MEF_{25}) measured in the terminal part of forced vital capacity (FVC), and a reduction of forced expiratory flow at 25–75% of FVC ($FEF_{25–75}$) (Pellegrino et al. 2005). The maximal mid-expiratory flow (MMEF) is equivalent to $FEF_{25–75}$ and this term will be used in the present article. The forced expiratory volume in 3 s to forced vital capacity ratio (FEV_3/FVC) is recommended by some authors as a routine spirometric measurement for identifying subjects with mild or peripheral airway obstruction. However, this ratio has not yet been approved by the American Thoracic Society (ATS) and European Respiratory Society (ERS) and is not widely used as a means to assess lung function (Morris et al. 2013). Further, correct and repeatable measurement results, according to the ATS/ERS standards, highly dependent on the patient's performance and thus are hardly achievable in approximately 20% of those aged over 65 (Kubota et al. 2009).

A relatively new method used for the evaluation of airflow limitation in peripheral airways is impulse oscillometry (IOS). This pulmonary function test is complementary to spirometry, with a much greater sensitivity to detect peripheral airway obstruction and requires tidal breathing as opposed to spirometric forced inspiratory/expiratory maneuvers. Thus, IOS method is recommended for elderly patients with physical or mental limitations, or with poor pulmonary function, who may have difficulty in carrying out the flow-volume spirometry, and is thus highly suitable for COPD patients (Janssens et al. 2001). The IOS enables to measure total respiratory resistance (R5) and proximal respiratory resistance (R20). The difference between the two (R5-R20) corresponds to small airway resistance (Jaranbäck et al. 2013; Crim et al. 2011). The present study seeks to define suitability of R5-R20 difference, MMEF, and FEV_3/FVC ratio in the assessment of small airway obstruction in COPD patients.

2 Methods

2.1 Patients

The study protocol was approved by the Ethics Committee of Poznan University of Medical Sciences in Poland and all patients gave informed consent. The study was conducted in

Table 1 Basic characteristics of study subjects

	COPD patients (n = 106)	Control subjects (n = 43)
Female/male	36/70	8/35
Age (year)	65 ± 10	56 ± 13
Body weight (kg)	78.9 ± 20.8	86.6 ± 11.6
Body height (cm)	166.5 ± 8.6	171.7 ± 10.3
BMI (kg/m^2)	28.3 ± 6.5	29.3 ± 2.1
Underweight (%)	3	0
Normal weight (%)	30	2
Overweight (%)	30	60
Obese (%)	37	38

COPD chronic obstructive pulmonary disease, *BMI* body mass index

106 stable COPD patients; F/M – 36/70, the mean age of 62 ± 10 years and BMI of 29.3 ± 2.1. The disease was diagnosed in accordance with the Global Initiative for Chronic Obstructive Lung Disease (GOLD 2016) recommendations that include the assessment of dyspnea according to the modified Medical Research Council scale (mMRC), a degree of bronchial obstruction, and the number of exacerbations and hospitalizations in the last 12 months. The control group consisted of 43 healthy subjects with normal spirometry; F/M – 8/35, the mean age of 56 ± 13 years and body mass index was (BMI) of 28.3 ± 6.5 (Table 1).

2.2 Respiratory Spirometry and Oscillometry

All subjects performed oscillometry and flow-volume spirometry (Jaeger MasterScreen; Erich Jaeger GmbH, Würzburg, Germany) according to European Respiratory Society and American Thoracic Society (ERS/ATS) recommendations (Pellegrino et al. 2005). Spirometry included the following: FEV_1/FVC and FEV_3/FVC, the ratio of forced expiratory volume in 1 s and 3 s, respectively, to forced vital capacity; FEV_1% pred, the ratio of forced expiratory volume in 1 s to its predicted value; FVC %pred, the ratio of forced vital capacity to its predicted value; MEF_{25}, maximal expiratory flow measured in the terminal part of FVC; MEF_{50}, maximal expiratory flow measured in mid-FVC; MEF_{75}, maximal expiratory flow measured in the proximal part of FVC; and MMEF, maximal mid-expiratory flow. The criterion for the diagnosis of bronchial obstruction was a fixed ratio of FEV_1/FVC of less than the 5th percentile. The severity of airway obstruction in COPD patients was defined on the basis of FEV_1%pred value according to the GOLD recommendations (GOLD 2016). The following degrees of airway obstruction were defined: mild ($FEV_1 \geq 80\%$ pred), moderate ($50\% < FEV_1 < 80\%$pred.), severe ($30\% < FEV_1 < 50\%$pred), and very severe ($FEV_1 < 30\%$pred). The predicted values of FEV_3/FVC and the lower limit of normal (LLN) were determined using the data elaborated by Hansen et al. (2006), based on the Third National Health and Nutrition Examination Survey (NHANES III). These values were calculated according to the following formulas: 100.63 – (0.1692 * age) and 95.00 – (0.1692 * age) for males, and 102.41 – (0.1826 * age) and 96.56 – (0.1826 * age) for females, respectively. The IOS assessed R5 at 5 Hz, consisting of extra-thoracic, central and peripheral airways, R20 at 20 Hz, consisting of mainly extra-thoracic and central airways, and the distal capacitive reactance X5 at 5 Hz, consisting of elastic lung and thorax components. The R5–R20 difference, corresponding to small airway resistance, was calculated. In addition, neurological condition and disability were assessed using a modified Rankin scale (Wilson et al. 2002). Mini-mental scale examination (MMSE) was performed to assess possible mental and cognitive deficits.

2.3 Statistical Elaboration

Continuous variables were described as means ±SD and categorical variables as percentages. The Kruskal-Wallis and Chi-squared tests were used for comparison between groups as appropriate. Spearman's correlation coefficient was used for the assessment of relationship between

pulmonary variables. Sensitivity, specificity, and positive predictive (PPV) and negative predictive value (NPV) were evaluated. A p-value <0.05 defined statistically significant differences. All analyses were conducted using STATISTICA 12.0 for Windows (Statsoft; Tulsa, OK).

3 Results

Spirometry confirmed mild airway obstruction in 16, moderate in 45, severe in 34, and very severe in 13 COPD patients. The mean FEV_3/FVC and MMEF values in COPD patients were lower than those in healthy subjects and were reduced with increasing bronchial obstruction. The mean R5-R20 difference was greater in COPD patients than that in controls and it increased with the severity of airway obstruction (Table 2). No appreciable neurological deficits were found in the patients; thus the Rankin score equaled zero. Nor were there any dementia symptoms substantiated (MMSE: 29 and 25–30 points; median and minimum-maximum, respectively).

We found a significant negative correlation between the R5-R20 difference and MMEF ($r = -0.45$; $p < 0.0001$) and between the FEV_3/FVC ratio and MMEF ($r = -0.28$; $p < 0.0001$) (Figs. 1 and 2; Table 3). Sensitivity and specificity of R5-R20 in reflecting the MMEF changes was 84.0% and 44.2%, respectively. On the other hand, FEV_3/FVC had a lower sensitivity of 52.1%, but 100% specificity with regard to MMEF, which translates into a high probability of identifying people without ventilatory disorders (Table 4). The evaluation of small airway obstruction in relation to COPD stage showed a good sensitivity of R5-R20 difference for detection of mild obstruction in COPD stage I as well as in more severe stages. The sensitivity of MMEF was low in mild obstruction and increased in moderate-to-severe stages and that of FEV_3/FVC ratio increased progressively with disease stage (Table 5).

4 Discussion

Characteristics of COPD patients in this study show that older age and low BMI correlated with increasing severity of airway obstruction, even though the mean BMI was not below the

Table 2 Comparison of pulmonary function tests between COPD patients and control subjects

	Patients in COPD stages				Controls	
	I (n = 16)	II (n = 43)	III (n = 34)	IV (n = 13)	(n = 43)	p-value
BMI (kg/m^2)	28.2 ± 6.0	29.5 ± 6.9	28.1 ± 6.3	24.5 ± 4.8	29.3 ± 2.1	0.0288
Spirometry						
FVC (L)	4.19 ± 0.99	2.65 ± 0.77	1.97 ± 0.71	2.52 ± 2.62	4.07 ± 1.36	<0.0001
FEV_1 (L)	2.76 ± 0.70	1.77 ± 1.49	0.91 ± 0.25	0.69 ± 0.11	3.18 ± 1.09	<0.0001
FEV_1%FVC ratio	65.6 ± 3.05	57.7 ± 8.0	48.2 ± 9.4	41.4 ± 6.4	78.2 ± 4.45	<0.0001
FEV_3%FVC ratio	88.9 ± 4.95	83.1 ± 8.4	78.4 ± 9.5	73.6 ± 8.75	94.9 ± 2.9	<0.0001
MEF_{75} (%pred)	65.7 ± 18.1	34.6 ± 13.7	15.7 ± 7.4	8.8 ± 2.0	92.2 ± 25.3	<0.0001
MEF_{50} (%pred)	50.8 ± 7.2	24.0 ± 9.2	11.7 ± 3.95	7.2 ± 1.9	84.7 ± 27.1	<0.0001
MEF_{25} (%pred)	41.7 ± 12.1	28.0 ± 12.4	19.4 ± 7.5	12.6 ± 3.6	72.2 ± 35.3	<0.0001
MMEF (%pred)	46.8 ± 7.6	24.7 ± 9.0	13.6 ± 3.9	8.8 ± 2.0	81.9 ± 27.6	<0.0001
Impulse oscillometry						
R5 (kPa s L^{-1})	0.49 ± 0.10	0.61 ± 0.27	0.71 ± 0.46	0.71 ± 0.34	0.46 ± 0.32	<0.0001
R20 (kPa s L^{-1})	0.39 ± 0.09	0.42 ± 0.16	0.37 ± 0.11	0.34 ± 0.09	0.34 ± 0.08	0.1107
X5 (kPa s L^{-1})	–0.14 ± 0.09	–0.24 ± 0.16	–0.36 ± 0.16	–0.47 ± 0.24	–0.12 ± 0.13	<0.0001
R5-R20 (kPa s L^{-1})	0.21 ± 0.10	0.31 ± 0.26	0.40 ± 0.46	0.44 ± 0.35	0.18 ± 0.32	<0.0001

COPD chronic obstructive pulmonary disease, *BMI* body mass index, *FVC* forced vital capacity, *FEV_1* forced expiratory volume in 1 s, *MEF* maximal expiratory flow, *MMEF* maximum mid-expiratory flow, *R* respiratory resistance at 5 Hz (R5) and 20 Hz (R20), *X5* respiratory reactance at 5 Hz; p-value pertains to the significance of inter-group differences for a given variable as assessed with the Kruskal-Wallis test

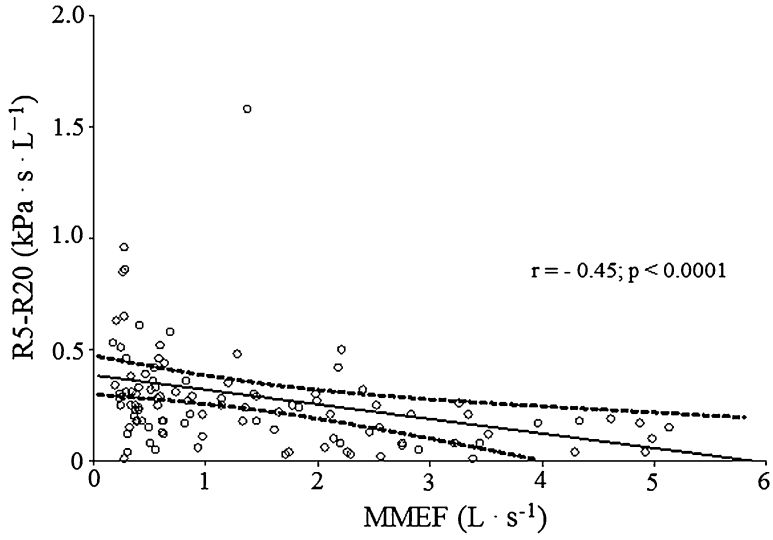

Fig. 1 Correlation between the difference in respiratory resistance at 5 Hz (R5) and 20 Hz (R20) and maximal mid-expiratory flow (MMEF)

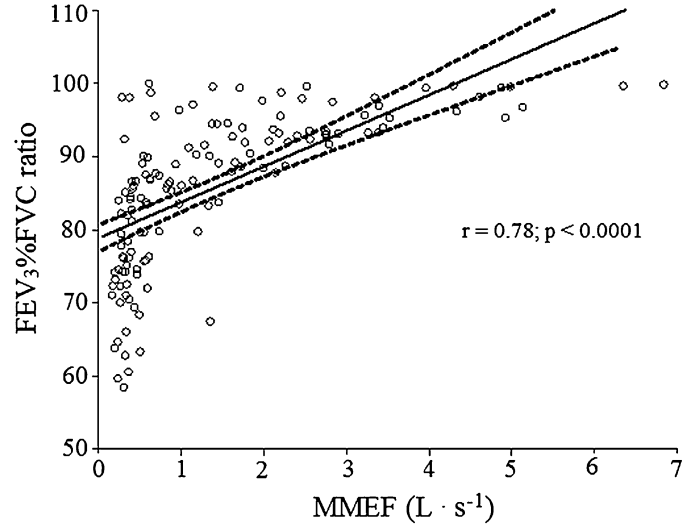

Fig. 2 Correlation between forced expiratory volume in 3 s to forced vital capacity ratio (FEV_3/FVC) and maximal mid-expiratory flow (MMEF)

Table 3 Correlations between pulmonary function variables in the entire study population (n = 149)

	R5		R20		X5		R5-R20	
	r	p-value	*r*	p-value	*r*	p-value	*r*	p-value
FEV_1 (L)	−0.49	<0.0001	−0.16	0.09	0.70	<0.0001	−0.42	<0.0001
FVC	−0.58	<0.0001	−0.29	0.01	0.75	<0.0001	−0.49	<0.0001
FEV_1%FVC ratio	−0.41	<0.0001	−0.02	0.81	0.53	<0.0001	−0.38	<0.0001
FEV_3%FVC ratio	−0.29	<0.0020	0.04	0.66	0.39	<0.0001	−0.28	<0.0001
MEF_{75} (%pred)	−0.49	<0.0001	−0.11	0.25	0.70	<0.0001	−0.43	<0.0001
MEF_{50} (%pred)	−0.49	<0.0001	−0.15	0.10	0.65	<0.0001	−0.44	<0.0001
MEF_{25} (%pred)	−0.48	<0.0001	−0.14	0.13	0.59	<0.0001	−0.39	<0.0001
MMEF (%pred)	−0.53	<0.0001	−0.14	0.12	0.67	<0.0001	−0.45	<0.0001

FVC forced vital capacity, *FEV_1* forced expiratory volume in 1 s, *MEF* maximal expiratory flow, *MMEF* maximal mid-expiratory flow, *R* respiratory resistance at 5 Hz (R5) and 20 Hz (R20), *X5* respiratory reactance at 5 Hz

Table 4 Sensitivity and specificity of FEV_3/FVC ratio and R5-R20 difference regarding the MMEF

	FEV_3%FVC	R5-R20
Sensitivity (%)	52.1	84.0
95% CI	41.6–62.5	73.7–91.4
Specificity (%)	100.0	44.2
95% CI	92.7–100.0	29.1–60.1
Positive predictive value (%)	100	72.4
95% CI	92.7–100.0	61.8–81.5
Negative predictive value (%)	52.1	61.3
95% CI	41.5–62.5	42.2–78.1

CI confidence interval, *FVC* forced vital capacity, FEV_1 forced expiratory volume in 1 s, FEV_3/FVC forced expiratory volume in 3 s to forced vital capacity ratio, *R* respiratory resistance at 5 Hz (R5) and 20 Hz (R20)

Table 5 Percentage of individuals in each COPD stage and in the control group in whom each of the three markers evaluated: FEV_3%FVC ratio, MMEF, and the R5-R20 difference detected small airway obstruction

	Patients in COPD Stages				Controls	
	I (n = 16)	II (n = 43)	III (n = 34)	IV (n = 13)	(n = 43)	p-value*
FEV_3%FVC	15%	33%	71%	92%	0%	0.0001
MMEF	54%	93%	97%	92%	7%	
R5-R20	82%	88%	86%	83%	50%	

*p-value signifies the presence of overall significant differences among the three markers of airway obstruction as assessed by the Chi-squared test.

normal level in each stage of the disease. Previous studies have reported that 38% of COPD patients are underweight (BMI < 18.5 kg/m^2), irrespective of disease severity. The mean BMI usually decreases with increasing COPD severity (De 2012).

The importance of spirometry in detecting and staging airway obstruction in patients with COPD is unquestionable. Clinical practice demonstrates that the evaluation of basic variables, such as FEV_1/FVC, FVC, and FEV_1 enables to assess airflow limitation in accord with the GOLD recommendations and helps make treatment decisions (GOLD 2016). It is underappreciated that there are many causes and sites in the airways that can be involved with obstruction (Johnston 2013). In contrast to airway obstruction in bronchial asthma, obstruction in COPD is progressive in nature, affects mainly peripheral bronchi of less than 2 mm in diameter, and requires the evaluation of variables additional to those outlined above. Small airway, peripheral, resistance makes up about 60% of total resistance in advanced stages of COPD (GOLD 2016; Jaranbäck et al. 2013). Changes in MMEF, used for the evaluation of small airway obstruction, are nonspecific and burdened with a high volatility. There is no justification for relating decreases in maximum expiratory flows to a distinct location of airflow obstruction. A reduction in these values is a result of changes in resistance and susceptibility of the surrounding lung parenchyma and lung capacity, and not in obstruction of a specific airway segment. The MMEF can also be misleading as it provides an unacceptably large number of probable false-negative and false-positive results (Hansen et al. 2006). In contrast to MMEF, which measures the mid-portion of exhalation ending at nearly the same time as FEV_1, the FEV_3/FVC ration is an excellent measure of small airway function. Several recently published studies, relating to FEV_3/FVC used for assessing small airway obstruction, refer to this ratio as an indicator of early detection of airway obstruction and lung injury (Guerreiro et al. 2015; Johnston 2013; Hansen et al. 2006). Those studies demonstrate that FEV_3/FVC is readily available and should be routinely reported in spirometry. The issues of FEV_3/

FVC has not yet been discussed in the ATS/ERS guidelines. Morris et al. (2013) have recommended the FEV_3/FVC as a routine spirometric measurement, in addition to FEV_1/FVC, and a standard for identifying subjects with mild airway obstruction when FEV_1/FVC is within normal limit. Previous studies have indicated that over 10% of patients with normal FEV_1/FVC have an abnormal FEV_3/FVC ratio, the result that enables to identify mild airway obstruction (Bhattarai et al. 2014). Johnston (2013) believes that FEV_3/FVC is clearly superior to MMEF and should replace the latter in the assessment protocols.

Flow-volume spirometry has a high repeatability of measurements expressed by a coefficients of variation, but requires a patient's cooperation, which in some cases is difficult achievable (Bellia et al. 2000). A cognitive decline limits the performance at spirometry. The mini-mental state examination and copying of intersecting pentagons have been employed to predict a difficulty in performing spirometry (Allen and Baxter 2009). Moreover, an easy to perform test enabling respiratory evaluation can also be considered for speech examination in neurodegenerative disorders, such as Parkinson's disease (Huber and Darling 2011). A noninvasive lung function test, IOS, introduced into clinical practice in recent years may be complementary to spirometry, and the method of choice in the evaluation of airway resistance in some cases of COPD (Schulz et al. 2013; Cooper 2005). The IOS is useful as a sensitive screening tool for the early detection of bronchial obstruction (Winkler et al. 2009). This particularly applies to elderly patients with accompanying physical and mental disorders (Miller et al. 2005), even though the repeatability of IOS measurements is slightly lower than that of spirometry in COPD (Oosteveen et al. 2003). Previous studies have also shown increases in R5 and R5-R20 difference in IOS with increasing severity of COPD obstruction (Piorunek et al. 2015; Anderson and Lipworth 2012; Tanaka et al. 2011). Therefore, IOS measurements seem a good alternative to spirometry in the assessment of respiratory dysfunction, particularly related to small airways. We believe we have confirmed that in the present study that compared the IOS indicators with classical spirometric variables.

5 Conclusions

We conclude that the R5-R20 difference is superior to spirometry in the assessment of small bronchi obstruction. A high sensitivity of R5-R20 in reflecting the MMEF changes makes the IOS method particularly useful for detection of mild lung injury, while a high specificity of the spirometric FEV_3/FVC ratio makes it useful to exclude obstruction of small airways. Both methods are thus complimentary.

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

References

Allen SC, Baxter M (2009) A comparison of four tests of cognition as predictors of inability to perform spirometry in old age. Age Ageing 38:537–541

Anderson WJ, Lipworth BJ (2012) Relationships between impulse oscillometry, spirometry and dyspnoea in COPD. J R Coll Physicians Edinb 42:111–115

Bellia V, Pistelli R, Catalano F, Antonelli-Incalzi R, Grassi V, Melillo G, Olivieri D, Rengo F (2000) Quality control of spirometry in the elderly. The SA.R.A. study. SAlute Respiration nell'Anziano = Respiratory Health in the Elderly. Am J Respir Crit Care Med 161:1094–1100

Bhattarai B, Ghosh M, Sinha A, Azad MR, Sivasambu B, Saha S, Wan SK, Pokharel S, Vadde R, Oke VV, Schmidt MF, Enriquez D, Quist J, Pandey A, Manhas S (2014) Can the FEV3/FVC ratio be reliably used to screen for lung injury in the absence of obstruction in African American population?. http://www.atsjournals.org/doi/abs/10.1164/ajrccm-conference. Accessed 10 Dec 2016

Cooper CB (2005) Assessment of pulmonary function in COPD. Semin Respir Crit Care Med 26:246–252

Crim C, Celli B, Edwards LD, Wouters E, Coxson HO, Tal-Singer R, Calverley PM (2011) Respiratory system impedance with impulse oscillometry in healthy and COPD subjects: ECLIPSE baseline results. Respir Med 105:1069–1078

De S (2012) Body mass index among patient with chronic obstructive pulmonary diseases. Indian J Physiol Pharmacol 56:353–358

GOLD (2016) Global initiative for chronic obstructive lung disease. http://www.goldcopd.org/guidelines-global-sreategy-for-diagnosis-management.html. Accessed 8 Dec 2016

Guerreiro C, Dionísio P, Ribeiro I, Moreira S, Valença J, Escaleira D, Bárbara C (2015) FEV_3/FVC ratio in early detection of airway obstruction. Eur Respir J 46:PA2280. doi:10.1183/13993003.congress-2015.PA2280

Hansen JE, Sun XG, Wasserman K (2006) Discriminating measures and normal values for expiratory obstruction. Chest 129:369–377

Huber JE, Darling M (2011) Effect of Parkinson's disease on the production of structured and unstructured speaking tasks: respiratory physiologic and linguistic considerations. J Speech Lang Hear Res 54:33–46

Janssens JP, Nguyen MC, Herrmann FR, Michel JP (2001) Diagnostic value of respiratory impedance measurements in elderly subjects. Respir Med 95:415–422

Jaranbäck L, Ankerst J, Bjermer L, Tufvesson E (2013) Flow-volume parameters in COPD related to extended measurements of lung volume, diffusion, and resistance. Pulm Med 11:1–10

Johnston R (2013) The FEV_3/FVC ratio, a useful tool for assessing early and mild airway obstruction. PFT Blog. Observations, opinions and ideas about pulmonary function testing. http://www.platform.com. Accessed 6 Oct 2013

Kubota M, Shirai G, Nakamori T, Kokubo K, Masuda M, Kobayashi H (2009) Low frequency oscillometry parameters in COPD patients are less variable during inspiration than during expiration. Respir Physiol Neurobiol 166:73–79

Miller MR, Crapo R, Hankinson J (2005) General considerations for lung function testing. Eur Respir J 26:153–161

Morris ZO, Coz A, Starosta D (2013) An isolated reduction of the FEV_3/FVC ratio is an indicator of mild lung injury. Chest 144:1117–1123

Oosteveen E, McLeod D, Lorino H (2003) The forced oscillation technique in clinical practice; methodology, recommendations and future developments. Eur Respir J 22:1026–1041

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R (2005) Interpretative strategies for lung function tests. Eur Respir J 26:948–968

Piorunek T, Kostrzewska M, Cofta S, Batura-Gabryel H, Andrzejczak P, Bogdański P, Wysocka E (2015) Impulse oscillometry in the diagnosis of airway resistance in chronic obstructive pulmonary disease. Adv Exp Med Biol 838:47–52

Schulz H, Flexeder C, Behr J, Heier M, Holle R, Hubner RM, Jörres RA, Nowak D, Peters A, Wichmann HE, Heinrich J, Karrasch S, KORA Study Group (2013) Reference values of impulse oscillometric lung function indices in adults of advanced age. PLoS One 8(5): e63366. doi:10.1371/journal.pone.0063366

Tanaka H, Fujii M, Kitada J (2011) Further examination of COPD. Using spirometry, respiratory function test, and impulse oscillometry. Nihon Rinsho 69:1786–1791

Wilson JT, Hareendran A, Grant M, Baird T, Schulz UG, Muir KW, Bone I (2002) Improving the assessment of outcomes in stroke: use of a structured interview to assign grades on the Modified Rankin Scale. Stroke 33:2243–2246

Winkler J, Hegert-Winkler A, Wirtz H, Schauer J, Kahn T, Hoheisel G (2009) Impulse oscillometry in the diagnosis of the severity of obstructive pulmonary disease. Pneumologie 63:266–275

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