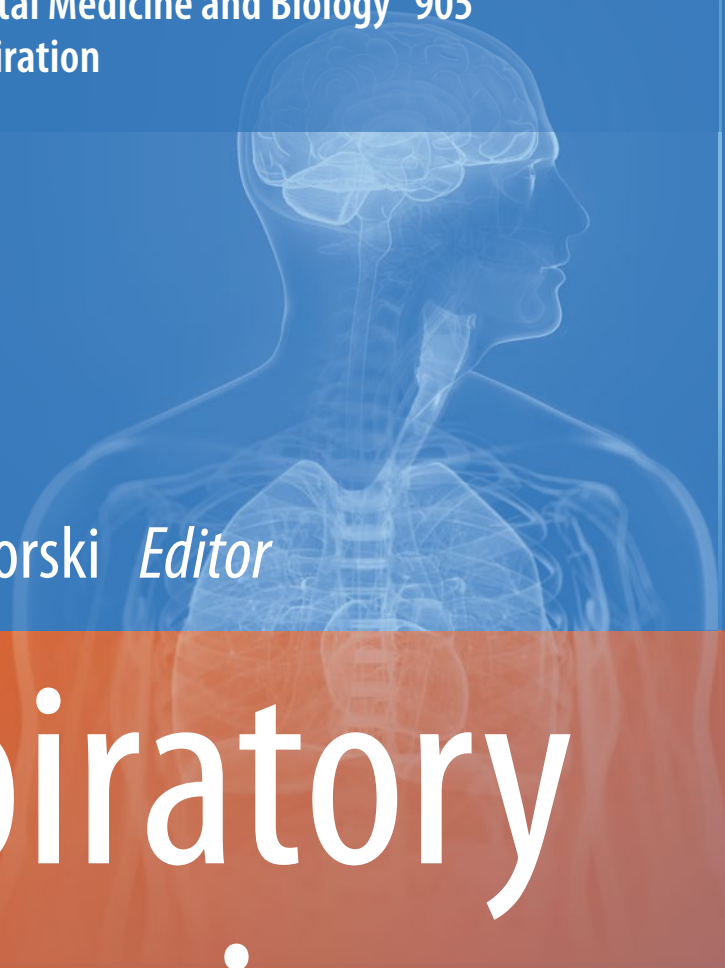
Advances in Experimental Medicine and Biology 905
Neuroscience and Respiration



Mieczyslaw Pokorski *Editor*

Respiratory Contagion

Springer

Advances in Experimental Medicine and Biology

Neuroscience and Respiration

Volume 905

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

Mieczyslaw Pokorski
Editor

Respiratory Contagion

Springer

Editor
Mieczyslaw Pokorski
Public Higher Medical Professional School in Opole
Institute of Nursing
Opole, Poland

ISSN 0065-2598 ISSN 2214-8019 (eBook)
Advances in Experimental Medicine and Biology
ISBN 978-3-319-30603-2 ISBN 978-3-319-30604-9 (eBook)
DOI 10.1007/978-3-319-30604-9

Library of Congress Control Number: 2016941177

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Preface

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

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

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

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

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

Opole, Poland Mieczyslaw Pokorski

Contents

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 1–4
DOI 10.1007/5584_2015_195

Published online: 25 February 2016

The Stress Reaction: A Historical Perspective

Oren Rom and Abraham Z. Reznick

Abstract

The history of stress research – milestones and people. Definitions and modern concepts of stress as well as the conflict between Hans Selye and the psychologists are described in this review. The molecular and physiological mechanisms of stress and their possible pharmacological intervention are introduced. The cycle of stress is presented as a new concept of the stress reaction, trying to bridge the gap between physiology and psychology. The cycle is a circular event in life, composed of 4 phases: (1) the resting ground phase, (2) the tension phase, (3) the response phase, and (4) the relief phase. In each phase, both physiological and psychological components can be assessed. These components are the basis for the proper handling of each phase and provide a unified model for the psychobiological response to stress. In addition, parameters of the cycle such as frequency, duration, and intensity can be measured, providing an effective tool for stress management. Finally, modern techniques and mechanisms for coping with stress are discussed like the Norwegian Gate Theory and Lazarus Dichotomy Model for the Stress Reaction. In the above models, specific examples of how people respond to the first time encounter of stressful events and how soldiers cope with stress are presented.

Keywords

Stress history • Stress research • Coping mechanisms • Gate theory • Stress physiology

O. Rom
Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa, Israel

A.Z. Reznick (✉)
Department of Anatomy and Cell Biology, Rappaport Faculty of Medicine, Technion; Efron St., P.O. Box: 9649, Bat Galim, Haifa 31096, Israel
e-mail: reznick@tx.technion.ac.il

1 Historical Introduction

1.1 Research Before Hans Selye

Several scientific giants have contributed to the foundations of stress research in the 19th century. Charles Darwin (1809–1892) in his monumental

book "The origin of species" had written that only those organisms that are capable of adapting to a changing environment will survive. Thus, what really Darwin was saying is that survival is the interaction of the biological world with the harsh and stressful environment. Claude Bernard (1813–1878) had stated that adaptation of an organism to a changing environment is possible by keeping the internal environment (milieu interieur) stable and constant. Walter B. Cannon (1871–1945), was the first to formulate some detailed concepts of the stress response and possible biological mechanisms involving emergency hormones (Cannon 1932). In addition, Cannon was the first to introduce some psychological aspects of stress by formulating the Fight or Flight model of the stress response. Finally, Cannon was the one who presented the concept of homeostasis as a basic mechanism of response to stress based on Bernard's idea of 'milieu interieur'.

1.2 Hans Selye (1907–1982)

Hans Selye is considered the founder of modern stress research. In the 1930s Selye advanced the concept of the General Adaptation Syndrome known also as the "GAS" Theory (Selye 1936). In his paper, Selye observed that in many long term exposures to various stressors the physiological responses followed a similar consistent pattern of three stages:

1. Alarm reaction (AR Stage)
2. Stage of resistance (SR Stage)
3. Stage of exhaustion (SE Stage)

These stages were further elaborated by Selye as based on neural and hormonal processes that are taking place in the body. Thus, the fast AR stage involves a neural response of the autonomic sympathetic nervous system which leads to rapid secretion of adrenaline followed by a slower SR stage which leads to increased levels of cortisol and other corticosteroids changing the body metabolism. Long term exposure to SE stage will eventually result in a damage to body systems such as the digestive, immune, or kidney systems. Altogether, two important ideas have been put forward by Selye: (i) stress is basically a physiological response and (ii) stress is a non-specific response of the body to any need or threat that it encounters (Selye 1974).

1.3 Controversy Between Selye and Psychologists

Selye's ideas were not accepted favorably by psychologists working in the stress field. J.W. Mason wrote in 1975: "In the psychological stress field it has been observed repeatedly that responses to any given psychological stimulus may vary widely from one individual to another or from one time to another in the same individual" (Mason 1975a, b). In a response paper, H. Selye tried to rebuttal this criticism by writing: "The fact that stressors or even the same stressor can cause different lesions in different individuals has been traced to what I have called 'conditioning factors' that can selectively enhance or inhibit one or the other stress effects" (Selye 1975). In addition, in his controversy with psychologists, Selye described a very famous experiment in animals in which he disconnected the brain cortex from the hypothalamus and avoided any emotional and psychological stimuli and still obtained the same physiological response to different stresses. Another psychologist, Susan R. Burchfield, wrote in 1979: "The research literature on failure to adapt to chronic stress suggests that maladaptation results from psychological not physiological exhaustion as was suggested by Selye" (Burchfield 1979). Despite the controversy, until his death in 1982, Selye strongly believed in his concepts of the stress reaction.

2 The Cycle of Stress

The above controversy emphasizes the diverse definitions, notions, and ideas that prevail in the stress field. Therefore, the concept of the stress cycle has been developed in order to try to

integrate many of the ideas that exist in this area into a unified model that would combine both physiological and psychological ingredients of stress and provide a comprehensive definition of the concept of stress (Reznick 1989). The cycle is a circular event in life, composed of four phases:

1. Resting ground phase
2. Tension phase
3. Response phase
4. Relief phase

In each phase, both physiological and psychological components can be assessed. These components are the basis for the proper handling of each phase and provide a unified model for the psycho-biological response to stress. In addition, parameters of the cycle such as frequency, duration, and intensity can be measured; providing an effective tool for stress management. In summary, the idea of the stress cycle is that it tries to define the stress response as a physiological and a psychological reaction at every stage of the cycle and integrates these into a cohesive definition of the stress reaction (Reznick 1989).

3 Coping with Stress: Modern Approaches to Stress Management

Finally, in the last section, we would like to discuss two relatively less known ideas how to cope with stress. The following models are elaborated.

3.1 The Norwegian Experiment

The group of Ursin et al. (1978) have performed a series of studies on young paratroopers that joined the Norwegian army. Prior to jumping from airplanes, the young men were trained on ground facilities which gave them some feelings and experience of sky jumping. The men were taken to a Norwegian army base where they exercised jumping from a tower of 12 m height. Before the first jump, some biochemical parameters of stress were measured in their blood, including the levels of adrenaline, insulin, glucose, and fatty acids and were designated as resting levels. Afterwards, the soldiers were asked to climb to the top of the tower to be hooked to special ropes and jump from the tower to the ground. Most of the fall is a free fall but as they approach the surface, the ropes slow down preventing them from hitting the ground. Immediately after the jump the above stress parameters were assessed again in their blood. They were jumping for 11 consecutive days and being assessed similarly every day. It was found that in the first 3 days, especially in the second day, the levels of the above parameters increased by 200–300 % above the resting levels. However, beginning after the fourth day of jumping and all the way to the last jump on day 11, the biochemical parameters of stress were gradually reduced, but did not return back to the resting levels of the pre-jumping period. The conclusion of the researchers was that by repeating the stressful experience of jumping over and over again, the soldiers were "getting used to" and by that they had developed coping mechanisms based on what is known in the literature as the gating mechanism. Accordingly, overcoming the initial fear means closing or narrowing the psychological gate in the brain which is followed by closing the physiological gate which, in turn, manifests by a milder or reduced biological reaction to stress, as shown in the Norwegian experiment.

3.2 The Dichotomy Model of Lazarus

Fear and threat are considered usually strong negative stressors. By overcoming them, the soldiers in the experiment outlined above have turned the negative stress into a positive reaction, and by that creating the psychobiological gate in the brain. However, most encounters of stress are such that the individual meets those events for the first time and does not have the opportunity to "practice" or repeat those situations many times.

Fig. 1 Dichotomy scheme of Lazarus

How does one react positively and create this gate when encounters, for the first time, a stressful situation? The dichotomy model of Lazarus (Lazarus et al. 1974) is a scheme that tries to provide such a mechanism by claiming that stress can be conceived in two different ways:

1. Conceived as a threat
2. Conceived as a challenge

When stress is conceived as a threat, one reacts strongly with an emotional response which dominates his reaction. Only after some time he may eventually refer to a logical response. Under such conditions he would have difficulties to create a positive psychobiological gate, needed for a mild logical response. On the other hand, if from the very first seconds he conceives the stress as a challenge, his emotional stage will be relatively short, while his logical response will be much longer; thus creating a positive psychobiological gate in the brain. The dichotomy scheme of Lazarus is shown in Fig. 1.

4 Summary and Conclusions

In this short review, a historical perspective on the long lasting scientific research into the stress field has been attempted. It is by no means comprehensive or complete, but it does emphasize that stress is a very complicated area of research with diverse definitions, concepts, and controversies. Nonetheless, the cycle of stress, elaborated in this paper, tries to bridge the gap between the physiologists and the psychologists by integrating many of the concepts, ideas, and discoveries into a cohesive model of the reaction to stress. Hopefully, the concept of the stress cycle will contribute to the proper handling of stress by human beings, having in mind that a disease, a hardly escapable encounter in everybody's life, is the most common form of stress as well.

Acknowledgments This study was supported by grants from the Rappaport Institute, the Krol Foundation of Barnegat N.J., the Myers-JDC-Brookdale Institute of Gerontology and Human Development, and ESHEL – The association for planning and development of services for the aged in Israel.

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

References

Burchfield SR (1979) The stress response: a new perspective. Psychosom Med 41:661–672

Cannon WB (1932) The wisdom of the body. W.W. Norton & Company, New York

Lazarus RS, Averill JR, Opton EM Jr (1974) The psychology of coping: Issues of research and assessment. In: Coelho GV, Hamburg DA, Adams JE (eds) Coping and adaptation. Basic Books, New York

Mason JW (1975a) A historical view of the stress field. J Human Stress 1(1):6–12

Mason JW (1975b) A historical view of the stress field. J Human Stress 1(2):22–36

Reznick AZ (1989) The cycle of stress—a circular model for the psychobiological response to strain and stress. Med Hypotheses 30:217–222

Selye H (1936) A syndrome produced by diverse nocuous agents. Nature 138(3479):32

Selye H (1974) Stress without distress, 1st edn. Lippincott Williams & Wilkin, Philadelphia

Selye H (1975) Confusion and controversy in the stress field. J Human Stress 1(2):37–44

Ursin H, Baade E, Levin S (1978) The psychobiology of stress – a study of coping men. Academic, New York/San Francisco/London

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 5–16
DOI 10.1007/5584_2015_181

Published online: 09 January 2016

The Environmental Domain of Quality of Life in Patients with Chronic Respiratory Diseases

Donata Kurpas, Katarzyna Szwamel, and Bożena Mroczek

Abstract

The literature lacks reports on the role played by the Environmental domain of quality of life (QoL) in care for patients with chronic respiratory diseases. Such information has a high potential for implementation in modern medicine based on a 'tailor-made' holistic healthcare model. The purpose of this study was to determine the components that shape the Environmental domain of QoL in patients with chronic respiratory diseases. The study group consisted of 305 adult patients (median age 65 years) with at least one chronic respiratory disease. The greatest contribution to a high value of QoL in the Environmental domain among patients with chronic respiratory diseases was made by the coexistence of high QoL levels in other domains and in satisfaction with QoL. Programs for preventing a decline in QoL in the Environmental domain should include patients with low scores for the above variables as well as those with a low level of education, those who have not shown an improvement in their psychological well-being in the past 12 months, those with a low level of positive mental attitudes or healthy eating habits, a low Camberwell index, and low levels of overall pro-health behavior.

Keywords

Chronic disease • Health care system • Preventive health services • Primary care • Pulmonary diseases • Social medicine • Social environment • Socioeconomic factors

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

Public Higher Medical Professional School, 68 Katowicka St., 45-060 Opole, Poland
e-mail: dkurpas@hotmail.com

K. Szwamel
Independent Public Health Care Team, Hospital Emergency Ward and Admissions, 2 Roosevelta St., 47-200 Kędzierzyn-Koźle, Poland

B. Mroczek
Faculty of Health Sciences, Department of Humanities in Medicine, Pomeranian Medical University, 11 gen. Dezyderego Chłapowskiego St., 70-103 Szczecin, Poland

1 Introduction

Chronic diseases and their related disabilities determine the level of well-being of people worldwide. The results of the Global Burden of Disease Study of 2013 (GBD 2015) show that people are living longer but with more diseases and with increased disability. From 1990 to 2013, the YLDs (years lived with disability) per person increased in 139 out of 188 countries, driven mainly by increases in pain, chronic respiratory disorders, and diabetes. Since healthcare systems and economies are not prepared to cope with these problems, and because of an increasing asymmetry between patients' needs and the capability of healthcare systems to meet them, efforts should be made to attain balanced development, based on the promotion of healthy lifestyles and well-being for people of all ages, as well as to create well-functioning, responsive and flexible healthcare systems (Atun 2015). Similar conclusions can be found in the European health report of 2012 (WHO 2012), which is an integral part of the European Health 2020 strategy. It was emphasized in this report that health and well-being are multidimensional and interactive concepts, and that they have one common determinant – namely, the efficacy of the healthcare system.

At present, we no longer evaluate the functioning and effectiveness of health care systems exclusively on the basis of the somatic status of chronically ill patients. Instead, we use elements of multidimensional structures, such as quality of life (QoL) (Pereira et al. 2006). In investigations of the QoL of chronically ill patients, we take into account not only health-related factors, such as good physical, functional, and emotional well-being (Jaracz et al. 2006), but also nonmedical aspects, including work, family, social contacts, and socioeconomic situation (Pereira et al. 2006; Bujok and Tombarkiewcz 2005). Thus, QoL analysis enables biopsychosocial evaluation, supports effective diagnostic and therapeutic interventions, and helps prevent social disorders. The QoL assessment is understood as an indicator of the efficiency, not only of medical services, but also of social support and political systems (Ostrowska 2009). The QoL level may reflect the need for satisfaction or indicate the impossibility of achieving happiness, self-realisation, and independence in the physical, social, and economic spheres (Pereira et al. 2006).

A lower QoL level is more often observed among patients who more often use medical services (Kurpas et al. 2013). The higher the QoL of patients, the lower are the direct medical expenses. What is especially emphasized is a significant relationship between the quality of medical services and patients' emotional well-being (Baernholdt et al. 2012). Studies of chronically ill elderly patients confirm that improvements in QoL lead to reductions in costs, due to lower healthcare utilization rates. It is worth emphasizing that the most important healthcare result or 'final medical point' that prevents the recurrence of symptoms, reduces their severity, and increases patients' satisfaction with healthcare is high QoL level (Faden and Leplege 1992).

The data provided by the Organization for Economic Cooperation and Development (OECD) encourage a wider look at the problem of investing in health (Davis 2015). These data highlight the role of social services as considerably improving health status while simultaneously reducing the costs of traditional and substantially more expensive medical care. This is because social services focus on the basic necessities of life, helping to ensure that individuals receive adequate nutrition, proper shelter, and a subsistence income, all of which is essential to maintaining good health.

The above-mentioned aspects can be regarded as elements of the Environmental domain of QoL. This domain, though until recently overlooked, has begun to play an increasingly prominent role in the development of effective healthcare systems – that is, of systems that meet not only the clinical requirements, but also social and economic needs and those related to patients' living and working environment. In a statistical model, Bowling et al. (2006) wrote that the perception of the surrounding environment may have effects on health status. The attitude to the problems, such as noise, delinquency, litter, traffic, graffiti, and poor air quality may all

contribute to a lower assessed health status. Henningsen and Priebe (2003) have described irritation as a 'new environmental illness', in which patients strongly believe that their symptoms are caused by environmental factors; even when such symptoms do not coincide with empirical data. A key component of the disease is the attitude of the patient toward environmental factors.

The literature lacks reports on the role played by the Environmental domain of QoL in shaping care for patients with chronic respiratory diseases. Such information has a high potential for implementation in modern medicine based on a 'tailor-made' holistic healthcare model. Considering this, the purpose of this study was to determine the components that shape the Environmental domain of QoL in patients with chronic respiratory diseases and to identify the most important factors in this group of patients.

2 Methods

This research was conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by the Bioethics Committee of the Medical University in Wrocław (approval no. KB-422/2014). The main inclusion criteria were age (at least 18 years old) and diagnosis of at least one respiratory chronic disease.

The study group consisted of 305 adult patients with chronic respiratory diseases. Ages ranged from 18 to 92 years, with the median of 65 years. Participants were recruited from among the patients of 135 general practitioners between June 2014 and April 2015. The patients who agreed to participate anonymously in the project signed an informed consent form and were given a questionnaire to complete at home and return in a stamped envelope.

Quality of life (QoL) was assessed using the Polish version of the World Health Organization Quality of Life Instrument Short Form (WHOQOL-BREF). The WHO Quality of Life (WHOQOL) is a generic QoL instrument that has been designed to be applicable to people living in different conditions and cultures (Jaracz et al. 2006). The WHOQOL-BREF measures the QoL within four domains: D1-Physical, D2-Psychological, D3-Social relationship, and D4-Environmental. Answers to all the questions in the WHOQOL-BREF are given on a five-point Likert-type scale, including the first two questions about satisfaction with QoL and health state. The reliability of the Polish version of the WHOQOL-BREF questionnaire, measured using the Cronbach-α coefficient, proved acceptable both for the parts that evaluate each domain (with coefficients ranging from 0.81 to 0.69) and for the questionnaire as a whole (coefficient 0.90).

The authors also used the Health Behavior Inventory (HBI) developed by Juczyński (2001). This instrument consists of 24 statements that measure four categories of pro-health behavior: healthy eating habits, preventive behavior, positive mental attitudes, and health practices. Respondents mark the frequency of the health behavior and the correct healthy activity: 1 – almost never; 2 – rarely; 3 – from time to time; 4 – often; and 5 – almost always. The sum of the results from all four scales gives the score for the general health behavior (range 24–120), where the higher the score, the healthier is behavior. The intensity of health behavior in the individual categories is the sum of all answers in the subscales divided by six. The HBI internal consistency, measured using Cronbach's α, equals 0.85.

The patients' adaptation to a lifestyle with a disease was assessed using the Acceptance of Illness Scale (AIS). The AIS consists of eight statements about the negative consequences of a health state, where every statement is rated on a five-point Likert-type scale. The value 1 denotes poor adaptation to a disease, and 5 its full acceptance. The score for illness acceptance is a sum of all points and can range from 8 to 40. Low scores (0–29) indicate a lack of acceptance and a lack of adaptation to a disease and the strong feeling of mental discomfort. High scores (35–40), on the other hand, indicate acceptance of the illness, manifesting as a lack of negative emotions associated with a disease. The scale can be used

to assess the degree of acceptance of each disease. The Cronbach's α coefficient was 0.85 for the Polish version and 0.82 for the original version.

The level of met and unmet needs was assessed using the Modified Short Camberwell Needs Assessment. This questionnaire focuses on 22 problem areas for patients with chronic somatic diseases (without severe mental disorders). In order to standardize the results, the following coding was established: 0 denotes unmet needs and 1 satisfied needs. Next, 24 questions present 22 needs to the respondents and enquire whether they are met (1) or unmet (0). In this way, the number of satisfied needs (M) of the total (N) needs could be established and the Camberwell index was calculated as the ratio M/N. The internal consistency of the Modified Short Camberwell Needs Assessment was given by a Cronbach's α of 0.96.

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

The index of services was calculated by summing up the services received and dividing by the number of types of the services received provided during visits to a doctor over the last 12 months.

2.1 Statistical Elaboration

None of the 52 variables were normally distributed, as was confirmed by the Shapiro–Wilk normality test. Medians and variability ranges (extremes) were calculated for measurable (quantitative) variables; for qualitative variables, the frequency (percentage) was determined. The analysis of qualitative variables was based on contingency tables and the chi-squared test or on Fisher's exact test for count data. The numerical variables were grouped into several ranges. The relationship between QoL levels in the Environmental domain and the other variables was analyzed by calculating Cramér's V coefficient, which reflects the strength of any relationship between two categorical units (0 – no relationship and 1 – the strongest relationship) and by calculating Spearman's rho rank correlation coefficient, which measures a linear relationship between two numerical variables (0 – no correlation and 1 – the strongest correlation). The V coefficient was calculated using categorized variables, and the *r* correlation coefficient was calculated using source variables (alternatively, nonnumeric variables are coded with numbers — for example, 'no' is replaced by '0' and 'yes' by '1'). A significant linear relationship (*r* coefficient significantly different from '0') was confirmed by the significant strength of the relationship, i.e., the V coefficient was significantly different from '0'. If there was no linear relationship, the strength of the relationship was small or insignificant.

Therefore, 23 variables that significantly correlated with the Environmental domain were selected for hierarchical cluster analysis, whose aim was to divide the initial group of variables into subgroups (clusters) of variables that correlate in a similar way with the Environmental domain. In the analysis of clusters, a feature according to which the objects will be classified needs to be defined. In our study, this feature was a variable (object) correlation degree with the Environmental domain measured using the *r* correlation coefficient. The absolute value of the difference between correlation coefficients was accepted as the measure of a distance between variables. Thus, the distance will be close to '0' between variables with similar correlation coefficients and higher between variables with different correlation coefficients. The division into classes was performed using Ward's method. Variables were divided into four clusters (Fig. 1).

The critical level of significance was taken to be $p < 0.05$. The R 3.1.3 statistical software for Mac OS X 10.10.4 was used for all data analyses.

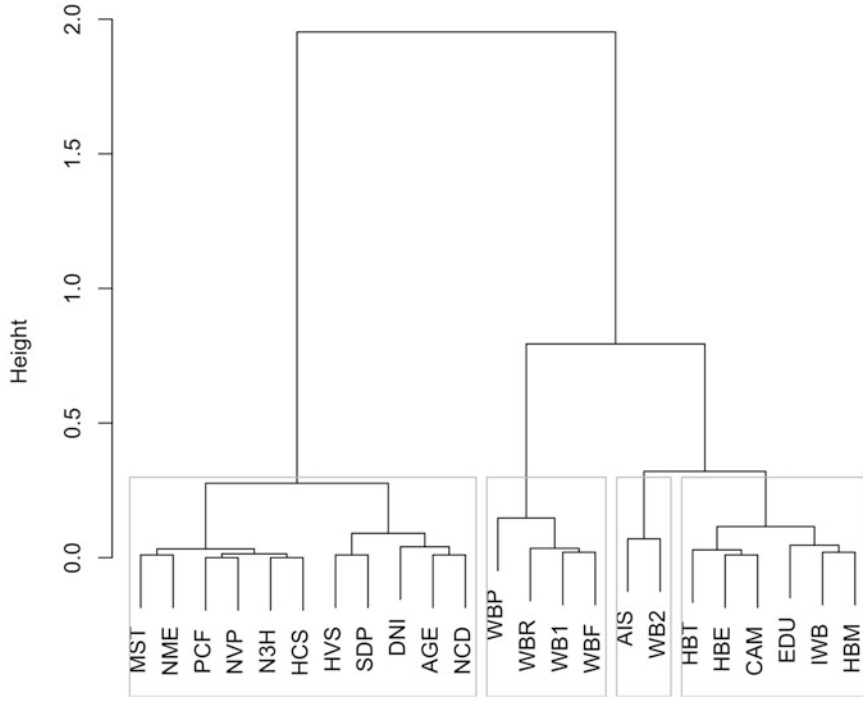


Fig. 1 Results of hierarchical cluster analysis for Environmental domain QoL in patients with chronic respiratory diseases. *HVS* number of home visits during the last 12 months, *DNI* number of district nurse interventions during the last 12 months, *IWB* improvement in psychological well-being in the past 12 months, *SDP* patient's subjective assessment of distance from primary care center, *PCF* patient's choice of family physician, *NVP* number of visits to primary care center during the last 12 months, *MST* marital status, *EDU* education, *AIS* level of illness acceptance, *WB1* satisfaction with QoL, *WB2* satisfaction with quality of health status, *WBF* level of QoL in Physical domain, *WBP* level of QoL in Psychological domain, *WBR* level of QoL in Social relationship, *HBT* pro-health behavior (total), *HBE* level of healthy eating habits, *HBM* level of positive mental attitudes, *AGE* age, *N3H* number of hospitalizations in the past 3 years, *CAM* camberwell index, *HCS* health care services index, *NME* number of medications, *NCD* number of chronic diseases

3 Results

The majority of patients were women (156, 51.5 %) aged 65 and over. Detailed sociodemographic data on the patients and their chronic diseases are shown in Table 1. The median number of chronic diseases was 3 (min–max: 1–15), median body mass index (BMI) was 27.3 kg/m^2 (min–max: 15.6–41.0 kg/m^2), and median somatic index was 0.4 (min–max: 0.0–1.0). The median Camberwell index was 0.8 (min–max: 0.2–1.0). The median disease acceptance level was low (26 points, min–max: 8–40). The median satisfaction with QoL was 4 (min–max: 1–5), while the median health state was 3 (min–max: 1–5). The highest median among QoL domains was found in the field of social relationships (14.0, min–max: 4.0–20.0); the Environmental domain was lower (median 13.5, min–max: 7.5–19.5); the lowest scores were found for the physical (13.1, min–max: 4.0–19.4) and the psychological (12.7, min–max: 4.0–19.3) domains.

Among the pro-health behaviors, the highest median was found for the preventive behaviors (3.8, min–max: 1.0–5.0); positive mental attitudes scored lower (median 3.7, min–max: 1.5–5.0); and the lowest values were for health practices (median 3.6, min–max: 1.5–5.0) and healthy eating habits (median 3.3, min–max: 1.0–5.0).

Table 1 Sociodemographic data and ICD-10 diagnoses[a] of patients[b]

	N = 214	n	%
Gender	Women	156	51.5
	Men	147	48.5
Age	24 and below	8	2.7
	25–44	29	9.7
	45–64	107	35.7
	65–84	139	46.3
	85 and above	17	5.7
Place of residence	Rural area	133	44.2
	Urban population		
	Below 5000	28	9.3
	5000–10,000	14	4.7
	10,000–50,000	58	19.3
	50,000–100,000	22	7.3
	100,000–200,000	21	7.0
	Over 200,000	25	8.3
Education	Primary	79	26.3
	Vocational	87	29.1
	Secondary	72	24.0
	Post-secondary	31	10.3
	Higher	31	10.3
Marital status	Single	34	11.3
	Married	192	63.6
	Separated	4	1.3
	Divorced	8	2.6
	Widowed	64	21.2
Diagnosis	J45 Bronchial asthma	116	38.0
	J44 Other chronic obstructive pulmonary diseases	98	32.1
	J42 Unspecified chronic bronchitis	43	14.1
	J43 Pulmonary emphysema	39	12.8
	J41 Chronic simple and mucous-purulent bronchitis	38	12.5
	J47 Bronchiectasis	14	4.6
Most common coexisting diseases	I10 Primary hypertension	118	38.7
	M47 Spondylosis	100	32.8
	I70 Atherosclerosis	59	19.3
	M15 Osteoarthritis of multiple joints	34	11.1
	I11 Hypertensive heart disease	33	10.8
Comorbidity	1 chronic disease	57	18.7
	2 chronic diseases	54	17.7
	3 chronic diseases	52	17.0
	4 chronic diseases	56	18.4
	5 chronic diseases	29	9.5
	>5 chronic diseases	57	18.7

[a]Some patients were diagnosed as having at least two pathological entities;
[b]The figures in Column *n* do not sum up to 305 due to gaps in the questionnaires completed by the patients

3.1 Significant Correlations

Patients with low QoL levels in the Environmental domain more often were of advanced age ($r = -0.29$, $p < 0.001$), were widowed ($r = -0.15$, $p = 0.012$), had only primary education ($r = 0.28$, $p < 0.001$), lived far from a primary care center ($r = -0.22$ $p < 0.01$), and had not chosen their family doctors themselves ($r = -0.14$, $p = 0.039$). These patients more

often had low levels of illness acceptance ($r = 0.44$, $p < 0.001$), were dissatisfied with their QoL ($r = 0.56$, $p < 0.001$), were dissatisfied with their health ($r = 0.37$, $p < 0.001$), had low QoL in the Physical domain ($r = 0.58$, $p < 0.001$), the Psychological domain ($r = 0.70$, $p < 0.001$), and the Social Relationship domain ($r = 0.60$, $p < 0.001$), had lower levels of health behaviors ($r = 0.17$, $p = 0.029$), including proper dietary habits ($r = 0.20$, $p = 0.004$) and positive mental attitudes ($r = 0.23$, $p = 0.001$), had a low level of satisfied needs (a low Camberwell index) ($r = 0.57$, $p < 0.001$), and had a high somatic index ($r = -0.20$, $p < 0.001$).

Low QoL levels in the Environmental domain were accompanied by more frequent visits to a family doctor during the last 12 months ($r = -0.14$, $p = 0.042$), a higher number of home visits ($r = -0.23$, $p < 0.001$), more interventions of a district nurse ($r = -0.26$, $p < 0.001$), more hospitalizations during the last 3 years ($r = -0.13$, $p = 0.025$), a lack of improvement in somatic ($r = 0.21$, $p < 0.001$) and mental well-being ($r = 0.25$, $p < 0.001$), a high healthcare utilization rate ($r = -0.13$, $p = 0.027$), a greater number of drugs taken ($r = -0.16$, $p = 0.005$), and a greater number of chronic diseases ($r = -0.30$, $p < 0.001$).

3.2 Results of Hierarchical Cluster Analysis

The results of the hierarchical cluster analysis are presented in Fig. 1. The Environmental domain had the strongest positive correlation with a cluster of the following variables: level of QoL in Psychological domain ($r = 0.70$, $p < 0.001$), level of QoL in Social relationship ($r = 0.60$, $p < 0.001$), level of QoL in Physical domain ($r = 0.58$, $p < 0.001$), and satisfaction with QoL ($r = 0.56$, $p < 0.001$). The next positively correlated clusters included satisfaction with quality of health status ($r = 0.37$, $p < 0.001$), and the level of illness acceptance ($r = 0.44$, $p < 0.001$).

The clusters that correlated positively but less strongly were made up of education ($r = 0.28$, $p < 0.001$), an improvement in psychological well-being in the past 12 months ($r = 0.25$, $p < 0.001$), positive mental attitudes ($r = 0.23$, $p = 0.001$), healthy eating habits ($r = 0.20$, $p = 0.004$), Camberwell index ($r = 0.19$, $p = 0.001$), and pro-health behaviors ($r = 0.17$, $p = 0.043$).

The clusters of negatively correlating variables included the number of chronic diseases ($r = -0.30$, $p < 0.001$), age ($r = -0.29$, $p < 0.001$), number of district nurse interventions during the last 12 months ($r = -0.26$, $p < 0.001$), number of home visits during the last 12 months ($r = -0.23$, $p < 0.001$), the patient's subjective assessment of distance from primary care center ($r = -0.22$, $p = 0.001$), number of medications ($r = -0.16$, $p = 0.005$), marital status ($r = -0.15$, $p = 0.012$), patient's choice of family physician ($r = -0.14$, $p = 0.039$), number of visits to a primary care center during the last 12 months ($r = -0.14$, $p = 0.042$), number of hospitalizations in the past 3 years ($r = -0.13$, $p = 0.025$), and the index of health care services ($r = -0.13$, $p = 0.027$).

4 Discussion

Ensuring a high QoL for healthy and chronically ill citizens is a major goal of both government and local community sectors. This goal can be achieved by means of a well-organized health care system that meets not only clinical requirements but, more importantly, patients' social needs and needs associated with their living and working environment (WHO 2012).

The study of Ross et al. (2013) suggests that a more integrated approach is needed for older adults with asthma, treating not only the physical aspects of asthma but the psychological and social aspects as well. That study suggests that people over 65 (the mean age was 73.3) with asthma have lower QoL levels than their healthy counterparts from the control group. In addition, worse overall functional status correlated with

poorer asthma QoL, and with living alone (Ross et al. 2013). However, contrary results were obtained by Szynkiewicz et al. (2013), who investigated a younger group of asthma patients with an average age of 44.9 ± 12.5 years. They concluded that the average QoL levels of single people were higher than those of married people. According to Kupcewicz and Abramowicz (2014), the age of COPD patients statistically significantly affected their QoL, as measured using the St. George's Respiratory Questionnaire (SGRQ) in the 'Symptoms' subscale. The youngest subjects (aged between 41 and 50 years) obtained a result of 51.24 ± 9.93 on a scale of 0–100, where 0 denotes the highest QoL level and 100 the lowest, as compared to the 64.42 ± 9.10 achieved by patients over 70 years of age.

The present study confirms that elderly widowers have low QoL levels. At the same time, it provides more details on this group of patients, showing that particularly low QoL scores are obtained for the Environmental domain. A QoL study (Kannan et al. 2015) of asthma patients conducted with the use of the Mini-Asthma Quality of Life Questionnaire (mAQLQ) demonstrated that the total mAQLQ (mean ± SD 5.4 ± 1.1) and Symptom, Emotional, and Activity domain scores were similar to those of younger populations, whereas the Environmental domain scores (4.4 ± 1.7) were lower. These conclusions are supported by the study of Kurpas et al. (2013) which investigated chronically ill elderly patients (71.60 ± 7.98 years old; n = 1974) and found a mean QoL score for the Environmental domain of the WHOQOL-BREF of 13.24 ± 2.21. Low QoL scores for this domain were obtained by the oldest group of patients, those with high BMI, and those who were not married. Isolated elderly individuals are significantly more vulnerable because of fewer resources and the lack of social support, and are more likely to manage their chronic conditions by using various strategies to remain independent (Ross et al. 2013). For this reason, it is important for the physician to consider living situation as a part of the standard evaluation of older adults with chronic respiratory diseases.

These studies show that both patients' age and marital status are important predictors of their QoL levels, especially in the Environmental domain. Geriatric programs and general social policy for home and hospital care addressed to senior citizens should be based on QoL assessments, with special attention paid to the Environmental domain (Pereira et al. 2006). Those living alone should be treated as a priority by decision-makers (Kurpas et al. 2013).

The present study demonstrated that the Environmental domain correlates most strongly with the QoL level in the Psychological domain and with satisfaction with QoL. It also revealed that patients with low QoL scores for the Environmental domain more often showed dissatisfaction with QoL and a low level of illness acceptance. In the study of asthma patients by Ross et al. (2013), both the QoL, as measured using the mAQLQ, and control of symptoms, as measured using the Asthma Control Questionnaire 6-item version (ACQ-6), were assessed as worse in subjects who scored higher on the Center for Epidemiological Studies Depression Scale (CES-D8). It has also been observed that the risk of depression in elderly people increased with the number of diseases, and partially as a result of functional limitations (Ross et al. 2013). Similarly, patients with COPD experienced negative consequences of the disease and accepted them moderately. In most cases, these patients had low levels of satisfaction with life. The average overall illness acceptance rate was 19.0 ± 6.9; this reflects a medium level of illness acceptance. As many as 54 % of the patients had a low level of satisfaction with life; the remaining 46 % had a medium level of satisfaction (Kupcewicz and Abramowicz 2015).

The physical environment, including pollution, noise, traffic, and climate, makes up one element of the Environmental domain (Jaracz et al. 2006). A study by Yen et al. (2006), which assessed the impact of the place of residence on an adult population with asthma in northern California, is interesting in this context. The authors investigated the relationship between QoL, depressive symptoms and physical functioning and various problems in the surrounding environment (traffic, noise,

pollution, smells, and fires). A higher level of perceived difficulties in the area was associated with a lower QoL, worse physical functioning, and an increase in depressive symptoms. Other authors have found that exposure to traffic pollution was the strongest predictor of poorer asthma-related QOL in older adults with asthma (Kannan et al. 2015). A study of Kurpas et al. (2014), comparing variables related to the QoL of primary care patients from rural ($n = 1239$) and urban areas ($n = 1886$), has demonstrated higher QoL scores for the Environmental domain among country dwellers (13.6 vs. 13.4, $p = 0.015$), with an OR (odds ratio) of overall QoL scores of 1.341 (95 % CI: 1.067–1.687). Ferreira et al. (2010), on the other hand, have shown higher values of QoL for patients with asthma from urban areas. This result may be explained by the fact that about 75 % of the respondents lived in urban or semiurban areas. However, Kupcewicz and Abramowicz (2014) have found that country dwellers obtained a result of 66.93 ± 10.71 (on a scale of 0–100, where 0 denotes the highest, and 100 the lowest, QoL level), which reflected a statistically significantly lower QoL level in the Symptoms domain than that of urban dwellers (58.34 ± 9.52); in that study, 32.3 % of the respondents were residents of rural areas and 67.7 % were residents of urban areas.

Comparison of the present results with those reported by other authors does not allow us to draw unambiguous conclusions about the influence of the place of living and the related exposure to physical environment on the QoL of patients with chronic respiratory diseases. Therefore, it is necessary to conduct further research on this group of patients.

Our study demonstrated a strong correlation between the QoL levels in the Environmental domain and the Physical domain (especially between low scores for the Environmental domain and high values of the somatic index). In the study group, low QoL scores for the Environmental domain were accompanied by more frequent visits to a family doctor during the last 12 months, a higher number of home visits and interventions of a district nurse, more frequent hospitalizations during the last 3 years, a high healthcare utilization rate, a greater number of drugs, and a greater number of chronic diseases. The report of Ross et al. (2013) cited earlier suggests that the lower QoL of patients with asthma is associated with the worsening of their general functional status and, consequently, with a higher number of unscheduled visits to a family doctor. As Ferreira et al. (2010) emphasize, factors such as limitations on activity, symptoms, emotional function, and environmental stimuli are also important constraints on the asthma patients' HRQoL. The fact that low QoL scores for the Environmental domain were obtained by the oldest group of chronically ill patients with a higher number of home visits, phone consultations, district nurse visits in the last 12 months, and hospitalizations in the last 3 years has also been confirmed by Kurpas et al. (2014). According to Kupcewicz and Abramowicz (2014), patients who were hospitalized at least five times during 1 year obtained a result of 71.36 ± 8.42 (on the scale from 0 to 100, where 0 denotes the highest, and 100 the lowest QoL level) and had statistically significantly lower QoL levels in the 'Symptoms' scale than those who were hospitalized less often (1–2 stays in hospital; 55.80 ± 7.87). According to Kannan et al. (2015), on the other hand, poorer mAQLQ scores among elderly asthma patients are significantly associated with emergency department visits (adjusted β [aβ] $= -1.3$, where β values indicate the strength and direction of association, $p < 0.0001$).

Both the present study and those cited above explicitly confirm a negative correlation between the QoL of patients with chronic respiratory diseases and the number of medical services provided for them, including the frequency of visits and the number of hospitalizations.

Another important element of the Environmental domain is the possibility of gaining new information and skills (Jaracz et al. 2006). Educational attainment is an important predictor of poor outcomes in COPD. Higher levels of formal education may have indirect impact on health by virtue of improved job opportunities and associated improved housing, access to health insurance, and income. Education may also directly impact health by facilitating improved health knowledge and the ability to navigate the

healthcare system (Omachi et al. 2013). Our results suggest that low scores in the Environmental domain are mainly obtained by subjects with only primary education. The results of the study of Szynkiewicz et al. (2013) show that, in all groups of asthma patients (with controlled, partially controlled and uncontrolled asthma), and also in the entire population, the level of QoL increases with the level of education. A significant increase was observed in the control group ($p < 0.05$) and in the whole population ($p < 0.0005$).

Independent of socioeconomic status, poor health literacy was associated with greater COPD severity, greater COPD helplessness, and worse respiratory-specific health-related QoL. Poorer health literacy was associated with greater odds of both COPD-related emergency department visits and hospitalizations. These results underline that COPD patients with poor health literacy may be at particular risk for poor health-related outcomes (Omachi et al 2013).

The QoL assessment is a sensitive indicator of the level of unsatisfied needs (not restricted to health-related needs) of chronically ill patients, as well as of direct and indirect medical expenses. When taking into consideration the QoL elements, it is good to think about them not only in terms of the current clinical status of chronically (somatically or mentally) ill patients and their satisfaction with their social relationships, but also in the context of their living and working environment (broadly understood) (Kurpas et al. 2015). In the analysis of the level of unsatisfied needs' among patients with chronic respiratory diseases ($n = 214$; median age: 65 years; min–max: 80–90 years), the median of values in the Environmental domain was set at 13.5 (min–max: 8.5–19.5) (Kurpas et al. 2015). Next to the Social Relationship domain, this was again the QoL component with the highest values. What is more, high QoL levels in the Environmental domain increased the chance of high levels of the patients' needs being satisfied by a factor of 410; to be compared with the factor of 94 for high values in the Psychological domain, 53 for the Social Relationship domain, and 33 for the Physical domain. A significant relationship between the QoL level and the level of satisfied needs has previously been confirmed; the higher the QoL level, the lower the level of unsatisfied needs (Kurpas et al. 2015). Nurmatow et al. (2012) also emphasizes the role of holistic care, thus drawing attention to the problem of QoL and the unsatisfied needs of patients with severe COPD. These authors emphasize that, despite the common belief, the major problems of such patients include not only dyspnea, but also social problems such as potential isolation caused by difficulties associated with leaving home. However, their review of the literature describing interventions used to deliver holistic care does not allow for a consensus to be reached or for interventions to be chosen on the basis of solid scientific evidence. It has, however, been found that social participation, strong family bonds, and good living conditions are all factors that have a positive impact on the QoL of chronically ill patients (Koligat et al. 2012).

One of the most important elements that affects an individual's life is his or her financial situation, since sufficient financial reserves allow people not only to satisfy their basic needs, but also to actively spend their free time, to have social contacts, and to feel more comfortable (Bąk-Drabik and Ziora 2010). The self-assessed QoL of employed people with asthma is higher than that of those who do not work (whether unemployed or retired) (Szynkiewicz et al. 2013). Ferreira et al. (2010) have shown that average monthly net income determines the self-assessment of QoL. Bąk-Drabik and Ziora (2010) have demonstrated that the low QoL of COPD patients is determined by income, current profession, and employment status. White-collar workers have significantly higher QoL levels than physical workers. Pensioners and retirees have significantly lower QoL levels than employed people. The authors demonstrate the influence of monthly income on the QoL of these patients. Income is one of important factors contributing to the QoL level in the following domains: Mental Health, Social Functioning, and Body Pain, and in the Symptoms subscale. Furthermore, a low socioeconomic status is a crucial risk factor for COPD development, and may sometimes negatively affect QoL to a larger extent than pathophysiological factors. In a study of

Kupcewicz and Abramowicz (2014), 21 % (n = 20) of the respondents claimed that clinical COPD symptoms caused them to give up a job, while 64 % (n = 61) believed that COPD symptoms had a negative effect on the quality of their work or forced them to change a job. As many as 70 % (n = 67) regarded themselves as invalids due to their breathing difficulties, while over a half of them thought that their disease was troublesome for their families, neighbors, and friends. An in-depth analysis showed that employed patients had significantly higher QoL levels in the 'Influence on life' subscale than those who were retired or living on a pension (Kupcewicz and Abramowicz 2014).

At present, there are a great number of people with chronic respiratory diseases. Approximately 34.1 million Americans and 300 million people worldwide have been diagnosed with asthma by a health professional during their lifetime, with nearly 3500 annual deaths being attributed to the disease in the United States (Ross et al. 2013). Scientists predict that the number of COPD patients will grow globally in the next two decades (Nurmatow et al. 2012). There is thus an urgent need for the development and evaluation of medical, social, and environmental interventions that emphasize social relationships and the living and working environment, and allow patients with chronic respiratory diseases to be independent, earn a living, and improve their QoL. Research on QoL, especially in the Environmental domain, may serve as a scientific basis for these interventions. The assessment of the Environmental domain may turn out to be essential for measuring the effectiveness of the healthcare system (Kurpas et al. 2015).

5 Conclusions

Among patients with chronic respiratory diseases, a high QoL level in the Environmental domain is most effectively produced by high QoL levels in the Psychological, Social, and Physical domain and by satisfaction with QoL. Satisfaction with the quality of health status and the level of illness acceptance is also of great importance.

Programs for preventing a decline in QoL in the Environmental domain should include patients with low scores for the above variables as well as those with a low level of education, those who have not shown an improvement in their psychological well-being in the past 12 months, those with a low level of positive mental attitudes or healthy eating habits, a low Camberwell index, and low levels of overall pro-health behavior. Additionally, any such program should include older widowed patients with chronic respiratory diseases and with several other chronic diseases, with more frequent district nurse interventions, with more home visits, with more visits to primary care centers in the last 12 months, with more hospitalizations in the last 3 years, with higher health care services indices, with negative subjective assessment of distance from primary care center, and with higher numbers of medications, as well as those who have not chosen their family doctors themselves.

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

References

Atun R (2015) Transitioning health systems for multimorbidity. Lancet Jun 5. pii: S0140-6736(14) 62254–622546. Available from: http://www.thelancet.com/pdfs/journals/lancet/PIIS0140-6736%2814%2962254-6.pdf. Accessed on: 24 Aug 2015

Baernholdt M, Hinton I, Yan G, Rose K, Mattos M (2012) Factors associated with quality of life in older adults in the United States. Qual Life Res 21:527–534

Bąk-Drabik K, Ziora D (2010) The impact of socioeconomic status on the quality of life in patients with chronic obstructive pulmonary disease. Pneumonol Alergol Pol 78:3–13

Bowling A, Barber J, Morris R, Ebrahim S (2006) Do perceptions of neighbourhood environment influence health? Baseline findings from a British survey of aging. J Epidemiol Community Health 60:476–483

Bujok G, Tombarkiewcz M (2005) Health-related quality of life as a new clinical problem. Wiad Lek 58:67–70

Davis K (2015) To lower the cost of health care, invest in social services. Health Aff. July 14, 2015. Available from: http://healthaffairs.org/blog/2015/07/14/to-lower-the-cost-of-health-care-invest-in-social-services. Accessed on 24 Aug 2015

Faden R, Leplege A (1992) Assessing quality of life: moral implications for clinical practice. Med Care 30:166–175

Ferreira LN, Brito U, Ferreira PL (2010) Quality of life in asthma patients. Rev Port Pneumol 16:23–55

GBD (2015) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 386(9995):743–800. doi:10.1016/S0140-6736(15)60692-4

Henningsen P, Priebe S (2003) New environmental illnesses: what are their characteristics? Psychother Psychosom 72:231–234

Jaracz K, Kalfoss M, Górna K, Baczyk G (2006) Quality of life in Polish respondents: psychometric properties of the Polish WHOQOL-Bref. Scand J Caring Sci 20:251–260

Juczyński Z (2001) Assessment tools in health promotion and health psychology. Psychological Tests Laboratory, Polish Psychological Association, Warsaw

Kannan JA, Bernstein DI, Bernstein CK, Ryan PH, Bernstein JA, Villareal MS, Smith AM, Lenz PH, Epstein TG (2015) Significant predictors of poor quality of life in older asthmatics. Ann Allergy Asthma Immunol, Jul 21. pii: S1081-1206(15)00412-3

Koligat D, Leszczyński P, Pawlak – Buś K, Koligat A, Zaprutko T, Kus K, Paczkowska A, Ratajczak P, Nowakowska E (2012) Impact of chronic diseases (osteoporosis and diabetes) on Health Related Quality – of – Life- a pilot study. Nowiny Lekarskie 81:122–128

Kupcewicz E, Abramowicz A (2014) Assessement of quality of life in chronic obstructive pulmonary disease patients. Hygeia Public Health 49:805–812

Kupcewicz E, Abramowicz A (2015) Influence of selected socio- demographic factors on degree of illness acceptance and on level of satisfaction with life in patients with chronic obstructive pulmonary disease. Hygeia Public Health 50:142–148

Kurpas D, Mroczek B, Bielska D (2013) The correlation between quality of life, acceptance of illness and health behaviors of advanced age patients. Arch Gerontol Geriatr 56:448–456

Kurpas D, Mroczek B, Bielska D (2014) Rural and urban disparities in quality of life and health-related behaviors among chronically ill patients. Rural Remote Health 14:2485

Kurpas D, Wroblewska I, Kassolik K, Andrzejewski W, Athanasiadou A, Mroczek B (2015) Unmet needs of patients with chronic respiratory diseases within primary healthcares. Adv Exp Med Biol 861:43–55

Nurmatow U, Buckingham S, Kendall M, Murray SA, White P, Sheikh A, Pinnock H (2012) Effectiveness of holistic interventions for people with severe chronic obstructive pulmonary disease: systematic review of controled clinical trials. PLoS One 7, e46433

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

Ostrowska A (2009) Disability, rehabilitation and social integration of people with disabilities. In: Ostrowska A (ed) Medical sociology. Efforts problems, categories of analysis. IFiS PAN, Warsaw

Pereira RJ, Cotta RMM, Franceschini SCC, Ribeiro RCL, Sampaio RF, Priore SE, Cecon PR (2006) Contribution of the physical, social, psychological and environmental domains to overall quality of life of the elderly. Revista de Psiquiatria do Rio Grande do Sul 28:27–38

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

Szynkiewicz E, Filanowicz M, Graczyk M, Cegła B, Jabłońska R, Napiórkowska – Baran K, Bartuzi Z (2013) Analysis of the impact of selected socio- demographic factors on quality of life of asthma patients. Adv Dermatol Allergol 30:218–225

WHO – European health report (2012) Available from: http://www.euro.who.int/en/data-and-evidence/european-health-report/european-health-report-2012. Accessed on: 27 Aug 2015

Yen IH, Yelin EH, Katz P, Eisner MD, Blanc PD (2006) Perceived neighborhood problems and quality of life, physical functioning, and depressive symptomps among adults with asthma. Am J Public Health 96:873–878

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 17–23
DOI 10.1007/5584_2015_185

Published online: 23 January 2016

Incidence and Clinical Course of Respiratory Viral Coinfections in Children Aged 0–59 Months

A. Nitsch-Osuch, E. Kuchar, A. Topczewska-Cabanek, K. Wardyn, K. Życińska, and L. Brydak

Abstract

Clinical data available on coinfections are contradictory concerning both the number of viruses involved and the severity of the condition. A total of 114 patients aged 0–59 months with symptoms of respiratory tract infection were enrolled into the study. Nasal and pharyngeal swabs were tested using the PCR method for the following 12 viruses: influenza A, influenza B, respiratory syncytial virus A (RSV A), respiratory syncytial virus B (RSV B), adenovirus, metapneumovirus, coronavirus 229E/NL63 (hCoV229), coronavirus OC43 (hCoVOC43), parainfluenza virus 1 (PIV-1), parainfluenza virus 2 (PIV-2), parainfluenza virus 3 (PIV-3), and rhinovirus A/B. Coinfections were detected in nine (8 %) patients. Five of the coinfections were related to influenza A (H3N2) virus associated with the following other, single or combined, respiratory viruses: influenza B in one case, hCoV229 in two cases, hCoV229, RSV A, and PIV-2 in one case, and PIV-1, PIV-2, RSV A, RSV B, and adenovirus in one case. The other four coinfections were caused by: adenovirus and hCoVOC43, adenovirus, and rhinovirus, RSV A and PIV-1, influenza B, and RSV B. We did not observe any significant differences in the clinical course of infections caused either by a single or multiple viral factors.

Keywords

Children • Coinfection • Etiology • Pathogenesis of infection • Respiratory tract disease • Virus

A. Nitsch-Osuch (✉), A. Topczewska-Cabanek, K. Wardyn, and K. Życińska
Department of Family Medicine, Warsaw Medical University, 1A Banacha St., 02-097 Warsaw, Poland
e-mail: anitsch@wum.edu.pl

E. Kuchar
Department of Pediatrics with Medical Assessment Unit, Warsaw Medical University, Warsaw, Poland

L. Brydak
National Influenza Center, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland

1 Introduction

Respiratory viral infections are a major source of morbidity and mortality in childhood. Children with respiratory infections are generally treated as outpatients and the etiology of their disease is usually not investigated. In case of hospitalization, the diagnostic techniques employed are often not sensitive enough (Jin et al. 2007). It is estimated that 50–85 % of pediatric acute respiratory tract infections are of viral origin. Experience and understanding of the role of viral coinfections in respiratory tract infections have grown in recent years due to the introduction of molecular techniques (Brunstein et al. 2008; Mahony et al. 2007). At present, clinical data available on confection are variable, sometimes contradictory, in terms of both the number of viruses involved and the severity of the condition. These discrepancies may be due to such factors as geographical region and detection methods (Kumar 2009). The detection of respiratory viruses in children using molecular methods can be challenging. The reason for this is that virus-virus coinfections and mixed viral-bacterial infections occur in 15–30 % of cases and viruses can be detected in 25–45 % of children in the absence of respiratory symptoms (Frobert et al. 2011; Peng et al. 2009; Raymond et al. 2009). Although, the effects of viral coinfections have been described and analyzed in the literature, the number of such studies is limited, especially in Central Eastern Europe, including Poland. The aim of this study was to analyze the incidence and clinical course of respiratory tract infections caused by more than one viral etiological factor among children aged 0–59 months.

2 Material and Methods

Ethical approval was obtained from the Medical University of Warsaw, Poland. Data and specimens were obtained from patients aged 0–59 months with respiratory tract infection symptoms: fever $> 38°C$, sore throat or cough lasting less than 96 h. The exclusion criteria were the following: symptoms lasting longer than 96 h, age > 59 months, ongoing antibiotic therapy, and the child's guardian refusal to take swabs. A total of 114 patients were enrolled into the study: 52 children hospitalized in the General Pediatric Ward and 62 children requiring ambulatory care; all with acute respiratory tract infections. Guardians of all the children enrolled were provided with written information regarding the study's aims and methods, and written consent was obtained from all the guardians. Two swabs were taken from the patients: one nasal and one pharyngeal. Viscose swabs were used to collect specimens that were stored for less than 24 h at a temperature of 2–8 °C and then transported to the National Influenza Center at the National Institute of Public Health – National Institute of Hygiene in Warsaw, Poland, where specimens from patients, stored at -80 °C, were tested by RT-PCR using a RV12 ACE Detection Kit (Seegene, Seoul, South Korea) for the detection of the following respiratory viruses: influenza A virus, influenza B virus, human respiratory syncytial virus A (RSV A), human respiratory syncytial virus B (RSV B), human adenovirus, human metapneumovirus, human coronavirus 229E/NL63 (hCoV229), human coronavirus OC43 (hCoVOC43), human parainfluenza virus 1 (PIV-1), human parainfluenza virus 2 (PIV-2), human parainfluenza virus 3 (PIV-3), and human rhinovirus A/B. A random hexamer primer for cDNA synthesis was used with the First Strand cDNA Synthesis Kit (Fermentas, York, UK). Each cDNA preparation was subject to the RV12 PCR procedure according to the manufacturer's instructions (Seegene, Seoul, South Korea). Afterwards, amplicons were detected using gel electrophoresis. The subtyping of influenza viruses was carried out using conventional multiplex RT-PCR (Influenza A/B OneStep Typing Set; Seegene, Seoul, South Korea). A panel of Seeplex RT-PCR assays was used to detect influenza A, influenza B, and the three subtypes of influenza A (H1, H3, and H1N1 2009).

A descriptive analysis of patients with coinfections was carried out, including age, gender and symptoms. A comparison of symptoms of infections caused by a single viral agent and

those caused by more than one agent was performed. The statistical analysis was conducted with a medical statistical calculator available at www.medcalc3000.com

3 Results

Nine patients (8 %) were infected by more than one viral agent; 64 (56 %) patients were infected by a single virus; 41 (36 %) samples were negative. Single etiological agents were as follows: influenza A (29 cases, 25 %), RSV B (18 cases, 16 %), RSV A (5 cases, 4 %), adenovirus (4 cases, 4 %), HCoVOC43 (3 cases, 3 %), hCoV229 (2 cases, 2 %), PIV-1 (1 case, 1 %) and PIV-2 (1 case, 1 %). Five cases caused by influenza A (H3N2) virus were associated with coinfections with other respiratory viruses, such as influenza B (1 case), hCoV229 (2 cases), hCoV229, RSV A, and PIV-2 combined (1 case), and PIV-1, PIV-2, RSV A, RSV B, and adenovirus combined (1 case). Figure 1 presents an image of PCR test results for a multiple coinfection with six respiratory viruses.

The remaining four were double infections caused by adenovirus and hCoVOC43, adenovirus and rhinovirus, RSV A and PIV-1, and influenza B and RSV B. The demographical and clinical characteristics of patients with coinfections are presented in Table 1. The incidence of viral coinfections was similar among hospitalized and non-hospitalized children with symptoms of respiratory tract infection (7.6 % *vs.* 8 %, respectively). Symptoms and clinical course of the disease caused by either single or multiple agents were similar; no significant differences were observed (Table 2).

4 Discussion

In the present study, the prevalence of viral coinfections among young children with acute respiratory tract infection was on the low side of 8 %. In the literature, coinfection rates appreciably vary depending on the study. Peng et al. (2009) found multiple etiological agents in 36 % of hospitalized children; coinfections occurred predominantly among children aged 3–6 years and the most common pathogens were influenza A, influenza B, and PIV-1. Renois et al. (2010) detected 17 % of multiple infections among patients with acute respiratory tract infections; they were combinations of influenza A/H1N1v virus with CoV, human bocavirus (HBoV), RSV or human rhinoviruses (HRVs). Bonzel et al. (2008) diagnosed viral coinfections in 16 % of samples, with RSV and hBoV being the most common combination. In that study, viral coinfection was found in 17 % of children with bronchitis and in 23 % of those with bronchiolitis, while in

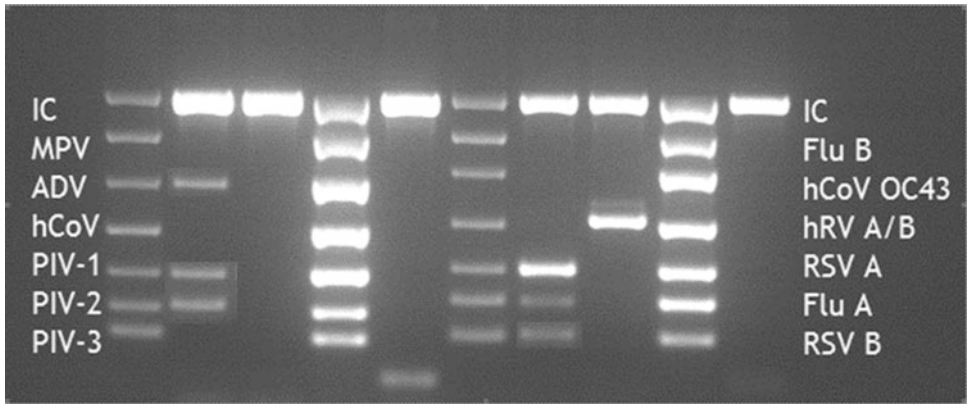


Fig. 1 PCR results for simultaneous infections with six respiratory viruses: influenza A (Flu A), parainfluenza 2 (PIV-1), parainfluenza 1 (PIV-2), respiratory syncytial virus A (RSV A), respiratory syncytial virus B (RSV B), and adenovirus (ADV)

Table 1 Characteristics of patients with coinfections

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
	Influenza A	Influenza A	Influenza A	Influenza A	Influenza A	Adenovirus	Adenovirus	RSVA	Influenza B
				Parainfluenza 2	Parainfluenza 2				
				hCoV229	Parainfluenza 1				
					RSV A				
					RSV B				
Etiology	Influenza B	hCoV229	hCoV229	RSV A	Adenovirus	hCoOC43	Rhinovirus	Parainfluenza 1	RSVB
Age (months)	11	12	16	5	3	4	3	1	2
Gender	M	M	M	F	M	F	F	M	F
Ambulatory care	Yes	Yes	Yes	Yes	Yes	No	No	No	No
Hospital care	No	No	No	No	No	Yes	Yes	Yes	Yes
Sore throat	Yes	No	Yes	No	Yes	No	No	No	No
Sneezing/coryza	No	No	No	Yes	Yes	No	No	No	
Cough	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
Dyspnea	No	Yes	No	No	No	No	No	No	No
Shortness of breath	No	No	Yes	No	No	No	No	No	No
Wheezing	No	No	No	No	No	No	No	No	No
Cyanosis	No	No	No	No	No	No	No	No	No
Tachycardia	No	No	Yes	No	No	No	No	No	No
Tachypnea	No	No	No	Yes	No	No	No	No	No
Abnormal pulmonary breathing sounds	Yes	No	Yes	No	Yes	No	No	No	No
Chills/convulsions	No	No	No	No	No	No	No	No	No
Vomiting/diarrhea	No	No	No	No	No	Yes	No	No	No

hCoV229 human coronavirus 229E/NL63, *hCoVOC43* human coronavirus OC43, *RSV A* respiratory syncytial virus A, *RSV B* respiratory syncytial virus B

Table 2 Clinical course and symptoms of infections caused by multiple and single agents

	Multiple viral agents (9 cases)	Single viral agents (64 cases)	p-value, OR, 95 % CI
Ambulatory care	5	30	$p > 0.05$, OR 1.41, 95 % CI 0.37–5.35
Hospital care	4	32	$p > 0.05$, OR 0.80, 95 % CI 0.21–3.03
Sneezing/coryza	2	31	$p > 0.05$, OR 0.30 95 % CI 0.06–1.41
Cough	6	50	$p > 0.05$, OR 0.56, 95 % CI 0.13–2.30
Sore throat	3	42	$p > 0.05$, OR 0.26, 95 % CI 0.06–1.06
Dyspnea	1	8	$p > 0.05$, OR 0.87, 95 % CI 0.12–6.32
Shortness of breath	1	10	$p > 0.05$, OR 0.67, 95 % CI 0.10–4.76
Wheezing	0	3	$p > 0.05$, OR 0.02, 95 % CI 0.02–9.74
Cyanosis	0	2	$p > 0.05$, OR 0.02, 95 % CI 0.01–14.77
Tachycardia	1	6	$p > 0.05$, OR 0.86, 95 % CI 0.17–9.02
Tachypnea	1	7	$p > 0.05$, OR 1.01, 95 % CI 0.14–7.41
Abnormal breathing sounds	3	7	$p > 0.05$, OR 2.32, 95 % CI 0.23–8.43
Chills/convulsions	0	8	$p > 0.05$, OR 0.02, 95 % CI 0.01–3.25
Vomiting/diarrhea	1	8	$p > 0.05$, OR 0.87, 95 % CI 0.12–6.32

OR odds ratio, *CI* confidence intervals

patients with pneumonia 33 % were positive for 2 or more viral pathogens. In the present study, the majority of coinfections were associated with influenza A infection and all of them occurred in children younger than 24 months.

We failed to not observe any significant differences in the clinical course of infections caused by either a single or multiple viral factors. Our data are in agreement with other studies that have reported no clinical differences between patients with respiratory infections caused by a single or multiple agents detected in nasopharyngeal aspirates from children (Martin et al. 2012; Nascimiento et al. 2010). Camargo et al. (2012) found coinfections with influenza A H1N1 in 22 % of patients with no greater morbidity or mortality. Suryadevara et al. (2011) diagnosed 28 % of multipathogen viral infections among febrile children younger than 24 months with acute respiratory infections. The most common viruses detected were RSV and rhinovirus/enterovirus. There were no differences in the severity of disease when comparing patients infected with one or multiple pathogens. However, there are some studies that show that coinfection may be a risk factor for poor evolution. Aberle et al. (2005) reported that airway obstruction was more severe when RSV contributed to coinfection and the hospital stay was lengthened when rhinovirus was involved. In another study, Greensill et al. 2003 showed that when coinfection consisted of RSV and metapneumovirus, evolution was worse. Further, Richard et al. (2008), who studied the relationship of coinfection with the need for admission to the pediatric intensive care unit (PICU), concluded

that the presence of two or more viruses increased the likelihood of admission. However, in another study conducted among patients admitted to the PICU, Ghani et al. (2012) found that bacterial coinfection was only associated with a longer hospital stay, with no increase in mortality. Calvo et al. (2008) reported multiple viral infections in hospitalized infants with respiratory tract disease in about 17 % cases and these coinfections were linked to higher fever, longer hospital stays, and a more frequent use of antibiotics compared with the cases of single RSV infections. It is worth noting that the detection of more than one virus may be due to the presence of viral fragments persisting for up to 5–6 weeks after the onset of symptoms, which may actually be irrelevant for the current clinical course. Further studies are required to confirm or exclude associations between co-detected respiratory viruses and to demonstrate the underlying mechanisms of such associations which may lead to cooperation or competition between viruses. Another important issue that remains unclear is the long-term effect that such coinfections may have on the development of chronic lung disease.

A limitation of the present study was a relatively small number of cases evaluated compared with the usually high numbers seen in pediatric practice. Another limitation was the lack of inclusion of bacterial coinfections. Predisposing factors for synchronous coinfection were not detected in our study, although there are some other data pointing to such a possibility. For instance, a high rate of codetection of HBoV with HAdV was reported and a prolonged shedding of the HBoV may potentially contribute to this phenomenon (Jartii et al. 2011). On the other hand, an advantage of the present study was that we described viral coinfections among young immunocompetent children, while other authors often report multiple infections among immunosupressed patients (Stefanska et al. 2013).

To conclude, viral respiratory coinfections do occur in young immunocompetent young children, but such infections do not seem to play an important role in the clinical presentation of acute respiratory infections in this age group. Studies involving larger numbers of patients and using improved quantitative detection techniques are needed to define the exact role of viral coinfections in the course of respiratory disease.

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

References

Aberle JH, Aberle SW, Pracher E, Hutter HP, Kundi M, Popow-Kraupp T (2005) Single versus dual respiratory virus infections in hospitalized infants: impact on clinical course of disease and interferon-gamma response. Pediatr Infect Dis J 24:605–610

Bonzel L, Tenenbaum T, Schroten H, Schildgen O, Schweitzer-Krantz S, Adams O (2008) Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. Pediatr Infect Dis J 27:589–594

Brunstein JD, Cline CL, McKinney S, Thomas E (2008) Evidence from multiplex molecular assays for complex multipathogen interactions in acute respiratory infections. J Clin Microbiol 46:97–102

Calvo C, García-García ML, Blanco C, Vázquez MC, Frías ME, Pérez-Breña P (2008) Multiple simultaneous viral infections in infants with acute respiratory tract infections in Spain. J Clin Virol 42:268–272

Camargo C, Guatura SB, Bellei N (2012) Respiratory viral coinfection among hospitalized patients with H1N1 2009 during the first pandemic wave in Brazil. Braz J Infect Dis 16:180–183

Frobert E, Escuret V, Javouhey E, Casalegno JS, Bouscambert-Duchamp M, Moulinier C (2011) Respiratory viruses in children admitted to hospital intensive care units: evaluating the CLARTR pneumovir DNA array. J Med Virol 83:150–155

Ghani AS, Morrow BM, Hardie DR, Argent AC (2012) An investigation into the prevalence and outcome of patients admitted to a pediatric intensive care unit with viral respiratory tract infections in Cape Town, South Africa. Pediatr Crit Care Med 13:e275–e281

Greensill J, McNamara PS, Dove W, Flanagan B, Smyth RL, Hart CA (2003) Human metapneumovirus in severe respiratory syncytial virus bronchiolitis. Emerg Infect Dis 9:372–375

Jartii T, Hedman K, Jartii L, Ruuskanen O, Allander T, Soderlund-Venermo M (2011) Human bocavirus- the first 5 years. Rev Med Virol 22:46–64

Jin YS, Kuak EY, Shin BM (2007) Detection of 12 respiratory viruses with two-set multiplex reverse transcriptase-PCR assay using a dual priming oligonucleotide system. Korean J Lab Med 27:420–427

Kumar RM (2009) The widely used diagnostics DNA microarrays – a review. Am J Infect Dis 5:207–218

Mahony J, Chong S, Merante F, Yaghoubian S, Shina T, Lisle C (2007) Development of respiratory virus panel test for the detection of twenty human respiratory viruses by using multiplex PCR and a fluid microbead-based assay. J Clin Microbiol 45:3056–3062

Martin ET, Kuypers J, Wald A, Englund JA (2012) Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. Influenza Other Respir Viruses 6:71–77

Nascimiento MS, Souza AV, Ferreira AV, Rodrigues JC, Abramovici S, Silva Filho LV (2010) High rate of viral identification and coinfections in infants with acute bronchiolitis. Clinics (Sao Paulo) 65:1133–1137

Peng D, Zhao D, Liu J, Wang X, Yang K, Xicheng H (2009) Multipathogen infections in hospitalized children with acute respiratory infections. Virol J 6:155. doi:10.1186/1743-422X-6-155

Raymond F, Carbonneau J, Boucher N, Robitaille L, Boisvert S, Wu WK (2009) Comparison of automated microarray detection with real-time PCR assays for detection of respiratory viruses in specimens obtained from children. J Clin Microbiol 47:743–750

Renois F, Talmud D, Huguenin A, Moutte L, Strady C, Cousson J (2010) Rapid detection of respiratory tract viral infections and coinfections in patients with influenza-like illnesses by use of reverse transcription-PCR DNA microarray systems. J Clin Microbiol 48:3836–3842

Richard N, Komurian-Pradel F, Javouhey E, Perret M, Rajoharison A, Bagnaud A (2008) The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. Pediatr Infect Dis J 27:213–217

Stefanska I, Romanowska M, Donevski S, Gawryluk D, Brydak B (2013) Coinfections with influenza and other respiratory viruses. Adv Exp Med Biol 756:291–301

Suryadevara M, Cummings E, Bonville CA, Bartholoma N, Riddell S, Kiska D (2011) Viral etiology of acute febrile respiratory illnesses in hospitalized children younger than 24 months. Clin Pediatr (Phila) 50:513–517

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 25–31
DOI 10.1007/5584_2015_198

Published online: 23 January 2016

Antibiotic Prescription Practices Among Children with Influenza

A. Nitsch-Osuch, E. Gyrczuk, A. Wardyn, K. Życinska, and L. Brydak

Abstract

The important factor in the development of resistance to antibiotics is their overuse, especially for viral respiratory infections. The aim of the study was to find out the frequency of the antibiotic therapy administrated to children with influenza. A total of 114 children younger than 59 months seeking care for the acute respiratory tract infection was enrolled into the study. The patients had influenza-like symptoms: fever $>$ 38 °C, cough, and sore throat of less than 4 days duration. Nasal and pharyngeal swabs were tested for influenza A and B virus with a real-time PCR. Thirty six cases of influenza were diagnosed: 34 of influenza A (H3N2) and 2 of influenza B. The rate of influenza infection was 32 % in the study group. The antibiotic therapy was ordered for 58 % patients with influenza. Antibiotics were given less frequently in the outpatient setting (33 %) compared with the hospitalized patients (93 %) ($p < 0.05$). The most often administrated antibiotics were amoxicillin with clavulanic acid, cefuroxime, and amoxicillin. None of the patients received oseltamivir. Antibiotics were overused, while antivirals were underused among children with influenza. To improve health care quality, more efforts in the diagnosis of influenza and the appropriate use of antimicrobials and antivirals are required.

Keywords

Antibiotics • Antimicrobials • Antivirals • Children • Infection • Influenza • Neuraminidase inhibitors • Resistance • Respiratory tract

A. Nitsch-Osuch (✉), E. Gyrczuk, A. Wardyn, and K. Życinska
Department of Family Medicine, Medical University of Warsaw, 1a Banacha St., 02-097 Warsaw, Poland
e-mail: anitsch@wum.edu.pl

L. Brydak
National Influenza Center, National Institute of Public Health – National Institute of Hygiene, Warsaw, Poland

1 Introduction

The antibiotic resistance to bacteria is an emerging threat to the public health. An important factor in the development of this resistance is overuse of antibiotics, especially for treatment of viral respiratory infections and influenza-like illness (ILI). It is estimated that 38 % of patients with the sole diagnosis of influenza received antibiotic prescriptions (Ciesla et al. 2004). Studies limited to children have demonstrated even higher rates of antibiotic treatment in patients diagnosed with viral infections, including influenza (Wilkes et al. 2009). One of the prime factors leading to the inappropriate prescription of antibiotics in children with influenza is a difficulty in making a reliable diagnosis. A misuse of antibiotic treatment for influenza infections and subsequent emergence of bacterial resistance can be reduced by limiting a viral spread through the hand hygiene and vaccination, using laboratory tests for confirmation of influenza cases, judicious use of antivirals, and by expanding the knowledge of physicians and parents regarding the appropriate use of antibiotics (Friedman et al. 2011). The aim of the present our study was to define the frequency and appropriateness of the antibiotic therapy administrated to children with a laboratory-confirmed influenza. This is one of first studies describing this problem in Central Europe where influenza vaccination rates are low, influenza incidence is underestimated and simultaneously the problem of overuse of antibiotics is increasing (Brydak and Nitsch-Osuch 2014; Skoczyńska et al. 2007).

2 Methods

The legal guardians of eligible children provided a written consent for the study participation. The study procedures, consent documents, and data collection sheets were reviewed and approved by the Ethics Committee of the Warsaw Medical University in Warsaw, Poland.

Children younger than 59 months seeking care for the acute respiratory tract infection (ARTI) in both inpatient and outpatient settings in the capital city of Warsaw were enrolled into the study. The patients were eligible for the study if they reported influenza-like illness (ILI) with fever $>$ 38 °C and cough or sore throat of less than 4 days duration. The enrollment took place when there was a confirmation of the presence of the influenza season in the period of January-March 2015. Respiratory specimens, one nasal and one pharyngeal swab, were taken and tested for influenza A and B virus with the real-time reverse transcription polymerase chain reaction (real-time PCR), described in detail elsewhere (Nitsch-Osuch et al. 2013b). The PCR results were not immediately available to clinicians, but collectively after all the samples were tested. Nonetheless, physicians had access to rapid influenza diagnostic tests, mainly for outpatients, or to other tests, such as X-ray examination, blood tests, and cultures, available mainly for inpatients. The patients' demographical data, symptoms, co-morbidities, and the information regarding antibiotic prescriptions were obtained from medical records. Statistical analysis was conducted using a statistical calculator available on www.medcalc3000.com. Categorical data were analyzed with a χ^2 test or Fisher's exact test when there were fewer than 10 cases. A p-value <0.05 was considered statistically significant. Odds ratio (OR) and 95 % confidence intervals (CI) were calculated using the Wald method.

3 Results

A total of 114 patients were enrolled into the study, 52 (45 %) inpatients and 62 (55 %) outpatients. There were 36 cases of influenza diagnosed; 34 cases of influenza A (H3N2) and 2 cases of influenza B virus infections. The prevalence of influenza in the study group was 32 %. There were 15 cases (42 %) of influenza diagnosed in the hospitalized children and 21 cases (58 %) diagnosed in ambulatory outpatients. Antibiotic therapy was prescribed for 21 (58 %) patients with influenza. The therapy was more frequently administered in the

hospitalized children than in those under ambulatory outpatient care (93 % *vs.* 33 % patients), while symptomatic treatment was more frequently conducted in the outpatients; the difference in the treatment mode between the two groups of patients was significant ($p < 0.05$; Table 1).

The most often administrated antibiotics for children with diagnosed influenza were amoxicillin with clavulanic acid, cefuroxime, and amoxicillin (Fig. 1). Amoxicillin alone was used only among ambulatory treated children (Table 2). Lower tract respiratory infections, including pneumonia (diagnosed in 12/21, 57 % patients), bronchitis (diagnosed in 4/21, 19 % patients), and otitis media (diagnosed in 5/21, 24 % patients) were the main reasons for administration of antibiotics. Pneumonia was a significantly reason of the introduction of antimicrobials in hospitalized children, while otitis media occurred mainly in children under ambulatory care ($p < 0.05$; Table 2).

Although we identified children with predisposing factors for a severe and complicated course of influenza, none of them received oseltamivir. Dehydration and decreased daily activity occurred statistically more often in the hospitalized children compared with the ambulatory treated ones ($p < 0.05$, Table 3). None of the children in this study group were vaccinated against influenza during the current season.

4 Discussion

In the present study, 58 % of children with a laboratory confirmed influenza received antibiotic therapy. The therapy was conducted mainly in the hospital setting. The crucial questions which should be addressed are the following. Is antibiotic therapy overused for children with influenza and how to increase the appropriateness of prescribing antibiotics for patients with influenza? Mazzaglia et al. (2003) have reported that 44 % of patients with influenza received antibiotics, while Ochoa et al. (2000) have shown that even more than 70 % cases of influenza found at the emergency department are treated with antibiotics. The present findings showing frequent prescribing of antibiotics for influenza treatment are in line with other studies. Interestingly, a majority of antibiotic courses was administrated to the hospitalized but not ambulatory treated children. A possible explanation for this finding may be a more frequent use of rapid influenza diagnostic tests (RIDTs) in ambulatory care settings, and not at hospital emergency units in Poland. Although RIDTs have both advantages and disadvantages, their use is recommended by WHO and CDC (Brydak et al. 2013). It has been previously shown that the use of RIDTs may lead to decreased use of antibiotics, even in up to 50 % of cases (Bonner et al. 2003). RIDTs can detect influenza A and influenza B antigens within 30 min, the tests are of variable sensitivity (median 70–75 %) and high specificity (90–95 %) (Nitsch-Osuch et al. 2013a). Significant false negative results have been reported (Wang et al. 2014). Nevertheless, RIDTs provide additional support for the initial diagnosis and may promote the rational use of antibiotics. Other popular tests, such as complete blood count and C-reactive protein measurements, may help recognize bacterial coinfections, secondary to primary viral infection, so that their results should be carefully taken under consideration while proscribing antibiotics for children with ARTI (Dugas et al. 2015; Friedman et al. 2011).

In the present study, a majority of hospitalized children with influenza had an admission diagnosis of pneumonia, which could explain

Table 1 Prevalence of antibiotic and symptomatic treatments in children with laboratory confirmed influenza

No. of influenza patients:	Ambulatory treatment	Hospital treatment	OR; 95 % CI
Symptomatic treatment	14	1[a]	0.04; 0.001–0.37
Antibiotic therapy	7	14[a]	28.00; 2.70–698.10

[a]$p < 0.05$ between the two treatment modes
OR odds ratio, *95 % CI* confidence intervals

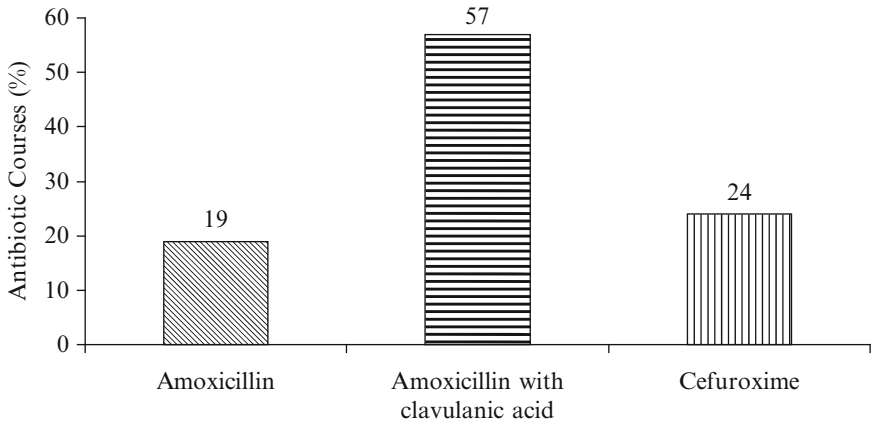


Fig. 1 Antibiotics prescribed for children with influenza

Table 2 Antibiotic therapy in hospitalized and ambulatory treated children with influenza

	Ambulatory treatment	Hospital treatment	OR; 95 % CI
Antibiotic therapy			
Amoxicillin	4	0[a]	3.28; 3.25–28.12
Amoxicillin + Clavulanate	2	10[a]	0.16; 0.02–1.08
Cefuroxime	1	4[a]	0.41; 0.05–3.72
Diagnosis			
Pneumonia	1	11[a]	0.05; 0.01–0.44
Bronchitis	2	2	2.40; 0.32–18.27
Otitis Media	4	1[a]	2.27; 2.27–27.13

[a]$p < 0.05$ between the two treatment modes; *OR*, odds ratio; *95 % CI*, confidence intervals
OR odds ratio, *95 % CI* confidence intervals

Table 3 Predictors of a severe and complicated course of influenza in children

	Ambulatory treatment (n = 21)	Hospital treatment (n = 15)	OR; 95%CI
Age < 24 months	12	12	0.33; 0.07–1.46
Comorbidities:	3	4	0.92; 0.17–4.79
Bronchial Asthma	2	4	
Obesity	1	0	
Feeding difficulties	1	4	0.13; 0.29–1.08
Dehydration	1	8[a]	0.44; 0.06–0.34
Breathing difficulties	1	2	0.35; 0.04–2.80
Decreased daily activity	1	5[a]	0.10; 0.01–0.77
Rapid symptomatic deterioration	1	3	0.20; 0.03–1.62
Neurological symptoms (convulsions)	1	1	0.70; 0.07–7.26

[a]$p < 0.05$ between the two treatment modes
OR odds ratio; *95 % CI* confidence intervals

administration of antibiotic therapy. However, several predisposing factors for influenza complications, predominantly young age of less than 5 years, were also present in our patients. We might expect that an earlier influenza diagnosis and oseltamivir treatment, a neuraminidase inhibitor, could have prevented some complications and antibiotic treatment. Whitley et al. (2001) have shown that the use of oseltamivir for children with influenza reduces the incidence of complications requiring antibiotics by 40 %, compared with placebo, and reduces the relative risk of otitis media by 44 %. Although there remains a possibility of triggering the development of resistant viral strains with antiviral treatment, the risk is much lower than that of the generation of resistance in bacteria following the use of antibiotics. The antiviral treatment benefits clearly outweigh the risk of bacterial resistance (Low 2008). The present study shows that antiviral therapy for influenza is not administrated frequently enough due to a lack of influenza diagnosis. The problem of the underuse of antivirals for influenza has also been reported by Havers et al. (2014) who show that only 16 % of influenza patients receive antivirals, while 30 % of them receive one of the three common antibiotics (amoxicillin, amoxicillin-clavulanate, or azithromycin). A neuraminidase inhibitor is recommended for patients who are at risk for influenza complications, including those who are younger than 2 years or older than 65 years of age, or have a chronic medical condition. The treatment should commence within two days after illness onset if possible, but a later treatment beginning also can help (Grohskopf et al. 2015).

In the present study, the main reason for administration of antibiotic therapy with penicillins or cephalosporins were the lower respiratory tract infections, such as pneumonia and bronchitis, in hospitalized children, and otitis media in children under ambulatory care. Although prescribing of antibiotics for pneumonia is justified, as we know that bacterial complications of influenza are present in 80 % of cases and only 20 % are of the primary viral origin (Crott et al. 2014), a need for antibiotic therapy for children with bronchitis is debatable, especially that the majority of them are hospitalized due mainly to feeding difficulties or dehydration. No benefit has been reported regarding antibiotic treatment for children with bronchitis. There is no evidence to support the use of antibiotics to prevent bacterial complications of influenza or to decrease the severity of persisting viral respiratory tract infections. On the other side, antimicrobial therapy is beneficial to children with underlying chronic pulmonary diseases such as cystic fibrosis, bronchopulmonary dysplasia, or ciliary dyskinesia (O'Brien et al. 1998).

Otitis media is the most common influenza complication among children, occurring in one third of cases. Acute otitis media (AOM) should be differentiated from otitis media with effusion (OME), since antimicrobial agents are not required for the latter unless the effusion persists for more than 3 months (Gisselsson-Solen 2015). The common pathogens of AOM include *Streptoococcus pneumoniae, Haemophilus influenzae,* and *Moraxella catarrhalis*; an increasing drug resistance to these pathogens creates a treatment dilemma (Short et al. 2013). An observational approach, without introducing antibacterial agents, lasting for 48–72 h, for uncomplicated AOM is an option for selected children, which should be based on diagnostic certainty, age, illness severity, and assurance of follow-up (Gisselsson-Solen 2015). This approach for AOM is worthy of consideration in view of the antibiotic resistance data in Poland, showing that 30 % of *Streptococcus pneumoniae* isolates are resistant to pencilllin (Skoczyńska et al. 2007).

The choice of antibiotics in the present study seemed rational and according the national recommendations. It also is in agreement with other researchers (Huang and Huang 2005). However, we should emphasize that the national guidelines encourage prevention of some infections, including influenza and its complications, by immune prophylaxis with vaccines. Kwong et al. (2009) have reported that after 10 years of universal influenza vaccination, there has been a 64 % decline in the

antibiotic prescribing practices. A need for vaccination against influenza should be underlined in Poland, where the coverage rate among the general population stands very low at 3.5 % (Brydak and Nitsch-Osuch 2014). Indeed, none of our patients with influenza was vaccinated during the current season.

We conclude that there are three following ways of responding to the challenge posed by influenza infections and the misuse of antibiotics: firstly, more common employment of laboratory diagnostic tests, including PCR and RIDTs, to identify patients with influenza; secondly employment of neuraminidase inhibitors, a causative treatment for influenza; and thirdly, prevention of influenza by vaccination. The first proposition is crucial, albeit pricey, the second one may be successfully introduced only when laboratory diagnosis is available and conducted, while the third one seems to be the most simple and effective. This complex approach could reduce the overuse of antibacterials and lead to more effective treatment of influenza and its complications.

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

References

Bonner AB, Monroe KW, Talley LI, Klasner AE, Kimberlin DW (2003) *Impact of the rapid diagnosis of influenza on physician decision-making and patient* management in the pediatric emergency department: results of a randomized, prospective, controlled trial. Pediatrics 112:363–367

Brydak LB, Nitsch-Osuch A (2014) Prevention of influenza infection – a Polish perspective. Adv Hyg Exp Med 68:137–144

Brydak LB, Wozniak-Kosek A, Nitsch-Osuch A (2013) Influenza diagnosis and vaccination in Poland. Respir Physiol Neurobiol 187:88–93

Ciesla G, Leader S, Stoddard J (2004) Antibiotic prescribing rates in the US ambulatory care setting for patients diagnosed with influenza, 1997–2001. Respir Med 98:1093–1101

Crott R, Pouplier I, Roch I, Chen YC, Closon MC (2014) Pneumonia and influenza, and respiratory and circulatory hospital admissions in Belgium: a retrospective database study. Arch Pub Heal 72:33

Dugas AF, Valsamakis A, Atreya MR, Thind K, Alarcon P, Faisal A, Gaydos CA, Rothman RE (2015) Clinical diagnosis of influenza in the ED. Am J Emerg Med 33:770–775

Friedman B, Schwabe-Warf D, Goldman R (2011) Reducing inappropriate antibiotic use among children with influenza infection. Can Fam Physician 57:41–43

Gisselsson-Solen M (2015) Acute otitis media in children-current treatment and prevention. Curr Infect Dis Rep 17:476–487

Grohskopf LA, Sokolow LZ, Olsen SJ, Bresee JS, Broder KR, Karron RA (2015) prevention and control of influenza with vaccines: recommendations of the advisory committee on immunization practices, United States, 2015–16 influenza season. Morb Mortal Wkly Rep 7:818–825

Havers F, Thaker S, Cliappard J (2014) Use of influenza antiviral agents by ambulatory care clinicians during the 2012–2013 influenza season. Clin Infect Dis 59:774–782

Huang Y, Huang Y (2005) Use of antimicrobial agents for upper respiratory tract infections in Taiwanese children. Chang Gung Med J 28:758–764

Kwong J, Maaten S, Upshur R, Patrick D, Marra F (2009) The effect of universal influenza immunization on antibiotic prescriptions: an ecological study. Clin Infect Dis 49:750–754

Low D (2008) Reducing antibiotic use in influenza: challenges and rewards. Clin Microbiol Infect 14:298–306

Mazzaglia G, Caputi A, Rossi A (2003) Exploring patient- and doctor-related variables associated with antibiotic prescribing for respiratory infections in primary care. Eur J Clin Pharmacol 59:651–657

Nitsch-Osuch A, Wozniak-Kosek A, Korzeniewski K, Zycinska K, Wardyn K, Brydak LB (2013a) Clinical features and outcomes of influenza A and B infections in children. Adv Exp Med Biol 788:89–96

Nitsch-Osuch A, Wozniak-Kosek A, Korzeniewski K, Zycinska K, Wardyn K, Brydak LB (2013b) Accuracy of rapid influenza detection test in diagnosis of influenza A and B viruses in children less than 59 months old. Adv Exp Med Biol 788:71–76

O'Brien KL, Dowell SF, Schartz B, Marcy SM, Philips WR, Gerber MA (1998) Cough illness/bronchitis-principles of judicious use of antimicrobial agents. Pediatrics 101:178–181

Ochoa C, Eiros JM, Inglada L, Vallano A, Guerra L (2000) Assessment of antibiotic prescription in acute respiratory infections in adults. The Spanish Study Group on Antibiotic Treatments. J Infect 41:73–83

Short KR, Reading PC, Brown LE, Pedersen J, Gilbertson B, Job ER, Edenborough KM, Habets MN, Zomer A, Hermans PW, Diavatopoulos DA, Wijburg OL (2013) Influenza-induced inflammation drives pneumococcal otitis media. Infect Immun 81:645–652

Skoczyńska A, Kadłubowski M, Waśko I, Fiett J, Hryniewicz W (2007) Resistance pattern of selected

respiratory tract pathogens. Clin Microbiol Infect 13:377–382

Wang L, Chang LS, Lee IK, Tang KS, Li CC, Eng HL, You HL, Yang KD (2014) Clinical diagnosis of pandemic A(H1N1) 2009 influenza in children with negative rapid influenza diagnostic test by lymphopenia and lower C-reactive protein levels. Influenza Other Respi Viruses 8:91–98

Whitley RJ, Hayden FG, Reisinger KS (2001) Oral oseltamivir treatment of influenza in children. Pediatr Infect Dis J 20:127–133

Wilkes JJ, Leckerman K, Coffin SE, Keren R, Metigan TA, Hodinka LR (2009) Use of antibiotics in children hospitalized with community-acquired. Laboratory-confirmed influenza. J Pediatrics 154:447–449

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 33–38
DOI 10.1007/5584_2016_216

Published online: 09 March 2016

Antigenic Drift of A/H3N2/Virus and Circulation of Influenza-Like Viruses During the 2014/2015 Influenza Season in Poland

K. Bednarska, E. Hallmann-Szelińska, K. Kondratiuk, and L.B. Brydak

Abstract

Morbidity rates of influenza could be greatly reduced due to vaccination. However, the virus is able to evolve through genetic mutations, which is why vaccines with updated composition are necessary every season. Their effectiveness depends on whether there is a good antigenic match between circulating viruses and vaccine strains. In Poland, the 2014/2015 influenza epidemic started in week 5 (January/February) of 2015 and continued until week 17 (April) of 2015. The influenza activity was moderate with the highest incidence of influence-like illness at week 10/2015 (March). During that season, antigenic drift of influenza virus A/H3N2/ occurred causing higher rates of A/H3N2/ infections. Among the 2416 tested specimens, 22.6 % of influenza cases were positive for A/H3N2/, while A/H1N1/pdm09 constituted 14.6 % cases. Influenza A viruses were detected in co-circulation with influenza B viruses; the latter amounted to 34.1 % of all influenza detections. Other detected causes of influenza-like illness consisted of respiratory syncytial virus (RSV), being predominant, and, sporadically, human coronavirus, parainfluenza 1–3, rhinovirus, and adenovirus. Despite low vaccine effectiveness of solely one component, A/H3N2/, the vaccine could mitigate or shorten the length of influenza infection and reduce the number of severe outcomes and mortality. Thus, vaccination against influenza remains the most effective way to prevent illness and possibly fatal outcomes.

K. Bednarska (✉), E. Hallmann-Szelińska, K. Kondratiuk, and L.B. Brydak
Department of Influenza Research, National Influenza Center, National Institute of Public Health – National Institute of Hygiene, 24 Chocimska St., 00-791 Warsaw, Poland
e-mail: kbednarska@pzh.gov.pl

Keywords
Antigenic drift • Epidemics • Infection • Influenza • Mismatch • Vaccine • Virus

1 Introduction

Influenza reappears every season and is caused by circulating influenza type A and type B viruses. These viruses evolve through genetic mutations and resulting antigenic changes, what allows them to evade host immunity. Influenza vaccines have to be updated periodically in order to be effective in the following season. Hence, the constant need for seasonal vaccination. As it takes approximately 6 months to produce influenza vaccines, recommendations need to be done in advance. The World Health Organization (WHO) convenes annual meetings in February and September each year to establish the composition of influenza vaccine for the forthcoming season in the northern and southern hemispheres, respectively (Chambers et al. 2015; WHO 2014a; Webster et al. 2013).

During the 2013/2014 season, influenza A/Texas/50/2012-like viruses were predominant among circulating influenza A/H3N2/ viruses. In consequence, this virus was recommended as a vaccine component in February 2014. New groups of A/H3N2/ viruses were detected in late March, after vaccine manufacturing had begun. That led to an antigenic mismatch between circulating viruses and viruses included in the vaccine for the 2014/2015 season (ECDC 2014; WHO 2014b).

2 Methods

2.1 Patient Population and Specimen Collection

In the epidemic season 2014/2015, 2,416 clinical specimens were tested in Poland. Additionally, selected specimens from the Voivodship Sanitary Epidemiological Stations (VSES) were tested at the National Influenza Center of the National Institute of Public Health – National Institute of Hygiene, as the Reference Laboratory in Poland, to verify the results obtained in those stations. Specimens for testing consisted mainly from nasal and throat swabs. The patients were categorized into seven age-groups: 0–4, 5–9, 10–14, 15–25, 26–44, 45–64, and $>$ 65 years old.

2.2 Extraction of Viral RNA

Viral RNA was extracted using a Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega Corporation, Madison, WI) from 200 µl of clinical samples in viral transport medium (PBS), in accordance with the manufacturer's instructions for low elution volume (LEV) cartridges. The RNA was eluted with 50 µl of RNase-free water.

2.3 Real-Time RT-PCR

The RNA virus samples were typed in the VSES, thus only subtyping of influenza viruses was achieved. The detection of subtypes was performed by one-step real-time RT-PCR reaction, performed using Roche Light Cycler 2.0 System (Roche Diagnostics, Rotkreuz, Switzerland). RT-PCR reactions were performed in capillary tubes of 20 µl volume with 0.5 µl (20 nM) primers and 0.5 µl (5 nM) probes for each reaction. Primers and probes were obtained through the Influenza Reagent Resource (IRR) program from the American Centers for Disease Prevention and Control The reaction mixture, containing reaction buffer, $MgSO_4$ buffer, BSA, RNase-free H_2O, and SuperScript® III/Platinum® *Taq* Mix ((Invitrogen by Life Technologies – Thermo Fisher Scientific, Carlsband, CA), was incubated with 5 µl RNA

sample per capillary tube. RNA from vaccine viruses A/California/7/2009(H1N1)pdm09 and A/Texas/50/2012(H3N2) were introduced as positive controls and RNase-free H_2O was utilized as a negative control sample. Before DNA amplification cycles were begun, the RNA templates were reverse transcribed to produce the corresponding cDNA templates during reverse transcription procedure: 50 °C for 30 min. DNA templates were then subjected to the initialization step (1 cycle at 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

Fifty five specimens from patients, stored at −80 °C, were tested by RT-PCR using RV12 ACE Detection Kit (Seegene; Seoul, South Korea) that detects the following respiratory viruses: influenza A and B viruses, human respiratory syncytial virus A, human respiratory syncytial virus B, human adenovirus, human metapneumovirus, human coronavirus 229E/NL63, human coronavirus OC43, human parainfluenza virus 1, human parainfluenza virus 2, human parainfluenza virus 3, and human rhinovirus A/B. Random hexamer–primed cDNA synthesis products were generated using the first strand cDNA Synthesis Kit (Fermentas, York, UK), according to the manufacturer's instructions, and stored at −20 °C until use.

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 2014/2015 influenza epidemic season in Poland started in week 5 (January/ February) of 2015 and continued until week 17 (April) of 2015. The influenza activity was moderate with the highest incidence of influenza-like illness (ILI) at week 10/2015 (March) (Fig. 1).

During this season, 2416 specimens were tested for influenza and influenza-like viruses, of which 21.2 % (n = 513) were positive for influenza. In detail, there were, 34.1 % (n = 175) influenza type B, 14.6 % (75) influenza A/H1N1/pdm09, 22.6 % (n = 116) influenza A/H3N2/, and 28.7 % (n = 147) non-subtyped influenza type A. There was observed a co-circulation of influenza type A and B viruses, with influenza A being the most prevalent one (Fig. 2).

Infections caused by influenza-like viruses accounted for 11.2 % (n = 270) of tested specimens. Respiratory syncytial virus (RSV) was the other predominant virus, constituting 87.0 % (n = 235) of all ILI cases, followed by sporadic cases of human coronavirus (n = 10, 3.7 %), parainfluenza virus-1 (n = 8, 3.0 %), rhinovirus (n = 6, 2.2 %), adenovirus (n = 5, 1.9 %), and parainfluenza viruses 2 and 3 (n = 3, 1.1 % each (Fig. 3)).

The highest percentage of influenza and ILI confirmed cases was noted in the 0–4 years

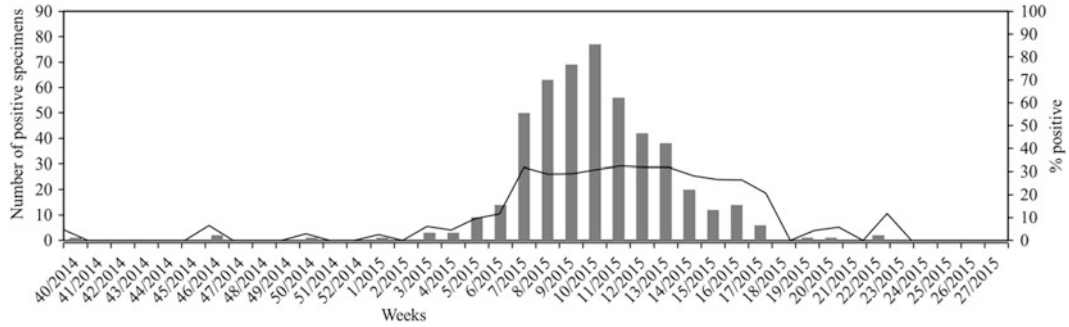


Fig. 1 Number and percentage of specimens positive for influenza each week of the 2014/2015 season

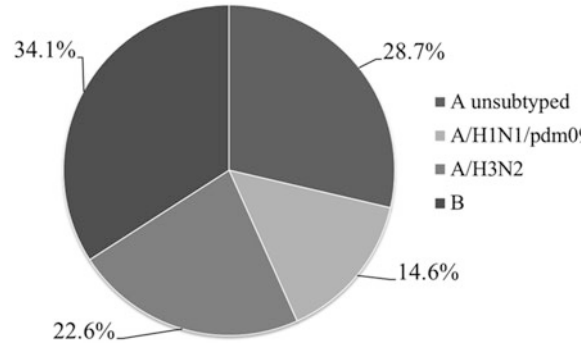


Fig. 2 Proportions of influenza viruses detected in the 2014/2015 season

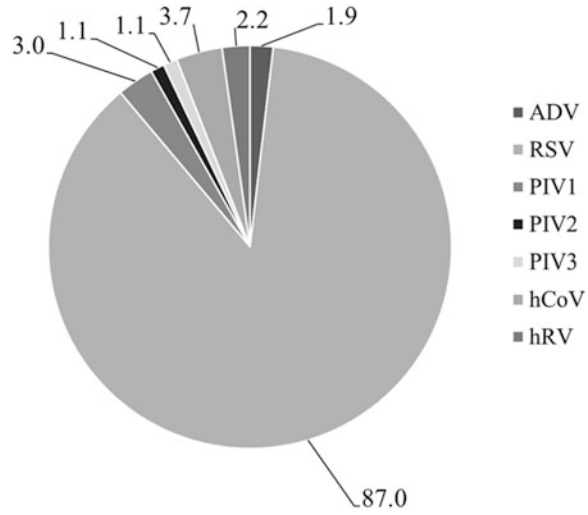


Fig. 3 Proportions of influenza-like viruses detected in the season 2014/2015

age-group (46.4 %), followed by the 45–64 years (12.3 %) and 26–44 years age-groups (11.6 %). The lowest morbidity was observed in the 15–25 years (4.9 %) and 10–14 years age-groups (5.4 %) (Fig. 4). The verification of specimen testing by VSES demonstrated a complete agreement with the results obtained at the National Influenza Center (Table 1).

4 Discussion

In the epidemic seasons 2013/2014 and 2014/2015 in Poland, the number of specimens received from primary care physicians was similar, although the percentage of influenza and influenza-like infection confirmations was 10 % higher in the latter season. The 2014/2015 morbidity in children aged 0–4 years was twice as much as it had been in the former epidemic season. The vast majority of infections in this group of children was induced by influenza-like viruses (67.2 %). In both seasons, in the case of influenza-like viruses, the predominant virus was RSV. Infections caused by other respiratory viruses occurred sporadically.

Influenza cases were caused mainly by influenza type A (83.2 %). The proportion of influenza B was significantly higher in the 2014/2015 season than that in the previous season where it amounted to 1.2 % of all influenza confirmations (Bednarska et al. 2015).

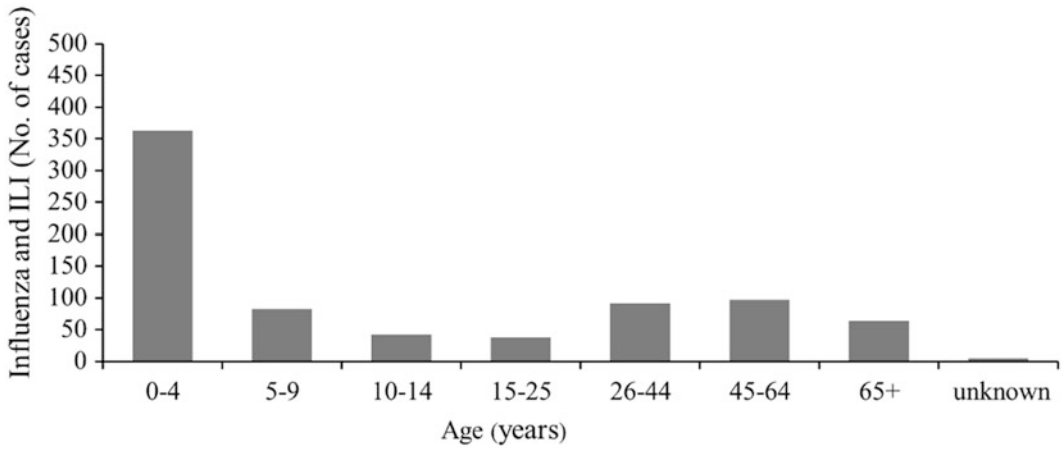


Fig. 4 Number of influenza and influenza-like infections (ILI) by age-groups in the 2014/2015 season

Table 1 Specimens from the Voivodship Sanitary Epidemiological Stations (VSES) which were verified at the Department of Influenza Research of the National Influenza Center of the National Institute of Public Health – National Institute of Hygiene in 2015

	Program	
Subtype	SENTINEL	Non-SENTINEL
A/H1N1/pdm09	5	0
A/H3N2/	39	42
B	51	17
Total	95	59

While comparing data from Poland and Europe, it is apparent that the proportion of circulating viruses of influenza type A and B remains similar. In Europe and the whole world alike, the dominant strain was A/H3N2/, but it co-circulated with influenza A/H1N1/pdm09. The predominance of the A/H3N2/ strain also was noted in Poland, even taking into account the fact that not all the specimens tested within the SENTINEL and non-SENTINEL influenza surveillance were subtyped (Broberg et al. 2015; ECDC/WHO 2015; WHO 2015; McCauley et al. 2014, 2015; Rolfes et al. 2014).

In the 2014/2015 season, antigenic drift of the subtype A/H3N2/ decreased the effectiveness of vaccine against influenza. Yet vaccination remains the most effective and the cheapest method of influenza prevention (Broberg et al. 2015). Since 1968, the Advisory Committee on Immunization Practices (ACIP) recommends trivalent vaccines against influenza. They contain two subtypes of influenza virus A and one lineage of influenza virus type B. It should be emphasized that people, particularly those who receive vaccination every season, substantially enhance their immunological memory.

Acknowledgements This work was funded in parts by research projects 2011/01/B/NZ7/06188 and NIPH-NIH's subject 5/EM.1. The authors would like to acknowledge physicians and employees of VSES participating in SENTINEL program for their input into the influenza surveillance in Poland.

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

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 2013/2014. Adv Exp Med Biol 857:1–7

Broberg E, Snacken R, Adlhoch C, Beaute J, Galinska M, Pereyaslov D, Brown C, Penttinen P, on behalf of the WHO European Region and the European Influenza Surveillance Network (2015) Start of the 2014/15 influenza season in Europe: drifted influenza A (H3N2) viruses circulate as dominant subtype. Euro Surveill 20(4):pii = 21023

Chambers BS, Parkhouse K, Ross TM, Alby K, Hensley S (2015) Identification of hemagglutinin residues

responsible for H3N2 antigenic drift during the 2014–2015 influenza season. Cell Rep 12:1–6

ECDC (2014) From European Centre for Disease Prevention and Control. Circulation of drifted influenza A (H3N2) viruses in the EU/EEA. http://ecdc.europa.eu/en/publications/Publications/RRA-InfluenzaA-H3N2-Dec-2014.pdf. Accessed 12 Sept 2015

ECDC/WHO (2015) From European Centre for Disease Prevention and Control (ECDC)/World Health Organization Regional Office for Europe (WHO/Europe). Flu News Europe. Archives. https://www.flunewseurope.org/Archives. Accessed 12 Sept 2015

McCauley J, Daniels R, Lin YP, Xiang Z, Gregory V, Whittaker L, Halai Ch, Cross K, Rattigan A, Ermetal B (2014) Report prepared for the WHO annual consultation on the composition of influenza vaccine for the Northern Hemisphere 2014/15. http://www.crick.ac.uk/media/221849/nimr-report-feb2014-web.pdf. Accessed 5 Oct 2015

McCauley J, Daniels R, Lin YP, Xiang Z, Gregory V, Whittaker L, Halai Ch, Cross K, Rattigan A, Ermetal B, Dai M (2015) Report prepared for the WHO annual consultation on the composition of influenza vaccine for the Northern Hemisphere 2014/15. http://www.crick.ac.uk/media/221813/nimr-report-feb2015-web.pdf. Accessed 5 Oct 2015

Rolfes M, Blanton L, Brammer L, Smith S, Mustaquim D, Steffens C, Cohen J, Leon M, Chaves SS, Abd Elal AI, Gubareva L, Hall H, Wallis T, Villanueva J, Xu X, Bresee J, Cox N, Finelli L (2014) Update: influenza activity – United States, September 28–December 6, 2014. MMWR 63(50):1189–1193

Webster RW, Monto AS, Braciale TJ, Lamb RA (2013) Textbook of influenza, 2nd edn. Wiley Blackwell, Hoboken, NJ. Chapter 16, 18, 21, pp 250–268, 283–297, and 327–336

WHO (2014a) http://www.who.int/influenza/vaccines/virus/recommendations/201402_qanda_recommendation.pdf. Accessed 18 Sept 2015

WHO (2014b) Recommended composition of influenza virus vaccines for use in the 2014–2015 northern hemisphere influenza season. Wkly Epidemiol Rec 10(89):93–104

WHO (2015) WHO FluNet charts. Available from http://gamapserver.who.int/gareports/Default.aspx?ReportNo=10. Accessed 12 Oct 2015

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 39–56
DOI 10.1007/5584_2015_184

Published online: 09 January 2016

Growing Antibiotic Resistance in Fatal Cases of Staphylococcal Pneumonia in the Elderly

Josef Yayan and Kurt Rasche

Abstract

Older people are often especially susceptible to pneumonia and bacteria may develop resistance to antibiotics quicker in the elderly, whose immune systems gradually diminish. This study analyses, retrospectively, resistance to antibiotics in high-risk elderly patients with fatal pneumonia. Records of all patients aged over 65 who did not survive a bout with pneumonia were gathered from the records of the Department of Pneumology of HELIOS Clinic in Wuppertal, Germany from the period of 2004–2014. Susceptibility testing was executed for the study population, whose pneumonia was triggered by various kinds of bacteria. We detected 936 pneumonia patients of the overall mean age of 68.0 ± 13.6 years, with the following pneumonia types: 461 (49.3 %) community-acquired, 354 (37.8 %) nosocomial-acquired, and 121 (12.9 %) aspiration pneumonia. There were 631 (67.4 %) males and 305 (32.6 %) females there. We identified 672 (71.8 %) patients who had a high risk for pneumonia, especially staphylococcal pneumonia ($p < 0.0001$). The elderly patients had a higher risk of dying from pneumonia (2.9 odds ratio, 95 % confidence interval 1.8–4.6; $p < 0.0001$); of the 185 pneumonia-related deaths, 163 (88.1 %) were in the elderly. In those with fatal staphylococcal pneumonia, a high antibiotic resistance rate was found for piperacillin-tazobactam ($p = 0.044$), cefuroxime ($p = 0.026$), cefazolin ($p = 0.043$), levofloxacin ($p = 0.018$), erythromycin ($p = 0.004$), and clindamycin ($p = 0.025$). We conclude that elderly patients with staphylococcal pneumonia show resistance to common antibiotics. However, no significant antibiotic resistance could be ascribed for other types of pneumonia in these patients.

J. Yayan (✉) and K. Rasche
Department of Internal Medicine, Division of Pulmonary, Allergy and Sleep Medicine, HELIOS Clinic Wuppertal, Witten/Herdecke University, Heusnerstrasse 40, 42283 Wuppertal, Germany
e-mail: josef.yayan@hotmail.com

Keywords
Antibiotics • Elderly • Mortality • Pneumonia • Sensitivity • Resistance

1 Introduction

Pneumonia is associated with an acute infection of the respiratory tissue, is usually of bacterial origin, and manifests with cough, fever, and shortness of breath. Young adults usually make a complete recovery from pneumonia. In older and inveterately unwell individuals, pneumonia can be lethal (Pepersack 2014; Chong and Street 2008). Community-acquired pneumonia in older people is commonly triggered by *Streptococcus pneumonia*, *Staphylococcus aureus*, *Enterobacteriaceae*, *Haemophilus influenzae*, *Pseudomonas aeruginosa,* or *Legionella* spp. (El-Solh et al. 2001). Pneumonia in the elderly is a major challenge for physicians due to a high frequency, variety of causes, atypical clinical presentation, and age-related factors. Despite the special management of pneumonia in the elderly, no specific recommendations for antimicrobial treatment are provided in the international guidelines (Petrosillo et al. 2015). With the increase in antibiotic resistance, questions about the clinical impact of pneumonia in the elderly have now been raised (Feikin et al. 2000). An increase in mortality in elderly pneumonia patients has been documented (Feldman 2001; Marrie 2000). Therefore, it is important to investigate antibiotic resistance in fatal pneumonia in the elderly, so that a timely antibiotic therapy can be initiated to shorten the patients' suffering and the duration of hospitalization, in addition to reduced mortality.

In the present study we set out to retrospectively identify antibiotic resistance in patients aged over 65 with fatal pneumonia over the last decade, according to the International Classification of Diseases (ICD) J15.0–J15.6 (WHO 2015).

2 Methods

The Ethics Committee of the Witten-Herdecke University in Germany approved the study, waiving the requirement for informed consent due to a retrospective nature of the study. The majority of patients' information was anonymized before investigation. This quality-control observational investigation retrospectively analyzed the resistance to antimicrobial agents commonly used in daily practice in patients over 65 years old with fatal community- or nosocomial-acquired pneumonia. Data were retrieved from clinical records at the Department of Pneumology of HELIOS Clinic, Witten/Herdecke University, in Wuppertal, Germany, during the period of 1 January 2004 to 20 September 2014. The underlying bacterial background was the following: group B *Streptococcus* (ICD J15.3), *Streptococcus pneumoniae* (ICD J15.4), *Staphylococcus* (ICD J15.2), *Pseudomonas* (ICD J15.1), *Escherichia coli* (ICD J15.5), Gram-negative bacteria (ICD J15.6), or *Klebsiella* (ICD J15.0). The study elderly population was compared with pneumonia inpatients less than 64 years of age with fatal outcomes, and with those over and under 65 years of age who survived (Table 1). All in patients with nosocomial-acquired pneumonia who were first treated for different medical reasons in different departments were enclosed in this trial. The elderly with acute infections, like urinary infections or gastroenteritis, were disqualified from this investigation as were the neurological inpatients due to a restricted access to their records.

The criteria were used for the designation of pneumonia were the infiltrations in X-ray examination, typical clinical symptoms, along with a minimum of two of the following: breathing difficulty, fever > 38 °C, sputum production, and coughing.

2.1 Antibiotics Examined

Sensitivity and resistance of *Streptococcus*, *Staphylococcus*, *Pseudomonas*, *Escherichia coli*, Gram-negative bacteria, and *Klebsiella* to penicillin, oxacillin, ampicillin, piperacillin, piperacillin-tazobactam, ampicillin-sulbactam, cefuroxime, cefazolin, cefepime, ceftazidime, cefotaxime, tetracyclin, meropenem, imipenem, ciprofloxacin, levofloxacin, erythromycin, co-trimoxazole, clindamycin, gentamicin, vancomycin, teicoplanin, linezolid, rifampicin, fosfomycin, fucidin, colistin, tigecycline, and amikacin were examined. The frequency of application of these antibiotics in the elderly patients with pneumonia in the clinical setting was noted. For bacterial susceptibility testing, diameter breakpoints in the inhibition zone were utilized according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI 2012), with a modification for European standards introduced by the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2011).

2.2 Susceptibility Testing

Streptococcus and *Staphylococcus* were identified based on growth on Columbia blood agar and chocolate agar (Becton Dickinson; Heidelberg, Germany) after 18–48 h at 37 °C using 5 % carbon dioxide and MALDI-TOF-MS (Bruker; Bremen, Germany). *H. influenzae* and Gram-negative bacteria were identified based on growth on chocolate agar with bacitracin (Becton Dickinson; Heidelberg, Germany). Gram-negative isolates and *H. influenzae* were further confirmed by use of the API NH system (biochemical reactions) for the identification of *Neisseria* and *Haemophilus* (bioMérieux; Marcy-l'Étoile, France). The panel selected to perform the evaluation for Gram-negative bacteria was NMIC/ID-76. BBL™ CHROMagar™ Orientation medium (Becton Dickinson; Heidelberg, Germany) was utilized to identify *Enterobacteriaceae*. The analyzed *Enterobacteriaceae* were *Escherichia coli*, *Enterobacter* spp., *Klebsiella*, *Proteus mirabilis*, *Citrobacter* spp., *Shigella*, *Serratia marcescens*, and *Yersinia*.

E. coli was grown on Columbia blood agar and MacConkey agar (Becton Dickinson; Heidelberg). The detection of *E. coli* by MALDI-TOF MS was executed on a Microflex LT device with FlexControl software (Bruker Daltonik; Bremen, Germany) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2–20 kDa. The antimicrobial susceptibility testing was achieved using the programed system BD PHOENIX (Becton Dickinson; Heidelberg, Germany). The measurement of the minimum inhibitory concentration (MIC) was executed by E-test for the antibiotics which showed resistances to carbapenems.

BD™ Pseudosel™ Agar was used for detection of *P. aeruginosa* from clinical specimens. The agar contains cetrimide, which is a selective agent against alternative microbial flora. Cetrimide also enhances the production of white pyocyanin and fluorescein pigments of *Pseudomonas*, which exhibit characteristic blue-green and yellow-green colors. *P. aeruginosa* colonies from agar plates were suspended in Phoenix ID broth (Becton Dickinson; Heidelberg, Germany) to a 0.5–0.6 McFarland standard. The identification was executed by either BD Phoenix™ automated system or by MALDI-TOF MS (Bruker Daltonik; Bremen, Germany). A BD Phoenix™ mechanized microbiology technique (Becton-Dickinson Diagnostic Systems; Sparks, MD) was used, equipped with software suitable for the interpretation of susceptibility testing. Fungi were grown and detected on BD™ Sabouraud Agar (Becton Dickinson; Heidelberg, Germany). Susceptibility testing was executed on Mueller-Hinton agar (BD, Heidelberg, Germany) by means of McFarland 0.5 from overnight cultures, followed by incubation at 35 °C for 16–18 h.

A disc diffusion method established by Bauer et al. (1996) was also used for susceptibility testing for screening purposes and in cases

when carbapenemase activity was ruled out, e.g., imipenem, meropenem, or ceftazidime resistance. A synergy testing or metallo-beta-lactamase E-test was performed when phenotypic metallo-beta-lactamase-producing Gram-negative bacteria were suspected.

2.3 Microbiology

The expectoration from the oropharyngeal space and windpipe was acquired in a number of ways, such as bronchial lavage, tracheal secretion, or throat smear. To perform bronchial lavage, approximately 20 mL of 0.9 % salt-water solution were infused following the administration of a local anesthetic and sucked back by means of the fiber-optic bronchoscope. Bronchial fluid was then deposited in three separate, sterilized 40-mL sample containers. Tracheal secretions were recovered also using flexible fiber-optic bronchoscopy by suctioning into aseptic 40-mL specimen traps (Argyle^{TM}, Covidien, Neustadt/Donau, Germany). The throat-smear was taken by applying slight turning pressure on the pharyngeal cotton swab, using a commercially available cotton throat-swab system (MEUS Srl, Piove di Sacco, Italy). Expectoration was collected by having the patients cough into 30-mL antiseptic sputum containers (Salivette®, SARSTEDT, Nümbrecht, Germany), which was later examined microbiologically.

A microscopic investigation was performed after Gram staining at magnification of 80–1000x in a minimum five viewing fields, according to the standards of Bartlett (1987). Adhering to the morphological and bacteriological standards actually produced higher than expected doubts in the microbiological assessment of bacteria.

For blood cultures, 20 mL of blood was taken through venipuncture and transferred into the BACTEC plus aerobic and anaerobic/F media (Becton, Dickinson and Company; Heidelberg, Germany). The samples were then incubated at body temperature and checked if positive for growing microorganisms after 5 days. Negative vials were discarded.

2.4 Statistical Analysis

Categorical variables were expressed as proportions, while continuous variables were given as means ± SD. 95 % confidence intervals (CI) were calculated. Odds ratios (OR) were calculated for the mortality in different age-groups and for the risk of pneumonia triggered by different types of bacteria in patients over 65 years old compared with younger patients. Antibiotics used in each kind of pneumonia were compared with a 4×2 chi-squared test, where samples were categorized as sensitive or resistant after evaluating the outcome of the antibiotic susceptibility testing. In cases where the number of samples was greater than 120, Fisher's exact probability test was used to classify an antibiotic as sensitive or resistant in the contingency table. Types of pneumonia were compared with a 4×3 chi-squared test. One-way analysis of variance ANOVA for independent samples was performed to compare various agents causing pneumonia. Two-tailed tests were performed, and $p < 0.05$ was taken as an indicator of significant differences.

Additionally, the number of deaths during hospital stay was calculated in pneumonia patients. Thereafter, survival probabilities were determined by means of the Kaplan-Meier method.

3 Results

Overall, 936 pneumonia cases were detected out of the total of 6,932 patients. One hundred and eighty five (19.8 %) patients of all age died from pneumonia (Table 1). The male patients older than 65 years were more likely to come down with pneumonia (Table 2). In addition, patients older than 65 years had an increased risk of dying from pneumonia, which was gender independent (Table 2). Pneumonia in the elderly was mostly of staphylococcal origin. Interestingly, fewer pre-senile patients suffered from pneumonia and they were not significantly more likely to die from pneumonia. In the elderly patients with fatal pneumonia, antibiotic resistance was not found in streptococcus pneumonia (Table 3).

However, increased antibiotic resistance was found in those who died from staphylococcal pneumonia. The resistance concerned in this case the following antibiotics: piperacillin-tazobactam, cefuroxime, cefazolin, levofloxacin, erythromycin, and clindamycin (Table 4). Some antibiotic resistance could be seen for pneumonia caused by *Pseudomonas* (Table 5). No appreciable resistance was detected for pneumonia caused by *E. coli* (Table 6), Gram-negative bacteria (Table 7), *Haemophilus* (Table 8), or *Klebsiella* (Table 9). The elderly were more likely to develop community-acquired pneumonia (Table 10). The bacteria were mainly discovered in the tracheal and bronchial secretions (Table 11). There were 163 (17.4 %, 95 % CI 15.0–19.8 %) deaths related to pneumonia in the elderly patients. Consequently, the survival rate in hospitalized patients with pneumonia in this investigation was 82.6 % (95 % CI 79.9–85.3 %).

4 Discussion

This observational study, which covered a 10-year period, shows that pneumonia had an overall mortality rate of 19.8 % in all age groups. Pneumonia continues to be feared by clinicians due to its high mortality rate for all ages, ranging between 10 and 25 %. Fatal outcomes from pneumonia are related, among other factors, to the advanced age of patients (Pachon et al. 1990). A high frequency of pneumonia in the elderly has been shown in several studies (Feldman 2001; Marrie 2000). Pneumonia in the elderly could be categorized for scientific reasons as community-acquired pneumonia, nursing home-acquired pneumonia, or hospital-acquired pneumonia (Watkins and Lemonovich 2011; Niederman et al. 2001; Marik 2001). Although this classification was not considered in this study, community-acquired pneumonia was the most frequent form of pneumonia in the elderly.

In contrast, aspiration pneumonia was found in all age groups in the present study. Aspiration pneumonia is characterized as the misrouting of gastric content into the lungs (Dikensoy et al. 2002). While initial clinical reports focused on aspiration pneumonia resulting from accidental foreign-body aspiration (Riquelme et al. 2008), the number of studies concerning aspiration pneumonia in the elderly has increased with aging populations in recent decades (Donowitz and Cox 2007; Gutiérrez et al. 2006). Since the definition of aspiration pneumonia remains imprecise, a confusion may arise in clinical reports.

There are sex differences in pneumonia caused by different bacteria. The incidence rate of pneumonia observed in the present study was higher in males and was higher than that reported in other studies (Millett et al. 2013; El-Solh et al. 2001). The male sex has been defined as a risk factor for pneumonia among nursing home residents (Cunha 2001). We found that the elderly was suffering predominantly from staphylococcal pneumonia. The frequency of different bacteriological causes of pneumonia in the elderly varied by the region. These observations are in line with those reported in some previous studies (Hashemi et al. 2010; Schito 2006).

S. aureus is frequently resistant to beta-lactams and also to beta-lactamase-resistant penicillins (Gin et al. 2007). Although piperacillin and tazobactam have a very broad spectrum of activity in both the Gram-positive and Gram-negative bacteria (Skov et al. 2002), an increased resistance to this beta-lactam combination was unraveled in this present study. The increased antibiotic resistance of staphylococci was mainly present in the elderly pneumonia patients. Increased resistance of staphylococci to cefuroxime, a second-generation cephalosporin, was also observed in the elderly whose illness was fatal. Cefuroxime has a broad spectrum of activity and it is used to combat pneumonia in clinical practice. In the Gram-positive range, cefuroxime acts against staphylococci, streptococci, and pneumococci, and others (Shoji et al. 2014; Jalil et al. 2008). Additionally, resistance to cefazolin, a first-generation cephalosporin, was found in the elderly who died from pneumonia. Cefazolin is effective against staphylococci (Mukae et al. 2014). According to the results of the present study, special care

Table 1 Number (%) of pneumonia cases caused by various bacteria according to age-group

	Streptococcus	*Staphylococcus*	*Pseudomonas*	*Escherichia coli*	Gram-negative bacteria	*Haemophilus*	*Klebsiella*	Total no. of cases (%)
Male	45 (67.2)	168 (64.4)	113 (67.3)	92 (68.1)	46 (62.2)	60 (73.2)	107 (71.8)	631 (67.4)
Female	22 (32.8)	93 (35.6)	55 (32.7)	43 (31.9)	28 (37.8)	22 (26.8)	42 (28.2)	305 (32.6)
No. of cases	**67 (7.2)**	**261 (27.9)**	**168 (17.9)**	**135 (14.4)**	**74 (7.9)**	**82 (8.8)**	**149 (15.9)**	**936 (100)**
Age (years)								
18–60	22 (32.8)	46 (17.6)	39 (23.2)	18 (13.3)	21 (28.4)	26 (31.7)	30 (20.1)	202 (21.6)
60–64	7 (10.4)	17 (6.5)	8 (4.8)	6 (4.4)	9 (12.2)	7 (8.5)	8 (5.4)	62 (6.6)
65–69	10 (14.9)	38 (14.6)	26 (15.5)	23 (17.0)	9 (12.2)	12 (14.6)	15 (10.1)	133 (14.2)
70–79	17 (25.4)	86 (33.0)	70 (41.7)	50 (37.0)	25 (33.8)	28 (34.1)	61 (40.9)	337 (36.0)
80–89	10 (14.9)	70 (26.8)	25 (14.9)	33 (24.4)	10 (13.5)	9 (11.0)	35 (23.5)	192 (20.5)
>90	1 (1.5)	4 (1.5)	0	5 (3.7)	0	0	0	10 (1.1)

Table 2 Mortality rate of pneumonia caused by various bacteria according to age-group; number of cases (%)

	Streptococcus	*Staphylococcus*	*Pseudomonas*	*Escherichia coli*	Gram-negative bacteria	*Haemophilus*	*Klebsiella*	Total no. of patients (%)	ORa	95 % CI	p
Age (years)											
18–60	1 (14.3)	5 (7.6)	2 (6.1)	2 (7.4)	4 (26.7)	0	2 (7.4)	16 (8.6)	0.1	0.1–0.2	<0.0001
60–64	1 (14.3)	3 (4.5)	0	1 (3.7)	1 (6.7)	0	0	6 (3.2)	1.2	0.5–3.3	0.662
65–69	4 (57.1)	9 (13.6)	6 (18.2)	6 (22.2)	2 (13.3)	2 (20.0)	2 (7.4)	31 (16.8)	3.5	1.8–6.8	0.0001
70–79	0	25 (37.9)	18 (54.5)	12 (44.4)	5 (33.3)	6 (60.0)	16 (59.3)	82 (44.3)	3.7	2.1–6.6	<0.0001
80–89	1 (14.3)	21 (31.8)	7 (21.2)	4 (14.8)	3 (20.0)	2 (20.0)	7 (25.9)	45 (24.3)	3.6	1.9–6.6	<0.0001
>90	0	3 (4.5)	0	2 (7.4)	0	0	0	5 (2.7)	11.6	3.0–44.4	0.0003
No. of fatal cases	**7 (3.8)**	**66 (35.7)**	**33 (17.8)**	**27 (14.6)**	**15 (8.1)**	**10 (5.4)**	**27 (14.6)**	**185 (100)**			
Male	5 (71.4)	45 (68.2)	16 (48.5)	19 (70.4)	8 (53.3)	8 (80.0)	20 (74.1)	121 (65.4)	0.9	0.6–1.3	0.515
Female	2 (28.6)	21 (31.8)	17 (51.5)	8 (29.6)	7 (46.7)	2 (20.0)	7 (25.9)	64 (34.6)	0.9	0.6–1.3	0.515
ORs	0.5	2.1	1.2	1.2	1.1	0.6	1	–	–	–	–
95 % CI	0.2–1.1	1.5–3.0	0.8–1.8	0.7–1.8	0.6–2.0	0.3–1.2	0.7–1.6	–	–	–	–
p	0.077	<0.0001	0.412	0.493	0.733	0.127	0.872	–	–	–	–

ORa odds ratio of age-group mortality, *ORs* odds ratio of species-related mortality, *CI* confidence intervals

Table 3 Susceptibility testing of common antibiotics in streptococcal pneumonia

		>65 deceased (n = 163)		<64 deceased (n = 22)		>65 survivor (n = 509)		<64 survivor (n = 242)		
Drug groups	Active substance	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	p
Penicillins	Penicillin	0	5/0	0	2/0	1	32/0	0	27/0	1.0
	Ampicillin	0	5/0	0	2/0	1	32/0	0	27/0	1.0
	Piperacillin	0	5/0	0	2/0	0	31/0	0	27/0	1.0
Penicillin + beta-lactamase inhibitors	Ampicillin + Sulbactam	0	5/0	0	2/0	11	32/0	9	27/0	1.0
	Piperacillin + Tazobactam	4	5/0	2	2/0	12	32/0	12	27/0	1.0
Cephalosporins	Cefuroxime	0	5/0	0	2/0	5	31/0	3	27/0	1.0
	Cefotaxime	0	5/0	0	2/0	0	32/0	0	27/0	1.0
Gyrase inhibitors	Ciprofloxacin	0	0/0	0	0/0	0	0/0	2	0/1	1.0
	Levofloxacin	0	1/0	1	0/0	0	11/0	1	5/1	0.389
Macrolide	Erythromycin	0	5/0	0	2/0	3	28/1	0	22/5	0.622
Trimethoprim + Sulfonamide	Co-trimoxazole	0	1/0	0	1/0	0	7/1	0	4/1	0.999
Lincosamide	Clindamycin	0	5/0	1	1/1	3	29/0	1	24/2	0.049
Aminoglycosides	Gentamicin	0	0/0	0	0/0	0	0/6	0	0/2	1.0
Glycopeptide	Vancomycin	0	5/0	0	2/0	0	32/0	0	27/0	1.0

Table 4 Susceptibility testing of common antibiotics in pneumonia caused by *Staphylococcus*

		>65 deceased (n = 163)		<64 deceased (n = 22)		>65 survivor (n = 509)		<64 survivor (n = 242)		
Drug groups	Active substance	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	p
Penicillins	Penicillin	0	5/51	0	1/7	0	21/115	0	12/42	0.398
	Oxacillin	0	25/31	0	6/2	1	84/54	0	38/15	0.025
	Ampicillin	0	6/49	0	0/7	0	23/115	0	11/43	0.356
	Piperacillin	1	5/30	0	1/5	1	11/87	0	7/29	0.666
Penicillin + beta-lactamase inhibitors	Ampicillin + Sulbactam	2	25/31	1	5/2	21	69/55	8	33/16	0.104
	Piperacillin + Tazobactam	33	26/31	6	6/2	79	83/54	26	38/16	0.044
Cephalosporins	Cefuroxime	7	25/32	0	6/2	23	83/54	11	38/16	0.026
	Cefotaxime	0	8/15	0	2/1	1	23/27	0	11/6	0.258
	Cefazolin	0	22/28	0	3/2	2	70/48	0	36/14	0.043
	Cefepime	0	5/10	0	2/0	1	20/13	0	7/3	0.121
Tetracycline	Tetracyclin	0	34/2	0	3/0	0	79/1	0	26/0	0.386
Gyrase inhibitors	Ciprofloxacin	3	2/34	2	0/4	5	4/60	5	2/17	0.840
	Levofloxacin	1	15/29	0	3/4	4	58/43	3	27/14	0.018
Macrolide	Erythromycin	4	22/34	0	7/1	3	80/57	3	37/17	0.004
Trimethoprim + Sulfonamide	Co-trimoxazole	1	54/3	0	8/0	0	130/7	2	49/3	0.923
Lincosamide	Clindamycin	1	26/30	0	7/1	3	85/53	1	38/16	0.025
Aminoglycosides	Gentamicin	2	48/9	0	7/1	4	127/10	5	50/4	0.285
	Tobramycin	0	8/11	0	2/0	0	47/18	0	15/5	0.054
Glycopeptide	Vancomycin	19	57/0	1	8/0	30	137/0	8	53/0	1.0
	Teicoplanin	0	11/1	0	2/0	0	36/0	0	10/1	0.203
Oxazolidinone	Linezolid	1	27/0	0	1/0	5	47/0	1	16/0	1.0
Rifamycin	Rifampicin	9	47/3	0	8/0	18	120/1	3	49/0	0.077
Epoxid	Fosfomycin	1	46/4	0	8/0	0	106/12	0	42/2	0.550
Fusidic acid	Fucidin	0	43/2	0	7/0	0	92/1	0	38/1	0.615

Table 5 Susceptibility testing of common antibiotics in pneumonia caused by *Pseudomonas*

		>65 deceased (n = 163)		<64 deceased (n = 22)		>65 survivor (n = 509)		<64 survivor (n = 242)		
Drug groups	Active substance	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using anti-biotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	p
Penicillins	Ampicillin	0	0/17	0	0/0	1	0/40	0	0/26	1.0
	Piperacillin	0	24/6	0	1/1	33	62/2	2	28/15	0.0001
Penicillin + beta-lactamase inhibitors	Ampicillin + Sulbactam	1	0/30	0	0/2	12	1/88	4	0/45	0.833
	Piperacillin + Tazobactam	13	25/6	1	2/0	40	65/23	29	29/14	0.501
Cephalosporins	Cefuroxime	3	0/23	0	0/1	3	0/61	2	0/34	1.0
	Cefotaxime	0	0/3	0	0/0	0	1/10	0	0/4	1.0
	Cefepime	0	22/5	0	2/0	3	75/11	0	33/11	0.317
	Ceftazidime	1	23/4	1	1/0	5	62/17	4	28/12	0.468
Carbapenem	Imipenem	4	25/6	0	1/1	16	67/22	3	26/19	0.091
	Meropenem	1	23/4	0	1/1	5	65/17	1	26/11	0.384
Gyrase inhibitors	Ciprofloxacin	4	22/8	0	2/0	0	60/28	6	30/14	0.755
	Levofloxacin	0	9/8	0	1/0	5	30/23	1	13/10	1.0
Trimethoprim + Sulfonamide	Co-trimoxazole	0	0/23	0	0/1	0	0/60	0	0/33	1.0
Aminoglycosides	Gentamicin	1	20/6	0	2/0	4	66/19	1	27/12	0.633
	Tobramycin	0	21/5	0	1/0	0	56/16	1	26/10	0.801
	Amikacin	0	17/1	0	2/0	0	41/4	0	23/5	0.244
Tetracycline	Tigecycline	0	0/5	0	0/1	0	1//22	0	0/8	1.0
Others	Fosfomycin	0	2//5	0	0/0	0	0/21	0	1/7	0.036
	Colistin	0	1/0	0	1/0	0	3/0	0	4/0	1.0
	Rifampicin	0	0/6	0	0/1	0	0/22	0	0/9	1.0

Table 6 Susceptibility testing of common antibiotics in pneumonia caused by *Escherichia coli*

		>65 deceased (n = 163)		<64 deceased (n = 22)		>65 survivor (n = 509)		<64 survivor (n = 242)		
Drug groups	Active substance	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	p
Penicillins	Ampicillin	0	7/17	0	0/3	0	35/50	0	8/12	0.387
	Piperacillin	0	8/15	0	0/3	1	39/47	0	10/11	0.352
Penicillin + beta-lactamase inhibitors	Ampicillin + Sulbactam	6	10/11	0	0/3	16	45/37	5	12/9	0.293
	Piperacillin + Tazobactam	11	17/4	2	1/1	48	68/12	14	15/2	0.538
Cephalosporins	Cefepime	0	14/4	0	1/2	0	66/9	0	13/4	0.058
	Cefotaxime	0	19/5	0	1/2	0	76/9	1	15/5	0.026
	Ceftazidime	1	18/4	0	1/2	1	75/9	0	15/4	0.030
	Cefuroxime	4	16/5	0	1/2	8	68/13	0	15/5	0.146
Glycylcycline	Tetracycline	0	3/3	1	0/0	0	6/7	0	1/0	1.0
	Tigecycline	0	10/0	0	2/0	0	26/0	0	6/0	1.0
Carbapenem	Imipenem	4	24/0	2	3/0	12	87/0	3	21/0	1.0
	Meropenem	0	19/1	0	2/1	1	80/0	2	18/0	<0.0001
Gyraseinhibitors	Ciprofloxacin	3	17/7	0	2/1	6	69/17	0	18/3	0.593
	Levofloxacin	1	15/5	0	2/1	2	64/18	0	16/3	0.859
Aminoglycosides	Amikacin	0	15/1	0	2/0	0	60/0	0	17/0	0.189
	Gentamicin	0	20/4	0	2/1	0	81/6	0	21/0	0.075
	Tobramycin	0	13/2	0	2/0	0	56/6	0	17/0	0.453
Tri-methoprim + Sulfamethoxazole	Co-trimoxazole	0	15/8	0	2/1	0	63/22	0	17/4	0.682
Polymyxin	Colistin	0	1/0	0	0/0	0	2/0	0	0/0	1.0
Others	Fosfomycin	0	12/0	0	2/0	0	58/1	0	12/2	0.180

Table 7 Susceptibility testing of common antibiotics in pneumonia caused by Gram-negative bacteria

		>65 deceased (n = 163)		<64 deceased (n = 22)		>65 survivor (n = 509)		<64 survivor (n = 242)		
Drug groups	Active substance	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	p
Penicillins	Ampicillin	0	2/5	0	0/5	0	2/31	0	0/5	0.202
	Piperacillin	1	5/3	0	3/2	1	23/8	0	3/2	0.737
Penicillin + beta-lactamase inhibitors	Ampicillin + Sulbactam	1	2/6	0	1/4	3	4/26	1	1/4	0.693
	Piperacillin + Tazo-bactam	6	6/3	3	3/1	15	26/6	3	3/1	0.778
Cephalosporins	Cefepime	0	6/0	0	5/0	0	27/1	0	5/0	1.0
	Cefotaxime	0	6/2	0	3/2	0	27/7	0	3/2	0.657
	Ceftazidime	0	6/3	0	4/0	2	25/4	0	4/0	0.449
	Cefuroxime	0	2/5	0	0/5	2	7/26	0	0/5	0.579
Glycylcycline	Tetracycline	0	0/5	0	1/2	0	1/2	0	1/2	0.382
	Tigecycline	0	1/0	0	0/0	1	2/1	0	0/0	1.0
Carbapenem	Imipenem	3	6/2	0	5/0	4	30/3	0	5/0	0.400
	Meropenem	1	8/2	0	5/0	1	30/2	0	5/0	0.318
Gyrase inhibitors	Ciprofloxacin	2	8/2	1	4/1	4	29/4	1	4/1	0.631
	Levofloxacin	0	6/1	0	1/1	6	20/3	0	1/1	0.170
Aminoglycosides	Amikacin	0	3/2	0	4/0	0	15/1	0	4/0	0.217
	Gentamicin	0	7/2	0	4/1	2	28/5	0	4/1	0.932
	Tobramycin	0	2/4	0	3/0	0	13/6	0	3/0	0.118
Trimethoprim + Sulfamethoxazole	Co-trimoxazole	0	9/1	0	4/1	1	28/5	0	4/1	0.999
Polymyxin	Colistin	0	2/0	0	0/0	0	2/0	0	0/0	1.0
Others	Fosfomycin	0	2/2	0	2/0	0	11/1	0	2/0	0.175

Table 8 Susceptibility testing of common antibiotics in pneumonia caused by *Haemophilus*

		>65 deceased (n = 163)		<64 deceased (n = 22)		>65 survivor (n = 509)		<64 survivor (n = 242)		
Drug groups	Active substance	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	p
Penicillins	Ampicillin	0	8/2	0	0/0	1	27/11	0	26/7	0.767
	Piperacillin	1	4/1	0	0/0	0	9/3	0	6/1	0.999
Penicillin + beta-lactamase inhibitors	Ampicillin + Sulbactam	1	9/1	0	0/0	14	36/3	13	29/2	0.999
	Piperacillin + Sulbactam	2	5/0	0	0/0	3	11/0	3	6/1	0.522
	Piperacillin + Tazobactam	5	10/0	0	0/0	12	37/1	7	30/1	0.999
Cephalosporins	Cefuroxime	0	9/1	0	0/0	2	35/2	1	29/4	0.543
	Cefotaxime	0	10/0	0	0/0	0	37/1	0	31/1	0.999
Gyrase inhibitors	Ciprofloxacin	2	9/0	0	0/0	0	39/0	3	33/0	1.0
	Levofloxacin	0	5/0	0	0/0	2	32/1	2	25/0	0.999
Macrolide	Erythromycin	0	5/4	0	0/0	1	21/15	0	17/12	1.0

Table 9 Susceptibility testing of common antibiotics in pneumonia caused by *Klebsiella*

		>65 deceased (n = 163)		<64 deceased (n = 22)		>65 survivor (n = 509)		<64 survivor (n = 242)		
Drug groups	Active substance	No. using anti-biotics	Sensitive/ resistant	No. using anti-biotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	No. using antibiotics	Sensitive/ resistant	p
Penicillins	Ampicillin	0	0/25	0	0/2	0	0/85	0	0/36	1.0
	Piperacillin	0	1/16	0	0/0	0	4/64	0	0/32	0.419
Penicillin + beta-lactamase inhibitors	Ampicillin + Sulbactam	1	13/11	0	2/0	13	56/27	6	23/13	0.466
	Piperacillin + Tazobactam	14	17/8	1	2/0	47	60/17	19	26/9	0.641
Cephalosporins	Cefuroxime	0	19/6	0	2/0	10	58/23	3	26/10	0.816
	Cefotaxime	0	19/6	0	2/0	0	69/15	0	28/8	0.775
	Cefepime	0	17/6	0	2/0	0	59/11	0	27/8	0.566
	Ceftazidime	1	16/6	0	2/0	1	60/12	1	27/8	0.591
Carbapenem	Imipenem	2	25/0	1	2/0	0	85/0	4	36/0	1.0
	Meropenem	1	25/0	0	2/0	0	77/0	0	36/0	1.0
Gyrase inhibitors	Ciprofloxacin	0	19/6	0	1/0	6	63/20	0	28/8	0.948
	Levofloxacin	0	13/5	0	2/0	1	48/17	0	25/6	0.860
Trimethoprim + Sulfo-namide	Co-trimoxazole	0	19/5	0	2/0	0	70/11	0	29/7	0.692
Aminoglycosides	Gentamicin	1	23/2	0	2/0	2	74/8	1	34/2	0.857
	Tobramycin	0	12/1	0	0/0	0	42/4	0	17/2	1.0
	Amikacin	0	11/0	0	0/0	0	42/1	0	18/0	0.999
Tetracycline	Tigecycline	0	2/0	0	0/0	0	14/1	0	7/0	0.999
	Tetracycline	0	8/3	0	1/0	0	24/5	0	13/2	0.773
Others	Fosfomycin	1	9/2	0	0/0	0	31/5	1	15/3	0.902

Table 10 Type of pneumonia according to age-group and mortality

	>65 deceased	<64 deceased	>65 survivor	<64 survivor	p
Community-acquired	73 (44.8)	9 (40.9)	242 (47.5)	137 (56.6)	
Nosocomial-acquired	65 (39.9)	5 (22.7)	213 (41.8)	71 (29.3)	
Aspiration	25 (15.3)	8 (36.4)	54 (10.6)	34 (14.0)	
Total no. of patients (%)	**163 (17.4)**	**22 (2.4)**	**509 (54.4)**	**242 (25.9)**	**0.0004**

Table 11 Specimen collection and bacterial species in patients with pneumonia; number (%) of patients

Specimen	>65 deceased	<64 deceased	>65 survivor	<64 survivor	p
Sputum	12 (7.4)	0	71 (13.9)	31 (12.8)	
Throat swab	3 (1.8)	1 (4.5)	3 (0.6)	2 (0.8)	
Tracheal secretion	92 (56.4)	14 (63.6)	231 (45.4)	116 (47.9)	
Bronchial secretion	40 (24.5)	4 (18.2)	136 (26.7)	61 (25.2)	
Arterial blood culture	2 (1.2)	0	6 (1.2)	4 (1.7)	
Venous blood culture	11 (6.7)	3 (13.6)	58 (11.4)	27 (11.2)	
Secretion drainage	3 (1.8)	0	4 (0.8)	1 (0.4)	0.110
Species					
Streptococcus pneumoniae	5 (3.1)	2 (9.1)	33 (6.5)	27 (11.2)	
Staphylococcus aureus	25 (15.3)	5 (22.7)	84 (16.5)	37 (15.3)	
Staphylococcus epidermidis	2 (1.2)	0	6 (1.2)	1 (0.4)	
Coagulase-negative staphylococci	3 (1.8)	0	5 (1.0)	4 (1.7)	
Staphylococcus haemolyticus	1 (0.6)	0	1 (0.2)	0	
Methicillin-resistant *Staphylococcus aureus* (MRSA)	27 (16.6)	3 (13.6)	40 (7.9)	11 (4.5)	
Staphylococcus capitis	0	0	3 (0.6)	0	
Staphylococcus hominis	0	0	1 (0.2)	1 (0.4)	
Staphylococcus warneri	0	0	0	1 (0.4)	
Pseudomonas aeruginosa	31 (19.0)	2 (9.1)	90 (17.7)	45 (18.6)	
Escherichia coli	24 (14.7)	3 (13.6)	87 (17.1)	21 (8.7)	
Enterobacter cloacae	1 (0.6)	1 (4.5)	3 (0.6)	1 (0.4)	
Serratia marcescens	4 (2.5)	3 (13.6)	16 (3.1)	12 (5.0)	
Proteus mirabilis	1 (0.6)	1 (4.5)	7 (1.4)	6 (2.5)	
Acinetobacter baumannii	2 (1.2)	0	2 (0.4)	0	
Stenotrophomonas maltophilia	1 (0.6)	0	2 (0.4)	3 (1.2)	
Citrobacter koseri	1 (0.6)	0	0	0	
Prevotella buccae	0	0	1 (0.2)	1 (0.4)	
Enterobacter aerogenes	0	0	1 (0.2)	2 (0.8)	
Proteus vulgaris	0	0	1 (0.2)	0	
Serratia plymuthica	0	0	1 (0.2)	0	
Haemophilus influenzae	7 (4.3)	0	21 (4.1)	18 (7.4)	
Haemophilus parainfluenzae	3 (1.8)	0	18 (3.5)	15 (6.2)	
Klebsiella pneumoniae	25 (15.3)	2 (9.1)	66 (13.0)	28 (11.6)	
Klebsiella ozaenae	0	0	1 (0.2)	1 (0.4)	
Klebsiella oxytoca	0	0	17 (3.3)	7 (2.9)	
Klebsiella granulomatis	0	0	2 (0.4)	0	
Total no. of patients (%)	**163 (17.4)**	**22 (2.4)**	**509 (54.4)**	**242 (25.9)**	

is required in the application of cefazolin for the treatment of pneumonia in the elderly.

The elderly patients with staphylococcal pneumonia in the present study also had high antibiotic resistance to levofloxacin, a gyrase inhibitor. Gyrase inhibitors have emerged as an important class of antibiotics in the treatment of pneumonia (Nasiri et al. 2013). A secondary development of resistance in staphylococci is possible during treatment with levofloxacin in pneumonia patients (Hidalgo et al. 2008). Another study demonstrates a very high rate of fluoroquinolone resistance in *S. aureus*, reaching almost 100 % in methicillin resistant isolates (Kim et al. 2004). Therefore, close monitoring of antimicrobial resistance is necessary during treatment with levofloxacin.

As a result of unnecessary application of macrolides, resistance to these antibiotics is frequently encountered. Erythromycin acts against a broad spectrum of Gram-positive bacteria, but its activity against staphylococci may be reduced due to the occurrence of resistance (Piątkowska et al. 2012). A past study shows this previously unidentified process of *S. aureus* resistance to erythromycin (Ying and Tang 2010). The present study confirmed a high frequency of resistance of staphylococci to erythromycin in the elderly pneumonia patients with a fatal outcome.

Resistance to antibiotics among staphylococci is a growing problem in everyday practice of treating pneumonia. This has prompted a renewed interest in the use of lincosamide antiinfection agents to treat *S. aureus* pneumonia (Guay 2007). Clindamycin is a clinically relevant representative of this group of antibiotics, which is usually effective against staphylococci (Prabhu et al. 2011). However, the extensive use of the antibiotics of this class has escalated staphylococci resistance to them (Colakoğlu et al. 2008; Yilmaz et al. 2007; Kader et al. 2005). The increased resistance to clindamycin was also demonstrated by the susceptibility testing in the present study, mainly in the elderly pneumonia patients who failed to survive. Since the primary clindamycin-resistant staphylococci may occur, sensitivity of staphylococci to clindamycin must be evidenced *in vitro* before the onset of pneumonia treatment.

A limitation of this study is that it was performed in a single university hospital. Since bacterial resistance to antibiotics may be regional, the results of this study may not be widely applicable and ought to be considered mostly for comparison with other studies in different regions. Also, after completion of the present study, it turned out that the antibiotics were not always evenly distributed in susceptibility testing.

In conclusion, bacterial resistance to antibiotics is a widespread problem. The emergence of resistance to piperacillin-tazobactam, cefuroxime, cefazolin, levofloxacin, erythromycin, and clindamycin in staphylococci pneumonia in the elderly may contribute to fatal outcomes. For that reason, close clinical and microbial monitoring of antibiotic resistance must be carried out during the treatment of pneumonia in patients over 65 years of age.

Conflicts of Interests The authors declare no competing financial or otherwise interests in relation to this study.

References

Barlett JG (1987) Diagnosis of bacterial infections of the lung. Clin Chest Med 8:119–134

Bauer AW, Kirby WM, Sherris JC, Turck M (1996) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 45:493–496

Chong CP, Street PR (2008) Pneumonia in the elderly: a review of severity assessment, prognosis, mortality, prevention, and treatment. South Med J 101(11):1134–1140

CLSI (2012) Clinical and Laboratory Standards Institute (2012) Performance standards for antimicrobial susceptibility testing. CLSI M100-S22. http://clsi.org/blog/2012/01/13/clsi-publishes-2012-antimicrobial-susceptibility-testing-standards/. Accessed 14 July 2015

Colakoğlu S, Alişkan H, Turunç T, Demiroğlu YZ, Arslan H (2008) Prevalence of inducible clindamycin resistance in Staphylococcus aureus strains isolated from clinical samples. Mikrobiyol Bul 42(3):407–412 (Article in Turkish)

Cunha BA (2001) Pneumonia in the elderly. Clin Microbiol Infect 7(11):581–588

Dikensoy O, Usalan C, Filiz A (2002) Foreign body aspiration: clinical utility of flexible bronchoscopy. Postgrad Med J 78(921):399–403

Donowitz GR, Cox HL (2007) Bacterial community-acquired pneumonia in older patients. Clin Geriatr Med 23(3):515–534

El-Solh AA, Sikka P, Ramadan F, Davies J (2001) Etiology of severe pneumonia in the very elderly. Am J Respir Crit Care Med 163(3 Pt 1):645–651

EUCAST (2011) European Committee on antimicrobial susceptibility testing breakpoints 2011–2014. http://www.eucast.org. Accessed 14 July 2015

Feikin DR, Schuchat A, Kolczak M et al (2000) Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995–1997. Am J Public Health 90(2):223–229

Feldman C (2001) Pneumonia in the elderly. Med Clin North Am 85(6):1441–1459

Gin A, Dilay L, Karlowsky JA, Walkty A, Rubinstein E, Zhanel GG (2007) Piperacillin-tazobactam: a beta-lactam/beta-lactamase inhibitor combination. Expert Rev Anti Infect Ther 5(3):365–383

Guay D (2007) Update on clindamycin in the management of bacterial, fungal and protozoal infections. Expert Opin Pharmacother 8(14):2401–2444

Gutiérrez F, Masiá M, Mirete C, Soldán B, Rodríguez JC, Padilla S, Hernández I, Royo G, Martin-Hidalgo A (2006) The influence of age and gender on the population-based incidence of community-acquired pneumonia caused by different microbial pathogens. J Infect 53(3):166–174

Hashemi SH, Soozanchi G, Jamal-Omidi S, Yousefi-Mashouf R, Mamani M, Seif-Rabiei MA (2010) Bacterial aetiology and antimicrobial resistance of community-acquired pneumonia in the elderly and younger adults. Trop Doct 40(2):89–91

Hidalgo M, Reyes J, Cárdenas AM, Díaz L, Rincón S, Vanegas N, Díaz PL, Castañeda E, Arias CA (2008) Resistance profiles to fluoroquinolones in clinical isolates of Gram positive cocci. Biomedica 28 (2):284–294 (Article in Spanish)

Jalil A, Niazi ID, Khan SU (2008) Evaluation of restoration of sensitivities of resistant Staphylococcus aureus isolates by using cefuroxime and clavulanic acid in combination. J Ayub Med Coll Abbottabad 20 (2):28–30

Kader AA, Kumar A, Krishna A (2005) Induction of clindamycin resistance in erythromycin-resistant, clindamycin susceptible and methicillin-resistant clinical Staphylococcal isolates. Saudi Med J 26 (12):1914–1917

Kim HB, Lee B, Jang HC, Kim SH, Kang CI, Choi YJ, Park SW, Kim BS, Kim EC, Oh MD, Choe KW (2004) A high frequency of macrolide-lincosamide-streptogramin resistance determinants in Staphylococcus aureus isolated in South Korea. Microb Drug Resist 10(3):248–254

Marik PE (2001) Aspiration pneumonitis and aspiration pneumonia. N Engl J Med 344(9):665–671

Marrie TJ (2000) Community-acquired pneumonia in the elderly. Clin Infect Dis 31(4):1066–1078

Millett ER, Quint JK, Smeeth L, Daniel RM, Thomas SL (2013) Incidence of community-acquired lower respiratory tract infections and pneumonia among older adults in the United Kingdom: a population-based study. PLoS One 8(9), e75131

Mukae H, Kawanami T, Yatera K, Yanagihara K, Yamamoto Y, Kakeya H, Tokimatsu I, Kadota J, Kohno S (2014) Efficacy and safety of levofloxacin in patients with bacterial pneumonia evaluated according to the new "Clinical evaluation methods for new antimicrobial agents to treat respiratory infections (Second Version)". J Infect Chemother 20 (7):417–422

Nasiri MI, Naqvi SB, Zaidi AA, Saeed R, Raza G (2013) Comparative study on resistant pattern of clinical isolates against levofloxacin and cefepime. Pak J Pharm Sci 26(2):415–419

Niederman MS, Mandell LA, Anzueto A, Bass JB, Broughton WA, Campbell GD, Dean N et al (2001) Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med 163:1730–1754

Pachon J, Prados MD, Capote F, Cuello JA, Garnacho J, Verano A (1990) Severe community-acquired pneumonia. Etiology, prognosis, and treatment. Am Rev Respir Dis 142(2):369–373

Pepersack T (2014) Specificities of pneumonia in geriatrics. Rev Med Brux 35(4):368–374 (Article in French)

Petrosillo N, Cataldo MA, Pea F (2015) Treatment options for community-acquired pneumonia in the elderly people. Expert Rev Anti Infect Ther 13 (4):473–485

Piątkowska E, Piątkowski J, Przondo-Mordarska A (2012) The strongest resistance of Staphylococcus aureus to erythromycin is caused by decreasing uptake of the antibiotic into the cells. Cell Mol Biol Lett 17 (4):633–645

Prabhu K, Rao S, Rao V (2011) Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. J Lab Phys 3(1):25–27

Riquelme OR, Riquelme OM, Rioseco ZML, Gómez MV, Cárdenas G, Torres C (2008) Community-acquired pneumonia in the elderly: clinical and nutritional aspects. Rev Med Chil 136(5):587–593 (Article in Spanish)

Schito GC (2006) The importance of the development of antibiotic resistance in Staphylococcus aureus. Clin Microbiol Infect 12(Suppl 1):3–8

Shoji T, Hirai Y, Osawa M, Totsuka K (2014) Cefazolin therapy for methicillin-susceptible Staphylococcus aureus bacteremia in Japan. J Infect Chemother 20 (3):175–180

Skov R, Frimodt-Møller N, Espersen F (2002) In vitro susceptibility of Staphylococcus aureus towards amoxycillin-clavulanic acid, penicillin-clavulanic

acid, dicloxacillin and cefuroxime. APMIS 110 (7–8):559–564
Watkins RR, Lemonovich TL (2011) Diagnosis and management of community-acquired pneumonia in adults. Am Fam Physician 83:1299–1306
WHO (2015) International Classification of Diseases (ICD). http://www.who.int/classification/icd/en/. Accessed 6 June 2015
Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I (2007) Detection and prevalence of inducible clindamycin resistance in staphylococci. J Med Microbiol 56(Pt 3):342–345
Ying L, Tang D (2010) Recent advances in the medicinal chemistry of novel erythromycin-derivatized antibiotics. Curr Top Med Chem 10(14):1441–1449

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 57–68
DOI 10.1007/5584_2015_193

Published online: 23 January 2016

Polymorphism of *FCGR2A, FCGR2C,* and *FCGR3B* Genes in the Pathogenesis of Sarcoidosis

M. Typiak, K. Rębała, M. Dudziak, J.M. Słomiński, and A. Dubaniewicz

Abstract

We have previously presented evidence that the polymorphism of the *FCGR3A* gene, encoding the receptor for Fc fragment of immunoglobulin G IIIa (FcγRIIIa) plays a role in the enhancement of circulating immune complexes (CIs) with the occurrence of *Mycobacterium tuberculosis* heat shock proteins in patients with sarcoidosis (SA). The immunocomplexemia might be caused by decreased affinity of CIs to Fcγ receptors, with the subsequently decreased receptor clearance by immune cells. In the present study we examined whether the polymorphisms of other related genes (*FCGR2A*, *FCGR2C, FCGR3B*) encoding other activatory Fcγ receptors, could have a similar effect. To this end, we genotyped 124 patients with sarcoidosis and 148 healthy volunteers using polymerase chain reaction with sequence-specific primers. We revealed a significant decrease in the percentage of the *FCGR2A* and *FCGR2C* variants that ensure effective CIs clearance, with a concomitant increase of less functional variants of these genes in Stages I/II, compared with Stages III/IV of SA. There was no aberration in *FCGR3B* allele/genotype frequencies. We conclude that the *FCGR2A* and *FCGR2C* polymorphisms may also contribute to immunocomplexemia present in SA. The assessment of *FCGR* genes could become a tool in presaging a clinical course of sarcoidosis and in its personalized therapy.

Keywords

FCGR genes • Immunoglobulin G • Polymorphism • Receptor • Sarcoidosis

M. Typiak, J.M. Słomiński, and A. Dubaniewicz (✉)
Department of Pulmonology, Medical University of Gdansk, 7 Debinki St, 80-211 Gdansk, Poland
e-mail: aduban@gumed.edu.pl

K. Rębała
Department of Forensic Medicine, Medical University of Gdansk, 23 Debowa St, 80-204 Gdansk, Poland

M. Dudziak
Non-invasive Cardiac Diagnostic Department, Medical University of Gdansk, 17 Mariana Smoluchowskiego St, 80-214 Gdansk, Poland

1 Introduction

Sarcoidosis (SA) is a multisystem, granulomatous disorder of unknown etiology. Infectious, genetic factors, and autoimmunity have been explored as potential causes of SA (American Autoimmune Related Diseases Association 2015; Spagnolo and Grunewald 2013; Baughman et al. 2011; American Thoracic Society et al. 1999). Due to similarities between SA and tuberculosis, *Mycobacterium tuberculosis* (Mtb) and its antigens, e.g., KatG, A85, SOD2, and heat shock proteins (Mtb-hsps), have been studied as causative factors for sarcoidosis (Dubaniewicz 2013; Dubaniewicz et al. 2012a; Dubaniewicz 2010; Kivity et al. 2009).

Mtb-hsps, involved in the formation of immune complexes (CIs), may be crucial in connecting infection and autoimmunity, both considered in sarcoidosis. Recently, we have shown the presence of immunocomplexemia with the involvement of Mtb-hsps in SA patients, especially of Mtb-hsp16 - the main marker of a dormant stage of mycobacteria. A high level of CIs suggests the occurrence of antigenemia, which may result from the persistent presence of phagocyted mycobacteria releasing Mtb-hsps. Mtb-hsps, in turn, presented through the human leukocyte antigen (HLA) system to T and B lymphocytes would trigger the cellular and humoral immune response (Dubaniewicz 2010; 2013; Dubaniewicz et al. 2012a; Baughman et al. 2011; Kivity et al. 2009). The immunocomplexemia may also be caused by altered elimination of antigen(s) by monocytes/macrophages and neutrophils. Both phagocytosis and clearance of CIs may be disrupted due to dysfunction of receptors, especially for Fc fragments of immunoglobulin G (FcγR): FcγRI, FcγRII, and FcγRIII. We have recently revealed an increased number of blood FcγRI^{+}-, FcγRII^{+}-, and FcγRIII^{+}-monocytes with a higher phagocytic activity in SA patients (Dubaniewicz 2013; Dubaniewicz et al. 2012b). Increased phagocytosis and immunocomplexemia in SA patients may result from dysfunction of FcγRI-III receptors due to their functional polymorphism. To this end we have revealed the *FCGR3A*-V158F polymorphism, responsible for decreased affinity of FcγRIIIa to CIs and decreased FcγRIIIa clearance in SA (Typiak et al. 2014). Similar findings have been presented by Maertzdorf et al. (2012) who report increased expression of genes connected with FcγR-mediated phagocytosis in SA patients, although the authors did not specify the receptors involved in the process.

The immunocomplexemia also may result from aberrant interaction of neutrophils with FcγRIIa, FcγRIIc and FcγRIIIb receptors, encoded by the polymorphic *FCGR2A, FCGR2C*, and *FCGR3B* genes (Behnen et al. 2014; Li et al. 2009; Tutor-Ureta et al. 2006; Schmekel et al. 1985). These receptors are important for the recognition and phagocytosis of IgG-opsonized pathogens, CIs, degranulation, antibody-dependent cell-mediated cytoxicity, release of proinflammatory cytokines, oxidative burst, and apoptosis. It has also been reported that neutrophils are among the first cells to arrive at the site of mycobacterial infection; often an underlying pathogenetic mechanism of sarcoidosis. Therefore, dysfunction of FcγRs on neutrophils may play a role in the CIs-mediated inflammatory process and thus in the induction of autoimmunity in sarcoidosis.

The *FCGR2A*-H131R, *FCGR2C*-X57Q, and *FCGR3B*-NA1/NA2/SH polymorphisms have been detected in different autoimmunological disorders, such as systemic lupus erythematosus, rheumatoid arthritis, and idiopatic thrombocytopenic purpura (Li et al. 2009), but not yet in SA. Therefore, the aim of the current study was to evaluate the polymorphism of these genes in patients with sarcoidosis.

2 Methods

Ethical approval for the study was granted by the Bioethics Committee for Scientific Research of the Medical University of Gdansk, Poland (NKEBN/337/2009). Written informed consent

was obtained from the study participants. Recruitment of patients, collection of blood samples, and genotyping all were conducted in the period January 2007 to June 2014.

2.1 Patients

Patients with sarcoidosis were followed by for at least three years to ensure the acquisition of data about disease recurrence and its chronic character (the average follow-up duration amounted to four years). There were 124 untreated patients (69 smokers, 55 non-smokers) with newly diagnosed pulmonary sarcoidosis enrolled into the study (Table 1). The diagnosis of SA was based on histological (scalenobiopsy of lymph nodes), clinical, and radiological evidence. Disease staging was performed using high resolution computed tomography according to the classification of Scadding (1961): Stage I – bilateral hilar lymphadenopathy (38 patients); Stage II – bilateral hilar lymphadenopathy and diffuse pulmonary infiltrations (60 patients); Stage III – diffuse pulmonary infiltrations (20 patients); Stage IV – fibrosis and cavities (6 patients). Twenty five patients had Löfgren's syndrome. Microbiological and cytological examination of lymph nodes and sputum samples revealed no acid-fast bacilli (PCR, culture of the *M. tuberculosis* strain), fungi, or atypical cells.

The control group consisted of 148 unrelated healthy volunteers (80 smokers, 68 non-smokers) recruited in the same time period as that for sarcoidosis patients (Table 1). All these individuals had normal chest radiographs, blood and serum analysis, as well as no acid-fast bacilli in sputum smears or sputum cultures of the *M. tuberculosis* strain. None of the controls or SA patients had a familial history of tuberculosis, sarcoidosis, or autoimmune disease. All participants of the study were free of HIV infection.

2.2 Sample Collection and DNA Isolation

Peripheral blood samples (10 ml) were collected into tubes with EDTA (Becton Dickinson Company, Franklin Lakes, NJ), and were stored at 4 °C for a maximum of 4 h and then at −20 °C for a maximum of one month. In case of patients, blood collection took place before treatment. Subsequently, samples were thawed in room temperature and DNA was isolated using a non-enzymatic method, according to the method of Lahiri and Nurnberger (1991). Analyzes were performed always by the same persons at the Department of Forensic Medicine of the Medical University of Gdansk in Poland.

2.2.1 Polymerase Chain Reaction for FCGR2 Genes Polymorphism

Polymerase chain reaction with the sequence specific primers (PCR-SSP) was used for the

Table 1 Comparative characteristics of patients with pulmonary sarcoidosis (SA) and healthy individuals (Controls)

	SA patients	Controls
	n = 124 (%)	n = 148 (%)
Age (year)		
Mean	41	42
Range	21–68	18–79
Gender		
Female	52 (42)	67 (45)
Male	72 (58)	81 (55)
BCG vaccination	124 (100)	148 (100)
Positive PPD skin test	0	0
Relapses	0	0
Symptoms		
Cough	58 (47)	0
Dyspnea	12 (10)	0
Fever	21 (17)	0
Night sweats	2 (2)	0
Weight loss	6 (5)	0
Erythema nodosum	25 (20)	0
Arthritis	25 (20)	0

gene polymorphism analysis in both groups (Table 2). The *FCGR2A*-H131R polymorphism, involving A > G at 519 nucleotide position, causing a change in H131R aminoacid in the second extracellular domain of FcγRIIa, resulting in the presence of two variants of the protein: 131H and 131R (rs1801274) was analyzed. To determine the genotype of a person, two separate PCR-SSP reactions were conducted for the two *FCGR2A* gene alleles according to the method of Edberg et al. (2002) with modifications, due to the same length of amplified products for 131H and 131R variants (371 bp). We used a reverse primer (5'-TCAAAGTGAAACAACAGCCTGACT-3') that ensured gene specificity for the *FCGR2A* and either a forward 131H allele-specific (5'-GGAAAATCCCAGAAATTCACACA-3') or 131R allele-specific primer (5'-GGAAAATCC-CAGAAATTCACACG-3'). To eliminate false negatives, each reaction was supplemented with an internal control of amplification. For this purpose, primers specific for human growth hormone (*hGH*) gene fragments were used after checking for and excluding unwanted primer interactions. Sequences of the primers for the amplification of the internal control (439 bp) were as follows: a forward primer 5'-CAGTGCCTTCCCAACCATTCCCTTA-3' and a reverse primer 5'-ATCCACTCACGGATTTCTGTTGTGTTTC-3'.

The PCR-SSP reaction for 131H (519A) allele was performed with 20 ng of genomic DNA in 1x Pol Buffer B, 1.75 mM $MgCl_2$, 0.3 mM of each dNTP, 0.2 μM of the *FCGR2A* gene-specific and 131H-specific primers, 0.16 μM of both *hGH*-specific primers and 1.0 U of Taq polymerase (EURx; Gdańsk, Poland) in a total volume of 10 μl. The PCR-SSP reaction for 131R (519G) allele was performed similarly, but with 30 ng of genomic DNA with 0.25 μM of both *FCGR2A* gene-specific and 131R-specific primers, and 0.32 μM of both *hGH*-specific primers. A temperature profile for the 131H allele comprised 2 min at 95 °C, 35 amplification cycles (1 min at 94 °C, 1 min of annealing at 56 °C, and 2 min at 72 °C), followed by a final elongation at 72 °C for 10 min. A temperature profile for the amplification of the 131R(519G) allele was identical except for a higher annealing temperature of 57 °C.

The *FCGR2C*-X57Q polymorphism involving T > C at 202 nucleotide position causing a change from STOP codone to a triplet coding for glutamine in the first extracellular domain of FcγRIIc, resulting in the presence of two variants of the protein: 57X (a shortened protein in the absence of the other variant) and 57Q (a full-length, functional receptor protein) was analysed (rs1801274). To determine the genotype of a person, two separate PCR-SSP reactions were performed for the two *FCGR2C* gene alleles according to the

Table 2 Location of single nucleotide polymorphism (SNPs) in *FCGR2A*, *FCGR2C*, and *FCGR3B* genes

Gene	Nucleotide position	Major allele	Minor allele	dbSNP ID	Aminoacid change	Allele esignation	Change in receptor protein
FCGR2A	519	A	G	rs1801274	H131R	131H/R	Structural change in the second extracellular domain.
FCGR2C	202	T	C	rs1801274	X57Q	57X/Q	Production of nonfunctional/ functional receptor protein.
FCGR3B	147	C	T	rs447536	L38L	NA1/ NA2	Structural change in the first extracellular domain
	141	G	C	rs403016	R36S		
	227	A	G	rs448740	N65S		
	277	G	A	rs428888	D82N		
	349	G	A	rs2290834	V106I		
	266	C	A	rs5030738	A78D	SH/NA2	

method of Su et al. (2002) with modifications, due to the same length of amplified products for 57X (202 T) and 57Q (202C) variants (124 bp). We used a reverse primer (5'-GAGATTCCCATTGTGGACCTACG-3') that ensured gene specificity for *FCGR2C* and either a forward 57X allele-specific (5'-GGCTGTGCTGAAACTGGAGACCT-3') or 57Q allele-specific primer (5'-GGCTGTGCTGAAACTGGAGCCAC–3'). To eliminate false negatives, we supplemented reactions with an internal control of amplification (439 bp). For this purpose, primers specific for the *hGH* gene fragments were used after checking for and excluding unwanted primer interactions (sequences of the *hGH*-specific primers were outlined above in the section describing the PCR-SSP for *FCGR2A*).

The PCR-SSP reaction for 57Q (202C) allele consisted of 10 ng of genomic DNA in 1x Pol Buffer B, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 1 μM of *FCGR2C* gene-specific and 57Q-specific primers and 1.25 U of Taq polymerase (EURx; Gdansk, Poland), in a total volume of 10 μl. The PCR-SSP for 57X (202 T) allele was performed similarly, but with 0.15 μM of both *FCGR2C* gene-specific and 57X-specific primers and 1.0 U of Taq polymerase. Both primers for the internal control of amplification (*hGH*) were added in the proper amount to reach the concentration of 0.1 μM. A temperature profile for the 57Q (202C) allele comprised 5 min at 95 °C, 37 amplification cycles (30 s at 94 °C, 30 s of annealing at 55 °C and 10 s at 72 °C), followed by a final elongation at 72 °C for 7 min. A temperature profile for the amplification of the 57X (202 T) allele was identical except for a higher annealing temperature of 57 °C and a lower number of amplification cycles of 35.

The *FCGR3B*-NA1/NA2/SH (HNA-1a/HNA-1b/HNA-1c) polymorphism was also analyzed. The NA1 and NA2 alleles differ in five nucleotide positions (C147T/L38L, rs447536 and four mutations leading to the following aminoacid changes: G141C/R36S, rs403016; A227G/N65S, rs448740; G277A/D82N, rs428888; G349A/V106I, rs2290834) in the sequence coding for the first extracellular domain of FcγRIIIb. The SH allele is similar to NA2, except for one nucleotide (C266A, rs5030738), which, however, changes the sequence of the receptor protein (A78D) in the above mentioned domain.

To determine the genotype of a person, one PCR-SSP reaction was performed for the three *FCGR3B* gene alleles according to the method of Siriboonrit et al. (2003) with modifications, due to different lengths of amplified products for the NA1 (140 bp), NA2 (219 bp), and SH variants (102 bp). Therefore, the internal control of amplification was unneeded. In every reaction we used a common reverse primer (5'-ATGGACTTCTAGCTGCAC-3') that ensured gene specificity for *FCGR3B*, and three forward primers: NA1-specific (5'-CAGTGGTTTCA-CAATGAGAA-3'), NA2/SH-specific (5'-CAATGGTACAGCGTGCTT-3'), and SH allele-specific primer (5'-TCGAGCTACTTCAT TGACGA-3').

The PCR-SSP reaction for all three alleles was performed with 10 ng of genomic DNA in 1x Pol Buffer B, 3 mM $MgCl_2$, 0.32 mM of each dNTP, 0,5 μM *FCGR3B* gene-specific primer, 0.3 μM of NA1-specific, 0.08 μM of NA2/SH-specific and 0.16 μM of SH-specific primers, and 0.5 U of Taq polymerase (EURx; Gdańsk, Poland) in a total volume of 10 μl. A temperature profile for the tested alleles comprised 2 min at 96 °C, 32 amplification cycles (30 s at 96 °C, 1 min of annealing at 63 °C, and 30 s at 72 °C), followed by a final elongation at 72 °C for 5 min.

Due to a close connection of the SH allele to NA2, which differ in only one of six nucleotides that define the allele, the PCR-SSP method used for the *FCGR3B* gene has a specific limitation. The NA2/SH-specific and *FCGR3B* gene-specific primers are substrates for the PCR product, which is specific for both NA2 and SH allele (NA2/SH product). It is worth emphasizing that supplementation of the PCR reaction with SH-specific primer allows obtaining a product that is specific only for the SH, and not NA2, variant. In the conjoint presence of both

NA2/SH- and SH-specific products, the method cannot differentiate the NA2/SH and SH/SH genotypes. Therefore, two values were given for the frequencies of NA2 and SH alleles, as well as of NA2/SH and SH/SH genotypes.

Electrophoresis of the amplified DNA samples was performed in 14 % polyacrylamide gels (acrylamide-bisacrylamide, 3 % of cross-linking). GeneRuler 100 bp DNA Ladder (Thermo Fisher Scientific, Waltham, MA) was used as a molecular weight marker (range 100–1000 bp). Gels were stained in 0.1 % silver nitrate solution.

2.2.2 Optimization of Methods

Our own contribution to the presented molecular methodology was a creation of a primer for the 57Q (202C) allele of *FCGR2C* and a changed sequence of the primer for the NA1 allele of *FCGR3B*, to enhance the specificity of connection of primers with DNA matrix. Further, to eliminate false negatives, we selected an internal control of amplification for the alleles of the *FCGR2A* and *FCGR2C* genes. The primers for an internal control were chosen on the basis of similarity of their length and annealing temperature to the primers amplifying the *FCGR* genes fragments and after excluding unwanted primer interactions (primer-dimer, hairpin structure). The use of internal control of amplification was unneeded in the method created for the *FCGR3B* gene analysis due to performing the amplification of all three gene alleles in one reaction (a lack of PCR product only in case of amplification error or gene deletion, which did not take place). In the PCR-SSP methods used we optimized the composition of reaction mixture (reaction buffer with ammonium sulfate, suitable concentrations of $MgCl_2$, dNTP, Taq polymerase, DNA, and primers for *FCGR* genes fragments and internal control of amplification) and temperature profile (annealing temperature, time of amplification steps, and number of amplification cycles). Finally, we also optimized the poliacrylamide gels concentration to achieve a clear distinction of the obtained PCR-products in the range of 100–1000 bp.

2.2.3 Statistical Analysis

The χ^2 test was used to compare the genotype and allele frequencies in the groups studied. Yates's correction was implemented when a number lower or equal to ten was included in the comparison. Odds ratios with 95 % confidence intervals were calculated for the *FCGRs* alleles and genotypes tested. Concordance with Hardy-Weinberg equilibrium was confirmed for each *FCGR* genotype using online software (Rodriguez et al. 2009). A p-value ≤ 0.05 was considered to indicate significant differences. Statistical elaboration was performed using STATISTICA for Windows ver.10.0 (StatSoft, Tulsa, OK).

3 Results

3.1 *FCGR2A* Gene

There was no significant difference in the *FCGR2A* allele or genotype frequency between the SA and control groups. Nor was there any difference in the *FCGR2A* allele frequency between the particular stages of SA and controls. However, in Stage III of SA, there was a significant increase in the occurrence of 131HH homozygotes and a decrease in 131HR heterozygotes compared with healthy individuals. In Stage II, in contrast, there was a significant decrease in 131HH homozygotes and an increase in the frequency of 131HR heterozygotes. In Stage I, we also found a lower presence of 131HH homozygotes and a higher percentage of 131HR heterozygotes than those in Stage III. Concerning the occurrence of particular genotypes of *FCGR2A* in patients with Stages I-IV of SA, we found a significant decrease in the percentage of 131HH homozygotes and an increase in 131HR heterozygotes in Stages I/II compared with Stages III/IV (Table 3).

Table 3 Frequency of alleles and genotypes of *FCGR2A* gene in patients with sarcoidosis (SA) and healthy controls; both SA (p = 0.43) and control (p = 0.70) groups were in Hardy–Weinberg equilibrium

	Number of alleles (%)		Number of genotypes (%)		
Groups	131H	131R	131HH	131HR	131RR
Sarcoidosis (n = 114)	130 (57)	98 (43)	35 (31)	35 (31)	19 (17)
Stage I (n = 36)	42 (58)	30 (42)	10 (28)[a]	22 (61)[b]	4 (11)
Stage II (n = 53)	56 (53)[c]	50 (47)	13 (24)[d]	30 (57)[e]	10 (19)
Stage III (n = 19)	27 (71)	11 (29)	11 (58)[f]	5 (26)[c]	3 (16)
Stage IV (n = 6)	5 (42)	7 (58)	1 (17)	3 (50)	2 (33)
Stage I/II (n = 89)	98 (55)	80 (45)	23 (26)[a]	52 (58)[e]	14 (16)
Stage III/IV (n = 25)	32 (64)	18 (36)	12 (48)	8 (32)	5 (20)
Controls (n = 142)	167 (59)	117 (41)	48 (34)	71 (50)	23 (16)

[a]p = 0.03 for Stage I *vs.* Stage III, Stage I/II *vs.* Stage III/IV
[b]p = 0.01 for Stage I *vs.* Stage III
[c]p = 0.05 for Stage III *vs.* controls, Stage II *vs.* Stage III
[d]p = 0.008 for Stage II *vs.* Stage III
[e]p = 0.02 for Stage II *vs.* Stage III, for Stage I/II I. Stage III/IV
[f]p = 0.04 for Stage III *vs.* controls

3.2 *FCGR2C* Gene

There was no significant difference in the *FCGR2C* allele or genotype frequency between the SA and control groups. In Stage III of SA, there were a significant increase in the occurrence of the 57Q allele and a decrease in the 57X allele compared with healthy individuals. Likewise, in Stages III/IV an increase in the 57Q allele and a decrease in the frequency of the 57X allele were found compared with healthy individuals. In Stages III and III/IV, we found a significant decrease in the percentage of 57XX homozygotes and an increase of 57XQ heterozygotes, which was in contrast to the findings in healthy individuals. In Stages I/II and II, there was a significant decrease in the percentage of 57XQ heterozygotes compared with Stage III. A significant decrease in the frequency of 57XQ heterozygotes was also detected in Stages I/II *versus* Stages III/IV (Table 4).

3.3 *FCGR3B* Gene

There were no significant differences in the *FCGR3B* allele or genotype frequency between the SA and control groups, particular stages of SA and controls, or between SA stages themselves (Table 5).

4 Discussion

In the current study, we found significant differences in the occurrence of alleles and genotypes of the *FCGR2A* gene in SA patients in Stages III and IV compared with Stages I and II, and with healthy individuals. In Stages III and IV there was a decrease in the frequency of *FCGR2A*-131HR heterozygotes and an increase in *FCGR2A*-131HH homozygotes compared with Stages II and II. We also found a lower frequency of *FCGR2A*-131H allele in Stage II than that in Stage III or IV. Further, a comparative analysis of the *FCGR2A*-H131R polymorphism in particular stages of SA *versus* healthy controls revealed an increased frequency of *FCGR2A*-131H allele and 131HH homozygotes in Stages III and IV. Concerning the *FCGR2C*-X57Q polymorphism, we found a decrease in the frequency of the 57XQ genotype with 57Q allele in Stages I and II compared with Stages III and IV. Moreover, in contrast to controls, a significant decrease in the frequency of the 57X allele and 57XX genotype, and an increase in the 57Q allele and 57XQ genotype were found in Stages III and IV. However, there were no appreciable differences in the frequency of *FCGR3B*-NA1/NA2/SH alleles or genotypes of the gene between SA patients and controls.

Table 4 Frequency of alleles and genotypes of *FCGR2C* gene in patients with sarcoidosis (SA) and healthy controls; both SA (p = 0.89) and control (p = 0.79) groups were in Hardy–Weinberg equilibrium

	Number of alleles (%)		Number of stages (%)		
Groups	57X	57Q	57XX	57XQ	57QQ
Sarcoidosis (n = 119)	193 (81)	45 (19)	78 (66)	37 (31)	4 (3)
Stage I (n = 37)	61 (82)	13 (18)	25 (67)	11 (30)	1 (3)
Stage II (n = 57)	94 (82)	20 (18)	40 (70)	14 (25)[a]	3 (5)
Stage III (n = 19)	28 (74)[b]	10 (26)[b]	9 (47)[c]	10 (53)[d]	0
Stage IV (n = 6)	10 (83)	2 (17)	4 (67)	2 (33)	0
Stage I/II (n = 94)	155 (82)	33 (18)	65 (69)	25 (27) [e,f]	4 (4)
Stage III/IV (n = 25)	38 (76)[g]	12 (24)[g]	13 (52)[d]	12 (48)[h]	0
Controls (n = 144)	249 (86)	39 (14)	108 (75)	33 (23)	3 (2)

OR odds ratio, *CI* confidence intervals
[a]p = 0.046 for Stage II *vs*. Stage III
[b]p = 0.038 for Stage III *vs*. controls (OR = 2.28, 95 % CI 1.03–5.06 for 57Q allele; OR = 0.44, 95 % CI 0.20-0.97 for 57X allele)
[c]p = 0.02 for Stage III *vs*. controls (OR = 0.27, 95 % CI 0.10–0.73, for 57XX to 57XQ proportion); for Stage III/IV *vs*. controls (OR = 0.33, 95 % CI 0.14–0.79, for 57XX to 57XQ proportion)
[d]p = 0.01 for Stage III *vs*. controls (OR = 3.64, 95 % CI 1.36–9.70, for 57XQ to 57XX proportion)
[e]p = 0.049 for Stage I/II *vs*. Stage III
[f]p = 0.04 for Stage I/II *vs*. Stage III/IV
[g]p = 0.056 for Stage III/IV *vs*. controls (OR = 2.02, 95 % CI 0.97–4.19 for 57Q allele; OR = 0.50, 95 % CI 0.24–1.03 for 57X allele)
[h]p = 0.009 for Stage III/IV *vs*. controls (OR = 3.02, CI 1.26–7.26, for 57XQ to 57XX proportion)

Table 5 Frequency of alleles and genotypes of *FCGR3B* in patients with sarcoidosis (SA) and healthy controls; both SA (p = 0.30) and control (p = 0.84) groups were in Hardy–Weinberg equilibrium. Due to similarities between NA2 and SH alleles two values of results were provided for them

	Number of alleles (%)					
Groups	NA1		NA2		SH	
Sarcoidosis (n = 124)	91 (37)		144/140 (58/56)		13/17 (5/7)	
Stage I (n = 38)	32 (42)		38/36 (50/47)		6/8 (8/11)	
Stage II (n = 60)	38 (32)		75/73 (62/61)		7/9 (6/7)	
Stage III (n = 20)	15 (37.5)		25/25 (62.5/62.5)		0/0	
Stage IV (n = 6)	6 (50)		6/6 (50/50)		0/0	
Stage I/II (n = 98)	70 (35.70)		113/109 (57.65/55.60)		13/17 (6.65/8.70)	
Stage III/IV (n = 26)	21 (40)		31/31 (60/60)		0/0	
Controls (n = 148)	113 (38)		168/167 (57/57)		15/16 (5/5)	
Groups	Number of genotypes (%)					
	NA1/NA1	NA2/NA2	SH/SH	NA1/NA2	NA1/SH	NA2/SH
Sarcoidosis (n = 124)	14 (11)	43 (35)	0/4 (0/3)	54 (44)	9 (7)	4/0 (3/0)
Stage I (n = 38)	5 (13)	9 (24)	0/2 (0/5)	18 (47)	4 (11)	2/0 (5/0)
Stage II (n = 60)	5 (8)	25 (42)	0/2 (0/3)	23 (39)	5 (8)	2/0 (3/0)
Stage III (n = 20)	3 (15)	8 (40)	0/0	9 (45)	0/0	0/0
Stage IV (n = 6)	1 (17)	1 (17)	0/0	4 (66)	0/0	0/0
Stage I/II (n = 98)	10 (10)	34 (35)	0/4 (0/4)	41 (42)	9 (9)	4/0 (4/0)
Stage III/IV (n = 26)	4 (15)	9 (35)	0/0	13 (50)	0/0	0/0
Controls (n = 148)	21 (14)	55 (37)	0/1 (0/1)	57 (39)	14 (9)	1/0 (1/0)

To the best of our knowledge, it is the first study of the polymorphism of the *FCGR2A, FCGR2C*, and *FCGR3B* genes in sarcoidosis and its particular stages; the stages that are, by some authors, considered as composing separate disease entities. However, studies of these genetic variations were performed in other autoimmune disorders, such as systemic lupus erythematosus (SLE), idiopathic trombocytic purpura (IPP), systemic sclerosis, rheumatoid arthritis (RA), idiopathic pulmonary fibrosis (IPF), granulomatosis with polyangiitis, giant cell arthritis (GCA), anti-glomerular basement membrane antibody disease, Kawasaki disease, microscopic polyangiitis, antiphospholipid syndrome, Sjögren's syndrome, Behçet's disease, Guillain-Barré syndrome (GBS), colitis ulcerosa, Crohn's disease, and myasthenia gravis (Li et al. 2014; Vigato-Ferreira et al. 2014; Wang et al. 2014; Haldorsen et al. 2013; Ji et al. 2013; Sanchez et al. 2011; Bournazos et al. 2010; Weersma et al. 2010; Asano et al. 2009; Bournazos et al. 2009; Li et al. 2009; Aksu et al. 2008; Morgan et al. 2006a; Morgan et al. 2006b; Hughes et al. 2004; Radstake et al. 2003; Manger et al. 2002).

In line with the present results concerning Stages I and II of SA, a decrease in the frequency of the *FCGR2A*-131HH genotype, with a simultaneous increase in 131RR homozygotes, was reported in SLE and GCA. The *FCGR2A*-131RR genotype has also been linked to earlier development of SLE and lupus nephritis. Further, the *FCGR2A*-131RR genotype, along with the presence of HLA-DRB1*04 allele, has been associated with a six-fold increase in the risk of developing GCA in Spanish patients, compared with individuals with the *FCGR2A*-131HH and 131HR genotype and a lack of DRB1*04 allele (Morgan et al. 2006a). Increased risk of disease development has also been reported in individuals having the *FCGR2A*-131R in GCA, SLE, antiphospholipid syndrome, ITP, granulomatosis with polyangiitis, and in RA (Vigato-Ferreira et al. 2014; Sanchez et al. 2011; Bournazos et al. 2009; Li et al. 2009; Morgan et al. 2006b; Manger et al. 2002), akin to what we found in Stage II of SA patients of the present study. In contrast to our results, however, the presence of *FCGR2A*-131R allele has been linked to a more severe course of disease in RA, myasthenia gravis, and in some patients with SLE. Increases in the frequency of 131H variant, like in our patients with Stage III of SA, and in 131HH homozygotes, like in our patients with Stages III and IV of SA have been reported in colitis ulcerosa, Crohn's disease, GBS, childhood-onset ITP, and in Chinese patients with Kawasaki disease (Wang et al. 2014; Ji et al. 2013; Weersma et al. 2010; Asano et al. 2009; Bournazos et al. 2009). In patients with Guillain-Barré syndrome having the *FCGR2A*-131HH genotype, increased risk of disease development has been reported, compared with individuals having the *FCGR2A*-131RR or 131HR genotype, which corresponds to our patients in advanced Stages III and IV of SA. However, some investigations of a genetic predisposition to develop RA in Caucasian and Asian populations, in European patients with systemic sclerosis, Japanese patients with microscopic polyangiitis, Greek patients with Kawasaki disease, and in Scandinavian patients with Sjögren's syndrome failed to connect the *FCGR2A*-H131R polymorphism to risk of disease development (Haldorsen et al. 2013; Bournazos et al. 2009; Radstake et al. 2003).

In a study on the *FCGR2C*-X57Q polymorphism in ITP patients *versus* healthy controls, increased percentage of the 57Q allele and 57XQ genotype has been reported (Bournazos et al. 2009), which corresponds to our patients with advanced Stages III and IV of SA. Likewise, the present results are in line with those concerning the *FCGR3B*-NA1/NA2/SH polymorphism reported in Chinese patients with anti-GBM disease, the majority of patients with RA and SLE from Europe and Asia, in Indian patients with GBS, and in Scandinavian patients with Sjögren's syndrome, where no association was revealed between this genetic variation and risk of disease development (Haldorsen et al. 2013; Li et al. 2009; Radstake et al. 2003; Manger et al. 2002). On the other hand, in Dutch and Norwegian patients with GBS, Spanish

patients with RA, in Turkish patients with Behçet's disease, or in Asian populations suffering from SLE, higher risk of disease development has been reported in those having the NA2 allele or NA2/NA2 genotype (Bournazos et al. 2009; Aksu et al. 2008; Morgan et al. 2006a). Some authors, however, have reported a decrease in the frequency of this genotype in Korean patients with SLE (Li et al. 2009). The *FCGR3B*-NA2 allele is linked to increased susceptibility to concomitant respiratory infections in some RA patients (Hughes et al. 2004). In contrast, increased frequency of the NA1 allele and NA1/NA1 genotype reported in patients with IPF plays a mitigating role in disease progression. A similar advantageous predisposition is reported in ITP patients having the *FCGR3B*-NA1 variant, whereas in patients with myasthenia gravis this variant seems associated with more severe disease course (Bournazos et al. 2010).

In the autoimmune disorders above outlined, the *FCGR2A*-H131R polymorphism affects FcɣRIIa affinity to IgG2, phagocytosis, and CIs clearance. The FcɣRIIa, with histidine (131H) in the second extracellular domain of the receptor protein, binds IgG2-CIs more effectively than the FcɣRIIa with arginine in this site (131R). Therefore, decreased presence of the 131H allele and 131HH genotype in Stages I and II of SA may lead to lower binding of IgG2 with bacteria or antigen in immune complexes, lower phagocytosis and clearance of CIs by monocytes/macrophages and neutrophils, and in consequence antigenemia and immunocomplexemia. As a result, an excessive (auto)immune, granulomatous reactions may arise in affected organs. Likewise, these reactions can be triggered by lower frequency of the 57Q allele of *FCGR2C* noted in our patients in Stages I and II of SA, since this allele shapes the expression of a full-length, functional FcɣRIIc receptor and effective CIs binding. In contrast, 57X allele produces a truncated, nonfunctional receptor protein. On the other hand, increased frequency of the *FCGR2C*-131H and *FCGR2C*-131HH variants in advanced Stages III and IV of SA may enhance FcγRIIa affinity to IgG2, its binding to bacteria or antigen, which accelerates CIs clearance. All these phenomena have been previously reported in SA patients (Dubaniewicz 2010; 2013; Dubaniewicz et al. 2012a; b; Bournazos et al. 2009; Kivity et al. 2009; Li et al. 2009).

After absorption of bacteria or antigen through FcγRIIa, their presence in phagocytes of SA patients is extended due to lower bactericidal activity of phagocytes, compared with tuberculosis patients. A dormant presence of *M. tuberculosis* in a phagocyte would subsequently cause heat shock protein secretion from both bacterial and host cells, which may cause extended antigenemia and immunocomplexemia with the subsequent (auto)antigen presentation to T and B lymphocytes in the context of HLA, their increased activation, proliferation, and formation of a sarcoid granuloma (Dubaniewicz et al. 2012a; Dubaniewicz 2010). Further, IgG2, through C1q binding, would initiate a classical pathway of complement activation, which greatly accelerates inflammatory reactions, present especially in patients with Stages III and IV of SA (Li et al. 2009). Increased occurrence of the 57Q allele and 57XQ genotype of *FCGR2C*, with the 57Q functional allele, in our patients with advanced Stages III and IV of SA, may also enhance phagocytosis of CIs by monocytes/macrophages and neutrophils and lead to excessive Hsp production, antigen presentation, and lymphocyte over-proliferation, causing granulomatous formation (Dubaniewicz et al. 2012b). Increased frequency of the NA1 allele of *FCGR3B*, with higher affinity to IgG1 and IgG3 bound in CIs, compared with the NA2 and SH alleles, could also enhance inflammatory processes accompanied by abundant neutrophil involvement (Bournazos et al. 2009; Li et al. 2009). Since the distribution of the NA1/NA2/SH variants of *FCGR3B* remained basically unchanged in the patients of the present study, the *FCGR2A* and *FCGR2C* polymorphisms found, and that of *FCGR3A* previously reported, may drive the excessive (auto) immune response in sarcoidosis (Typiak et al. 2014). This plausibility is supported by the finding that FcγRIIa, but not FcγRIIIb, induces L-selectin shedding of neutrophils to

enable their arrival at the site of infection (Kocher et al. 1997). Additionally, solely FγRIIa-mediated neutrophil interactions with CIs result in the formation of neutrophil extracellular traps in tissues, a proinflammatory process that is linked to autoimmunity (Chen et al. 2012).

Differences in the allele and genotype distribution concerning the *FCGR2A* and *FCGR2C* genes may suggest the presence of different pathomechanisms underlying less severe Stages I/II and more advanced, parenchymal Stages III/IV of sarcoidosis. Furthermore, variations in the distribution of polymorphic *FCGR2A*, *FCGR2C*, along with the previously reported *FCGR3A*, may explain the immunocomplexemia combined with the presence of Fcγ receptors on the surface of phagocytes, observed in patients with sarcoidosis. The genotyping analysis may help anticipate the clinical run of sarcoidosis, i.e., the transition from initial mild to later advanced phases, and thus may help personalize therapy.

Acknowledgements The study was funded by the Ministry of Science and Higher Education and the National Science Centre in Poland (grant number 5160/B/P01/2010/39). The funding source had no role in the study design, collection, analysis, and interpretation of data.

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

References

Aksu K, Kitapcioglu G, Keser G, Berdeli A, Karabulut G, Kobak S, Ozmen M, Inal V, Kabasakal Y, Oksel F, Kocanaogullari H, Doganavsargil E (2008) FcgammaRIIa, IIIa and IIIb gene polymorphisms in Behçet's disease: do they have any clinical implications? Clin Exp Rheumatol 26:S77–83

American Autoimmune Related Diseases Association (2015) List of autoimmune and autoimmune-related diseases. Available from: www.aarda.org/autoimmune-information/list-of-diseases. Accessed on 11 June 2015

American Thoracic Society, European Respiratory Society, World Association of Sarcoidosis and Other Granulomatous Disorders (1999) Statement on sarcoidosis. Am J Respir Crit Care Med 160:736–755

Asano K, Matsushita T, Umeno J, Hosono N, Takahashi A, Kawaguchi T, Matsumoto T et al (2009) Genome-wide association study identifies three new susceptibility loci for ulcerative colitis in the Japanese population. Nat Genet 41:1325–9

Baughman R, Culver D, Judson M (2011) A concise review of pulmonary sarcoidosis. Am J Respir Crit Care Med 183:573–581

Behnen M, Leschczyk C, Moller S, Batel T, Klinger M, Solbach W, Laskay T (2014) Immobilized immune complexes induce neutrophil extracellular trap release by human neutrophil granulocytes via FcγRIIIB and Mac-1. J Immunol 193:1954–65

Bournazos S, Bournazou I, Murchison JT, Wallace WA, McFarlane P, Hirani N, Simpson AJ, Dransfield I, Hart SP (2010) Fcγ receptor IIIb (CD16b) polymorphisms are associated with susceptibility to idiopathic pulmonary fibrosis. Lung 188:475–81

Bournazos S, Woof J, Hart S, Dransfield I (2009) Functional and clinical consequences of Fc receptor polymorphic and copy number variants. Clin Exp Immunol 157:244–54

Chen K, Nishi H, Travers R, Tsuboi N, Martinod K, Wagner DD, Stan R, Croce K, Mayadas TN (2012) Endocytosis of soluble immune complexes leads to their clearance by FcγRIIIB but induces neutrophil extracellular traps via FcγRIIA *in vivo*. Blood 120:4421–31

Dubaniewicz A (2010) Mycobacterium tuberculosis heat shock proteins and autoimmunityin sarcoidosis. Autoimmun Rev 9:419–24

Dubaniewicz A (2013) Microbial and human heat shock proteins as 'danger signals' in sarcoidosis. Hum Immunol 74:1550–8

Dubaniewicz A, Holownia A, Kalinowski L, Wybieralska M, Dobrucki IT, Singh M (2012a) Is mycobacterial heat shock protein 16 kDa, a marker of the dormant stage of Mycobacterium tuberculosis, a sarcoid antigen? Hum Immunol 74:45–51

Dubaniewicz A, Typiak M, Wybieralska M, Szadurska M, Nowakowski S, Staniewicz-Panasik A, Rogoza K, Sternau A, Deeg P, Trzonkowski P (2012b) Changed phagocytic activity and pattern of Fcγ and complement receptors on blood monocytes in sarcoidosis. Hum Immunol 73:788–4

Edberg JC, Langefeld CD, Wu J, Moser KL, Kaufman KM, Kelly J, Bansal V, Brown WM, Salmon JE, Rich SS, Harley JB, Kimberly RP (2002) Genetic linkage and association of Fcγ receptor IIIA (CD16A) on chromosome 1q23 with human systemic lupus erythematosus. Arthritis Rheum 46:2132–40

Haldorsen K, Appel S, Le Hellard S, Bruland O, Brun JG, Omdal R, Kristjansdottir G et al (2013) No association of primary Sjögren's syndrome with Fcγ receptor gene variants. Genes and Immunity 14:234–7

Hughes LB, Criswell LA, Beasley TM, Edberg JC, Kimberly RP, Moreland LW, Seldin MF, Bridges SL (2004) Genetic risk factors for infection in patients with early rheumatoid arthritis. Genes and Immunity 5:641–7

Ji Y, Zhang H, Lin S (2013) Single nucleotide polymorphism of *FCGR2A* gene in Han Chinese children with

Kawasaki disease. Chinese Journal of Contemporary Pediatrics 15:196–200

Kivity S, Agmon-Levin N, Blank M, Shoenfeld Y (2009) Infections and autoimmunity – friends or foes? Trends Immunol 30:409–14

Kocher M, Siegel M, Edberg J, Kimberly R (1997) Cross-linking of Fc gamma receptor IIa and Fc gamma receptor IIIb induces different proadhesive phenotypes on human neutrophils. J Immunol 159:3940–8

Lahiri D, Nurnberger J Jr (1991) A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19:5444

Li R, Peng H, Chen GM, Feng CC, Zhang YJ, Wen PF, Qiu LJ, Leng RX, Pan HF, Ye DQ (2014) Association of FCGR2A-R/H131 polymorphism with susceptibility to systemic lupus erythematosus among Asian population: a meta-analysis of 20 studies. Arch Dermatol Res 306:781–91

Li X, Ptacek T, Brown E, Edberg JC (2009) Fcɣ receptors: structure, function and role as genetic risk factors in SLE. Genes and Immunity 10:380–9

Maertzdorf J, Weiner J 3rd, Mollenkopf HJ, TBornot TB, Network, Bauer T, Prasse A, Muller-Quernheim J, Kaufmann SH (2012) Common patterns and disease-related signatures in tuberculosis and sarcoidosis. Proc Natl Acad Sci USA 109:7853–7858

Manger K, Repp R, Jansen M, Geisselbrecht M, Wassmuth R, Westerdaal NA, Pfahlberg A, Manger B, Kalden JR, van de Winkel JG (2002) Fcgamma receptor IIa, IIIa, and IIIb polymorphisms in German patients with systemic lupus erythematosus: association with clinical symptoms. Ann Rheum Dis 61:786–92

Morgan AW, Barrett JH, Griffiths B, Subramanian D, Robinson JI, Keyte VH, Ali M et al (2006a) Analysis of Fcγ receptor haplotypes in rheumatoid arthritis: *FCGR3A* remains a major susceptibility gene at this locus, with an additional contribution from *FCGR3B*. Arthritis Research & Therapy 8:R5

Morgan AW, Robinson JI, Barrett JH, Martin J, Walker A, Babbage SJ, Ollier WE, Gonzalez-Gay MA, Isaacs JD (2006b) Association of *FCGR2A* and *FCGR2A-FCGR3A* haplotypes with susceptibility to giant cell arteritis. Arthritis Research & Therapy 8: R109

Radstake T, Petit E, Pierlot C, van de Putte LB, Cornelis F, Barrera P (2003) Role of Fcgamma receptors IIA, IIIA, and IIIB in susceptibility to rheumatoid arthritis. J Rheumatol 30:926–33

Rodriguez S, Gaunt T, Day I (2009) Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. Am J Epidemiol 169:505–14

Sanchez E, Comeau ME, Freedman BI, Kelly JA, Kaufman KM, Langefeld CD, Brown EE et al (2011) Identification of novel genetic susceptibility loci in African American lupus patients in a candidate gene association study. Arthritis Rheum 63:3493–3501

Scadding JG (1961) Prognosis of intrathoracic sarcoidosis in England. Br Med J 2:1165–72

Schmekel B, Hakansson L, Hallgren R, Nou E, Stalenheim G, Venge P (1985) Neutrophil phagocytosis in sarcoidosis. Reduced C3b receptor-mediated phagocytosis in active and silent sarcoidosis. Clin Exp Immunol 60:191–5

Siriboonrit U, Tsuchiya N, Sirikong M, Kyogoku C, Bejrachandra S, Suthipinittharm P, Luangtrakool K et al (2003) Association of Fcγ receptor IIb and IIIb polymorphisms with susceptibility to systemic lupus erythematosus in Thais. Tissue Antigens 61:374–83

Spagnolo P, Grunewald J (2013) Recent advances in the genetics of sarcoidosis. J Med Genet 50:290–7

Su K, Wu J, Edberg JC, McKenzie SE, Kimberly RP (2002) Genomic organization of classical human low-affinity Fcγ receptor genes. Genes Immunity 3: S51–6

Tutor-Ureta P, Citores MJ, Castejon R, Mellor-Pita S, Yebra-Bango M, Romero Y, Vargas JA (2006) Prognostic value of neutrophils and NK cells in bronchoalveolar lavage of sarcoidosis. Cytometry Part B Clin Cytometry 70:416–22

Typiak M, Rabala K, Dudziak M, Dubaniewicz A (2014) Polymorphism of *FCGR3A* gene in sarcoidosis. Hum Immunol 75:283–8

Vigato-Ferreira IC, Toller-Kawahisa JE, Pancoto JA, Mendes-Junior CT, Martinez EZ, Donadi EA, Louzada-Júnior P, Del Lama JE, Marzocchi-Machado CM (2014) FcγRIIa and FcγRIIIb polymorphisms and associations with clinical manifestations in systemic lupus erythematosus patients. Autoimmunity 47:451–8

Wang D, Hu S, Cheng X, Yang JY (2014) FCGR2A rs1801274 polymorphism is associated with risk of childhood-onset idiopathic (immune) thrombocytopenic purpura: evidence from a meta-analysis. Thromb Res 134:1323–7

Weersma RK, Crusius JB, Roberts RL, Koeleman BP, Palomino-Morales R, Wolfkamp S, Hollis-Moffatt JE et al (2010) Association of FcgR2a, but not FcgR3a, with inflammatory bowel diseases across three Caucasian populations. Inflamm Bowel Dis 16:2080–9

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 69–78
DOI 10.1007/5584_2015_182

Published online: 09 January 2016

Chronic Cough as a Female Gender Issue

N. Kavalcikova-Bogdanova, T. Buday, J. Plevkova, and W.J. Song

Abstract

Cough accompanying acute respiratory tract disorders is a self-limiting phenomenon, and it usually does not require sophisticated management. Chronic cough, in contrast, is a bothersome problem, considerably influencing the quality of life of affected individuals. Specialized cough clinics report that substantial proportion of their patients are middle aged-to-postmenopausal females who cough for years in response to otherwise non-tussigenic stimuli, without a clear underlying disease reason. A newly established entity – 'cough hypersensitivity syndrome' explains pathogenesis of this problem. However, the syndrome has not been generally accepted, and the guidelines regarding the diagnostic protocols and treatment are not yet available. The reason why females cough more than males do is unclear, but the analysis of literature and experience with the chronic cough patients allows selecting three main targets of hormonal background which can contribute to the enhanced coughing in females. They are as follows: increased activity of transient receptor potential (TRP) channels expressed on vagal C-fibers mediating cough, laryngeal hypersensitivity and laryngeal dysfunction with paradoxical vocal cord movement, and mast cells which are known to express receptors for female sexual hormones and are frequently found in the bronchoalveolar lavage in chronic cough patients. In this review we analyze the potential contribution of the factors above outlined to excessive cough in female subjects.

N. Kavalcikova-Bogdanova and T. Buday
Jessenius Faculty of Medicine, Department of Pathophysiology, Comenius University, Mala Hora 4C, 036 01 Martin, Slovakia

J. Plevkova (✉)
Jessenius Faculty of Medicine, Simulation Education Center, Comenius University, Novomeskeho 7A, 036 01 Martin, Slovakia
e-mail: plevkova@jfmed.uniba.sk

W.J. Song
Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea

Keywords
Cough • Female gender • Laryngeal dysfunction • Mast cells • Respiratory tract • Transient receptor potential channels • Vagus nerve

1 Cough as Gender Problem?

Chronic cough is an important health issue leading to numerous consultations which in many cases do not provide satisfactory explanation of the underlying health problem, and thus cannot lead to appropriate treatment or improvement of quality of life. Epidemiologic data show that patients visiting specialized cough clinics worldwide are mainly female (Song et al. 2015). A new concept of cough hypersensitivity brought useful insight into the 'pathogenetic' classification of chronic cough patients, excluding the patients with known cases and focusing on those with unexplained increased airway sensitivity (Morice et al. 2015). A typical chronic cough patient is an otherwise healthy female in her 50s to 60s and chronic cough is her only problem. The patient has unremarkable chest X-ray and lung function tests and she has already visited, on average, 6–8 different specialists to find a solution for her condition. She has taken a lot of medications, with a partial or null effect. Coughing is provoked easily by a number of stimuli such as perfumes, cleaners, cosmetics, cold air, exercise, speech, or laughter. Patients' testimonies provide further insight into this problem documenting a seriously decreased quality of life, starting from family and social life and affecting psychological well-being (Escamilla and Roche 2014; Pavord and Chung 2008).

It has been suggested that the female preponderance to exaggerated cough reflex has developed as an evolutionary mechanism to protect airways against aspiration of the refluxate during pregnancies (Brooks 2011), which would be an advantageous mechanism. This hypothesis seems to have a logical background, because the incidence of the gastroesophageal reflux is increased during pregnancy (Ramya et al. 2014). However, there are no data on the objective changes in cough sensitivity in females with different parity. Another important insight into the issue has been introduced by pain physiologists. They argue that female preponderance to chronic cough is part of enhanced or overdeveloped visceral sensitivity compared with males, as a consequence of evolutionary selection process (Kvachadze et al. 2015).

2 Historical Notes on Gender Differences in Cough

The female preponderance for cough was identified for the first time in a study of angiotensin converting enzyme (ACE) inhibitor-induced cough, which affects approximately 5–20 % of patients treated with these drugs (Gibson 1989). Irrespective of ACE inhibitor-induced cough, Fujimura et al. (1992) have also found that chronic persistent non-productive cough resistant to bronchodilators is more frequent in females. In a study of cough sensitivity to tartaric acid by the same authors it has been shown that females are more sensitive than males (Fujimura et al. 1990). In yet another study, the same group of authors have focused on capsaicin-induced cough, which is dependent on C-fiber activation. The aim of the study was to observe how gender, age, and pulmonary function influence cough reflex sensitivity. The results show that capsaicin cough threshold was significantly lower in females than males, in both young and middle-aged subjects. Cough threshold was weakly but significantly correlated with height, weight, forced volume capacity (FVC) and forced expired volume in 1 s (FEV1) in the entire population of subjects. Moreover, multiple regression analysis revealed that gender difference was the single

most important predicting factor for cough threshold (Fujimura et al. 1998).

Choudry and Fuller (1992), on the other hand, have failed to show gender-related differences in capsaicin-induced cough using single breath inhalation of increasing concentrations of capsaicin. This conflicting result has been explained by the authors as a consequence of a different methodological approach as they used single breath inhalation as opposed to Fujimura et al. (1998) who used 15 s tidal breathing inhalation. The most convincing evidence on the gender differences in capsaicin cough sensitivity has been published by Dicpinigaitis and Rauf (1998). The difference is believed to be of hormonal origin and this hypothesis has been strongly supported by Varechova et al. (2008), who have shown that a greater female sensitivity to cough appears already at puberty. However, if gender difference in cough sensitivity is a consequence of female sexual hormones, one would expect it to decrease after menopause, which is at odds with the well-defined population of postmenopausal females with increased capsaicin cough sensitivity or 'cough hypersensitivity syndrome'. Increased cough sensitivity after menopause remains to be elucidated. Gender differences in cough sensitivity are confirmed in a population of chronic cough patients as well (Kastelik et al. 2002).

3 Maturation of Cough Reflex

Cough as a defensive reflex is not present at birth because it requires extremely high flow rates to clean the airways, which is hardly achievable in liquid filled lungs in newborns. In contrast, newborns are equipped with pharyngeal and laryngeal protective mechanisms against potential hazards. For example, low chloride solutions such as water, gastric fluid, or saliva have a potential to provoke laryngeal chemoreflex as one of the reflex tools of the airway protection. During the neonatal period, cough is usually associated with disease states such as tuberculosis, pertussis and chlamydia pneumonia infections, or aspiration of a foreign body (Boileau and Dschildre 2010). A study performed in sleeping human infants has shown that instillation of 0.1 ml bolus of water through the nasal catheter induces swallows in 55 %, obstructed breaths in 40 %, apnea in 40 %, arousal in 18 %, and cough in just 1–2 % of children. With maturation of a newborn, apnea and swallowing components of laryngeal chemoreflex decrease, while cough becomes increasingly prominent (Thach 2007). These findings are compatible with the maturation of airway defenses in animal species (Korpas and Tomori 1979).

Cough develops quickly as a substantial airway defensive reflex and its presence as the most prominent symptom of airway diseases is of importance during entire childhood. The process of maturation of the cough reflex is very sensitive to neuroplastic changes, and it is believed that different factors during the maturation process may influence the definite status outcome of cough reflex physiology and regulation. Neural pathways responsible for the regulation of cough may undergo changes in structure and function in response to exposure to cigarette smoke, inflammation, and allergens (Undem et al. 2002; Li et al. 1999). Maturation process is also influenced by other factors such as exposure to air pollutants, airway inflammatory diseases, and endogenous factors related to the gender and puberty. Capsaicin cough sensitivity is similar in pre-pubertal and early pubertal boys and girls. The gender-related difference of capsaicin cough sensitivity between girls and boys appears not until late pubertal age (Varechova et al. 2008). Puberty and gender have a significant influence on the cough threshold to capsaicin. Investigations in children show that girls reveal a greater extent of cough plasticity. Their susceptibility to modulation of cough reflex sensitivity to capsaicin is higher, compared with boys of the same pubertal development. Cough reflex in girls responds significantly more than that in boys to the exposure to air pollution and lower respiratory tract infection. These findings could be taken as indirect indicators of increased cough plasticity in girls.

4 Hypersensitivity of Cough Neural Pathways in Females

As mentioned above, a typical 'coughing' female patient coughs in response to stimuli which are not tussigenic to others. This means that airway afferents mediating cough are hypersensitive and respond with the action potential discharge to subthreshold stimuli. Hypersensitivity, which can be easily tested in clinical conditions by a capsaicin single breath test, is a hall mark of excessive coughing.

Cough reflex is a unique vagal phenomenon, with the polysynaptic pathway comprising the airway afferent nerves, central nervous system network, efferent pathways, and the effectors, which produce cough motor pattern in response to the activation of 'cough sensors'. Gender dimorphism has been identified at the level of airway afferents and also of central processing of airway afferent information leading to cough (Morice et al. 2014; Dicpinigaitis and Rauf 1998).

Afferent fibers innervating the airways belong to the vagus nerve. However, there are recent data suggesting that airways are also innervated by fibers derived from the dorsal root ganglia. They are the most studied parts of the cough reflex arch. Vagal neurons innervating the airways are located in the vagal nodose and jugular ganglia, while their terminals are broadly distributed in the mucosa of larynx, trachea, and bronchi; the tussigenic regions (Canning 2006). There are two types of vagal afferents depending on how they respond to different stimuli. The sensation of a mechanical stimulus is mediated mainly *via* low-threshold mechanosensors of Aδ-type, which are also sensitive to acid, but not to other irritants, e.g., capsaicin. Activation of these fibers provokes cough also under anaesthesia. The main role of Aδ-driven cough is to prevent the aspiration of foreign bodies (mechanosensitivity) or refluxate (sensitivity to low pH).

Gender differences are mainly related to the airways C-fibers; polymodal non-myelinated endings capable of detecting chemical substances and temperature changes. The endings, with chemoreceptor-like properties, are broadly distributed all over airways and lungs. They are not activated during a regular breathing cycle, but rather by different chemical stimuli such as inflammatory mediators, oxidizing substances, or air-born irritants and their activation leads to release of neuropeptides (Mazzone 2005). The ability to sense chemical substances is determined by the expression of different ion channels activated by temperature and endogenous and exogenous ligands, e.g., transient receptor potential (TRP) V1/A1 channels. C-fiber-mediated cough is believed to be part of adaptive responses to pathological conditions such as airway diseases or exposure to pollution. Nonetheless, the role of C-fibers in cough remains controversial and needs to be further clarified. Chemical activators of airway chemosensors such as bradykinin, citric acid, and capsaicin are also known as the most potent tussive agents in humans and animal models. That is the case, however, only in the state of wakefulness (Karlsson 1996), which has led to the assumption that cough induced by chemical agents depends on cortical activity. Cough induced by chemical substances would not be 'strictly' of reflex origin, but it would have a voluntary component related to conscious perception of airway irritation. That notion is also in accord with the urge-to-cough phenomenon, a specific sensation present in man, having to do with the activation of jugular C-fibers by stimuli that do not reach the threshold for induction of cough motor pattern. That is a complex cortical interpretation of airway nociceptive signaling which precedes the motor response to cough (Davenport 2008).

According to the guidelines of the European Respiratory Society (ERS), cough sensitivity should be evaluated in clinical settings by a capsaicin single breath test, which is believed to produce coughing by activation of vagal C-fibers, usually with the urge-to-cough preceding the onset of cough motor pattern (Morice et al. 2007). These fibers are activated by capsaicin *via* TRPV1 channel. The role of TRP channels in cough has been extensively studied, and, outside of TRPV1, also TRPA1 channels are known to play a role in activation of cough

related fibers by natural ligands, air-born irritants, and endogenous signaling molecules produced in inflammation and oxidation stress (Grace et al. 2014). Patients with chronic cough have an increased expression of TRPV1 receptors in their airways (Mitchell et al. 2005).

TRPV1 expressing vagal fibers also appear to play a role in the cough gender dimorphism. The influence of gonadal sex hormones on ion channels has long been recognized in human physiology. It is known that females are predominantly affected by functional disorders involving ion channels. That may be exemplified by their being more prone to drug induced arrhythmias; female gender in itself is an independent risk factor for sudden cardiac death (Patberg et al. 2012). Evidence supporting female preponderance for hypersensitivity to cough is that estrogen influences TRPV1 activation/sensitization, leading to a gain in channel excitability (Patberg 2011). It has been documented that activation of TRPV1 channels in pelvic nerves depends on the estrus cycle. Activation of C-fibers by capsaicin is significantly greater in pro-estrus state (high estradiol, low progesterone) than in the metestrus phase (low estradiol, high progesterone) favoring estradiol over progesterone as a hormonal mediator for TRPV1 (Peng et al. 2008). Also, more than 75 % of C-fiber neuronal bodies expressing TRPV1 co-express estrogen receptor α, suggesting a regulatory role for estrogen. TRPV1 channel expressing fibers in ovarectomized rats are activated only in the case of hormonal substitution therapy (Yan et al. 2007).

It is hardly feasible to extrapolate data obtained on sensory pelvic nerves to the function of vagus nerves. Therefore, it is noteworthy that one of our studies that investigated capsaicin cough sensitivity in healthy young females with physiological menstrual cycles and those on hormonal contraceptive pills. We found that cough sensitivity was higher in the luteal (not follicular) phase of the menstrual cycle, and from all hormonal indices tested (follicular stimulating hormone, luteinizing hormone, estrogen, progesterone, and testosterone) the strongest correlation was present between cough sensitivity and estrogen blood level. The contraceptive group failed to show cyclic changes. These data cannot be extrapolated to subjects with chronic cough or the population of postmenopausal females, but they clearly show that TRPV1 expressing airway sensors are under the hormonal influence (Kavalcikova-Bogdanova 2015).

There is also an interesting study that recorded functional brain activity in response to capsaicin inhalation in healthy males and females to assess possible differences in the neural processing in coughing. The authors showed that capsaicin inhalation activates a neural network in the human brain in response to an urge-to-cough. Females were more sensitive to capsaicin challenge and despite a lower concentration of the stimulus, the magnitude of somatosensory cortex activation was approximately twice as high as that in males. The region of the somatosensory cortex displaying the sex-related differences is known to receive airway sensory inputs and is activated in close correlation with an individual's perceived urge-to-cough intensity. A larger somatosensory response in healthy females, despite a lower provoking stimulus, suggests a possible explanation for the observed sex-related distribution of patients presenting at cough clinics (Morice et al. 2014).

5 Vocal Cord Dysfunction and Laryngeal Hypersensitivity

Laryngeal hypersensitivity and vocal cord dysfunction are factors that contribute to a higher prevalence of chronic cough in females. Female gender seems to be more frequently affected by vocal cord dysfunction, with a reported female-to-male ratio of 2–3: 1 (Campainha et al. 2012).

Laryngeal problems are increasingly recognized as being part of the chronic cough syndrome, and besides coughing these subjects manifest specific symptoms, such as dyspnea, hyperresponsiveness of extrathoracic airways with enhanced glottal stop reflex, reduced inspiratory airflow following a provocative challenge, and a paradoxical vocal cord movement (PVCM). These symptoms are part of vocal cord dysfunction. Paroxysmal periods of vocal

cords adduction during inspiration/expiration, restricting the airway opening, lead to episodic dyspnea, wheezing, or stridor and cough, which may easily be mistaken with asthma. PVCM may be precipitated by exercise or stress, but it also occurs spontaneously (Morris et al. 2006; Brugman 2003). The most common reasons leading to PVCM are psychogenic stress, proximal gastroesophageal reflux, inhalation of irritants, and conditions associated with asthma. Both vocal cord dysfunction and chronic cough are often accompanied by the gastroesophageal reflux or exposure to irritants. It seems, therefore, likely that both are underlain by hypersensitive airways manifesting as either in response to triggers. A link between laryngeal hypersensitivity and chronic cough is confirmed by the finding in PVCM subjects in randomized trials that speech therapy, ameliorating laryngeal dysfunction, also is effective in patients with chronic cough (Gibson and Vertigan 2009; Ryan et al. 2009). A specific type of therapy for chronic cough, consisting of the education on using cough suppressant strategies, vocal hygiene, and of the psychological counseling has been developed and validated. The therapy significantly reduces cough sensitivity and cough frequency and improves clinical outcomes concerning cough and laryngeal symptoms in patients with chronic cough.

Vocal cord dysfunction is a recognized disease entity by speech therapy professionals, and it usually worsens in the late phase of menstrual cycle, giving rise to the so-called voice premenstrual syndrome. The condition is of particular concern to voice performers, such as singers, teachers, or media anchors. These subjects report hoarseness and voice fatigue, which has to do with low estrogen and high progesterone levels in the late menstrual phase. The analysis of variations in respiratory symptoms, such as shortness of breath, wheezing, and cough has shown that coughing was enhanced just after putative ovulation in subjects with asthma, with body mass index greater than 23 kg/m^2, and in smokers, as well as just before the expected onset of menses. An increased prevalence of laryngeal hypersensitivity and PVCM in females, along with the time correlation between the menstrual cycle and the symptoms outlined above, points to the possibility of an appreciable laryngeal contribution to the pathogenesis of chronic cough (Macsali et al. 2013).

Early studies that explored the laryngeal contribution to cough were based merely on subjective impressions of voice quality. Recent studies use more objective methods including histologic examinations, stroboscope, electroglottography, and computerized acoustic analyses. In these studies, the larynx has been shown to be a hormonal target organ and as such, its function may be affected by sexual hormones (Amir and Biron-Shental 2004). Estrogen sensitive cells are present in the larynx and alterations of estrogen/progesterone balance cause laryngeal water retention, edema of interstitial tissues, and venous dilatation. Edema is not considered a direct trigger of cough *via* C-fibers or Aδ-fibers, but the rapidly adapting receptors (RARs) in airways, located in apposition to the vasculature, are highly sensitive to changes in the pulmonary extra-vascular space produced, e.g., by mild elevations of left atrial pressure. Activation of RARs ensues respiratory stimulation, an increase in tracheal tone, and cough. Also a reflex diuresis is evoked in response to stimulation of these receptors by pulmonary lymphatic obstruction. It is proposed that RARs function as a sensory component of the pulmonary defense mechanism (Ravi and Kappagoda 2002), which preserves the 'milieu interior' (De Corso et al. 2013). There are also documented cases of stridor and cough provoked by edema of arytenoid mucosa, which sheds new light on the possible mechanisms of chronic refractory cough.

6 Role of Mast Cells

Airway biopsies taken from the non-asthmatic phenotype of chronic cough patients show that their submucosal features of inflammation are distinctly different from those in the asthmatic type of chronic cough. Non-asthmatic cough is

characterized by the increased number of submucosal mast cells, whereas in asthmatic cough, as expected, there is a significant increase in submucosal eosinophils and neutrophils (Niimi et al. 2005). The increase in mast cells in the submucosa of chronic cough patients raises the possibility that these cells may be important in the pathogenesis of chronic cough. These data are in accord with the increase in mast cells and histamine in bronchoalveolar lavage fluid of coughers without asthma (McGarwey et al. 2000). In chronic cough, there are increased levels of mediators with protussive effects, in particular in the mast cell-derived histamine and prostaglandin D, suggesting a role for mast cell activation in chronic cough. In addition, mast cells in bronchoalveolar lavage of patients with chronic cough are hyperresponsive to the neuropeptides neurokinin A and calcitonin gene-related peptide (Gibson 2004). The neuropeptides induce significantly more histamine release from mast cells in chronic cough and this effect is seen in chronic cough associated with asthma as well as cough associated with postnasal drip syndrome, gastroesophageal reflux, and idiopathic chronic cough (Birring et al. 2003). These findings demonstrate that activation of airway mast cells is a feature of all types of chronic cough, even those associated with extrapulmonary diseases.

Female sex hormones have long been suspected to have an effect on mast cells function. This assumption is based on the expression of hormone receptors in mast cells and on the fact that many mast cell-related pathophysiological alterations have a different prevalence in females and males. A much higher asthma prevalence is present in women at reproductive age compared with men. Serum levels of estradiol and progesterone directly correlate with the clinical and functional features of asthma. Approximately 30–40 % of women who have asthma experience a worsening of their symptoms during the perimenstrual phase, the so-called perimenstural asthma, and in some of chronic cough patients in reproductive age we also see the 'perimenstrual' worsening of cough.

A key characteristic of mast cells appears to be the ability to span the division between neural and immune systems with the cells exhibiting variably functional aspects of both systems. Mast cells can be activated by a range of neurotransmitters and, reciprocally, a variety of molecules including histamine and serotonin that are synthesized and released by mast cells, can influence neuronal activity, while mast cell-derived cytokines, including tumor necrosis factor (TNF) and neural growth factors (NGF), lower the threshold for activation of local neurons and promote nerve fiber growth (Forsythe and Bienenstock 2012).

The effect of mast cells on cough is mediated *via* neuroimmune interactions in the tissue (Moon et al. 2010). There is a strong bidirectional relationship between mast cells and 'cough-related' airway afferents, non-myelinated C-fiber nerve endings play a major role in. This interaction and its effect in chronic cough hypersensitivity syndrome has recently been reviewed (Song and Chang 2015). There is anatomical evidence for mast cell associations with peripheral myelinated and unmyelinated nerves. Close apposition of mast cells and neurons containing substance P, calcitonin gene-related peptide (CGRP), or both has been described in the rat and human gastrointestinal tract, the rat trachea and peripheral lung, the urinary bladder and several other tissues. These interactions underlie the classical inflammatory axon reflex, where antigen or noxious stimuli causes stimulation of sensory C-fibers, which, in turn, through collateral axons, provides an efferent route for the lateral spread of inflammatory signals. The neuropeptides neurokinin A and CGRP co-localize in the unmyelinated C-fibers of airway sensory nerves. In the airway epithelium, these nerves participate in the afferent limb of the cough reflex and are believed to be fundamental to sensory hyperresponsiveness that characterizes chronic cough. In the chronic cough accompanying eosinophilic bronchitis, mast cells also accumulate in the airway epithelium where C-fibers end. This raises the possibility of a link between mast cells and sensory nerves in the airway epithelium in chronic cough.

Apart from a variety of stimuli of immunogenic and non-immunogenic character that can activate mast cells, the female hormones

estradiol and progesterone activate them as well (Zierau et al. 2012). The expression of estradiol and progesterone receptors on mast cells has been confirmed in the mouse, rat, and man (Jensen-Jarolim and Untersmayr 2008). From the functional standpoint, it is worth noting that tamoxifen, a tissue specific estrogen receptor antagonist, blocks degranulation of mast cells (Zaitsu et al. 2007). An interesting insight into mast cell activation by environmental estrogen-like molecules has been recently published. The molecules, called xenoestrogens, are present as environmental pollutants, mainly in water and food. They are able not only to activate mast cells, but also enhance degranulation upon allergen cross-linking of IgE, which may explain a global rise in allergic diseases of late (Narita et al. 2007).

7 Conclusions

Epidemiologic data clearly confirm that hypersensitive cough is more frequent in females. Intrinsic factors, predominantly a generally increased female viscerosensitivity, along with hypersensitivity of airway afferents and relevant parts of somatosensory cortex, seems to form the background of a higher prevalence of chronic cough in females than men. Hormonal influences can further contribute to this difference *via* the modulation of nerve excitability, laryngeal hypersensitivity, and mast cell function. These factors combined likely contribute to a significant gender difference in the proportion of chronic cough patients, with a preponderance of females.

Acknowledgements Supported by VEGA No. 1/0107/2014 and Biomed ITMS: 26220220187

Conflicts of Interest Authors declare no conflicts of interest in relation to this study.

References

Amir O, Biron-Shental T (2004) The impact of hormonal fluctuation on female vocal folds. Curr Opin Otolaryngol Head Neck Surg 3:180–184

Birring SS, Berry M, Brightling CE, Pavord ID (2003) Eosinophilic bronchitis: clinical features, management and pathogenesis. Am J Respir Med 2:169–173

Boileau S, Deschildre A (2010) Acute respiratory distress in the newborn and the child. Foreign bodies of the upper respiratory tract. Rev Prat 60:715–720

Brooks SM (2011) Perspective on the human cough reflex. Cough 7:10. doi:10.1186/1745-9974-7-10

Brugman S (2003) The many faces of vocal cord dysfunction. What 36 years of literature tell us. Am J Respir Crit Care Med 167A588

Campainha S, Ribeiro C, Guimarãe M, Lima R (2012) Case report vocal cord dysfunction: a frequently forgotten entity. Case Rep Pulmonol 2012:525493

Canning BJ (2006) Anatomy and neurophysiology of the cough reflex: ACCP evidence-based clinical practice guidelines. Chest 129:33–47

Choudry NB, Fuller RW (1992) Sensitivity of the cough reflex in patients with chronic cough. Eur Respir J 5:296–300

Davenport PW (2008) Urge-to-cough: what can it teach us about cough? Lung 186:S107–S111

De Corso E, Pandolfini M, Battista M, Della Marca G, Scarano E (2013) Management of a rare case of arytenoid mucosa oedema inducing stridor and cough. Int J Pediatr Otorhinolaryngol 77:1593–1595

Dicpinigaitis PV, Rauf K (1998) The influence of gender on cough reflex sensitivity. Chest 113:1319–1321

Escamilla R, Roche N (2014) Cough hypersensitivity syndrome: towards a new approach to chronic cough. Eur Respir J 44:1103–1106

Forsythe P, Bienenstock J (2012) The mast cell-nerve functional unit: key component of physiologic and pathophysiologic responses. Chem Immunol Allergy 98:196–221

Fujimura M, Sakamoto S, Kamio Y, Matsuda T (1990) Sex difference in the inhaled tartaric acid cough threshold in non-atopic healthy subjects. Thorax 45:633–634

Fujimura M, Sakamoto S, Matsuda T (1992) Bronchodilator-resistive cough in atopic patients: bronchial reversibility and hyperresponsiveness. Intern Med 31:447–452

Fujimura M, Kasahara K, Yasui M, Myou S, Ishiura Y, Kamio Y, Hashimoto T, Matsuda T (1998) Atopy in cough sensitivity to capsaicin and bronchial responsiveness in young females. Eur Respir J 11:1060–1063

Gibson GR (1989) Enalapril-induced cough. Arch Intern Med 149:2701–2703

Gibson PG (2004) Cough is an airway itch? Am J Respir Crit Care Med 169:1–9

Gibson PG, Vertigan AE (2009) Speech pathology for chronic cough: a new approach. Pulm Pharmacol Ther 22:159–162

Grace MS, Baxter M, Dubuis E, Birrell MA, Belvisi MG (2014) Transient receptor potential (TRP) channels in the airway: role in airway disease. Br J Pharmacol 171:2593–2607

Jensen-Jarolim E, Untersmayr E (2008) Gender-medicine aspects in allergology. Allergy 63:610–615

Karlsson JA (1996) The role of capsaicin-sensitive C-fibers afferent nerves in the cough reflex. Pulm Pharmacol 9:315–321

Kastelik JA, Thompson RH, Aziz I, Ojoo JC, Redington AE, Morice AH (2002) Sex-related differences in cough reflex sensitivity in patients with chronic cough. Am J Respir Crit Care Med 166:961–964

Kavalcikova-Bogdanova N (2015) The effect of female hormonal profile on selected cough parameters. In: Fifth American cough conference, Washington, DC, 5–6 June 2015

Korpas J, Tomori Z (1979) Cough and other respiratory reflexes. S Karger, Basel, pp 56–70

Kvachadze I, Tsagareli MG, Dumbadze Z (2015) An overview of ethnic and gender differences in pain sensation. Georgian Med News 238:102–108

Li JS, Peat JK, Xuan W, Berry G (1999) Meta-analysis on the association between environmental tobacco smoke (ETS) exposure and the prevalence of lower respiratory tract infection in early childhood. Pediatr Pulmonol 27:5–13

Macsali F, Svanes C, Sothern RB, Benediktsdottir B, Bjørge L, Dratva J, Franklin KA, Holm M, Janson C, Johannessen A, Lindberg E, Omenaas ER, Schlünssen V, Zemp E, Real FG (2013) Menstrual cycle and respiratory symptoms in a general Nordic-Baltic population. Am J Respir Crit Care Med 187:366–373

Mazzone SB (2005) An overview of the sensory receptors regulating cough. Cough 1:2

McGarvey L, Heaney L, MacMahon J, Ennis M (2000) Eosinophilic bronchitis is an important cause of chronic cough. Am J Respir Crit Care Med 161:1763–1764

Mitchell JE, Campbell AP, New NE, Sadofsky LR, Kastelik JA, Mulrennan SA, Compton SJ, Morice AH (2005) Expression and characterization of the intracellular vanilloid receptor (TRPV1) in bronchi from patients with chronic cough. Exp Lung Res 31:295–306

Moon TC, St Laurent CD, Morris KE, Marcet C, Yoshimura T, Sekar Y, Befus AD (2010) Advances in mast cell biology: new understanding of heterogeneity and function. Mucosal Immunol 3:111–128

Morice AH, Fontana GA, Belvisi MG, Birring SS, Chung KF, Dicpinigaitis PV, Kastelik JA, McGarvey LP, Smith JA, Tatar M, Widdicombe J, ERS TASK FORCE (2007) ERS guidelines on the assessment of cough. Eur Respir J 29:1256–1276

Morice AH, Jakes AD, Faruqi S, Birring SS, McGarvey L, Canning B, Smith JA, Parker SM, Chung KF, Lai K, Pavord ID, van den Berg J, Song WJ, Millqvist E, Farrell MJ, Mazzone SB, Dicpinigaitis P, Chronic Cough Registry (2014) A worldwide survey of chronic cough: a manifestation of enhanced somatosensory response. Eur Respir J 44:1149–1155

Morice AH, Millqvist E, Belvisi MG, Bieksiene K, Birring SS, Chung KF, Dal Negro RW, Dicpinigaitis P, Kantar A, McGarvey LP, Pacheco A, Sakalauskas R, Smith JA (2015) Cough hypersensitivity syndrome: clinical measurement is the key to progress. Eur Respir J 45:1509–1510

Morris MJ, Allan PF, Perkins PJ (2006) Vocal cord dysfunction: etiologies and treatment. Clin Pulm Med 13:73–86

Narita S, Goldblum RM, Watson CS, Brooks EG, Estes DM, Curran EM, Midoro-Horiuti T (2007) Environmental estrogens induce mast cell degranulation and enhance IgE-mediated release of allergic mediators. Environ Health Perspect 115:48–52

Niimi A, Torrego A, Nicholson AG, Cosio BG, Oates TB, Chung KF (2005) Nature of airway inflammation and remodeling in chronic cough. J Allergy Clin Immunol 116:565–570

Patberg KW (2011) The female preponderance to cough hypersensitivity syndrome: another clue pointing to the role of TRPV1 in cough. Lung 189:257–258

Patberg KW, de Groot JR, Blaauw Y (2012) Camphor, an old cough remedy with a new mechanism. Am J Respir Crit Care Med 185:343. doi:10.1164/ajrccm.185.3.343

Pavord ID, Chung KF (2008) Management of chronic cough. Lancet 371:1375–1384

Peng HY, Huang PC, Liao JM, Tung KC, Lee SD, Cheng CL, Shyu JC, Lai CY, Chen GD, Lin TB (2008) Estrous cycle variation of TRPV1-mediated cross-organ sensitization between uterus and NMDA-dependent pelvic-urethra reflex activity. Am J Physiol Endocrinol Metab 295:E559–E568

Ramya RS, Jayanthi N, Alexander PC, Vijaya S, Jayanthi V (2014) Gastroesophageal reflux disease in pregnancy: a longitudinal study. Trop Gastroenterol 35:168–172

Ravi K, Kappagoda CT (2002) Airway rapidly adapting receptors-sensors of pulmonary extra-vascular fluid volume. Indian J Physiol Pharmacol 46:264–278

Ryan NM, Vertigan AE, Gibson PG (2009) Chronic cough and laryngeal dysfunction improve with specific treatment of cough and paradoxical vocal fold movement. Cough 5:4. doi:10.1186/1745-9974-5-4

Song WJ, Chang YS (2015) Cough hypersensitivity as a neuro-immune interaction. Clin Translat Allergy 5:24. doi:10.1186/s13601-015-0069-4

Song WJ, Chang YS, Faruqi S, Kim JY, Kang MG, Kim S, Jo EJ, Kim MH, Plevkova J, Park HW, Cho SH, Morice AH (2015) The global epidemiology of chronic cough in adults: a systematic review and meta-analysis. Eur Respir J 45:1479–1481

Thach BT (2007) Maturation of cough and other reflexes that protect the fetal and neonatal airway. Pulm Pharmacol Ther 20:365–370

Undem BJ, Carr MJ, Kollarik M (2002) Physiology and plasticity of putative cough fibers in the guinea pig. Pulm Pharmacol Ther 15:193–198

Varechova S, Plevkova J, Hanacek J, Tatar M (2008) Role of gender and pubertal stage on cough sensitivity in childhood and adolescence. J Physiol Pharmacol 59:719–726

Yan T, Liu B, Du D, Eisenach JC, Tong C (2007) Estrogen amplifies pain responses to uterine cervical distension in rats by altering transient receptor potential-1 function. Anesth Analg 104:1246–1250

Zaitsu M, Narita SI, Lambert KC, Grady JJ, Estes DM, Curran EM, Brooks EG, Watson CS, Goldblum RM, Midoro-Horiuti T (2007) Estradiol activates mast cells via a non-genomic estrogen receptor-α and calcium influx. Mol Immunol 44:1977–1985

Zierau O, Zenclussen AC, Jensen F (2012) Role of female sex hormones, estradiol and progesterone, in mast cell behavior. Front Immunol 3:169. doi:10.3389/fimmu.2012.00169

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 79–86
DOI 10.1007/5584_2015_183

Published online: 09 January 2016

Treatment Options for Central Sleep Apnea: Comparison of Ventilator, Oxygen, and Drug Therapies

Josef Yayan and Kurt Rasche

Abstract

Central sleep apnea (CSA) is a sleep-related disorder characterized by pauses in breathing during sleep when the brain respiratory network momentarily interrupts transmission of impulses to the respiratory musculature. CSA presents significant problems being an independent risk factor for cardiovascular events and death. There are several available treatment options according to CSA severity. Currently, adaptive servo-ventilation is considered best for CSA patients. The goal of the present study was to retrospectively investigate different treatment methods employed for CSA, such as different modes of ventilation, oxygen therapy, and drugs to determine the most effective one. Data were obtained from hospital records during 2010–2015. The diagnosis of CSA and the optimal treatment method were supported by polysomnography examinations. Devices used during sleep to support breathing included continuous positive airway pressure, bi-level positive airway pressure, or adaptive servo-ventilation. We classified 71 (2.9 %) patients as having CSA from 2,463 patients with sleep-disordered breathing. Of those 71 patients, 54 (76.1 %, 95 % CI 66.2–86.0 %) were male and 17 (23.9 %, 95 % CI 14.0–33.8 %) were female, and they had a mean age of 67.1 ± 14.1. Four (5.6 %) patients underwent a combination therapy, 39 (54.9 %) received a ventilator in proper ventilation mode, 25 (35.2 %) received oxygen therapy, 7 (9.9 %) received medication, and 4 (5.6 %) received no treatment. We conclude that although the majority of patients needed treatment for central sleep apnea, a clear advantage in using ventilators when compared to oxygen therapy or drug therapy could not be found.

J. Yayan (✉) and K. Rasche
Department of Internal Medicine, Division of Pulmonary, Allergy, and Sleep Medicine, HELIOS Clinic Wuppertal, Witten/Herdecke University, Heusnerstr. 40, 42283 Wuppertal, Germany
e-mail: josef.yayan@hotmail.com

Keywords
Central sleep apnea • Continuous positive airway pressure • Bi-level positive airway pressure • Adaptive servo-ventilation • Oxygen therapy • Drugs

1 Introduction

Central sleep apnea (CSA) is a sleep disorder characterized by the occurrence of apneas of more than 10 s during sleep as a result of reduced or absent stimulation from the respiratory center in the brain. There are different clinical presentations of CSA, and symptoms may include daytime sleepiness, repeated awakenings, and insomnia (Muza 2015).

The gold standard in the treatment of sleep disorders is continuous positive airway pressure (CPAP). The different forms of treatment for central breathing disorders include CPAP, bi-level positive airway pressure (BPAP), and adaptive servo-ventilation (ASV). ASV represents the most modern method of treatment for Cheyne-Stokes respiration and ventilation support, with variable airflow through the respiratory tract that changes depending on synchronization with the respiratory rate of the patient. ASV has the most favorable impact on the long-term prognosis of sleep disorders (Kazimierczak et al. 2013).

Oxygen therapy is an alternative therapy for CSA (Momomura 2012). In cases where CPAP fails, drug therapy is also recommended for the treatment of CSA according to the International Classification of Sleep Disorders, Third Edition (ICSD-3) (AASM 2014). Medicinal treatment with acetazolamide and theophylline has restricted supporting evidence but may be considered for the treatment of CSA related to congestive heart failure if standard medical therapy has not been effective. Zolpidem or triazolam may be prescribed for the management of CSA after the exclusion of causal risk factors for respiratory depression (ICSD-3) (AASM 2014). However, clear, evidence-based recommendations for the treatment of CSA have not been published in the most recent issue, and the suggestions given as to the most effective treatment options of CSA were relative.

The aim of present study was to seek the best method of treatment for CSA by comparing the influence on the apnea-hypopnea index (AHI) of different ventilation modes, oxygen therapy, and drug therapy. Starting the optimal form of treatment as early as possible can enhance effectiveness of therapy and reduce patients' suffering.

2 Material and Methods

2.1 Setting and Patients

Patients' data were anonymized prior to analysis. The Ethics Committee of the University of Witten-Herdecke in Germany approved the study. The requirement for written, informed consent of the patients was waived by the Ethics Committee because of the retrospective nature of the study.

The study examined optimal treatment methods in patients with CSA using records gathered at the Department of Pneumology, HELIOS Clinic, University of Witten/Herdecke, in Wuppertal, Germany over the period 1 January 2010 – 20 July 2015. The Clinic is the largest university hospital in the Bergisch Land, which is a low mountain range region within the state of North Rhine-Westphalia in Germany and has 967 beds and 26 departments. The hospital treats approximately 550 inpatients in the sleep lab each year. The records concerned patients with CSA according to the International Classification of Diseases (ICD) G47.30 (WHO 2015), examined in the sleep lab.

Three study groups were formed relating to the severity of CSA according to the AHI. The

patients were over 18 years old, but their age varied. The presumption of CSA arose from the patients' history and information from their partners, and the diagnosis of CSA was made principally by a pronounced daytime sleepiness and a number of other symptoms and secondary diseases.

2.2 Cumulative Impact Case Study

The case definition included those with clinical symptoms of CSA, such as excessive daytime sleepiness, snoring, episodes of breathing cessation during sleep witnessed by another person, abrupt awakenings accompanied by shortness of breath, awakening with a dry mouth or sore throat, morning headache, difficulty staying asleep, or concentration problems, who tested positive for CSA by polysomnography. The selection of cases was made independently using hospital chart data; individual matching was not undertaken.

CSA was defined by a tendency to experience apnea during sleep due to insufficient activity of the respiratory center, weak respiratory muscle activity, and failure of the diaphragm and lungs. A clinically relevant CSA was diagnosed if more than 55 % of the total number of respiratory events were central (White 1985). The symptoms of sleep-disordered breathing were classified as breathing pauses, snoring, daytime sleepiness, concentration disturbance, performance degradation, insomnia, dry mouth, headache, and dizziness.

2.3 Diagnosis

The diagnosis of CSA included various investigations, with a focus on interviewing the participants regarding their sleep habits, daily condition, and medical histories. In addition, physical examinations that included an examination of the ear, nose, and throat were performed. In the present study, a Masimo Radical^{TM} Signal Extraction Pulse Oximeter with Finger Sensor (Masimo Europe Ltd., Puchheim, Germany) was used through the night to measure and record the patients' blood oxygen saturation levels and pulse rates. A more comprehensive investigation was performed in case of evident pathological findings on these tests.

Polysomnography was performed by standard procedures (Sleep Diagnostic System ALICE 4®, Heinen + Löwenstein, Bad Ems, Germany). The examination by polysomnography was carried out several times until the optimal application of therapy could be found for the patients with CSA.

The AHI, which is used to indicate the severity of CSA (Punjabi 2015), is represented by the number of apnea and hypopnea events per hour of sleep. The apneas must last for at least 10 s and be associated with dips in blood oxygenation. Combining the AHI and oxygen desaturation gives an overall sleep apnea severity score that evaluates both the number of sleep disruptions and the degree of oxygen desaturation. The AHI was calculated by dividing the number of apnea events by the number of hours of sleep. AHI values were categorized as follows: 0–4 was normal; 5–14 was mild sleep apnea; 15–29 was moderate sleep apnea; and 30 or more was severe sleep apnea (Thornton et al. 2012). Three study groups were formed according to the AHI calculation corresponding to the severity of CSA.

The Epworth Sleepiness Scale (ESS 2015) was used to assess daytime sleepiness, which was measured with a short questionnaire (Johns 1991). The questionnaire asked to rate the increasing probability of falling asleep on a scale from 0 to 3 for eight different situations that most people engage in during their daily lives, though not necessarily every day. A total score of 0–9 was considered normal, while that of the 10–24 range indicated that expert medical advice should be sought. Scores of 11–15 were taken as indicators of mild-to-moderate sleep apnea, and above 16 indicated the possibility of severe sleep apnea or narcolepsy.

2.4 Ventilation Therapy

Continuous Positive Airway Pressure (CPAP) was used a breathing method in patients with CSA during sleeping, which supports

spontaneous breathing by positive pressurization in the inspiratory phase. CPAP provides continuity of the functional residual capacity of the lungs to make adequate oxygenation possible. The ratio of ventilation to perfusion is significantly improved, and the work of breathing is reduced.

AutoSet CS™ Pace Wave™: Adaptive Servo-Ventilator (ASV) for patients with CSA. This technology continuously monitors the ventilation of patients with CSA and automatically adapts to the exact volume of required pressure support and expiratory positive airway pressure (EPAP) in response to the patient's needs stemming from episodes of apnea, respiratory flow limitations, and snoring in order to stabilize the upper airway.

Bi-level positive airway pressure S/T (BPAP S/T) is a method of pressure-controlled ventilation combined with spontaneous breathing through a respirator. This breathing method allows for the spontaneous breathing of a patient without interrupting the set ventilation rate. BPAP S/T is suitable for the treatment of CSA at a medium pressure level. In central apnea, the device switches to ventilation mode and thus ensures the necessary supply of air. BPAP S/T provides two different pressure levels for inhalation and exhalation.

Oxygen therapy was used with either 1 or 2 L of oxygen per minute through a nasal cannula, nasogastric tube, or a mask. The medications used for the treatment of CSA were L-dopa (100 mg) + benserazide (25 mg), prothipendyl (40 mg), mirtazapine (15 mg), tilidine (50 mg), tilidine (50 mg) + naloxone (4 mg), zopiclone (7.5 mg), and clozapine (6.5 mg). The length of patients' hospital stay was compared between the different forms of CSA.

2.5 Statistical Analysis

Data were expressed as proportions, and means ± standard deviations (SD) wherever appropriate. We calculated 95 % confidence intervals (CI) for the total number of patients with CSA. Fisher's exact test for three independent standard normal variables of two probabilities was used to compare associations among the three study groups between sex difference, treatments by breathing apparatus, oxygen therapy, number of patients using medications, and the number of patients who did not receive treatment. One-way analysis of variance (ANOVA) for three independent samples was performed to compare the number and severity of CSA cases, age difference, and duration of hospital stay, ESS, and AHI among the study groups. Two-tailed tests were performed, and a p value of < 0.05 was considered statistically significant.

3 Results

We found 71 (2.9 %) patients with CSA among 2,463 patients with sleep apnea who had been treated in the sleep lab. The mean age of CSA patients was 67 ± 14 years, of whom 54 (76.1 %, 95 % CI 66.2–86.0 %) were males and 17 (23.9 %, 95 % CI 14.0–33.8 %) were females. The male sex was significantly more likely to suffer from CSA ($p = 0.034$). No significant difference in age were found between the three study groups ($p = 0.168$). Severe CSA cases predominated (Table 1; $p < 0.0001$).

Although more than half of the CSA patients were prescribed a ventilator, a clearly advantageous ventilatory mode could not found (Table 1). Only were small proportions of patients treated with oxygen therapy for sleeping at night or with drugs. There was a significant difference in the AHI ($p < 0.0001$), but no difference in the length of hospital stay among the three groups (Table 1; $p = 0.453$).

4 Discussion

Although every year a relatively large number of patients were examined for sleeping disorders, CSA was seen in only 2.9 % of those who had been identified with sleep apnea. CSA was a rare sleep disorder. However, different CSA prevalence is quoted in the literature. The prevalence may be up to 5 % of all patients examined in the sleep lab (Inönü et al. 2014) and it is believed that CSA most often affects men (Sinn et al. 1999).

Table 1 Comparison of gender, forms of ventilation, oxygen therapy, drug treatment, Epworth sleepiness scale (ESS), and duration of hospital stay in three study groups according to their apnea-hypopnea index (AHI) scores

	AHI 5–14 (%)	AHI 15–29 (%)	AHI > 30 (%)	p-value
Gender				
Male (N = 54)	5 (50)	16 (69.6)	33 (86.8)	0.034
Female (N = 17)	5 (50)	7 (30.4)	5 (13.2)	0.034
Total no. of patients	10 (14.1)	23 (32.4)	38 (53.5)	
Breathing apparatus				
CPAP	0	4 (17.4)	3 (7.9)	0.337
AutoSet CS-A	3 (30)	3 (13.0)	10 (26.3)	0.416
BPAP S/T	1 (10)	1 (4.3)	8 (21.1)	0.169
ASV	0	2 (8.7)	4 (10.5)	0.851
Total N = 39 (54.9 %)	4 (5.6)	10 (14.1)	25 (35.2)	0.126
Oxygen therapy				
Oxygen therapy with 1 liter per minute	0	5 (21.7)	6 (15.8)	0.354
Oxygen therapy with 2 liters per minute	0	7 (30.4)	7 (18.4)	0.137
Total N = 25 (35.2 %)	0	12 (16.9)	13 (18.3)	0.010
Drug treatment				
L-Dopa 100 mg + Benserazide 25 mg	0	0	1 (1.4)	0.999
Prothipendyl 40 mg	1 (10)	0	0	0.141
Mirtazapine 15 mg	0	1 (4.3)	0	0.465
Tilidine 50 mg	1 (10)	0	0	0.141
Tilidine 50 mg + Naloxone 4 mg	1 (10)	0	0	0.141
Zolpidem 10 mg	1 (10)	0	0	0.141
Clozapine 6.5 mg	0	1 (4.3)	0	0.465
Total N = 7 (9.9 %)	4 (5.6)	2 (2.8)	1 (1.4)	0.006
No. treatment N = 4 (5.6 %)	3 (30)	1 (4.3)	0	0.003
ESS (mean score ± SD)	8.4 ± 7.0	9.2 ± 6.3	7.8 ± 5.0	0.639
AHI (mean % ± SD)	9.4 ± 3.6	22.1 ± 6.1	51.9 ± 19.5	<0.001
Duration of hospital stay (mean days ± SD)	2.6 ± 2.3	2.3 ± 1.1	3.5 ± 4.8	0.453

To date, the exact prevalence of people suffering from sleep apnea is unknown (Aurora et al. 2012). Perhaps due to the rare appearance of CSA, no adequate therapies have been developed. In the present study, the male gender was more frequently diagnosed with CSA. CSA occurs due to a disturbance of the brain respiratory network during sleeping, resulting in apnea. The affected people do not realize that their breathing pauses during sleep. Only later, during the day, do the people affected feel the symptoms of CSA, such as sleepiness. This could be one explanation for the late detection and treatment of CSA. While CSA was detected mainly in the elderly in this investigation, the effects of age on the frequency and severity of CSA have not yet been clarified (Bixler et al. 1998).

The gold standard for the examination and diagnosis of CSA is polysomnography (Costanzo et al. 2015). As a baseline control to investigate the relative success of each treatment, all the patients in the present study were subjected to polysomnography, whether being under ventilation, oxygen, or drug therapy. Since the adverse effects of CSA intensify with an increasing number of apneic episodes, reducing the AHI must be the focus of any treatment. The AHI index not only describes the severity of CSA but also serves as an indicator of the success of CSA treatment.

The most frequent recommendation for the treatment of CSA according to the American Academy of Sleep Medicine is CPAP (AASM 2014). This recommendation relates mainly to patients with CSA and heart failure, but other

types of CSA also respond to CPAP treatment (Aurora et al. 2012). However, a definite advantage of CPAP over other treatment modalities was not observed in the present study. That could be explained by the heterogeneity of the various causes of CSA, for which treatment with CPAP would not work equally well. Treatment with CPAP was proposed in patients with mixed CSA and Cheyne-Stokes respiration CSA (Hsu and Lo 2003). Although we did not divide the patients into subtypes of CSA due to a small number of patients, we could find no real advantage of treatment with CPAP in this form of CSA.

CPAP technology has recently been expanded to provide the ASV ventilation mode for the non-invasive treatment of sleep-disordered breathing. In CSA patients, ASV measurements are collected continuously, and this information is used to continuously adjust the EPAP and the levels of pressure support from the device. ASV is particularly appropriate for patients with heart failure, who are especially prone to developing CSA (Brown and Javaheri 2014). Respiration devices with ASV mode were used in a few patients with CSA in the present study; two out of the six patients who were treated with ASV devices had heart failure. The AutoSet CS-A is a type of ASV that opens the upper airway and automatically adjusts EPAP (Wisskirchen and Teschler 2000). This form of respiration has been increasingly used in patients with CSA in the present study. However, further studies are needed to verify the benefits of the AutoSet CS-A in CSA patients.

Flow-targeted BPAP ventilatory support is designed to normalize breathing in CSA patients, mainly those with Cheyne-Stokes respiration. The flow-targeted dynamic BPAP device offers a minimum EPAP to the upper airway to eliminate obstructive apneas and hypopneas. In addition, the device modulates the inspiratory positive airway pressure to obtain a target intake airflow and eliminate central apneas and hypopneas (Arzt et al. 2008). BPAP was prescribed only for a few patients with severe CSA in the present study. The appropriate ventilation mode for an individual depends on specific needs of each patient.

According to the recommendation of the American Academy of Sleep Medicine, oxygen therapy is designated as a standard treatment for CSA associated with heart failure (AASM 2014). The majority of the patients with CSA in this investigation did not need oxygen therapy. In contrast, nocturnal home oxygen therapy improved the quality of life in patients with CSA and heart failure in a recent study (Nakao et al. 2014).

In the present study, periodic leg movement during sleep was reduced by a combination of L-dopa and benserazide in a patient with CSA and restless legs syndrome who had not tolerated therapy with ventilators. This result and previous publications support the hypothesis that restless legs syndrome and periodic movements in sleep lead to reduced central dopaminergic activity, possibly resulting in reduced sensitivity of postsynaptic dopaminergic receptors (Karatas 2007). This case shows that treatment of polyneuropathy can lead to an improvement in sleep disorders in patients with CSA.

In the absence of non-organic insomnia, we initiated a treatment with the sleep induction medication prothipendyl in a patient with CSA and suspected depression. Controlled studies in patients with CSA and primary insomnia are completely absent; therefore, experts warn against the uncritical and uncontrolled prescription of neuroleptics due to significantly increased mortality rates (Schreinzer et al. 2001).

In the present study we observed a significant reduction of leg movement and good sleep efficiency in a patient with CSA who was treated with tilidine. That patient showed no benefit from ventilation therapy, so after a neurological examination, a therapeutic trial with tilidine was launched yielding a positive therapeutic effect. While tilidine has been previously studied for the management of restless legs syndrome and periodic limb movement disorder (Vignatelli et al. 2006), there has so far been no investigation of tilidine in CSA patients. In another patient zolpidem was used with a significant improvement in sleep as a proportion of sleep pauses to total sleep time was reduced to 6.6 %; thus, central apneas were reduced, although not

significantly. ICSD-3 recommends using zolpidem for treatment of primary CSA only if the patient does not have underlying risk factors for respiratory depression (Aurora et al. 2012). Although an improvement in CSA with the use of zolpidem has also been shown in another study, the drug is generally not recommended due to the current lack of control studies (Quadri et al. 2009). In yet another patient with CSA and Parkinson's disease in the present study, treatment with clozapine accompanied by nightly oxygen therapy was used. Clozapine has been recommended for patients with Parkinson's disease with excessive daytime sleepiness (Askenasy 2003).

A limitation of the present study was that it examined a small number of patients with CSA in one hospital department during a relatively short study period. Further, distinction of CSA in complex or mixed sleep apneas, which could help improve the clarity of study groups was not performed.

Nevertheless, we have shown that none of the ventilation modes, such as CPAP, ASV, AutoSet CS-A, or BPAP shows a clear advantage over another in treatment of CSA patients. We conclude that treatment method for CSA patients should be individually determined after completion of polysomnographic examinations.

Conflicts of Interests The authors report no conflicts of interest in relation to this work.

References

AASM – American Academy of Sleep Medicine (2014) International classification of sleep disorders, 3rd edn. American Academy of Sleep Medicine, Darien

Arzt M, Wensel R, Montalvan S, Schichtl T, Schroll S, Budweiser S, Blumberg FC, Riegger GA, Pfeifer M (2008) Effects of dynamic bilevel positive airway pressure support on central sleep apnea in men with heart failure. Chest 134(1):61–66

Askenasy JJ (2003) Sleep disturbances in Parkinsonism. J Neural Transm 110(2):125–150

Aurora RN, Chowdhuri S, Ramar K, Bista SR, Casey KR, Lamm CI, Kristo DA, Mallea JM, Rowley JA, Zak RS, Tracy SL (2012) The treatment of central sleep apnea syndromes in adults: practice parameters with an evidence-based literature review and meta-analyses. Sleep 35(1):17–40

Bixler EO, Vgontzas AN, Ten Have T, Tyson K, Kales A (1998) Effects of age on sleep apnea in men: I. Prevalence and severity. Am J Respir Crit Care Med 157(1):144–148

Brown LK, Javaheri S (2014) Adaptive servo-ventilation for the treatment of central sleep apnea in congestive heart failure: what have we learned? Curr Opin Pulm Med 20(6):550–557

Costanzo MR, Khayat R, Ponikowski P, Augostini R, Stellbrink C, Mianulli M, Abraham WT (2015) Mechanisms and clinical consequences of untreated central sleep apnea in heart failure. J Am Coll Cardiol 65(1):72–84

ESS (2015) The official website of the Epworth sleepiness scale. http://epworthsleepinessscale.com/about-epworth-sleepiness/. Accessed 20 July 2015

Hsu AA, Lo C (2003) Continuous positive airway pressure therapy in sleep apnoea. Respirology 8 (4):447–454

Inönü KH, Kanbay A, Köktürk O (2014) The treatment of central sleep-apnea syndrome, updated information, and review of the literature. Tuberk Toraks 62 (1):68–78 (Article in Turkish)

Johns MW (1991) A new method for measuring daytime sleepiness: the Epworth sleepiness scale. Sleep 14 (6):540–545

Karatas M (2007) Restless legs syndrome and periodic limb movements during sleep: diagnosis and treatment. Neurologist 213(5):294–301

Kazimierczak A, Krzesiński P, Krzyżanowski K, Gielerak G (2013) Sleep-disordered breathing in patients with heart failure: new trends in therapy. Biomed Res Int 2013:459613

Momomura S (2012) Treatment of Cheyne-Stokes respiration-central sleep apnea in patients with heart failure. J Cardiol 59(2):110–116

Muza RT (2015) Central sleep apnoea-a clinical review. J Thorac Dis 7(5):930–937

Nakao YM, Ueshima K, Yasuno S, Sasayama S (2014) Effects of nocturnal oxygen therapy in patients with chronic heart failure and central sleep apnea: CHF-HOT study. Heart Vessels. 2014 Oct 28 [Epub ahead of print]

Punjabi NM (2015) Counterpoint: is the AHI the best way to quantify the severity of sleep disordered breathing? No. Chest. doi:10.1378/chest.14-2261

Quadri S, Drake C, Hudgel DW (2009) Improvement of idiopathic central sleep apnea with zolpidem. J Clin Sleep Med 5(2):122–129

Schreinzer D, Frey R, Stimpfl T, Vycudilik W, Berzlanovich A, Kasper S (2001) Different fatal toxicity of neuroleptics identified by autopsy. Eur Neuropsychopharmacol 11(2):117–124

Sin DD, Fitzgerald F, Parker JD, Newton G, Floras JS, Bradley TD (1999) Risk factors for central and obstructive sleep apnea in 450 men and women with

congestive heart failure. Am J Respir Crit Care Med 160(4):1101–1106
Thornton AT, Singh P, Ruehland WR, Rochford PD (2012) The new AASM criteria for scoring respiratory events: interaction between apnea senor and hypopnea definition. Sleep 35(3):425–432
Vignatelli L, Billiard M, Clarenbach P, Garcia-Borreguero D, Kaynak D, Liesiene V, Trenkwalder C, Montagna P, EFNS Task Force (2006) EFNS guidelines on management of restless legs syndrome and periodic limb movement disorder in sleep. Eur J Neurol 13(10):1049–1065
White D (1985) Central sleep apnea. Med Clin North Am 69(6):1205–1219
WHO. International Classification of Diseases (ICD). http://www.who.int/classification/icd/en/. Accessed 20 July 2015
Wisskirchen T, Teschler H (2000) Central sleep apnea syndrome and Cheyne-Stokes respiration. Ther Umsch 57(7):458–462 (Article in German)

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 87–95
DOI 10.1007/5584_2016_217

Published online: 18 March 2016

The Guinea Pig Sensitized by House Dust Mite: A Model of Experimental Cough Studies

T. Buday, S. Gavliakova, J. Mokry, I. Medvedova, N. Kavalcikova-Bogdanova, and J. Plevkova

Abstract

The guinea pig sensitized by ovalbumin is the most widely used model to study cough experimentally, as the neurophysiology of the vagus nerve in the guinea pig is closest to humans. Nonetheless, the choice of the antigen remains questionable, which influences the translation of results into clinical medicine. The present study seeks to develop an alternative model of cough study using house dust mite sensitization (HDM). Thirty guinea pigs were divided into the HDM group, ovalbumin (OVA) group, and control group based on their cough response to 0.4 M citric acid. In the HDM group animals were sensitized by 0.25 %HDM aerosol, which they inhaled for 5 min over 5 days, followed by inhalation of 0.5 %HDM in the same protocol. Sensitization was confirmed by a skin test. Symptoms of allergic rhinitis were induced by intranasal application of 15 μl 0.5 %HDM and cough challenges with citric acid were performed. Airway resistance was measured *in vivo* by Pennock's method. We found that both HDM and OVA-sensitized groups showed a significantly enhanced nasal reactivity and cough response compared with controls. The airway resistance data did not show significant differences. We conclude that the HDM cough model replicates functional aspects of the OVA model, which may make it an alternative to the latter. However, the superiority of the HDM model for experimental cough studies remains to be further explored.

Keywords

Airway reflexes • Allergy • Cough • House dust mite • Ovalbumin • Sensitization

T. Buday (✉), S. Gavliakova, N. Kavalcikova-Bogdanova, and J. Plevkova
Department of Pathophysiology and Biomedical Center Martin, Jessenius Faculty of Medicine, Mala Hora 4C, 036 01 Martin, Slovakia
e-mail: buday@jfmed.uniba.sk

J. Mokry and I. Medvedova
Department of Pathophysiology and Biomedical Center Martin, Jessenius Faculty of Medicine, Mala Hora 4C, 036 01 Martin, Slovakia

1 Introduction

Animal models are extensively used in the biomedical research with the purpose to improve the understanding of disease mechanisms, to find early and effective diagnostic tools, and to develop novel treatment options. The rationale for using animal models is that they are likely to remain necessary until alternative models and systems, which would be equally sound and robust, are developed (Chow et al. 2008). The data obtained from animal models should be compared and translated to the conditions of human diseases.

Cough is the most important airway defensive mechanism and a symptom of the majority of respiratory diseases including asthma and COPD. Despite extensive animal research, there is still a lack of sufficient treatment for cough, regardless of its cause. The inability to translate and replicate the results from preclinical animal models to humans can stem not from the medication or the animal used, but from the model itself, which should mimic the natural pathogenesis of disease processes. Most preclinical studies of neural pathways involved in cough reflex and its pharmacological regulation have been conducted in guinea pigs, rats, rabbits, cats, or dogs (Belvisi and Bolser 2002). The literature shows that the most useful animal model to study cough is the conscious guinea pig.

Extensive work in this field clearly shows that the neurophysiology and neuropharmacology of the guinea pig vagus nerves are very similar to those of man. A great deal of research on the modulation of cough as a defensive mechanism and on the neural background involved in the cough regulatory circuits have been obtained from animal models using ovalbumin (OVA)-induced airway inflammation (Mokry et al. 2013; Sutovska et al. 2013; Hori et al. 2011; Brozmanova et al. 2008). Although the animals sensitized to OVA exhibit positive skin reactivity to the allergen and they develop early and late phase allergic responses after OVA exposure, accompanied by eosinophilia, the way of sensitization (intraperitoneal) and the antigen itself (chicken egg protein) are not as natural as human inhalation allergies.

The elucidation of mechanisms which are responsible for airway hypersensitivity can significantly increase the effectiveness of cough treatment and contribute to better quality of life of the affected patients. Effective therapy can simultaneously decrease the costs associated with diagnostics and more or less effective therapy. In the UK alone, there are around two million prescriptions issued by general practitioners for medications used for cough treatment every year, which translates into £1,314,000 (British Thoracic Society 2006). These costs are underestimated, as many cough treatment medications can be obtained over the counter.

The main objective of this study was to develop a model of house dust mite (HDM) antigens inhalation allergy in the guinea pig and to compare selected functional parameters with those that are usually measured in cough reflex studies employing OVA in the conscious guinea pig. The HDMs are complex antigens which sensitize the subjects *via* immunogenic epitopes, fecal pellets, lipopolysaccharides, beta-glucans, and chitin. They are used to study allergy and asthma (Yasue et al. 1998), but have never been used in the cough research field. We hypothesized that HDM-induced airway inflammation could also be an alternative model to study the cough response in the guinea pig.

2 Methods

2.1 General Notes

Animal care was provided and the experiments were conducted in agreement with the Animal Welfare Guidelines of the Comenius University and the statutes and rules of the Slovak Republic legislation. The study was approved by the Ethics Committee of Jessenius Faculty of Medicine (permit no. 2999/07-221). Male Dunking Hartley guinea pigs were used, obtained from an accredited breeding facility (L. Sobota, Městec Králové, Czech Republic). The animals were housed at a controlled room temperature of 21–22 °C, humidity of 60–70 %, 12-h light–dark cycle, and had free access to water and standard chow.

The animals were adapted twice to the laboratory conditions. To keep the stress to the minimum, they stayed in a plethysmography chamber to get familiarized with the environment and the laboratory personnel responsible for the experimental manipulations. In the chamber, the animals were exposed to an aerosol of buffered saline for 2 min, which corresponded to the future experimental procedure. Later on, the animals were challenged with 0.4 M citric acid and based on the response, they were divided into the control, OVA, and HDM groups, 10 animals each. All the groups involved an equal count of normo-, hypo-, and hyperreactors. The reason for this pattern of group assignment was that the cough response to citric acid in conscious animals shares extremely high variability, present even among healthy animals.

2.2 Sensitization

There were the following sensitization procedures.

1. Sham-sensitized animals in the control group, injected with physiological saline – 1 ml;
2. OVA-sensitized animals, injected with 10 μg of OVA, suspended in the aluminium hydroxide adjuvant, in 1 ml of saline; sensitization was confirmed by a skin prick test 21 days later on. All injections were intraperitoneal.
3. HDM-sensitized animals. First line sensitization was achieved by inhalation of 0.25 % HDM aerosol (Greer Labs; Lenoir, NC) for 5 consecutive days for 5 min each in the plethysmography chamber. Second line (boosting) sensitization was achieved by inhalation of 0.5 % HDM aerosol for 5 consecutive days for 5 min each. Successful sensitization was confirmed 14 days later by a skin prick test (15 μl of 0.5 % HDM; intradermal application) and the animals were observed throughout the early and late phases of allergic reaction.

2.3 Assessment of Nasal Symptoms After Antigen Challenge

The intensity of symptoms was evaluated using a nasal symptom score system that matches symptoms' intensity to numeric values (maximum 6 and minimum 0), according to the method of Brozmanova et al. (2006). The number of sneezes was tallied and the following symptoms were scored: (i) nasal discharge – lack 0, mild/moderate 1, dropping from the nostrils 2, (ii) eye/conjunctival reaction – lack 0, hazy eyes 1, visible lacrimation 2, and (iii) nasal crackles: lack 0, audible 1, audible from a distance 2. The main objective of this procedure was to confirm the ability of the allergen to induce upper airway response after a topical antigen challenge. To this end, the OVA group received 0.15 ml of 0.5 % OVA and the HDM group received 0.15 ml of 0.25 % HDM into both nostrils.

2.4 Citric Acid-Induced Cough

Conscious guinea pigs were individually placed in the body box (type 855, Hugo Sachs Electronik, March, Germany) consisting of head and body chambers. The opening between the two chambers was equipped with a plastic collar sealing the animal's neck to prevent communication between the chambers. The appropriate collar size was chosen for each animal to prevent neck compression. The head chamber was connected to a nebulizer (Provokation Test I-Menzel; PARI GmbH, Starnberg, Germany), having the following specification: output 5 l/min and particle mass median aerodynamic diameter 1.2 μm. A suction device adjusted to the input of 5 l/min was connected to the head chamber to maintain constant airflow through the chamber during the aerosol administration. The airflow was measured with a Fleisch head connected to the head chamber. Data were recorded with the acquisition system ACQ Knowledge (Biopack, Santa Barbara, CA). Respiratory sounds, including sounds during coughing and sneezing, were recorded with a microphone placed in the roof of

the head chamber and connected to a preamplifier and MP3 recorder. The pneumotachograph and microphone output were simultaneously recorded for off-line analysis.

The cough challenge was performed using an inhalation of 0.4 M citric acid for 10 min. Cough was defined as an expiratory airflow interrupting the basic respiratory pattern accompanied by a coughing sound. Coughs were analyzed by two trained persons; both being blind to the procedures carried out. Their results were compared and averaged if no statistically significant differences occurred.

To assess the cough threshold, the animals were challenged by gradually increasing concentrations of citric acid (0.05–1.60 M; 30 s inhalation separated by 1 min intervals) conducted at least 3 days apart. Parameters of C2 and C5 were obtained, corresponding to the concentrations of citric acid inducing 2 and more coughs or 5 or more coughs, respectively. The dose response curves also were constructed. Cough responses were measured after nasal allergen and saline challenges as based on the upper airway cough syndrome model validated by Brozmanova et al. (2006).

2.5 Measurement of Specific Airway Resistance

Specific airway resistance (Raw) was measured by a non-invasive plethysmographic method of Pennock et al. (1979). Conscious animals were placed in the body box as outlined above. Nasal airflow was measured in the head chamber and thoracic airflow was calculated from the pressure change in the lower chamber. Both measurements, made with differential pressure transducers (Simsoft; Martin, Slovakia), were fed into PC equipped with Pulmodyn Pennock W software for further analysis. The Raw is proportional to the phase difference between nasal and thoracic airflows and is based on the Lissajous loop representation of the two airflows on the X and Y axes. The Raw was measured after inhalation of histamine and methacholine aerosols.

3 Results

3.1 Skin Prick Tests

Skin tests were performed in all groups and early and late phase responses were observed on the skin after OVA and HDM intradermal injections, with saline and histamine pricks used as negative and positive controls, respectively (Fig. 1a). All OVA-sensitized animals exhibited the acute phase response with the presence of flare and edema, which developed within 15 min after antigen administration. The acute phase was observed up to 1 h. After 4–8 h, late phase was observed with the presence of flare (3–5 mm on average), edema, and induration, which was detected in 8/10 OVA-sensitized animals. In the HDM group, acute phase of the skin response

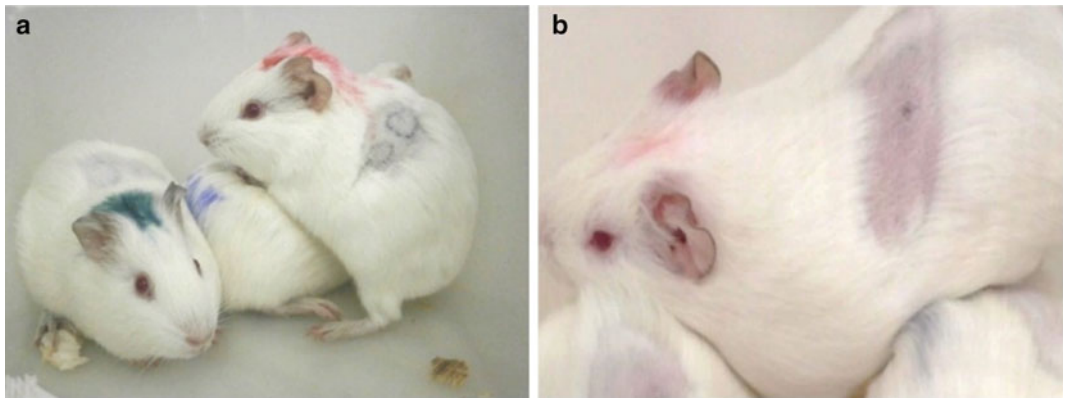


Fig. 1 Guinea pigs with positive and negative pricks (**a**) and detail of a scratch injury and scab (**b**)

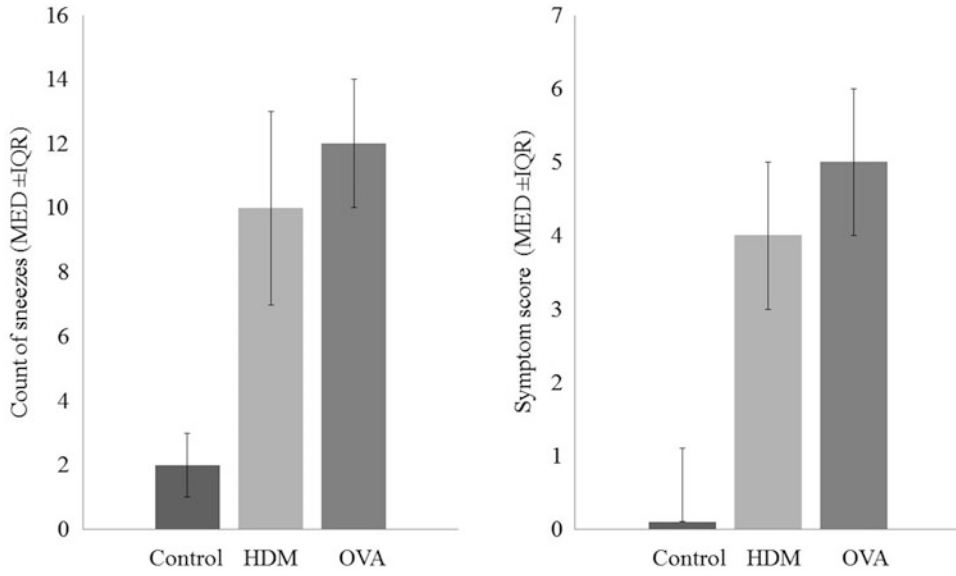


Fig. 2 Count of sneezes and nasal symptom score in the early phase. *MED* median, *IQR* interquartile range, *HDM* house dust mite sensitization, *OVA* ovalbumin sensitization

was significantly weaker; redness with edema developed only in 4/10 animals. The late phase response, however, was considerably stronger when compared with the OVA group as it was present in 10/10 animals. Intensive scratching was observed in all animals a day after sensitization and scratch injuries and scabs were observable (Fig. 1b).

3.2 Nasal Symptoms

Animals in all groups were challenged by intranasal saline, OVA, or HDM. Control (saline) challenge induced no considerable symptoms, animals sneezed once or twice immediately after or during the insertion of a catheter. The animals of OVA and HDM groups were sneezing significantly more during the whole observation time of 1 h: 2 ± 1 *vs.* 12 ± 4 *vs.* 10 ± 2, $p < 0.05$, in the control, OVA, and HDM groups, respectively. In the last two groups, there also were present nasal discharge and nasal acoustic phenomenon typical for breathing through obstructed nasal passages. The OVA, but no HDM group, developed a conjunctival reaction. A total symptom score was comparable in these two groups: 5 ± 1 *vs.* 4 ± 1 points, which was significantly higher from the lack of symptoms in the control group ($p < 0.05$) (Fig. 2).

3.3 Cough Response to Inhalation of Citric Acid

Cough response to citric acid, the most frequently used tussive agent, was analyzed during the acute phase of the allergic response induced by nasal challenges of saline, HDM, and OVA. It is generally known that an inflammatory process in the nose/sinuses considerably upregulates the cough response. A total cough count obtained during 10-min lasting inhalation of citric acid was 9 ± 2 in controls *vs.* 16 ± 3 in HDM and 15 ± 2 coughs in OVA groups. The cough response in both sensitized groups, OVA and HDM, was significantly higher than that in controls ($p < 0.05$). However, the OVA and HDM groups achieved very similar results for the total cough count. This pattern was present also for the cough latency, which is the time from the start of exposure to a tussigen to the onset of the first cough bout. In controls, the first cough appeared after 180 ± 8 s, while in the OVA and

HDM groups it appeared after 86 ± 8 and 80 ± 10 s, respectively, after the start of citric acid exposure (Fig. 3).

3.4 Specific Airway Resistance *In Vivo*

No significant changes were observed in specific airway resistance measured *in vivo* in response to histamine or methacholine, as compared with saline, in the HDM group (Table 1).

4 Discussion

The present study demonstrates that the house dust mite antigens can be used to model airway hypersensitivity/hyperreactivity in the guinea pig, with the possible application of this model in studies on cough reflex. Sensitization to HDM was confirmed indirectly by positive skin tests after intradermal administration of HDM, and also by the onset of nasal symptoms after topical intranasal HDM challenges in sensitized animals. We were able to reproduce functional changes, especially the upregulation of cough reflex in HDM-sensitized guinea pigs after topical administration of the allergen. Some of the data were comparable to the functional changes observed in the OVA-sensitized animals. However, we failed to show a significantly modified cough reflex sensitivity, which is well known for the OVA model (Brozmanova et al. 2006).

An animal model suitable to study cough in conscious animals, along with the effectiveness of a medication, should be characterized by an enhanced cough response to tussive stimuli when compared with control subjects, which reflects the upregulation of cough. This is achieved by the mechanisms of either peripheral or central neuroplasticity. The effectiveness of an antitussive medication should be studied on the background of upregulated cough response, which enables to notice a reduction of coughing. Further, such a model should be characterized by increased permeability of airway mucosa to tussive stimuli, which allows using lower concentrations of tussive aerosols. This strategy mimics the situation in humans, especially when the cough response is triggered by subthreshold stimuli. Increased permeability of airway mucosa is typical for airway inflammation. The animals used to model cough responses should be in the

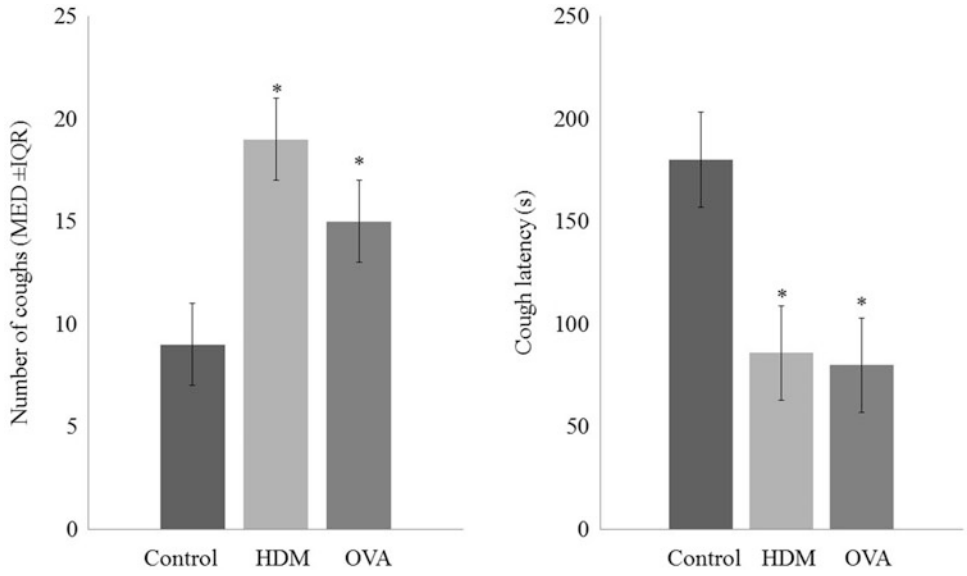


Fig. 3 Citric acid induced cough. *MED* median, *IQR* interquartile range, *HDM* house dust mite sensitization, *OVA* ovalbumin sensitization

Table 1 Specific airway resistance (Raw; ml/s) *in vivo* in the house dust mite (HDM)-sensitized, compared with control, guinea pigs

Controls	Saline	Histamine	Methacholine
	3.16 ± 1.29	3.42 ± 0.23	3.75 ± 1.59
p *vs.* saline	–	0.40	0.38
HDM	Saline	Histamine	Methacholine
	3.67 ± 2.07	4.20 ± 1.68	4.39 ± 1.95
p *vs.* saline	–	0.24	0.33

Data are means ± SD

state of consciousness to mimic the cough reflex in humans that cannot be studied in anesthetized subjects, when the essential contribution of the suprapontine neural mechanisms is missing.

Another important issue is to limit bronchoconstrictive response, which is a usual accompaniment of different models of airway hyperreactivity. Bronchoconstriction influences breathing pattern of an animal and a total amount of inhaled tussigen and its deposition in airways. Therefore, none or mild bronchoconstriction would benefit a cough study, whereas models in which animals respond to airway challenge with wheezing and breathing difficulties are not suitable for this purpose. In the present study, we used the 0.25 % and later 0.50 % HDM antigen concentrations to sensitize the guinea pig. These concentrations are lower than those used in the mouse asthma models where they are in a range of 10 mg HDM extract per ml (Yasue et al. 1998).

In modeling, it is important to consider what the expected outcomes would be. Intraperitoneal sensitization, followed by intranasal and aerosol challenges, is relatively easy to conduct, induces strong Th2 responses, and it is more suitable from the immunological standpoint. On the other side, repeated aerosol challenges as a way of sensitization are more suitable to develop airway hyperreactivity and airway remodeling. Therefore, we set out to use the aerosol HDM challenges to develop our model. The downside of the HDM use is that it represents increased risk for the development of occupational allergy or asthma in laboratory personnel (Ruoppi et al. 2005), whereas this risk is virtually null when using intraperitoneal sensitization.

The question arises of whether the HDM as a whole extract or Der p 1 protein alone should be employed for sensitization? The Der p 1 allergen is the most immunogenic house mite protein of the extract, but not the only one. Accordingly, we found the sensitization to be far less effective with Der p 1 alone. Also, no one is exposed to Der p 1 alone. Therefore, unless one desires to study the allergenic capacity of just this allergen or to work on a specific desensitization, the whole HDM extract is more advisable (Hammad et al. 2010).

Cough is the most important defensive reflex of airways and it remains the most common symptom of airway diseases. Nonetheless, extensive research in the cough field has by far failed to bring satisfactory results which could possibly be translated into clinical applications in chronic cough. Neural circuits responsible for the mediation and modulation of cough reflex are studied in both anesthetized and conscious animals using either *naïve* animals or animals with airway inflammation. These models use different approaches to induce inflammation and thus modulate neurophysiological properties of cough mediating fibres in airways. This process, known as peripheral cough plasticity, is believed to be responsible for upregulation of cough reflex and troublesome persistent coughing (Poliacek et al. 2009a,b).

The overview of the literature suggests that the most frequently used model is based on ovalbumin sensitization with different patterns of secondary airway challenges (intranasal, inhalational) after the primary sensitization phase, which is usually intraperitoneal. Ovalbumin models of acute and chronic airway inflammation have been extensively reviewed by Kumar et al. (2008), with the listing of advantages and disadvantages regarding the clinical translation of findings as well as the description of limitations of OVA use. The OVA, however, is seldom implicated in humans, and other researchers have used alternative allergens that may have a greater clinical relevance, e.g., house

dust mite or cockroach extracts (Nials and Uddin 2008).

The antigenic potential of HDM is complex, and this aggregate of allergens can induce an inflammatory response *via* multiple mechanisms compared with OVA. Proteases, fecal pellets, chitin, lipopolysaccharide, and others belong to the spectrum of pro-inflammatory signals released by the HDM. In general, currently used HDM models rely on topical administration of HDM into the airways (usually intranasally) over a course of weeks, which results in airway inflammation accompanied by eosinophilia, but there is not enough evidence to show the allergic nature of inflammation underscored by HDM-specific IgE antibodies, B-, or T-cells. Therefore, it is unclear whether the inflammation is truly allergic or just a consequence of repeated nasal insults.

Cough studies *in vivo* are usually based on the observation and analysis of the cough response to tussive challenges. Citric acid or capsaicin aerosols are used to induce reliable and reproducible cough responses to study either the mechanisms of cough or effectiveness of anti-cough medications. The HDM could play a role in such studies. It contains a number of proteolytic enzymes: cysteine proteases (group 1: Der p 1 and Der f 1) and serine proteases (groups 3, 6, and 9: Der p 3, Der p 6, Der p 9, Der f 3, Der f 6, and Der f 9); the enzymes are also present in HDM fecal pellets (Chapman et al. 2007). Collectively, they are responsible for 79 % of proteolytic activity of HDM (Stewart et al. 1994). Der p 1 and Der f 1 disrupt the intercellular tight junction proteins occludin and claudin-1 (Wan et al. 2001; Wan et al. 1999), which enables the delivery of an antigen to submucosal antigen-presenting cells. This impairment of the barrier function of the epithelium, present in poorly controlled asthmatic patients (Bhure et al. 2009), has not been replicated in a murine *in vivo* model after a single dose of HDM (Turi et al. 2011), which suggests that it results from chronic exposure to HDM rather than from an acute insult. To induce coughing, tussive aerosols must penetrate the airway mucosa to reach afferent nerve endings mediating cough. It is supposed that the HDM, with its protease activity, can destroy tight junction proteins connecting the airway epithelial cells, which may, in turn, increase mucosal permeability for different irritants, including tussive stimuli. Increased permeability of airway mucosa may facilitate cough induction with the use of lower concentrations of tussive substances, which possibly better emulates the natural conditions in which coughing appears.

In conclusion, HDM antigens appear to be a promising way to study the cough reflex. Further research is required to validate and optimize the HDM model of hypersensitive, cough producing airways.

Acknowledgements This study was supported by VEGA No. 1/0107/2014 and Biomed ITMS: 26220220187

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

References

Belvisi MG, Bolser DC (2002) Summary: animal models for cough. Pulm Pharmacol Ther 15(3):249–250

Bhure UN, Bhure SU, Bhatt BM, Mistry S, Pednekar SJ, Chari VV, Desai SA, Joshi JM, Paidhungat AJ (2009) Lung epithelial permeability and inhaled furosemide: added dimensions in asthmatics. Ann Nucl Med 23 (6):549–557

British Thoracic Society (2006) Burden of lung disease – a statistics report from British Thoracic Society 2006, 2nd edn. Available from https://www.brit-thoracic.org.uk/document-library/delivery-of-respiratory-care/burden-of-lung-disease/burden-of-lung-disease-2006. Accessed 12 Aug 2015

Brozmanova M, Calkovsky V, Plevkova J, Bartos V, Plank L, Tatar M (2006) Early and late allergic phase related cough response in sensitized Guinea Pigs with experimental allergic rhinitis. Physiol Res 55(5):577–584

Brozmanova M, Plevkova J, Tatar M, Kollarik M (2008) Cough reflex sensitivity is increased in the guinea pig model of allergic rhinitis. J Physiol Pharmacol 59 (Suppl 6):153–161

Chapman MD, Wünschmann S, Pomés A (2007) Proteases as Th2 adjuvants. Curr Allergy Asthma Rep 7(5):363–367

Chow PKH, Ng RTH, Ogden BE (2008) Using animal models in biomedical research: a primer for the investigator. World Scientific, Hackensack

Hammad H, Plantinga M, Deswarte K, Pouliot P, Willart MA, Kool M, Muskens F, Lambrecht BN (2010) Inflammatory dendritic cells – not basophils – are necessary and sufficient for induction of Th2 immunity to inhaled house dust mite allergen. J Exp Med 207(10):2097–2111

Hori A, Fujimura M, Ohkura N, Tokuda A (2011) Involvement of nitric oxide (NO) in cough reflex sensitivity between non-sensitized and OVA-sensitized guinea pigs. Cough 7(1):5

Kumar RK, Herbert C, Foster PS (2008) The "classical" ovalbumin challenge model of asthma in mice. Curr Drug Targets 9(6):485–494

Mokry J, Joskova M, Mokra D, Christensen I, Nosalova G (2013) Effects of selective inhibition of PDE4 and PDE7 on airway reactivity and cough in healthy and ovalbumin-sensitized guinea pigs. Adv Exp Med Biol 756:57–64

Nials AT, Uddin S (2008) Mouse models of allergic asthma: acute and chronic allergen challenge. Dis Model Mech 1(4–5):213–220

Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH (1979) A noninvasive technique for measurement of changes in specific airway resistance. J Appl Physiol Respir Environ Exerc Physiol 46(2):399–406

Poliacek I, Jakus J, Simera M, Barani H, Visnovcova N, Halasova E, Tomori Z (2009a) Excitability and rhythmicity of tracheobronchial cough is altered by aspiration reflex in cats. J Physiol Pharmacol 60(Suppl 5):105–110

Poliacek I, Tomori Z, Simera M, Barani H, Visnovcova N, Halasova E, Donic V, Jakus J (2009b) Provocation of aspiration reflexes and their effects on the pattern of cough and reflex apnea in cats. J Physiol Pharmacol 60 (Suppl 5):99–104

Ruoppi P, Koistinen T, Pennanen S (2005) Sensitisation to mites in laboratory animal workers with rhinitis. Occup Environ Med 62(9):612–615

Stewart GA, Boyd SM, Bird CH, Krska KD, Kollinger MR, Thompson PJ (1994) Immunobiology of the serine protease allergens from house dust mites. Am J Ind Med 25(1):105–107

Sutovska M, Adamkov M, Kocmalova M, Mesarosova L, Oravec M, Franova S (2013) CRAC ion channels and airway defense reflexes in experimental allergic inflammation. Adv Exp Med Biol 756:39–48

Turi GJ, Ellis R, Wattie JN, Labiris NR, Inman MD (2011) The effects of inhaled house dust mite on airway barrier function and sensitivity to inhaled methacholine in mice. Am J Physiol Lung Cell Mol Physiol 300(2):L185–L190

Wan H, Winton HL, Soeller C, Tovey ER, Gruenert DC, Thompson PJ, Stewart GA, Taylor GW, Garrod DR, Cannell MB, Robinson C (1999) Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J Clin Invest 104(1):123–133

Wan H, Winton HL, Soeller C, Taylor GW, Gruenert DC, Thompson PJ, Cannell MB, Stewart GA, Garrod DR, Robinson C (2001) The transmembrane protein occludin of epithelial tight junctions is a functional target for serine peptidases from faecal pellets of Dermatophagoides pteronyssinus. Clin Exp Allergy 31(2):279–294

Yasue M, Yokota T, Suko M, Okudaira H, Okumura Y (1998) Comparison of sensitization to crude and purified house dust mite allergens in inbred mice. Lab Anim Sci 48(4):346–352

Advs Exp. Medicine, Biology - Neuroscience and Respiration (2016) 20: 97–98
DOI 10.1007/5584_2015

Index