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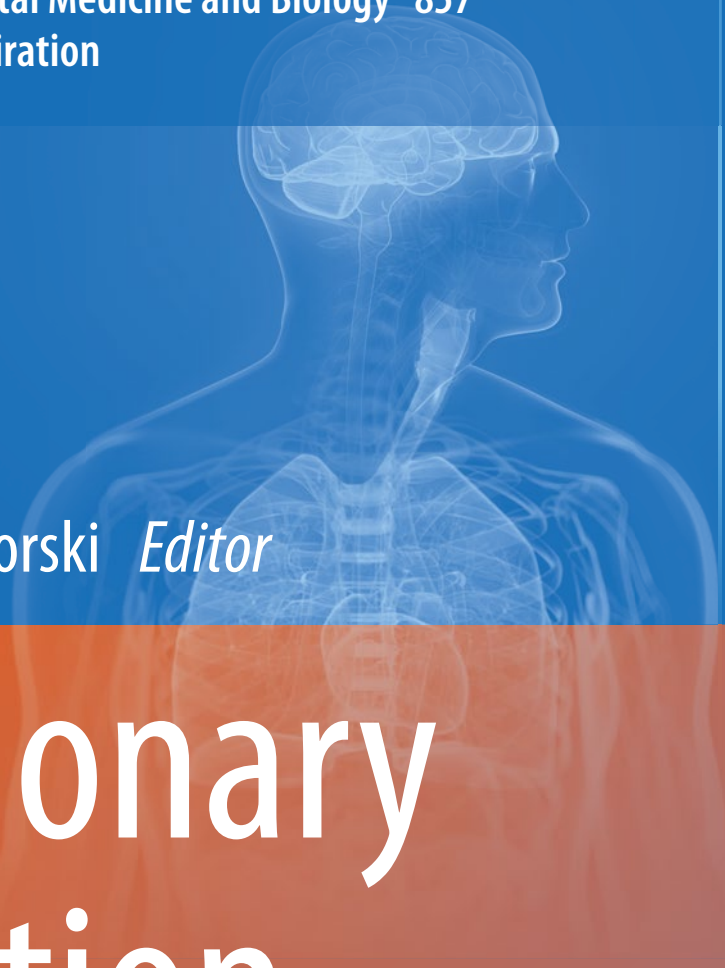

Mieczyslaw Pokorski *Editor*

Pulmonary Infection

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Pulmonary Infection

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Preface

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

Volume 12: Pulmonary Infection

The respiratory tract is a frequent target of infections caused by a wide range of organisms. The book provides reader-friendly information on aspects of pulmonary infections, including comprehensive accounts of bacterial and viral diseases, therapeutic approaches, molecular and classical culture-related techniques of diagnosis, and explaining the basic cell biological mechanism. The role of oxidative stress, both helpful in fighting invading pathogens and detrimental in yielding to infection is detailed as it is often undervalued and needs focus. The chapters encompass latest developments and applications in bacteriology and virology, preventive and therapeutic tips, and raise attention to implementation of proper antibiotic policies by medical institutions to decrease resistance to antimicrobials. The perennial problem of low influenza vaccination coverage rate is rationalized. The volume will be of interest to both clinicians and biomedical researchers engaged in this exciting field.

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Evaluation of the Activity of Influenza and Influenza-Like Viruses in the Epidemic Season 2013/2014

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Abstract

Infections caused by respiratory viruses can have different clinical symptoms, while specific set of symptoms can be induced by different viruses. Despite usually mild course of disease, some viruses causing certain disease entity can result in serious complications. Therefore, quick and appropriate diagnostic is crucial for administering proper treatment. In the epidemic season 2013/2014, 2,497 specimens were tested. Infections caused by influenza viruses were confirmed in 9.8 %, while infections caused by influenza-like viruses (ILI) in 13.2 %. The co-domination of A/H1N1/pdm09 (29.4 %) with A/H3N2/ (30.6 %) was observed among circulating subtypes of influenza virus type A. Analysis of positive specimens categorized into 7 age groups indicated that most of morbidity to influenza was noted in the age intervals: 26–44 (22.9 %) and 45–64 years old (21.6 %). Considering infections caused by influenza-like viruses, the highest amount of positive cases was registered in the age group 0–4 years old (92.7 %) with the highest ratio of RSV (87.9 %) and PIV-3 (10.5 %). Judging by the epidemiological and virological indicators, the 2013/14 influenza season was mild and only low virus activity was reported in Poland as well as in most European countries. Still, 9,000 hospitalizations and 17 deaths were registered in Poland during this epidemic season.

Keywords

Detection • Flu • Incidence • Infection • Upper respiratory tract

1 Introduction

Respiratory tract infections consist one of the major health problem of society, because they lead to high rates of hospitalizations, morbidity, and mortality. Essential feature of respiratory

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viruses is their easiness of transmission, especially in places with great density of people (schools, governments, mall centers, and means of public transport) (Brydak 2007). Elder people, children up to 24 months and persons from high risk groups are particularly exposed to respiratory viruses' infections. The most frequently circulating respiratory viruses are: influenza virus type A and B, respiratory syncytial virus A (RSV-A) and B (RSV-B), parainfluenza viruses 1–3 (PIV-1,-2,-3), human coronavirus (hCoV), adenovirus (ADV), human rhinovirus (hRV), and human metapneumovirus (hMPV). Infections caused by respiratory viruses can have different clinical symptoms, while specific set of symptoms can be induced by different viruses. Despite usually mild course of disease, some viruses causing certain disease entity can result in serious complications. Furthermore, not only viruses can cause particular illness, but also bacteria. Therefore, quick and appropriate diagnostic is crucial for administering proper treatment (Stefańska et al. 2013). What is more, organism attacked by one pathogen has reduced immunity and becomes vulnerable to the subsequent ones. In this way numerous co-infections occur, which can result in death (Brydak 2008, 2011).

2 Methods

2.1 Patient Population and Specimen Collection

In the epidemic season 2013/2014, 2,471 clinical specimens were tested in Poland. Additionally, National Influenza Center (NIC), Department of Influenza Research at National Institute of Public Health – National Institute of Hygiene as a Reference Laboratory in Poland – tested selected specimens from Voivodeship Sanitary Epidemiological Stations (VSES) to verify the results obtained in these labs. Mainly, materials for testing consisted from nasal and throat swabs, while in NIC also bronchus-associated lymphoid fluid (BALF) was tested. The age of the patients ranged from 1 day to 87 years of age. The patients were categorized into 7 age groups: 0–4, 5–9, 10–14, 15–25, 26–44, 45–64, and >65 years of age.

2.2 Extraction of Viral RNA

The 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 Polymerase Chain Reaction (RT-PCR)

Because RNA virus samples were typed in Voivodeship Sanitary Epidemiological Stations, only subtyping of influenza viruses was achieved. The detection of subtypes was performed by RT-PCR. One-step RT-PCR analysis was performed using Roche Light Cycler 2.0 System. RT-PCR reactions were performed in capillary tubes in 20 µl volumes with 0.5 µM primers and 0.2 µM probe for each reaction. According to WHO recommendations. The primers GRswH1-349Fw, GRswH1-601Rv, GRsw-N1-975Fw, GRswN1-1084Rv, H3h-319Fw, H3h-377Rv, N2h-1150bFw, N2h-1344Rv, and probes GRswH1-538, GRswN1-10465b, H3h-358, N2h1290 were used (WHO 2014). The reaction mixture, containing reaction buffer, $MgSO_4$ buffer, BSA, RNase-free H_2O, and SuperScript® III/Platinum® *Taq* Mix, was incubated with 5 µl RNA sample per capillary tube. RNA from vaccine viruses A/California/7/2009(H1N1) pdm09 and A/Victoria/361/2011(H3N2) were introduced as the positive control sample and RNase-free H_2O was utilized as the 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: 45 °C for 15 min. DNA templates were then subjected to an initialization step (1 cycle at 95 °C for 3 min), followed by 50 cycles of

amplification: denaturation at 95 °C for 10 s, annealing at 55 °C for 10 s and elongation at 72 °C for 20 s.

2.4 Conventional Multiplex RT-PCR

Thirty six specimens from patients, stored at −80 °C, were tested by RT-PCR using RV12 ACE Detection Kit (Seegene, Seoul, South Korea) that enables detection of following respiratory viruses: Influenza A virus, influenza B virus, 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

In the epidemic season 2013/2014, 2,497 specimens were tested. 23.0 % were positive for influenza and influenza-like viruses. Infections caused by influenza viruses were confirmed in 9.8 %, while infections caused by influenza-like viruses in 13.2 % (Table 1).

The majority of influenza-like cases – 57.4 % was caused by RSV (83.9 %). Infections of upper respiratory tracts were also caused by viruses such as: PIV-3 (11.2 %), rhinovirus (1.8 %), adenovirus (1.2 %), human coronavirus (0.6 %), metapneumovirus (0.6 %), PIV-1 (0.3 %), and PIV-2 (0.3 %). Taking into account infections caused only by influenza viruses, they amounted to 42.6 % of all laboratory confirmed cases. The predominant type of influenza virus was type A which made up 98.8 % of all influenza cases. In the epidemic season 2013/2014, co-domination of A/H1N1/pdm09 (29.4 %) with A/H3N2/ (30.6 %) was observed among circulating subtypes of influenza virus type A. Influenza virus type B comprised 1.2 % of influenza virus confirmations.

Analysis of positive specimens categorized into 7 age groups indicated, that most of morbidity to influenza was noted in age groups: 26–44 (22.9 %) and 45–64 years of age (21.6 %), whereas the influenza was the rarest in the age group 10–14 years of age (5.3 %) (Table 2). If it goes about influenza laboratory confirmed cases in high risk groups, 14.7 % of specimens were positive in >65 years age group and 17.6 % in 0–4 years age group. In all seven age groups, a co-domination between two subtypes of influenza virus type A was present, i.e. A/H1N1/pdm09 and A/H3N2/. Considering infections caused by influenza-like viruses, the biggest amount of positive cases was registered in the age group 0–4 years of age (92.7 %), with the highest ratio of RS virus (87.9 %) and PIV-3 (10.5 %). The lowest rate of infections caused by influenza-like viruses was observed in age groups: >65 (0.3 %) and 10–14 years of age (0.3 %).

Verification of results received by VSESs demonstrated complete consistency with the results obtained NIC (Table 3). In NIC, infections caused by influenza viruses comprised 50 % of laboratory confirmed cases and the other 50 % were those caused by influenza-like viruses. In the event of influenza viruses, the predominant subtype was A/H3N2/ (50 %), while A/H1N1/pdm09 made up 18.8 % of confirmations.

Taking into consideration only influenza-like viruses, it was noted that RSV (31.3 %) and Rhinovirus (37.5 %) were dominating in this group. Moreover, eight co-infections were registered in the framework of conducted research. Five of them were between influenza virus and influenza-like viruses (A + ADV, 2 A + RSV + hRV, A/H3N2/ + RSV B, A/H3N2/ + hCoV), whereas three co-infections were detected only among influenza-like viruses (RSV A + hRV, RSV B + PIV-3, ADV + PIV-1-3 + hCoV). All co-infections detected in epidemic season 2013/2014 in Poland are presented in Table 4.

Table 1 Confirmations of influenza and influenza-like viruses in the epidemic season 2013/2014 in Poland

Epidemic season 2013/2014															
Age group	A	A/H1N1/pdm09	A/H3N2/	A Total	B	A + B Total	ADV	RSV	PIV 1	PIV 2	PIV 3	hCoV	RhV	hMPV	ILI Total
0–4	25	5	12	42	1	**43**	0	269	1	0	32	0	3	1	**306**
5–9	6	6	13	25	0	**25**	1	2	0	0	0	0	0	0	**3**
10–14	5	2	6	13	0	**13**	0	0	0	0	1	0	0	0	**1**
15–25	11	3	4	18	1	**19**	2	0	0	0	1	1	1	1	**6**
26–44	22	21	13	56	0	**56**	0	2	0	0	1	1	1	0	**5**
45–64	13	24	16	53	0	**53**	1	4	0	1	1	0	1	0	**8**
>65	13	11	11	35	1	**36**	0	0	0	0	1	0	0	0	**1**
Total no of detections	**95**	**72**	**75**	**242**	**3**	**245**	**4**	**277**	**1**	**1**	**37**	**2**	**6**	**2**	**330**
Percent of total (%)	**3.8**	**2.9**	**3.0**	**9.7**	**0.1**	**9.8**	**0.2**	**11.1**	**0.0**	**0.0**	**1.5**	**0.1**	**0.2**	**0.1**	**13.2**
Percent of flu/ILI (%)	**38.8**	**29.4**	**30.6**	**98.8**	**1.2**	**100**	**1.2**	**83.9**	**0.3**	**0.3**	**11.2**	**0.6**	**1.8**	**0.6**	**100**

Table 2 Number of specimens tested and the percentage of influenza and influenza-like virus (ILI) detections in each age group

Age group	Influenza detections	Percentage of detections (%)	ILI	Percentage of detections (%)
0–4	43	17.6	306	92.7
5–9	25	10.2	3	0.9
10–14	13	5.3	1	0.3
15–25	19	7.8	6	1.8
26–44	56	22.9	5	1.5
45–64	53	21.6	8	2.4
>65	36	14.7	1	0.3
Total	245	100	330	100

Table 3 Selected specimens from VSES that were verified in NIC NIPH-NIH

	Subtype			
Program	A/H1N1/pdm09	A/H3N2/	A/H1N1/pdm09 + A/H3N3	Total
Sentinel	11	25	1	37
Non-sentinel	25	34	1	60

VSES Voivodeship Sanitary Epidemiological Stations, *NIC NIPH-NIH* National Influenza Center, National Institute of Public Health – National Institute of Hygiene

Table 4 Co-infections of respiratory viruses in the epidemic season 2013/2014 in Poland

Type of co-infection	
Influenza viruses	1. A/H1N1/pdm09 + A/H3N2/ (39 years old)
	2. A/H1N1/pdm09 + A/H3N2/ (7 years old)
Influenza viruses and Influenza-like viruses	1. Influenza A + ADV (20 years old)
	2. Influenza A + RSV (60 years old)
	3. Influenza B + PIV-3 (70 years old)
	4. Influenza A/H3N2/ + hCoV 229/NL63 (35 years old)
	5. Influenza A/H3N2/ + RSV B (7 years old)
	6. Influenza A + RSV A + hRV A/B **(30 days old)**
	7. Influenza A + RSV A + hRV A/B **(1 day old)**
Influenza-like viruses	1. RSV A + hRV A/B (20 years old)
	2. RSV B + PIV-3 (32 years old)
	3. PIV-(1–3) + ADV + hCoV 229E/NL63 (24 years old)

4 Discussion

In the epidemic season 2013/2014 in Poland, 2,497 specimens were tested, 23.0 % of which consisted positive specimens, which was, for comparison, about one third of the 6,949 tests performed in the preceding season. In order to get detailed virological analysis, laboratory data were categorized into 7 age groups: 0–4, 5–9, 10–14, 15–25, 26–54, 45–64, and >65 years old. Influenza viruses made up 42.6 %, while influenza-like viruses 57.4 % of all positively confirmed cases. Non-subtyped influenza virus type A was present in 39.3 % specimens and quite close percentages concerned subtypes A/H3N2/ – 31.0 % and A/H1N1/pdm09 – 29.8 %. In the season, three types of co-infections were registered: co-infections within influenza viruses, influenza viruses with influenza-like viruses, and co-infections within influenza-like viruses. Detailed information is presented in Table 1. Co-infection combinations encountered, especially the one combining five influenza-like viruses, were rather unprecedented and registered for the first time in Poland.

In the epidemic season 2012/2013, the dominant type of influenza virus was type A (86.8 %), while influenza virus type B was confirmed in 9.8 %. A significantly lower number of circulating influenza virus type B was noted in Poland compared with many a previous epidemic season. The same observations were made in Europe and worldwide. Explicitly fewer cases of infections caused by influenza virus type B were registered in the epidemic season 2013/2014, than in previous seasons – only 1.2 % of confirmations. Concerning influenza virus type A, 66.7 % of confirmations were A/H1N1/pdm09, whereas A/H3N2/ amounted merely to 11.7 %. Non-subtyped influenza viruses type A constituted 21.6 % of influenza type A confirmations. Among influenza-like viruses, RSV was the prevalent one (87.0 %).

In every epidemic season, infections caused by influenza virus and influenza-like viruses are registered. In the epidemic season 2013/2014, the highest number of influenza confirmations was registered in the age group 26–44 years old (22.8 %), followed by 45–64 years old (21.6 %), and the lowest percentage was in 10–14 years old (5.3 %). In case of children aged 0–4 years old, an alarming issue is that the percentage of vaccinated children has been remaining at a very low level, namely 0.6–0.9 %, throughout many epidemic seasons. Despite seasonal recommendations of Advisory Committee on Immunization Practices and many other international scientific societies, the percentage of vaccinated children in Poland remains disappointingly low (Epimeld 2014; Epperson et al. 2014; Grohskopf et al. 2014).

Judging by the epidemiological and virological indicators, the epidemic season 2013/14 was mild and only low virus activity was reported in Poland as well as in most European countries. The occurrence of influenza has been delayed in Europe in the epidemic season 2013/2014, with a different timing across European countries. In the first four affected countries (Bulgaria, Greece, Portugal, and Spain), A/H1N1/pdm09 virus dominated. The peak of incidence to influenza and influenza-like infections in Europe occurred in the third week of 2014 (13–19. 01. 2014), whereas in Poland it was in the eleventh week of 2014 (10–16. 03. 2014) (Fig. 1). At the beginning of

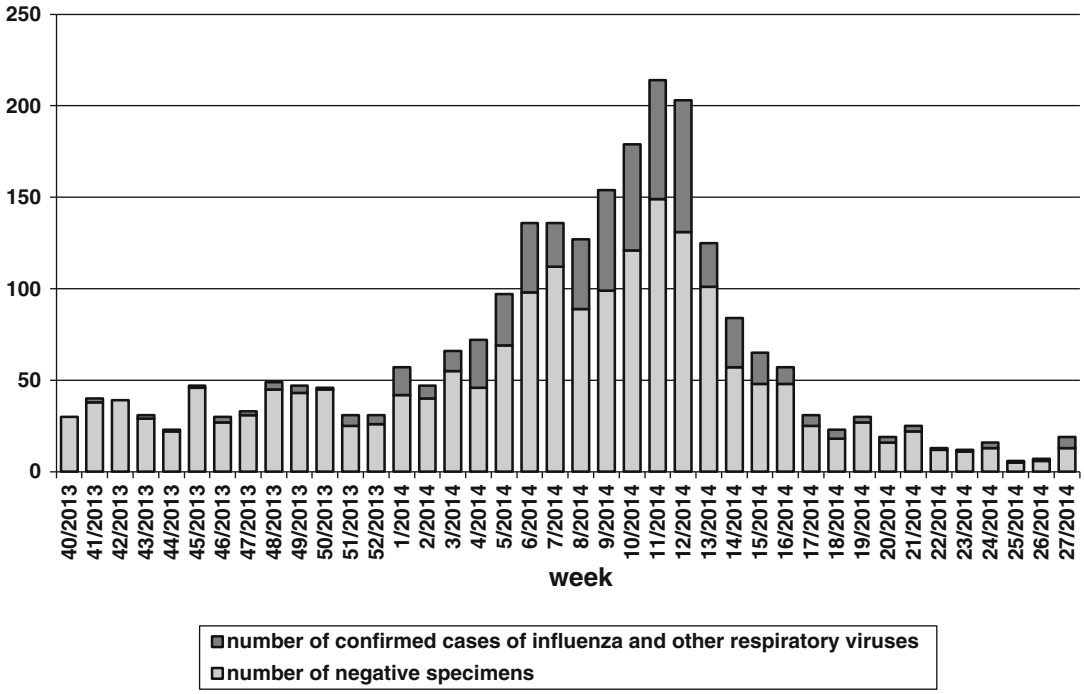

Fig. 1 Weekly number of the processed specimens and the proportion of the confirmed cases of influenza and influenza-like infections in the epidemic season 2013/2014 in Poland

the season, A/H1N1/pdm09 virus dominated, while in the last weeks of the season, A/H3N2/ virus gained prevalence.

According to the NIPH-NIH information, 9,000 hospitalizations and 17 deaths were registered in Poland in 2013/2014. However, data regarding deaths are definitely underestimated, because only 3.8 % of population was vaccinated against influenza. In Europe, 430 deaths of influenza were noted with the highest number in Spain (ECDC 2014).

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

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Necrotizing Pneumonia and Its Complications in Children

Katarzyna Krenke, Marcin Sanocki, Emilia Urbankowska, Grażyna Kraj, Marta Krawiec, Tomasz Urbankowski, Joanna Peradzyńska, and Marek Kulus

Abstract

Necrotizing pneumonia (NP) is an emerging complication of community acquired pneumonia (CAP) in children. This study aimed at the evaluation of etiology, clinical features, treatment, and prognosis of NP. The institutional database of children with CAP treated between April 2008 and July 2013 was searched to identify children with NP. Then, data on the NP characteristics were retrospectively reviewed and analyzed. We found that NP constituted 32/882 (3.7 %) of all CAPs. The median age of children with NP was 4 (range 1–10) years. The causative pathogens were identified in 12/32 children (37.5 %) with *Streptococcus pneumoniae* being the most common (6/32). All but one patient developed complications: parapneumonic effusion (PPE), pleural empyema or bronchopleural fistula (BPF), which required prompt local treatment. The median duration of hospital stay and antibiotic treatment was 26 (IQR 21–30) and 28 (IQR 22.5–32.5) days, respectively. Despite severe course of the disease no deaths occurred. A follow-up visit after 6 months revealed that none of the patients presented with any signs and symptoms which could be related to earlier pneumonia. Chest radiographs showed complete or almost complete resolution of pulmonary and pleural lesions in all patients. We conclude that necrotizing pneumonia is a relatively rare but severe form of CAP that is almost always complicated by PPE/empyema and/or BPF. It can be successfully treated with antibiotics and pleural drainage without major surgical intervention.

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Keywords
Bronchopleural fistula • Community acquired pneumonia • Empyema • Pediatrics

1 Introduction

Necrotizing pneumonia (NP) is one of the most severe complications of community acquired pneumonia (CAP) in children. Although the number of patients with NP is relatively low, an increasing incidence of this form of pneumonia has been reported in the last decades (Lemaître et al. 2013; Bender et al. 2008; Sawicki et al. 2008; Hsieh et al. 2004). The key pathologic feature of NP, i.e. lung necrosis, is caused by toxins produced by invasive bacterial strains and secondary vascular changes, including vasculitis and intravascular thrombosis (Hsieh et al. 2006). *Streptococcus pneumoniae* and *Staphylococcus aureus* are the main etiologic factors of NP in children (Lemaître et al. 2013; Sawicki et al. 2008; Ramphul et al. 2006). Albeit NP can present with highly variable clinical course, the majority of children demonstrate severe disease with high, prolonged fever, dyspnea, and clinical and radiological signs of extensive consolidation of lung parenchyma. In a high percentage of children, NP is complicated by parapneumonic effusion (PPE)/empyema, bronchopleural fistula (BPF), septicemia, and respiratory failure. Thus, the disease requires aggressive, systemic and local treatment.

Since NP is relatively uncommon, our knowledge on the entity is based mainly on case reports and small series (Hasan et al. 2011). The number of publications reporting large series is low (Sawicki et al. 2008) and only one larger European study was published to date (Lemaître et al. 2013). Therefore, we undertook this study to evaluate the etiology, clinical features, treatment strategies, and prognosis of NP in children managed in the largest referral children's pneumology center in the capital city of Warsaw, Poland.

2 Methods

The study protocol was accepted by the institutional Review Board of Medical University of Warsaw, Poland. The children with NP aged from 1 month to 18 years, treated in a tertiary referral center from April 2008 to July 2013 were included in the study. The cases of NP were selected from a specific electronic database of patients with pneumonia which has been running in our institution since 2003. Numerous clinical, laboratory, and radiological data of all patients have been prospectively collected during patient hospitalization and follow-up. To select a study group, an initial search of database was performed with the identification of all patients suspected to have NP. Then, plain chest radiographs and thorax CT scans of the selected patients were reviewed by two study clinicians (KK, MS). NP was diagnosed in case of typical radiological pattern in a patient with signs and symptoms of pneumonia. Radiographic criteria for NP were as follows: areas of parenchymal consolidation, lucencies within the area of consolidation and multiple thin-walled cavities (Hodina et al. 2002). Data on etiology (based on the results of blood and pleural fluid culture), prior medical history, clinical features (fever, tachypnea, cough, chest pain, and abdominal pain), physical examination, treatment strategies (systemic antibiotic and local treatment), and outcome were collected from electronic databases and completed from medical records, when necessary. The results of the study were summarized by standard descriptive statistics. A nonparametric Mann-Whitney U test or Fisher exact test were used to assess the difference between quantitative variables in different groups. A p-value <0.05 was regarded as significant.

3 Results

Eight hundred eighty two children with CAP were hospitalized in our institution during the study period. One hundred ninety eight (22.4 %) of them had complicated pneumonia (PPE/empyema, lung abscess, or NP). Necrotizing pneumonia was diagnosed in 32 patients and these children were selected as the study group. Thus, the rate of NP among all CAP patients requiring hospital admission was estimated as 3.7 %. The annual number of patients with NP treated in our center ranged between 4 (2010) and 11 (2009).

The study group included 18 girls and 14 boys; median age 4 years (range between 1 and 10 years). Twenty six children with NP were previously treated in other hospitals and transferred to our referral center because of disease progression or its complications. Comorbidities or underlying diseases were found in seven patients, while the remaining children were otherwise healthy. There were no children with primary or secondary immunodeficiency. Two children had viral infection prior to NP. The most common symptoms on admission were fever, tachypnea, and cough. The median duration of fever before hospital admission and during hospital treatment was 6 days (range 1–10 days) and 9 days (range 0–22 days), respectively. Detailed data on medical history and clinical presentation of NP are summarized in Table 1.

In all children, areas of lung consolidation, lucencies within consolidation areas and multiple thin–walled cavities were identified in plain chest radiograph or CT scans. In 18 and 10 children the abnormal radiographic appearance was limited to the right and left lung, respectively, while 4 patients presented with bilateral lung involvement. Pleural effusion was revealed in 31 patients and pneumothorax in 2 cases (Figs. 1 and 2).

The results of laboratory tests on admission are presented in Table 2.

There were significantly elevated acute phase reactants, with the median CRP value of 18.2 mg/dl (normal range <1 mg/dl) (IQR 15.2–24.1). The median WBC count was 21.3×10^9/L (IQR 17.7–25.2) with the polymorphonuclear cell predominance. Anemia was a common laboratory finding (median Hb concentration – 89.0 g/L, IQR 84.0–99.0 g/L). Fourteen children (43.8 %) required packed red blood cell transfusion. One child had a reduced platelet count, while the remaining children presented

Table 1 Selected clinical data on patients with NP (n = 32)

Medical history	n (%)
Viral infection prior to NP	2 (6.25 %)
Tonsillar hypertrophy	2 (6.25 %)
Asthma	2 (6.25 %)
Systemic hypertension and obesity	1 (3.13 %)
Recurrent upper respiratory tract infections	1 (3.13 %)
Congenital heart disease (atrial septal defect)	1 (3.13 %)
Clinical presentation	
Fever on admission	30 (93.75 %)
Tachypnea	28 (90.3 %)
Cough	24 (77.4 %)
Abdominal pain	14 (45.2 %)
Chest pain	11 (36.6 %)
Physical examination	
Dullness to percussion	30 (93.8 %)
Decreased breath sounds	30 (93.8 %)
Crackles	20 (62.5 %)
Bronchial breath sounds	7 (21.9 %)

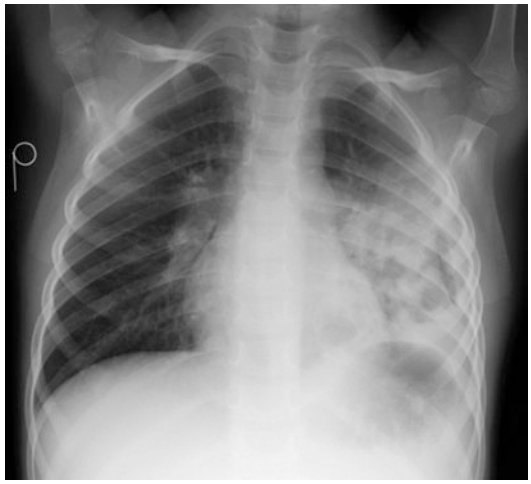

Fig. 1 Plain chest radiograph. Extensive consolidation with multiple irregular lucency areas in the middle and lower field of the left lung

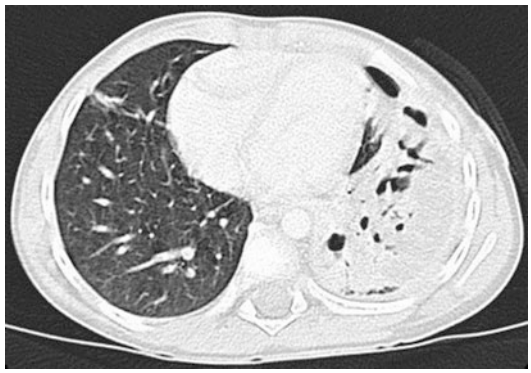

Fig. 2 Thorax computed tomography. Consolidation of the left lower lobe with multiple air filed cavities

with thrombocytosis (median platelet count 818.5 $\times$ 10^9/L, (IQR 557.3–1069.3)). There was a significant percentage of children with declined total serum protein and albumin concentration (56.3 % and 84.4 %, respectively).

All children had blood culture taken on admission. In 20 children, additional blood culture results from regional hospitals were available for analysis. Microbiological studies of pleural fluid were performed in all 31 patients presenting with pleural effusion. Positive results of blood and pleural fluid analysis were found in only 6 (18.8 %) and 7 (22.5 %) children, respectively. Ultimately, blood and pleural fluid cultures enabled identification of the pathogens responsible for NP in 12 patients (1 patient had both blood and pleural fluid cultures positive). The most commonly identified species was *Streptococcus pneumoniae* (n = 8), followed by *Staphylococcus aureus* (n = 2), *Streptococcus milleri* (n = 1), *Staphylococcus epidermidis* (n = 1) and *Stenotrophomonas maltophilia* (n = 1). All *Streptococcus pneumoniae* strains were susceptible to penicillin and *Staphylococcus aureus* strains were sensitive to methicillin (MSSA).

In all children, antibiotic therapy had been initiated before admission to the referral center. Amoxicillin, ampicillin, amoxicillin/clavulanic acid and cefuroxime were most commonly applied as the first line of treatment. There was no difference between the duration of pre-admission antibiotic therapy in children treated at home (median 3 days; IQR 1.75–4.25) and transferred from other hospitals (median 3 days; IQR 2–6). Due to prolonged symptoms, the

Table 2 Selected laboratory results in children with NP, data presented as median and IQR

Laboratory test	Result	Normal range
CRP (mg/dl)	18.2 (15.2–24.1)	<1.0
WBC ($\times$ 10^9/L)	21.3 (17.7–25.2)	4.5–13
Hemoglobin (g/dl)	8.9 (8.4–9.9)	10.9–14.2
Neutrophils (%)	70 (47.4–95.6)	Age-dependent
Platelets ($\times$ 10^9/L)	818.5 (557.3–1069.3)	150–400
Total serum protein (g/L)	53.0 (49.0–58.0)	60.0–80.0
Serum albumin (g/L)	25 (23.5–27.5)	35.0–52.0

Table 3 Complications of NP

Complications of NP	n (%)
Parapneumonic effusion/empyema	31 (96.9 %)
Bronchopulmonary fistula	8 (25 %)
Pneumothorax	2 (6.25 %)
Mortality	0 (0 %)

Table 4 Pleural fluid characteristics; data presented as median and IQR

pH	7.3 (7–7.5)
Specific gravity	1.020 (1.015–1.020)
Protein concentration (g/L)	38.0 (34.5–44.0)
Lactate dehydrogenase (U/l)	8670 (2827.5–14253.5)
Glucose (mg/dl)	50 (32–55)

initial therapy was switched to the second-line antibiotic treatment in all patients after admission to our center. The second-line therapy included cefotaxime, ceftriaxone, clindamycin, vancomycin, and carbapenems. At least a two drug-regimen was used in all patients. The median duration of antibiotic treatment was 28 days (IQR 22.5–32.5). All but one patient had NP associated complications with PPE/empyema being the most common (Table 3).

Thus, 31 children required additional local treatment (see below). The results of pleural fluid analysis are summarized in Table 4. Two children only required therapeutic thoracentesis, while in 29 (93.5 %) children the chest tube insertion and pleural drainage were necessary. Due to inadequate pleural fluid drainage one patient was subsequently treated with video- assisted thoracoscopic surgery (VATS). Intrapleural fibrynolitic treatment with urokinase was applied in 25 (78.1 %) children. The median duration of pleural drainage was 8.6 days (IQR 6–11.25, range 2–27).

In two children with PPE/pleural empyema small/medium pneumothorax (pyopneumothorax) was found. Neither of them demonstrated an air leak after the chest tube insertion and both were successfully treated with pleural drainage.

Eight children (25 %) with PPE/empyema developed signs of BPF during treatment with pleural drainage. In all these patients, spontaneous healing of BPF was achieved and none of the children required surgical treatment. The median duration of air leak was 10.5 days (IQR 7–17, range 5–20). The duration of pleural drainage in children with BPF was significantly longer than in children without BPF (16 *vs.* 7 days, $p < 0.001$).

No deaths occurred in the study group. The duration of hospital stay ranged between 13 and 44 days (median 26). All children had follow-up visits 1 and 6 months after hospital discharge. At the first visit, physical examination revealed asymmetry of the chest and decreased breath sounds in the previously affected areas in 10 (31.3 %) and 14 (45.2 %) children, respectively. Chest radiographs showed residual pulmonary and pleural lesions in all patients. Five months later, none of the children revealed any abnormality on physical examination. Moreover, in all patients chest radiograph showed complete or almost complete resolution of pulmonary and pleural lesions.

4 Discussion

Our study of 32 patients with NP showed that this entity affects mainly immunocompetent children with no underlying disorders and the most common causative organism is *Streptococcus pneumoniae*. The clinical course of NP is usually complicated by PPE/empyema, BPF, and pneumothorax. Nevertheless, NP can be successfully treated with antibiotics and pleural drainage.

Although pneumonia with associated lung necrosis was reported as early as in the nineteenth century, the first detailed description of four pediatric cases of NP caused by *Streptococcus pneumoniae* was published only 20 years ago (Kerem et al. 1994). Since then, a significant increase in the incidence of this entity has been reported (Lemaître et al. 2013; Bender et al. 2008; Sawicki et al. 2008; Hsieh et al. 2004). Bender et al. (2008) have found a more than five-fold increase in the incidence of NP caused by *Streptococcus pneumoniae* between 1997–2000 and 2000–2006. An increasing incidence (two-fold) of NP caused by *Streptococcus pneumoniae* was

also reported in a Taiwan study covering the period from 2001 to 2010 (Hsieh et al. 2011). The incidence of NP shows seasonal variations. The majority of cases in our study were diagnosed during the fall and winter seasons (15 and 12 cases, respectively). Substantial year-to-year variations in the number of new cases were also found with an unexpectedly high number of children with NP (11) in 2009. It might be speculated that this was related to influenza A [H1N1] pandemic occurring in the same period of time. A similar observation was noticed by Lemaître et al. (2013).

Streptococcus pneumoniae is regarded as the most common etiologic factor of NP in children (Tsai and Ku 2012; Ramphul et al. 2006; Hacimustafaoglu et al. 2004). Sawicki et al. (2008) have been able to establish the etiology of NP in 38 (48 %) of 80 children treated in a Children's Hospital in Boston, MA. *Streptococcus pneumoniae* was responsible for 18 (22 %) of all cases and for 47.4 % of cases with known etiology. In the present study, the causative organism was identified in 12 children (37.5 %), and pneumococcal predominance was even more significant. *Streptococcus pneumoniae* was diagnosed as the etiologic factor in more than 58 % of children with NP of known etiology.

On the other hand, the increasing incidence of NP caused by other organisms has also been demonstrated. This was the case in the study by Sawicki et al. (2008), where methicillin resistant *Staphylococcus aureus*, and Streptococcal spp. such as *S. milleri* were increasingly identified during the study period (1990–2005). In our study, *Staphylococcus aureus* was responsible for two cases of NP and both strains were sensitive to methicillin. In a recently published French study which included 41 children the etiology of NP was established in 51 % (21 cases). The most common cause of NP was *Staphylococcus aureus* (13 cases, 61.9 %). All *Staphylococcus aureus* strains encoded genes of Panton –Valentine leucocidin and all but one were sensitive to methicillin (Lemaître et al. 2013). Other organisms were also incidentally isolated from patients with NP. Those included *Pseudomonas aeruginosa*, *Fusobacterium* spp., *Streptococcus pyogenes*, *Staphylococcus epidermidis* (Lemaître et al. 2013; Sawicki et al. 2008). Among atypical bacteria, *Mycoplasma pneumoniae* seems responsible for rare cases of NP in children (Wang et al. 2004; Wong et al. 2000). Anaerobic bacteria are considered to play a synergistic role in causing NP, but this data come from adult NP cases (Tsai and Ku 2012; Palmacci et al. 2009).

Necrotizing pneumonia should be suspected in all severely ill children with prolonged fever and significantly elevated serum inflammatory markers. Imaging studies, including chest radiograph, and CT scan play a crucial role in the diagnosis of NP. This, in particular, refers to the chest CT scan, which can show necrotizing/cavitary lesions not visible in plain chest radiographs. Thus, CT scan is regarded as the most sensitive diagnostic tool in patients with NP (Tsai and Ku 2012; Sawicki et al. 2008). On the other hand, justification to perform thorax CT scan in all children with cavities in a consolidated lung, a typical appearance of NP seen in the chest radiograph, might by a matter of controversy. It seems that in the era of increasing awareness of radiographic and clinical features of NP, initial chest CT scan is not always a necessary prerequisite for the adequate treatment. This is of particular importance in view of the fact that a significant proportion of children may require subsequent thorax CT scanning due to systemic or local treatment failure. Repeated thorax CT imaging may result in increased radiation exposure.

Despite the undeniable progress in the treatment of NP, controversies regarding the most effective treatment strategies still exist. As to date, no randomized trial comparing different treatment modalities have been performed. Data on treatment efficacy come exclusively from observational studies. Due to a significant variability in the natural course of NP, including its local complications, comparison of the effectiveness of different therapeutic regimens is particularly difficult. Intravenous antibiotics are the cornerstone of the effective treatment. In nearly all cases empirical antibiotic therapy

is initially administered. The choice of antibiotics should be based on local epidemiological and microbiological data. Positive results of the microbiological studies identifying etiological factors might be expected only in 11–51 % of patients (Lemaître et al. 2013; Sawicki et al. 2008; Hacimustafaoglu et al. 2004; Wong et al. 2000). In our study, this was the case in 12 (37.5 %) of patients. These results should be used as a guide for further antibiotic treatment. Nevertheless, we did not find differences in the outcome measures in children in whom the causative pathogen was identified and those in whom it was not. Prolonged antibiotic treatment is recommended. In our study, the median duration of antibiotic therapy was 28 days. Similar duration was reported by other authors (Sawicki et al. 2008). Broad-spectrum penicillins, second or third generation of cephalosporins, clindamycin, and vancomycin have been the most commonly used.

Necrotizing pneumonia in children is associated with a very high risk of local complications. In our study, PPE, or pleural empyema were found in as many as 31/32 (97 %) of all patients. The incidence of pleural effusion reported in other studies has been as similar and ranged between 63 and 94 % (Lemaître et al. 2013; Hacimustafaoglu et al. 2004). Thus, it might be stated that pleural complications belong to the typical clinical characteristics of the disease. Treatment of NP associated pleural effusion does not differ from that presented in the guidelines on management of pleural effusion/empyema and include therapeutic thoracentesis, pleural drainage (with or without intrapleural instillation of fibrynolytic agents) and VATS (Balfour-Lynn et al. 2005). The choice between these methods is mainly based on illness severity and local anatomical conditions (pleural fluid volume, its location, the presence of adhesions, etc.). However, personal and hospital experience is an important factor affecting the management strategy. Although an early VATS is certainly justified in some patients' management, pleural drainage with chest tube (with or without intrapleural fibrinolytics) is probably a sufficient therapeutic option in the vast majority of children. This was shown in our study. Pleural drainage was applied in 28/32 (87.5 %) of our patients and it was shown to be an effective method of treatment in 27 (96.4 %) of them. None of our patients was primarily scheduled for VATS, and only one patient required surgical intervention when local treatment with pleural drainage failed. As NP is a relatively rare condition, no randomized trial comparing the treatment efficacy of pleural drainage or pleural drainage plus intrapleural fibrinolitics *vs*. VATS has been published to-date. This is not surprising in the context of only several randomized studies comparing the efficacy of these methods in all children with PPE/pleural empyema (Krenke et al. 2010). The second most common local complication of NP is BPF. The incidence of BPF has been reported between 15 and 67 % (Sawicki et al. 2008; Hacimustafaoglu et al. 2004). In our study, BPF was diagnosed in 8/32 (25 %) of children. Interestingly, pneumothorax was not revealed in the chest radiograph taken before chest tube insertion in any of these children, and in all eight patients BPF developed during pleural drainage applied to treat PPE/empyema. It should be noted that the incidence of BPF has been associated with the duration of pleural drainage (Sawicki et al. 2008). Thus, the duration of pleural drainage is a risk factor for the development of BPF and the chest tube inserted to treat PPE/empyema in children with NP should be removed as early as possible and prolonged pleural drainage in these patients should be avoided. Likewise, the present study showed that the duration of pleural drainage in children with BPF was significantly longer than that in children without BPF. The duration of air leak ranged between 5 and 20 days and the hospital stay was significantly longer in children with BPF than without it. In all our patients, BPF healed spontaneously during pleural drainage and none of these children required surgical intervention. This is consistent with the observations of other authors (Sawicki et al. 2008). It might be speculated that adequate systemic (antibiotics) and local treatment (pleural drainage) result in BPF healing and its closure.

Our analysis confirms the observations of other centers that despite severe clinical course

of NP in children, the prognosis is good. No deaths occurred in our study group. Likewise, favorable outcome has been demonstrated in three other respective large series which included 80, 41, and 21 patients (Lemaître et al. 2013; Sawicki et al. 2008; Wong et al. 2000). However, as some fatal cases have been also reported, one should be aware of the risk of death due to NP (Al-Saleh et al. 2008; Hacimustafaoglu et al. 2004). Li et al. (2011) have reviewed the factors associated with increased risk of fatal outcome in patients with community-acquired NP caused by *Staphylococcus aureus* and found that influenza-like symptoms, hemoptysis, and leucopenia are the predictors of unfavorable prognosis. As adolescent patients represented only 10 % of the study group, these findings should not be directly extrapolated to the entire pediatric population.

There are data suggesting that early surgical resection of the affected lung might be associated with less favorable outcome. In a study by Westphal et al. (2010), the mortality rate in 20 children who underwent surgical intervention was as high as 20 %. A high percentage of complications after surgery was also reported with BPF being the most common (4 children – 20 %). Further studies on the relationship between surgical lung resection and outcome in children with NP are warranted. According to some larger series, including the present study group, adequate conservative treatment results in a very low mortality rate; surgical lung resection should thus be limited to a very small number of carefully selected cases.

To conclude, the present study shows that NP affects mainly immunocompetent children with no underlying disorders and the most common causative organism is *Streptococcus pneumoniae*. Although, the clinical course of NP is usually complicated by PPE/empyema. BPF and pneumothorax, the entity and its complications can be successfully treated with antibiotics and pleural drainage without major surgical intervention.

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

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Progress in the Diagnosis and Control of Ebola Disease

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Abstract

Ebola hemorrhagic fever is one of numerous viral hemorrhagic fevers. It is a severe, often fatal disease in humans and nonhuman primates (gorillas and chimpanzees). This article discusses the history of Ebola disease, already known routes of infection together with defining prevention methods and treatment trials. The importance of increasing awareness of the risk of disease among people who do not inhabit endemic regions is emphasized. This risk is associated especially with the increasing popularity of tourism to African countries, even to those where the virus is endemic. The research conducted over the years shows that three species of frugivorous bats are subjected to contamination by Ebola, but the infection is asymptomatic in them. It is believed that the saliva of these mammals and other body fluids may be a potential source of infection for primates and humans. In the laboratory, infection through small-particle aerosols has been demonstrated in primates, and airborne spread among humans is strongly suspected, although it has not yet been conclusively demonstrated. The importance of this route of transmission remains unclear. Poor hygienic conditions can aid the spread of the virus. These observations suggest approaches to the study of routes of transmission to and among humans.

Keywords

Ebola virus • Epidemiology • Diagnosis • Prevention

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1 Introduction

Ebola virus disease is a hemorrhagic fever with a mortality of up to 90 %. It is caused by a virus of the same name, which belongs to the Filoviridae family. Classification of the risk group organisms for these viruses requires the conditions provided by the fourth grade safety laboratory (BSL-4) (Bente et al. 2009). This group includes microorganisms for which there is no effective prophylaxis or therapeutic protection in case of infection. Five types of these viruses are distinguished, named after the places where they were isolated for the first time:

- Ebola Sudan (SEBOV),
- Ebola Zaire (ZEBOV),
- Ebola Cote d'lvore (Côte d'Ivoire) (CIEBOV),
- Ebola Reston (REBOV),
- Ebola Bundibugyo (BEV), detected as the last type.

This article discusses the historical background of Ebola virus disease and the so far recognized routes of infection as well as identifies the ways of prevention and treatment attempts. The study underscores the importance of making people aware of increasing risk of disease among those who do not live in endemic regions. This risk is particularly associated with the increasing popularity of tourism to African countries, including those where Ebola virus disease is endemic.

The reports, which have been released this year, indicate another epidemic of Ebola hemorrhagic fever in West Africa. The disease has not been reported there for 10 or 15 years. The first cases of infection were observed in Guinea and in the neighbouring countries soon afterward. At least 6,263 infections and 2,917 deaths were reported until 21 September 2014. It has been the largest epidemic so far, both in terms of the number of cases and deaths. To prevent further spread of the epidemic, many humanitarian organizations as well as the American Centers for Disease Prevention and Control of Infectious Diseases (2001), the European Commission, and the Economic Community of West African States have provided financial and medical support.

2 Historical Outline

Hemorrhagic fevers probably occurred in antiquity. The Greek historian Thucydides, who lived in the years 460–396 B.C., described the illness which occurred in the Athenians and began with a sudden deterioration of health accompanied by fever with severe chills, cough, and reddening of the mouth. Additional symptoms included intense red rash progressing to blisters and ulcers (Olson et al. 1996). The disease picture closely corresponds to changes encountered in today's hemorrhagic fevers. The Ebola virus was isolated and described in 1976 during an epidemic in Southern Sudan (the villages of Nazar, Maridi, and Ambuku) and Zaire (near the Ebola River, hence the name of the virus and the disease).

3 Epidemiology

Macaques, chimpanzees, and bats are the known reservoirs of Ebola virus. Research has shown that three species of fruit-eating bats may be infected with Ebola virus, although the infection is asymptomatic in these species. It is believed that saliva or other bodily fluids of these mammals may be a potential source of infection for primates and humans. In humans, the infection spreads primarily through direct contact, digestive system, and inhalation of the air containing aerosol of virus's particles. Also, small skin lesions may be an entry way for the infection. It is believed that infection may spread during ritual funeral rites (kissing of dead bodies infected with hemorrhagic fever). Figure 1 displays geographic distribution of Ebola virus disease outbreaks in humans and animals.

4 Course of Infection

The research and observation of infected people as well as experiments in monkeys made it possible to develop a model of the infection course. The incubation period of the disease is from 2 to

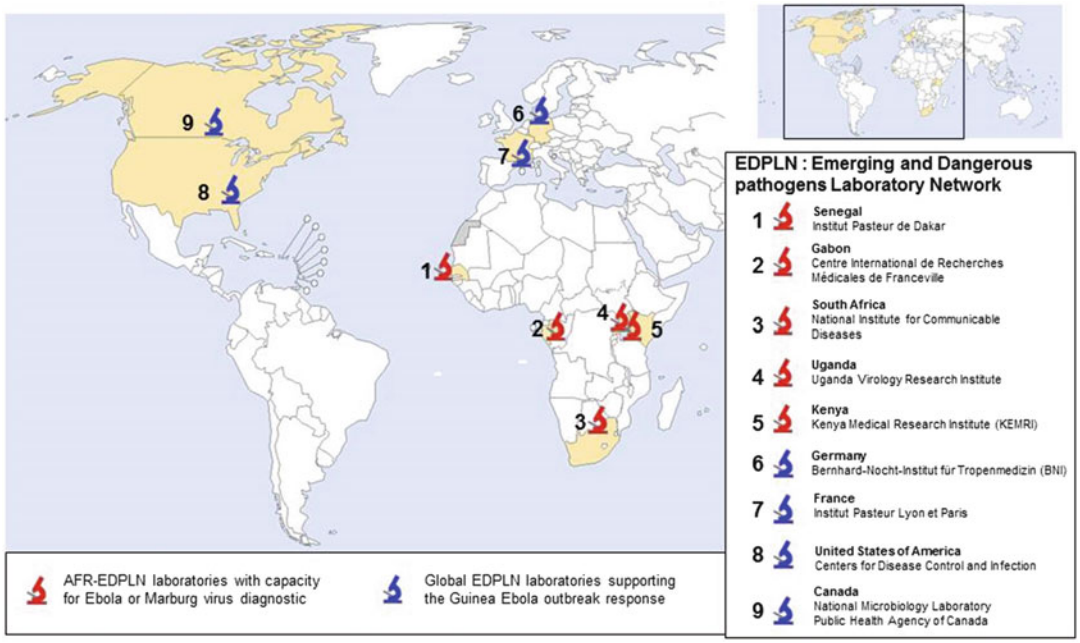

Fig. 1 Ebola virus disease in West Africa. EDPLN laboratories for Ebola or Marburg virus diagnostic. WHO, 10 April 2014 (GAR 2014; reproduced with permission)

more than 20 days. Usually, the initial symptoms of hemorrhagic fever can be confused with malaria or diseases caused by bacteria, for example with typhoid fever, shigellosis, cholera, or colibacillosis. The disease generally begins with high fever accompanied by chills, arthralgia, and muscle, abdomen, and chest pain. Nausea and loss of appetite also may appear. After about 5 days, lumpy rash appears on the skin, which transforms after 1 day into large confluent limited lesions of the spotted-follicular character. They may also assume a hemorrhagic form. As a result of distorted of blood vessels integrity, bleeding arises from the nose, vagina, gums, and bloody vomiting, hemoptysis, or tarry stools may appear. Later on, Ebola virus affects the nervous system causing headache, agitation/fatigue, confusion, or even coma. Sometimes, it is possible to reign in the further development of the disease and the healing occurs approximately 2 weeks after the first symptoms. The vast majority of patients infected with Ebola virus die on the 7–16th day of the clinically developed disease. Multiple organ failure is an immediate cause of death associated with a decrease in arterial blood pressure and progressive tissue necrosis (Feldmann and Geisbert 2011; Jaax et al. 1996).

5 Diagnostics

The properly taken medical history is the basis for diagnosis, with particular emphasis on the journeys made recently and the contact with wild animals (bats and primates). The isolation of the virus from specimens taken from the patient confirms the diagnosis.

There are several methods for detection of Ebola virus. The presence of the virus in the material is confirmed by the occurrence of the cytopathic effect in cell culture of human adrenocortical cancer. This study gives 100 % certainty of its presence. Breeding and serotyping are not widely used because of a high risk of infection of laboratory workers and the requirement of a class 4 safety laboratory (BSL4) (Gonzalez et al. 2007). Transmission electron microscopy is another method which allows for detection of viral particles in the blood and body fluids of the patient as well as of nucleocapsids

(resulting from multiplication of the virus in the cytoplasm of infected cells). The use of this method, and also of the classic breeding method (Gear et al. 1978), requires a well-equipped laboratory, which makes it hardly available in African countries where the disease is endemic. Skin immunoassay is another sensitive, specific, and safe method. It is used to test skin samples taken from the deceased persons. Viral antigens are detected in the skin using monoclonal antibodies. The test can be carried out even on the skin preserved with formalin (Bwaka et al. 1999). Other available methods include immunoenzymatic assays (ELISA) based on the detection of viral antigen and anti-EBOV antibodies. The presence of antibodies may be confirmed by indirect immunofluorescence, radioimmunoprecipitation, or western-blots (Ksiazek et al. 1992). Currently, the best way to confirm the infection is to use molecular biology techniques with a variety of modified PCR, which allows for detection of Ebola virus nucleic acid. These techniques enable to detect RNA in mononuclear blood cells of infected patients, even before symptoms arise.

6 Prevention and Treatment

Due to high infectivity of the virus and the lack of effective vaccines and drugs, it is paramount that the principles of non-specific prevention be applied, i.e. isolation of patients, blocking the roads of the infection spread, equipment sterilization, the absolute use of goggles, gloves, aprons, and masks with HEPA filters.

There has so far been no known effective treatment for hemorrhagic fever caused by Ebola virus. Symptomatic treatment includes the use of analgesics and antipyretics. The loss of fluids, electrolytes, and blood and plasma clotting factors is supplemented in the event of bleeding diathesis. In case of severe infection, all the above described methods remain, unfortunately, little or no effective and may at best delay death for a few days (Ksiazek et al. 1999; Zaki et al. 1999). Experimental trials have included treatments with convalescent serum and human interferon in combination with IgG taken from a horse, goat, or hyperimmune sheep. These methods tested in various animals have turned out effective only for guinea pigs and mice (CDC 2001; Zaki et al. 1999). It is difficult to determine the effectiveness of these methods in human therapy, because promising results have been obtained only in a small number of patients. Recent studies have reported the ability of cyanovirins to slow down the development of viruses. Cyanovirins connect with the cell membrane and inhibit the viral entry into cells (WHO 1978). Another promising method of therapy appears to be the use of siRNA (small interfering RNA), which is responsible for silencing the activity of RNA polymerase. However, the beneficial therapeutic effect has been obtained only in non-primate mammals (Mupapa et al. 1999).

7 Experimental Vaccines

The following types of vaccines were experimentally tested in animals:

- vaccine containing a bare DNA strand with the genes encoding the viral proteins NP, GP, and sGP cloned on plasmids (Jahrling et al. 1999);
- vaccine made on the basis of various other viruses containing the genes encoding the EBOV protein. Here, vaccine production employed adenoviruses, attenuated equine encephalitis virus, and vaccinia virus (Kudoyarova-Zubavichene et al. 1999). To this end, an interesting approach has recently been reported using recombinants of human parainfluenza virus type 3 expressing the Ebola virus proteins GP alone or in combination with NP, which were inoculated *via* the intranasal or tracheal route in rhesus monkeys. This kind of topical application of vaccine, particularly when applied twice, appeared highly immunogenic and protective against intraperitoneal lethal challenge with Ebola virus (Bukreyev et al. 2007);
- subunit vaccine which contains the Ebola virus proteins VP24, VP30, VP35, VP40 (Barrientos et al. 2003);

- vaccine containing the Ebola virus inactivated with formalin;
- vaccine containing liposomes with the Ebola virus inactivated by irradiation. Here, the presence of lipid A liposomes would be of great importance in the formation of permanent immunity (Feldmann 2001).

8 Routes of Infection

The Ebola virus is extremely virulent and contagious. Apart from obvious direct inoculation, like monkey's bite or contact with body fluids and excretions of infected persons, experimental studies confirmed that transmission of Ebola infection may take place *via* oral or conjunctival routes (Jaax et al. 1996). Aerosol droplets have been less certain as a possible route of infection transmission. Recently, however, transmission of Ebola virus has also been validated for the aerosol (Johnson et al. 1995). Airborne droplets carrying viruses can easily gain access to mucosal membranes of conjunctivas or upper and lower respiratory tract. Therefore, it is highly probably that the epithelial layer of the respiratory tract may also be the entry way for the Ebola virus. The pulmonary route of infection has since long been pondered due to reports of catching the infection 'at a distance', without having direct contact. Jaax et al. (1995) have reported unexpected infection and death of two rhesus monkeys housed about three meters away from monkeys infected with Ebola virus. This kind of infection was substantiated in later studies in which the infection was transmitted from purposely inoculated pigs to macaques that were moved to the pig pen, even though the two species remained physically separated (Weingartl et al. 2012 and Weingartl 2011). Likewise, Kobinger et al. (2011) have reported virus transmission from infected to naive pigs, when the latter were put into the same living space, albeit remained physically separated. Further, the predominant feature of the infection in both reports was the involvement of the respiratory tract, ending up with fulminant interstitial pneumonia. Such observations give credence to the possibility of airborne transmission of the Ebola virus. Such transmission has recently been verified by Reed et al. (2011) who infected monkeys with Zaire Ebola virus by inhalation. The animals died and the courses of infection after inhalational and parenteral inoculation of the virus were alike. It seems that Ebola virus is an omnipotent virus capable of initiating infection through a variety of entry ways into the susceptible host and a variety of cell types. The Ebola virus, as any other virus, is totally dependent on living cells and only can it replicate in such cells. Thus, the skin which is covered the stratum corneum consisting of a layer of dead cells filled with keratin constitutes seemingly an impenetrable barrier for the virus. That may be illusory, since all too often microabrasions, invisible by naked eye, make corneocytes pervious to viruses. The extremely high risk level of infection with Ebola virus is a compelling reason to use the most elaborate protection gear, particularly in the health care setting.

9 Summary

Despite advances in the diagnosis and control of Ebola virus disease, there is still a constant danger of accidental dragging of the condition to developed countries or its use in a bioterrorism attack. Therefore, further tests are carried out which aim at understanding the construction of the virus and especially proteins, which are likely to be chief targets of the action of potential drugs. Although the knowledge of diagnostics and immunology of the disease has also been expanded, much remains to be explained. Despite achievements, medicine has no effective remedies and in any event the threat of new infections and epidemics cannot be underestimated as evidenced by the 2014 epidemic of Ebola virus disease in several West African countries.

Conflicts of Interest Authors declare no conflicts of interests in relation to this article.

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Pathophysiology of Clinical Symptoms in Acute Viral Respiratory Tract Infections

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Abstract

In this article we discuss the pathophysiology of common symptoms of acute viral respiratory infections (e.g., sneezing, nasal discharge, sore throat, cough, muscle pains, malaise, and mood changes). Since clinical symptoms are not sufficient to determine the etiology of viral respiratory tract infections, we believe that the host defense mechanisms are critical for the symptomatology. Consequently, this review of literature is focused on the pathophysiology of respiratory symptoms regardless of their etiology. We assume that despite a high prevalence of symptoms of respiratory infection, their pathogenesis is not widely known. A better understanding of the symptoms' pathogenesis could improve the quality of care for patients with respiratory tract infections.

Keywords

Common cold • Coronavirus • Flu • Influenza • Rhinovirus • RSV

1 Introduction

1.1 Respiratory Tract Infections

The literature reports that each year up to 25 million people in the US visit their doctor because of respiratory tract infections. The upper respiratory tract infections, better known as common colds, are the most common clinical presentation of said infections (Gonzales et al. 2001). Viral lower respiratory tract infections, bronchitis, bronchiolitis, and pneumonia; e.g., with respiratory syncytial virus (RSV) or influenza are generally more common in infants, young children, and in patients with chronic medical conditions, whereas older children and healthy adults usually suffer from

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milder upper respiratory tract infections (Eccles 2005). Common cold was the third most common diagnosis made during ambulatory care visits by patients of all ages in the US (Hsiao et al. 2010). It has been estimated that an average adult suffers from 2 to 4 colds per year and a schoolchild suffers from 6 to 10 colds per year (Spector 1995; Johnston and Holgate 1996; Winther et al. 1998; Eccles 2005). Therefore, respiratory tract infections represent a significant clinical and therapeutic problem, and an economic burden (Wat 2004). Upper respiratory tract infections are generally mild, self-limiting, and viral in their origin (Johnston and Holgate 1996; Snow et al. 2001; Turner 2011; Kennedy et al. 2012). In experimental studies, the infections have been defined as a short mild illness, with the early symptoms being headaches, chills, sneezing, and a sore throat, and delayed symptoms being nasal obstruction or discharge, cough, and malaise (Jackson et al. 1958). The duration of symptoms varies from 7 to 10 days, with a peak occurring on the 2–3rd day. However, some symptoms may be observed up to 3 weeks after the onset of the infection (Heikkinen and Järvinen 2003; Eccles 2005). Common colds are triggered by rhinovirus (RV) coronavirus, influenza and parainfluenza viruses, and RSV (Wat 2004; Eccles 2005; Kennedy et al. 2012). The diagnosis of upper respiratory tract infections is based on symptoms and, with the exception of antivirals which may be used in influenza, treatment remains symptomatic. Studies on different viruses responsible for the upper respiratory tract infections have shown that it is not possible to identify the virus based on the symptoms (Johnston and Holgate 1996; Eccles 2002). Not only is the clinical presentation of these infections similar regardless of their etiology, but the order in which the symptoms develop is also similar. If the etiology of infections cannot be determined on the basis of clinical symptoms, the host reaction must play a major role in their pathogenesis. The similarities in the clinical presentation of viral upper respiratory tract infections stems from the same immune response pattern to different etiologic agents (Eccles 2005). Furthermore, in acute respiratory tract infections a routine diagnosis to determine the etiology is not usually carried out in primary care settings.

Advances in molecular biology help explain the mechanisms that generate the symptoms of viral respiratory tract infections. Nevertheless, the practical use of the pathophysiology of common symptoms seems relatively poor compared with the amount of scientific knowledge available.

1.2 Symptomatology of Respiratory Tract Infections

Clinical manifestations of respiratory tract infections are familiar and well-known (Eccles 2005; Turner 2011). Although the symptomatology depends, to some extent, on the type and location (e.g., pharyngitis, rhinitis, sinusitis, and bronchitis), the etiology of respiratory infection (viral or bacterial), patient's age, general health, co-morbidities, immunity, and on whether the infection is primary or secondary, e.g., in RSV or influenza there is a great amount of variation and overlap in both etiology and symptoms of individual infections. Consequently, even defining the exact syndrome, like common cold or influenza-like morbidity, is difficult and problematic (Eccles 2005).

The most significant signs of viral respiratory tract infections include sneezing, rhinorrhea (runny nose and nasal discharge), nasal congestion, cough, tachypnea, and fever. Subjective symptoms include a sore throat, malaise, shivering (chills), shortness of breath, muscle aches and weakness, fatigue, and a loss of appetite and headaches (Snow et al. 2001; Wat 2004; Eccles 2005; Kennedy et al. 2012). Febrile seizures are a rare but important symptom in young children up to 6 years of age (Schuchmann et al. 2011). Symptoms of upper respiratory tract infections have been traditionally classified as early or late (Jackson et al. 1958; Eccles 2005). The early symptoms are those that develop quickly and resolve rapidly after 1–2 days, like headaches, sneezing, chills, sore throat, and malaise. In children a high fever may be observed, complicated by seizures in some cases (Monto et al. 2000; Schuchmann et al. 2011). Late symptoms include nasal discharge, nasal obstruction, and cough. The later symptoms develop over several days and are present one week after

experimental infection (Jackson et al. 1958; Eccles 2005). The development of sneezing before coughing in patients with a common cold may be partly explained by the involvement of the upper airways first and the infection subsequent spread to the lower respiratory tract (Eccles 2005).

The aim of this review is to discuss the pathophysiology of symptoms of respiratory tract infections. We focused on the most significant symptoms of acute viral respiratory infections: sneezing, nasal discharge and obstruction, sore throat, coughing, muscle pains, malaise and mood changes, fever, and febrile seizures in children. We believe that despite a high prevalence of the symptoms, their pathogenesis is not widely known and a better understanding should have a beneficial effect on the therapeutic approach and should improve the quality of patient care.

2 Methods of Literature Selection

We searched the literature to identify relevant data. Medline, Scopus, Web of Science, Cochrane databases and respiratory-specific journals such as Respiratory Physiology & Neurobiology, American Journal of Respiratory and Critical Care Medicine, Thorax, Chest, Journal of Allergy, Asthma and Clinical Immunology, European Respiratory Journal, American Journal of Respiratory Cell and Molecular Biology, American Journal of Physiology – Lung Cellular and Molecular Physiology, BMC Pulmonary Medicine, Respiratory Research, Current Opinion in Pulmonary Medicine, Expert Review of Respiratory Medicine, Respiratory Research, Seminars in Respiratory, Critical Care Medicine, and Lancet Infectious Diseases were searched with the following key words: (Pathophys* or Pathogen*) and (Resp* symptom or Resp* infection or Common cold) from January 1990 through May 2014. If possible the results of the search were sorted according to 'relevance'. Due to a large number of articles, exceeding 20,000 (e.g., PubMed), only the first 250 were analyzed. References from relevant articles were also identified. Studies were included if they met the following two criteria: published in English and containing valid, consistent, and relevant data. The first two authors of the present review independently assessed all titles and abstracts to determine whether the inclusion criteria were satisfied. If either of the assessors considered the abstract potentially suitable, full-text articles were retrieved and then assessed by both assessors for their suitability for inclusion. Eventually, 129 publications were selected and studied by each of the authors before the manuscript was prepared.

3 Results

3.1 Significant Etiologic Viral Agents of Upper Respiratory Tract Infections

3.1.1 Rhinoviruses

Rhinoviruses are the most common etiologic agents of common cold. They are responsible for one-third to half of all upper respiratory tract infections reported in adults annually (Proud et al. 1990; Hendley 1998; Heikkinen and Järvinen 2003; Ruuskanen et al. 2013). Improved knowledge about the structure and function of rhinoviruses has been acquired in recent years using virus culture techniques and new molecular genetics methods available. Currently, more than 100 serotypes have been identified with HRV-A and HRV-B being the most important of them all (Heymann et al. 2005; Peltola et al. 2008; Bochkov et al. 2011; Kennedy et al. 2012). It has been demonstrated that the pathomechanism of symptoms in rhinoviral respiratory tract infections does not result from the cell damage, unlike influenza virus or RSV action (Winther et al. 1990). Rather, rhinovirus disrupts the tight junctions of the epithelial barrier, which facilitates the translocation of pathogens and stimulates the host's innate and adaptive immune responses (Rezaee et al. 2011; Kennedy et al. 2012). Over 90 % of rhinovirus serotypes enter the nasal epithelial cells through the host receptor with the intercellular adhesion molecule-1 (ICAM-1) being a glycoprotein immunoglobulin expressed on non-ciliated epithelial cells and on the basal surface of the

ciliated epithelium of nasopharyngeal mucosa, while only around ten rhinovirus serotypes use a minor group of receptors, low-density lipoprotein (LDL) (Bardin et al. 1994; Winther et al. 1997; Whiteman et al. 2003; Bella and Rossmann 2000; Vlasak et al. 2005; Kennedy et al. 2012). Newly discovered and sequenced human rhinovirus-C (HRV-C) is somehow unique as the virus isolates do not grow in typically used cell cultures, e.g., embryonic lung fibroblasts. *In vitro* growth of HRV-C was successfully performed using the sinus mucosal tissue as a substrate (Bochkov et al. 2011; Kennedy et al. 2012). Furthermore, studies on the structure and function of HRV-C have shown that the virus enters the cells using neither the ICAM-1 nor LDL receptor and the pathomechanism of the HRV-C infection remains unclear (Arden and Mackay 2010; Simmonds et al. 2010; Bochkov and Gern 2012; Lee et al. 2012; Ruuskanen et al. 2013).

In otherwise healthy individuals, rhinovirus infections are generally limited to the upper respiratory tract with rhinorrhea and nasal obstruction being the most prominent symptoms (Kennedy et al. 2012). Entry of the rhinovirus triggers the release of interleukin-8 (IL-8) a major cytokine in the recruitment of polymorphonuclear cells (Hendley 1998). IL-8, which is produced locally, increases the production of nasal secretions and causes an influx of neutrophils (Douglass et al. 1994). Oxidative stress caused by viral infection is probably responsible for the cellular mechanisms that lead to the production and release of IL-8 (Zhu et al. 1997). Studies of volunteers infected with a rhinovirus show a local infection in a small proportion (1–2 %) of nasopharyngeal epithelial cells (Turner et al. 1982; Bardin et al. 1994; Arruda et al. 1995). The higher the nasopharyngeal viral load the more severe is the disease (Esposito et al. 2014). Vasoactive kinin peptides are other important mediators produced on-site in nasal mucosa and submucosa in rhinovirus-infected humans. Kinins released in the nose following plasma exudation increase the symptoms of rhinoviral infection and cause an increase in vascular permeability, vasodilatation, and glandular secretion. Bradykinin applied to the nasal mucosa causes symptoms that mimic the common cold, including rhinitis, nasal obstruction, and sore throat (Proud et al. 1988, 1990). Symptom scores correlate with an increase in the kinin concentration. While nasal secretion in adults with symptomatic experimentally-induced rhinovirus infection contain significantly higher concentrations of bradykinin and lysyl-bradykinin, asymptomatic infections do not result in increased kinin concentrations (Naclerio et al. 1988). Interestingly, the presence of rhinovirus has been detected by RT-PCR in 20 % of asymptomatic people who had an infected family member (Jartti et al. 2008).

3.1.2 Coronaviruses

The coronavirus (CoV) is the second etiologic agent of upper respiratory tract infections (Eccles 2005; Mesel-Lemoine et al. 2012). The most common human-infecting coronaviruses include 229E, OC43, SARS-CoV, and the recently discovered NL63 and HKU1 (Arden et al. 2005; Esper et al. 2005; Vabret et al. 2005; Pyrc et al. 2007). The virus is transmitted by aerosol inhalation and reinfections often occur due to a short-lived immunity (Callow et al. 1990; Wat 2004). As a result, coronavirus accounts for 15–30 % of upper respiratory tract infections in humans. These infections are limited predominantly to the upper respiratory tract and rarely spread to lower airways and lungs (Mesel-Lemoine et al. 2012). The coronavirus infection can occasionally involve other organs (Arbour et al. 2000; Collins 2002; Desforges et al. 2006). It has been reported that the two new members of the coronavirus family, NL63 and HKU1, especially the NL63, could also trigger severe lower respiratory tract infections and abdominal disorders such as pain and diarrhea (Arden et al. 2005; Esper et al. 2005; Vabret et al. 2005; Pyrc et al. 2007). Epidemics caused by CoV-NL63 and CoV-HKU1 have been observed every 2–3 years (Kahn 2006; Jartti et al. 2012). Studies have confirmed that the infection with CoV-NL63 is associated with croup and acute respiratory disease mostly in children, the elderly, and patients with chronic diseases, with some fatal cases being reported (van der Hoek et al. 2005; Han et al. 2007; Wu

et al. 2008; Oosterhof et al. 2010; Sung et al. 2010; Milewska et al. 2013). The growth of coronaviruses using standard tissue cultures is poor and advanced molecular methods are needed to isolate the virus, so that most infections may remain undiagnosed (Walsh et al. 2013). Human aminopeptidase N (hAPN), a zinc-binding protein with endopeptidase activity, is used by CoV-229E for entry into the epithelial cells, whereas CoV-OC43 uses hemagglutinin-esterase (HE) and spike (S) – its own surface glycoproteins – to enter the cell (Tyrrell et al. 1993; Künkel and Herrler 1996; Wat 2004). Recent studies have shown the ability of CoV-229E to destroy the dendritic cells, which are essential components of the respiratory tract's immune system, which may explain multiple reinfections with the same type of CoV (Mesel-Lemoine et al. 2012). Although the pathogenesis of infection caused by the two main groups of CoV – 229E and OC43 – is different, the clinical symptomatology is similar (Tyrrell et al. 1993; Wat 2004).

3.1.3 Respiratory Syncytial Virus

Respiratory syncytial virus (RSV) is responsible for many flu-like illnesses (Zambon et al. 2001). RSV replication starts in the nasopharynx and then the virus infects the bronchiolar epithelium presumably by cell-to-cell spread or aspiration of secretions. The virus spares the basal cells and subsequently extends to the alveolar pneumocytes. The pathologic findings of RSV are characterized by necrosis of epithelial cells, infiltration with T cells and monocytes around arterioles and with neutrophils between the vascular structures and small airways (Johnson et al. 2007). This leads to airway obstruction, air trapping, increased airway resistance, and is also associated with neutrophilia in bronchoalveolar lavage (Everard et al. 1994). RSV has never been isolated from blood (Peebles and Graham 2005). The immune response to RSV, especially cytokines and chemokines, seems to be responsible for the symptoms and severity of bronchiolitis (Garofalo et al. 2001; Legg et al. 2003). The cytokines IL-8, IL-6, TNF-alpha, and IL-1 beta were detected in respiratory secretions from infected children and high IL-6 concentrations are associated with severe manifestations of the disease (Matsuda et al. 1995; Noah et al. 1995; Smyth et al. 1997). Respiratory secretions from infected children contain chemokines expressed and secreted by T-cells (chemokine ligand 3 – CCL3, i.e. macrophage inflammatory protein-1 – MIP-1 alpha; chemokine ligand 2 – CCL2, i.e. monocyte chemoattractant protein-1 – MCP-1; chemokine ligand 11 – CCL11 – eotaxin, and chemokine ligand 5 – CCL5, i.e. RANTES; regulated on activation). MIP-1 alpha, and to a lesser extent other beta-chemokines, primarily secreted by activated immune cells, are associated with severe manifestations of the disease (Noah et al. 1995; Welliver et al. 2002; Garofalo et al. 2005). Experimental infection of explanted polarized respiratory epithelium in tissue culture generates IL-8 and CCL5 (Mellow et al. 2004). Nonetheless, it is unknown whether the cytokines and chemokines are the cause of disease or are by-products of enhanced inflammatory responses (Barr and Graham 2014).

3.1.4 Influenza Viruses

Whether respiratory tract infections caused by influenza viruses present as common colds with typical symptoms or as severe lower respiratory tract diseases depends on the type of virus, pre-existing immunity, the patient's underlying disorders, and multiple other factors (Wat 2004). The phenomena such as antigenic shift or drift have led to the formation of more recent and increasingly virulent variations of the influenza virus and, consequently, to more serious clinical manifestations (Gething et al. 1980; Treanor 2004). As an example, pandemic influenza A (H1N1)pdm09 affected all age groups, but it was more prevalent in younger patients and children in whom there was the highest rate of hospitalization and pneumonia (Kuchar et al. 2013). It has previously been shown that coughing and fever are the best predictive factors of influenza infections having a positive predictive value (PPV) of 79 % (Monto et al. 2000). However, neither symptom is sufficiently predictive in

children aged 1–4 (Ohmit and Monto 2006). Overall, influenza viruses are generally responsible for 5–15 % of acute upper respiratory tract infections in humans (Wat 2004). The influenza virus causes damage to the epithelial cells and its replication occurs in the airways with predilection to the lower respiratory tract (Hers and Mulder 1961; Hers 1966; Wat 2004). The two main glycoproteins of the influenza virus surface – hemagglutinin (HA) and neuraminidase (NA) – play an essential role in the infection spread. HA targets cells for infection binding to the epithelial sialylated glycans (specific for upper or lower airways) (Shinya et al. 2006; de Wit et al. 2010; Fukuyama and Kawaoka 2011), while NA is responsible for effective viral replication (Pappas et al. 2008). Another viral protein, nonstructural protein 1 (NS1) is also important due to its counteracting IFN-α production in infected cells (Fukuyama and Kawaoka 2011). Viral replication is possible in host cells due to activation of nuclear factor kappa B (NF-κB) and the Raf/MEK/ERK cascade, and then proinflammatory cytokines are produced with interleukin 6 (IL-6) being the most important of them (Kaiser et al. 2001; Pinto et al. 2011; Wine and Alper 2012). IL-6, tumor necrosis factor–α (TNF-α), interferon-α (IFN-α), IL-8, and IL-1β increase significantly in response to the viral invasion resulting in the development of fever, nasopharyngeal mucous production, and respiratory and systemic symptoms. Viral replication and the intensity of the main influenza symptoms are correlated with the level of cytokines, particularly with IL-6 and TNF-α (Hayden et al. 1998; Kaiser et al. 2001).

4 Pathophysiology of Common Respiratory Signs and Symptoms

Evidence presented in our review of the pathophysiology of signs and symptoms in the four most common viral upper respiratory tract infections suggest that the immune system's response to infection rather than the virus-specific damage to the respiratory tract is responsible for the symptomatology (Turner 1997; Hendley 1998; Eccles 2005). Studies on different viruses responsible for upper respiratory tract infections have shown that it is not possible to identify the virus based on the symptoms (Eccles 2005). The pathology of rhinovirus infections consists of the influx of polymorphonuclear leukocytes at the beginning of the infection (Winther et al. 1984). Macrophages play a key role in triggering an acute phase response with the production of cytokines (Beutler 2003), while the release of proinflammatory cytokines and other mediators cause upper respiratory tract infection symptoms (Eccles 2000a, b). Cytokines are responsible for the systemic symptoms (e.g., fever) and bradykinin plays a major role in local symptoms of respiratory tract infections (e.g., sore throat and nasal congestion) (Proud et al. 1988; Shibayama et al. 1996; Conti et al. 2004).

4.1 Sore Throat

A sore throat, irritation, and pain in the pharynx usually appear at the beginning of a respiratory tract infection. A sore throat is most likely caused by the action of prostaglandins and bradykinin on sensory nerve endings in the upper respiratory tract. Intranasal administration of bradykinin causes symptoms of rhinitis and a sore throat, so it is likely to be responsible for these symptoms (Rees and Eccles 1994; Proud 1998). The sensation of pain is mediated by the cranial nerves supplying the nasopharynx. Similar symptoms have been observed in bacterial upper respiratory tract infections, pharyngitis, and tonsillitis (Georgitis 1993).

4.2 Nasal Congestion

Nasal congestion is a subsequent symptom of respiratory infection that develops over the first week of symptoms (Tyrrell et al. 1993). The mechanism of nasal congestion relies on the dilation of the venous sinuses in the nasal epithelium in response to the vasodilator mediators such as

bradykinin (Proud et al. 1990; Widdicombe 1997). Symptom scores increase as kinin concentrations rise (Proud et al. 1990). Dilatation of the sinuses in the narrow nasal valve region causes obstruction of the nasal airway (Eccles 2000b). The so-called nasal cycle (alternating congestion and decongestion of the nasal passages controlled by the sympathetic vasoconstrictor nerves) is accentuated and the asymmetry of nasal airflow is more pronounced during respiratory infection (Eccles et al. 1996).

4.3 Rhinorrhea

A watery nasal secretion often accompanied by sneezing is an early symptom of a respiratory tract infection. Nasal discharge in respiratory infections is a complex mixture of plasma and glandular exudates with cellular elements (e.g., goblet cells, plasma cells, and neutrophils) of variable composition that changes over the course of the infection and severity of the inflammatory response (Eccles 1983). The first phase of nasal discharge consists of a glandular secretion reflex caused by stimulation of the upper airway's trigeminal nerves. Studies have demonstrated that intranasal administration of ipratropium inhibits nasal secretions in the first 4 days of a common cold (e.g., when it is caused by coronavirus) (Akerlund et al. 1993; Hayden et al. 1996). The color of the nasal discharge may change from watery clear to yellow and green during the course of the respiratory tract infection and this reflects the severity of the inflammatory response rather than the etiology of the infection (Stockley et al. 2001). The green or yellow color of nasal discharge is often regarded as a clinical marker of bacterial superinfection and clinical indication to antibiotic treatment, but there is no evidence that supports this concept (Murray et al. 2000). The color change is related to the recruitment of leukocytes into the airway lumen (Stockley et al. 2001). Neutrophils and activated monocytes contain chromatic, green granules (azurophil granules) containing myeloperoxidase with heme pigment. The more leukocytes present in nasal discharge the more colorful the nasal discharge appears (Stockley et al. 2001). Although the literature is related to sputum color changes, the same mechanisms apply to nasal discharge.

4.4 Sneezing

A watery nasal secretion in the infection's early stage is often accompanied by sneezing. Sneezing together with a sore throat are the early symptoms of respiratory tract infections. Sneezing is a reflex mediated by the trigeminal nerves which supply the nasal epithelium (Leung and Robson 1994; Eccles 2005). The sneeze center in the brainstem coordinates the sneeze reflex. Sneezing is related to inflammatory responses in the nose and nasopharynx that stimulate the trigeminal nerves (Eccles 2005). As intranasal administration of histamine causes sneezing, sneezing is probably mediated by histamine receptors on the trigeminal nerves (Mygind et al. 1983; Eccles 2005).

4.5 Cough

Coughing is the most common clinical symptom and the most frequent reason for visits to see a doctor (McGarvey and Morice 2006). Coughing is a protective reflex that prevents the aspiration of food and fluids into the airway and cleans the respiratory tract of mucus and other foreign bodies. The reflex is mediated exclusively by the vagus nerve (Eccles 2005). Coughing is initiated in the airway through stimulation of the sensory nerves in the larynx or below (Widdicombe 1995). The airway inflammation associated with rhinitis must reach the larynx to cause coughing. The external ear and esophagus are also supplied by the vagus nerve and coughing can also be triggered by gastroesophageal reflux (Morice 2002). Respiratory tract infections are often accompanied by redundant, dry, and unproductive coughing in the first days. The unproductive coughing may be caused by the inflammatory process spreading to the larynx since nasal inflammation causes sneezing rather than

coughing. Coughing in respiratory tract infections is believed to be mediated by hyperreactivity of the cough reflex due to the effects of inflammatory mediators on the airway's sensory nerve endings (Lee et al. 2002; Eccles and Lee 2004). When the larynx is inflamed and hyperreactive, coughing may occur spontaneously or in response to stimuli that would not normally cause coughing, e.g., cold air. It may persist for three weeks or longer. Some coughs may be voluntary and related to airway irritation (Lee et al. 2002). Productive coughing usually occurs later in the course of respiratory tract infection and is related to the mucus production associated with inflammation of the lower airways (Eccles 2005). Rhinovirus and coronavirus do not usually cause significant damage to the airway cells and the common cold is typically associated with little, if any, coughing while the influenza virus may cause substantial cellular damage to the respiratory epithelium and an influenza infection is usually associated with coughing (Monto et al. 2000).

4.6 Malaise and Mood Changes

Respiratory tract infections are associated with impaired psychomotor function (Smith et al. 1998). Mood changes and malaise may be explained by the unpleasant objective symptoms of respiratory tract infections such as nasal congestion, rhinorrhea, and coughing (Eccles 2005). These symptoms may cause discomfort and lower the patient's quality of life. However, there is increasing evidence that mood changes may also be caused by the effects of cytokines on the central nervous system (Mahoney and Ball 2002). Interferon alpha treatment for chronic hepatitis B and C is associated with flu-like adverse effects similar to those observed in respiratory tract infections: malaise, fever, myalgia, and mood changes (Schaefer et al. 2002). Psychiatric adverse effects such as depression, irritability, impaired concentration, and psychoses have been reported with interferon alpha therapy. It has been reported that cytokines, e.g., tumor necrosis factor-α (TNF-α) and interleukins 1, 2, and 6 cause mood changes with anhedonia, cognitive dysfunction, anxiety, irritability, psychomotor slowing, fatigue, anorexia, sleep alterations, and a lower pain threshold (Capuron and Miller 2004). The production of these cytokines is also associated with respiratory tract infections which may mediate mood changes associated with these infections. The exact mechanisms of cytokine action in the brain are poorly understood, but there is a growing body of evidence suggesting that anorexia associated with respiratory infections is mediated by cytokines that act directly on the feeding center in the hypothalamus (Langhans 2000).

4.7 Headache

Headaches are a common early symptom of respiratory tract infections. The majority (60 %) of patients with respiratory tract infections with a sore throat reported headaches in a clinical trial (Eccles et al. 2003). The mechanism of a headache associated with a respiratory tract infection is unknown but headaches may be triggered by cytokines released in response to a viral infection (Smith 1992). It has been shown that the administration of some cytokines such as tumor necrosis factor and interferons cause headaches (Smith 1992; Gold et al. 2005; van Zonneveld et al. 2005).

4.8 Muscle Pain

Myalgia is a common symptom of respiratory tract infections. Around half of patients with a common cold complain about muscle pain (Eccles et al. 2003; Eccles 2005). Myalgia occurs in the acute immune response to infection phase and is related to the effects of cytokines on skeletal muscles (Baracos et al. 1983). Proinflammatory cytokines including TNF-α have been implicated in the breakdown of muscle proteins (Kotler 2000). Fever and myalgia associated with respiratory tract infection may be caused by the production of prostaglandin E2 in response to cytokines (Baracos

et al. 1983). The cytokine-related synthesis of prostaglandin E2 and the breakdown of skeletal muscle has been inhibited *in vitro* by non-steroidal anti-inflammatory agents and similarly fever and myalgia accompanying acute respiratory infection are relieved with acetylsalicylic acid, a classic anti-inflammatory agent (Eccles et al. 2003). Since prostaglandin E2 is a pain mediator, its increased synthesis may explain the myalgia associated with acute respiratory tract infections.

4.9 Fever and Chills

Fever is a classic response to infection. It is a manifestation of cytokine release in response to a variety of stimuli. It is believed to be beneficial for the host's response to infection (Cabanac 1990; Eccles 2005) and is usually associated with novel or severe viral infections such as influenza (Monto et al. 2000). Consequently, fever is a common symptom in infants, probably because viruses responsible for acute respiratory tract infections are new to the infant and induce a strong immune response. However, in adults who had been exposed to numerous common cold viruses in the past, subsequent infections do not elicit a strong cytokine response and fever is a rare symptom of a common cold in adults (Eccles 2005). On the contrary, some patients experience a transient fall in body temperature during the early stages of acute benign respiratory tract infection and about 1/3 of all patients experience chills (Eccles et al. 2003). Chills associated with a fall in skin temperature related to vasoconstriction of the skin's blood vessels may be explained as an initial stage of fever, but chills may also be unrelated to changes in skin temperature. Chills have developed after administration of exogenous pyrogens, even if the subjects maintained a neutral skin temperature (34.5 °C in water) in experimental human studies (Guieu and Hellon 1980). Chills occur together with shivering and the latter symptom is probably related to the cerebral cortex influence over the shivering control. Both chills and shivering may be caused by cytokines acting on the temperature control center of the hypothalamus. Many cytokines act as endogenous pyrogens and are released from leukocytes in response to infection (Conti et al. 2004). The proinflammatory cytokines (IL-1 and IL-6) are regarded as the most important cytokines for causing fever (Netea et al. 2000; Leon 2002). They are believed to cross the blood-brain barrier and increase the thermal set point in the temperature control center. The hypothalamus then induces shivering, constriction of the skin's blood vessels, and chills (Eccles 2005).

4.10 Febrile Seizures

Febrile seizures are a rare but significant symptom of acute viral respiratory infections in children. They occur in 2–5 % of all children and the majority of febrile seizures are triggered by respiratory tract infection (Schuchmann et al. 2011). Febrile seizures are defined as occurring in children aged 6–60 months with a temperature $\geq$38.0 °C with no central nervous system infection, metabolic disturbance, or history of afebrile seizure. They are the most common type of seizure in children under 60 months (American Academy of Pediatrics 2011; Graves et al. 2012). Cytokines seem to play a crucial role in febrile seizures, however there is a lot of confusion about the relationship between proinflammatory and anti-inflammatory cytokines and the febrile seizure risk. Is it generally accepted that the genotype IL-1α-889 1/1 and IL-1β-511 T/T homozygote as well as the serum concentration of IL-6 are associated with an increased risk of febrile seizures (Saghazadeh et al. 2014).

5 Therapeutic Points

The treatment of acute viral respiratory tract infections remains primarily supportive. There is evidence that medications like acetaminophen (paracetamol) and non-steroidal anti-inflammatory agents such as ibuprofen or aspirin relieve some symptoms of acute respiratory tract infections (fever, sore throat, pain, and malaise),

but it is debatable whether symptomatic treatment could speed up recovery (Eccles 2005). Many other common advices like drinking plenty of fluids or steam inhalation have not been scientifically proven. Some new agents seem to be promising. For example in a study by Asada et al. (2012) L-carbocysteine reduced the baseline and RS virus infection-induced secretion of pro-inflammatory cytokines, including IL-1β, IL-6, and IL-8 as well as virus titers in the supernatant of human tracheal epithelial cells culture. Although a virus-orientated approach and the development of anti-viral agents should be more beneficial, the diverse etiology makes the development of universal antivirals highly unlikely (Passioti et al. 2014).

6 Conclusions

Since clinical symptoms are not sufficient to determine the etiology of acute viral respiratory tract infections, we believe that the host defense mechanisms are critical to the symptomatology. Immune response seems to be fundamental for understanding the pathomechanisms of these infections. Inflammatory mediators such as prostaglandins and bradykinin are responsible for the local symptoms of nasal congestion and rhinorrhea, while cytokines are responsible for systemic symptoms. A better understanding of the immune response including cytokines interactions will eventually allow for better treatments to be developed and should improve the quality of care for patients with acute respiratory tract infections.

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Stagnating Low Influenza Vaccine Coverage Rates in the Polish Elderly Population in 2008–2013

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Abstract

Although annual vaccination is the most effective way of preventing the disease and its severe outcomes, influenza vaccine coverage rates have always been at suboptimal levels in Poland. A retrospective analysis was conducted on influenza vaccine coverage rates among patients older than 65 years at local and national levels. Influenza vaccine coverage rates among the elderly in the capital city of Warsaw ranged from 20.5 % in 2013 to 31.5 % in 2010 and these rates were higher than those reported at the national level (from 7.6 % in 2012 to 11.3 % in 2009). At a local level the proportion of vaccines given to the elderly compared to all vaccinated individuals varied from 40 to 52 % which was comparable to the proportions reported at the national level (37–48.5 %). 69 % of the elderly were only vaccinated once during the observation period, and only 0.5 % of them repeated the vaccination in each subsequent year. The chance of being vaccinated against influenza more than once was statistically higher among women than men (OR 4.9; 95 % CI 4.2–5.8). Influenza vaccine coverage rates are low at both local and national levels and ought to be improved in Poland in future.

Keywords

Elderly • Flu • Immunization • Prophylaxis • Risk group

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1 Introduction

Influenza is an acute infectious viral disease spreading through droplets or contact with seasonal attack rates ranging from 5 to 20 % (increasing to 50 % in pandemic years). At the global level, these epidemics cause about 3–5 million severe cases of the disease and about 0.3–0.5 million deaths each year (Barr

et al. 2014). Despite its attack rate being highest among younger adults, influenza's effects are more significant in the elderly. It has been recognized that people aged 65 and over are at greater risk of serious complications from influenza compared with young, healthy adults. It is estimated that 90 % of seasonal influenza-related deaths and more than 60 % of seasonal influenza-related hospital admissions in the US each year occur in people aged 65 and older (Thompson et al. 2004). Influenza should not just be considered as a serious health condition but the disease's social and financial aspects should also be estimated. A decline in measurable functional status has been observed 3–4 months after infection in at least one major function (e.g., bathing, dressing, or mobility) in 25 % of older patients residing in nursing homes as compared with 16 % of controls (randomly selected residents living in the same facility not contracting influenza or influenza-like illness) during the same outbreak (Barker and Mullooly 1980). Molinari et al. (2007) estimated that the total financial burden of the seasonal influenza infection in the US amounts to $10.4 billion a year and that the older population is responsible for 64 % of the total economic burden. It has been estimated that direct costs of influenza in the elderly population in Poland can be very high (Kovacs et al. 2014). Therefore, efforts to reduce the disease's burden in the elderly population would have a substantial influence on the costs of seasonal influenza (Ryan et al. 2006).

Although an annual influenza vaccination for the elderly is recommended in many countries, including Poland, because it has been proven to be the most effective way to prevent the disease and its severe outcomes, influenza vaccination coverage rates have always been at suboptimal levels in almost all countries. For instance, the coverage rates among the elderly in 20 developed nations in 2008 ranged from 21 to 78 % (median 65 %) (Blank and Szucs 2009). In the US, influenza vaccination levels among the elderly population appeared to reach a plateau of about 70 % in the late 1990s (CDC 2006). A 2008 survey showed 40-fold differences between vaccination coverage in various European Union countries in individuals aged ≥65 years, ranging from less than 2 % to more than 80 % in the 2006–2007 season (Blank et al. 2009). Poland is one of the countries with a low influenza vaccination coverage rate, both in the general population and in age-related risk groups; in the 2013/2014 season, only 3.8 % of the entire population was vaccinated against influenza (NIPH-NIH 2013).

There is still limited literature on vaccination coverage rates in Central Eastern European countries, including Poland. The last report on influenza vaccine coverage rates in age-related risk groups, including the elderly population in Poland, analyzed the years 2004–2007 and indicated very low rates, ranging from 7 to 14 % throughout the country (Nitsch-Osuch and Wardyn 2009). The aim of the current study was to describe influenza vaccine coverage rates among the elderly aged >65 years in a single primary care setting in Warsaw (Poland) over a 6-year period (2008–2013) and to compare them with the official influenza vaccine coverage rates at the national level.

2 Methods

Approval from the local Ethics Committee was obtained prior to the study. A retrospective analysis of influenza vaccine coverage rates among patients aged over 65 from a single primary care clinic in Warsaw (Poland) was conducted and compared to influenza vaccine coverage rates among the elderly at the national level in years 2008–2013. The vaccine coverage rates were calculated as a percentage of vaccinated individuals among all patients over the age of 65 years, at both local and national level. At the national level, official data regarding the number of vaccinated patients were collected by National Institute of Public Health – National Institute of Hygiene (NIPH-NIH). These data, concerning the number of doses of vaccines given to patients and the age of vaccinated persons, were collected from reports prepared by Regional Sanitary-Epidemiological Stations. Data from these reports are supported by medical records obtained from general practitioners

administering flu vaccines. The official national data are published annually as the 'Vaccinations in Poland' bulletin, available on www.pzh.gov.pl. Demographic data (the number of persons aged >65 years in Poland in the years 2008–2013) were obtained from the Central Statistical Office (www.stat.gov.pl). The national data does not provide any information on the gender of vaccinated patients or the number of patients repeating the vaccination in subsequent years. At the local level, the medical history and vaccination records of patients aged over 65 years were analyzed with special attention being paid to the gender of patients receiving vaccinations and their willingness to repeat flu vaccines in subsequent epidemic seasons. In the primary care clinic the influenza vaccine for the elderly was paid for by a local government, while at the national level the vaccine was either reimbursed by local authorities or paid for by patients. The inactivated trivalent split vaccine was used for all vaccinated patients in a primary care setting, while both split and subunit inactivated trivalent vaccines were used in the country as a whole. Statistical calculations (OR and 95 % CI) were conducted using the medical statistical calculator available on www.medcalc3000.com. These calculations were required to find and describe the influence of the gender of vaccinated patients on their willingness to repeat the flu vaccine in subsequent seasons.

3 Results

In the period analyzed, the influenza vaccine coverage rates among the elderly in the primary care clinic ranged from 31.5 % in 2010 to 20.5 % in 2013. Every year the coverage rates were two to three times higher compared with the coverage rates reported at the national level (Table 1). At both local and national levels, the highest influenza coverage rates were reported in 2009 and 2010; the influenza A (H1N1) pdm09 pandemic year and the first post-pandemic year (Table 1). Persons aged >65 years represented between 37 % in 2009 and 48.5 % in 2012 and 2013 of all vaccinated individuals at the national level, while these proportions were slightly higher at the local level (43 % in 2008 and 52 % in 2013) (Table 2). Each year, more women than men were given an influenza vaccine at the local level (Table 3). Among the total of 4,459 flu vaccines given to 3,096 elderly patients, the majority of them (2,140) were only given in one single year, and patients never repeated the vaccination in subsequent years (Table 4). More women than men decided to be vaccinated against influenza in subsequent years (Table 4). The chance of being vaccinated against influenza more than once during subsequent seasons was statistically higher among women than men (OR 4.9; 95 % CI 4.2–5.8).

Table 1 Influenza vaccine coverage rates among the elderly at local and national levels in 2008–2013

	Primary care setting in the city of Warsaw			Poland		
Year	Number of individuals aged >65	Number of vaccinated individuals aged >65	Influenza vaccine coverage rate in individuals aged >65	Number of individuals aged >65	Number of vaccinated individuals aged >65	Influenza vaccine coverage rate in individuals aged >65
2008	3,135	721	23.0 %	5,146,287	464,755	9.0 %
2009	3,158	758	24.0 %	5,161,470	584,128	11.3 %
2010	3,232	1,018	31.5 %	5,184,564	480,951	9.3 %
2011	3,259	717	22.0 %	5,325,015	455,988	8.5 %
2012	3,238	680	21.0 %	5,487,713	418,905	7.6 %
2013	3,243	665	20.5 %	5,672,608	427,808	7.5 %

Table 2 Proportion of influenza vaccinations conducted among the elderly compared with the general population at local and national levels in 2008–2013

	Primary care setting in the city of Warsaw			Poland		
Year	Total number of vaccinated individuals[a]	Number of vaccinated individuals aged >65	% vaccines given to individuals aged >65 compared with all vaccinated individuals	Total number of vaccinated individuals[a]	Number of vaccinated individuals aged >65	% vaccines given to individuals aged >65 compared with all vaccinated individuals
2008	1,677	721	43.0 %	115,878	464,755	40.0 %
2009	1,895	758	40.0 %	584,128	584,128	37.0 %
2010	2,317	1,018	44.0 %	1,168,432	4,800,951	41.0 %
2011	1,593	717	45.0 %	1,061,111	455,988	43.0 %
2012	1,333	680	51.0 %	861,204	418,905	48.5 %
2013	1,279	665	52.0 %	880,904	427,808	48.5 %

[a]Each age

Table 3 The gender distribution of flu vaccines given to the elderly in a primary care setting in the city of Warsaw in 2008–2013

Number of flu vaccines given	Number (%) of vaccinated women	Number (%) of vaccinated men
2008	407 (56 %)	314 (44 %)
2009	421 (56 %)	337 (44 %)
2010	567 (56 %)	451 (44 %)
2011	406 (57 %)	311 (43 %)
2012	372 (55 %)	308 (45 %)
2013	361 (55 %)	304 (45 %)

Table 4 The gender distribution of flu vaccines among single and repeated users of the vaccine in a primary care setting in the city of Warsaw

Number of flu vaccines given	Number (%) of vaccinated patients	Number (%) of vaccinated women	Number (%) of vaccinated men
One	2,140 (69 %)	725 (34 %)	1,415 (66 %)
Two	675 (22 %)	486 (72 %)	189 (18 %)
Three	173 (5.5 %)	112 (65 %)	61 (35 %)
Four	90 (3 %)	74 (82 %)	16 (12 %)
Five	18 (0.5 %)	12 (67 %)	6 (33 %)

4 Discussion

In 2003, the World Health Assembly (WHA) issued a resolution for the prevention and control of influenza pandemics and annual epidemics, which urges the European Union's 25 Member States (EU-25) to establish and implement strategies to increase vaccination coverage among all high-risk groups, including the elderly and people with an underlying disease, with the aim of achieving vaccination coverage of the elderly population of at least 50 % by 2006 and 75 % by 2010 (WHA 2003). This resolution was reinforced by the EU Member States which agreed to make additional efforts to improve vaccination coverage in their territory in accordance with their own recommendations and to achieve the WHO target of 75 % in high risk groups before 2010. Today we know that the proposed and expected goals have not been reached in many countries, including Poland.

As previously described (Blank et al. 2009; Nitsch-Osuch and Wardyn 2009), influenza vaccination coverage levels among the Polish elderly population were well below the recommended limits (<15 %). The current official Polish data from the years 2008–2013 do not indicate any significant changes in influenza vaccine coverage rates among the elderly (rates from 11.3 % in 2009 to 7.0 % in 2012). However, higher influenza vaccine coverage rates (31.5 % in 2010 and 20.5 % in 2013) were reported in a single Warsaw primary care setting. Indeed, influenza vaccine coverage rates at a local level were two to three times higher compared with those reported nationwide. This difference may be explained by the fact that our study was conducted in a selected urban area. Warsaw is the country's capital city, where inhabitants have the highest income and access to healthcare services is fairly good. It also appears to be significant that

influenza vaccinations have been offered to the elderly free-of-charge in Warsaw by a local government for 10 years. Indeed, reimbursement for the influenza vaccine may have the greatest influence on higher coverage rates at a local level. Unfortunately, only one third of Polish local authorities provide free influenza vaccinations for the elderly. The highest influenza vaccination rate was achieved at both levels in 2009 and 2010, and we think this may be a result of an increased interest in vaccinations during and just after a pandemic year (especially given that the pandemic vaccine against influenza A (H1N1) pdm09 was not available in Poland). The proportion of the elderly among all individuals vaccinated against influenza in 2008–2013 increased at the national level; it varied between 37 and 48 % compared with the previous period of 2004–2007, when this proportion varied between 35 and 36 % (Nitsch-Osuch and Wardyn 2009). The proportion of vaccinated seniors among all those vaccinated against influenza at a local level was similar to the proportion reported at the national level.

The official data reported at the national level do not support any information on patients who continue vaccinations against influenza during subsequent seasons. In the present study we found that 69 % of vaccinated seniors received only a single flu vaccine during the 6 years' period analyzed. It should be noted that only a small fraction of the seniors (0.5 %) repeated flu vaccines every year. As the vaccine uptake patterns in the Polish elderly population are little known, this problem should be further investigated. Fitchett et al. (2014) identified several factors associated with recurrent non-uptake of the seasonal influenza vaccine in elderly patients: gender (elderly men were vaccinated less often than women), allergies, and the fear of adverse reactions. We also found that women are more likely than men to repeat the influenza shot in subsequent seasons. This finding indicates that special strategies should be implemented to encourage older men to get vaccinated.

Our data indicate that influenza vaccine coverage rates among the elderly are still at very low levels and must be improved. Reimbursement, while very important, is not the only possible factor increasing the coverage rate. Other possible methods for increasing influenza vaccine coverage rates have been described and their efficacy has already been evaluated. It is well known that "good intentions are not enough" (Litt and Lake 1993). Vaccination coverage rates can be improved by 10–30 % by the use of the risk register and reminder systems. Actions that have appeared effective were: financial incentives for patients, auditing and feedback, clinician reminders, financial incentives for clinicians, and team changes (more effective when nurses administered influenza vaccinations independently) (Litt and Lake 1993). The most powerful motivation for getting vaccinated in all countries was advice from a family doctor (58.6 %) and the perception of influenza as a serious illness (51.9 %). The major reasons why individuals do not get vaccinated were the feeling that catching influenza was not likely (39.5 %) and the issue of never considering the need for being vaccinated (35.8 %) (Lau et al. 2012). Negative patients' attitudes and the lack of organized and systematic approach are still identified as major barriers to improving vaccination rates (Lau et al. 2012; Litt et al. 1998). In Poland, systematic approach to influenza vaccines and vaccinations started in 2012 and resulted in creation of the National Influenza Program focusing on the ways to increase influenza vaccine coverage rates mainly among at-risk groups, including children, elderly, and healthcare workers.

Our study has some limitations. Firstly, we did not evaluate the health and social status and risk factors for a severe course of influenza in vaccinated patients. Secondly, we did not investigate the factors underlying motivation or its lack for conducting vaccination. Finally, we did not evaluate the possible co-administration of influenza vaccine with other vaccines, including pneumococcal vaccine. However, the advantage of this study is that it is the first, to the best of authors' knowledge, which compares influenza vaccination rates at local and national levels. The analysis of local data revealed that gender may influence the willingness of elderly patients to repeat flu shots in subsequent years.

5 Conclusions

Influenza vaccine coverage rates among the Polish elderly population are at a stagnating low level. The higher influenza vaccine coverage rates at a local level indicate that reimbursement for the vaccine, only available in some districts of the country, may play a crucial role in the acceptance and performance of the flu vaccine in the Polish elderly population.

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

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Incidence of Circulating Antibodies Against Hemagglutinin of Influenza Viruses in the Epidemic Season 2013/2014 in Poland

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Abstract

The aim of this study was to determine the level of antibodies against hemagglutinin of influenza viruses in the sera of people in different age groups in the epidemic season 2013/2014 in Poland. The level of anti-hemagglutinin antibodies was determined by hemagglutination inhibition test (HAI). A total number of 1,050 randomly selected sera was tested in seven age groups. The level of antibodies against influenza viruses was very low, which indicates that the people have not been vaccinated against influenza in the epidemic season 2013/2014. The value of protection rate against influenza in the Polish population is very low. These results are worrying, because complications of influenza may be harmful to health and even life-threatening to persons who are not vaccinated. Furthermore, these results confirm the circulation of three antigenically different influenza virus strains, two subtypes of influenza A virus – A/California/7/2009/(H1N1)pdm09 and A/Victoria/361/2011 (H3N2) - and B/Massachusetts/2/2012.

Keywords

Anti-HA antibodies • Flu • GMT • Influenza protection • Vaccination

1 Introduction

Influenza is an infectious disease caused by influenza viruses from the *Orthomyxoviridae* family. The continuing evolution of the virus is the cause of seasonal epidemics and, from time to time, of pandemics in the human population. Furthermore, due to appearance of influenza-like illnesses, diagnosis of this disease on the basis of clinical symptoms is possible only during the outbreak. It should be noted that in case of respiratory infections various clinical symptoms can be caused by the same virus and, on the other hand, the same set of symptoms can be caused by more than 200 different viruses (e.g. parainfluenza, adenovirus, rhinovirus, and coronavirus). For this reason, the laboratory

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confirmation of influenza virus infection is crucial in the influenza surveillance and is essential for evaluating the effectiveness of vaccines and antiviral drugs. Laboratory diagnosis of influenza includes confirmation of the presence of influenza virus antigen in the material collected from the patient and serological evidence of infection with influenza virus by detecting the increase of specific antibodies in patient's serum (Brydak 2008).

The major line of defense from influenza infection are antibodies directed against two glycoproteins exposed on the surface of the virion – hemagglutinin (HA) and neuraminidase (NA) (Johansson et al. 1989). Furthermore, evolution of influenza virus is most evident in the case of these surface proteins. HA and NA antigens are constantly changing due to antigenic pressure. They both are highly immunogenic, and antibodies produced in response to viral infection are specific for a particular subtype of hemagglutinin (H1-H18) and neuraminidase (N1-N11) and do not give complete protection against all influenza virus strains (Nicholson et al. 1998). The presence of hemagglutinin antibodies (anti-HA) provides not only protection against infection with specific strains of influenza virus, but also might alleviate symptoms of the disease in case of infection with another variant of the virus. This is caused by the occurrence of so-called cross-reactive antibodies. It has been shown that subtype-specific anti-HA antibodies can decrease the infectivity of the virus of other subtype by disruption of proliferation and release of viral particles during infection (Ekiert et al. 2011; Epstein and Price 2010; Thorsby et al. 2008). For this reason, regular vaccinations are essential for reducing the impact of seasonal influenza and influenza prevention. Seasonal vaccinations protect from infection with currently circulating viruses and give cross-protection, which can reduce viral replication, accelerate viral clearance, and thus reduce the severity of disease. The World Health Organization leads the global influenza surveillance and gives seasonal recommendations for influenza virus strains included in the vaccine for the upcoming epidemic season.

An anti-HA antibody titers of 1:40 has been determined as corresponding to a 50 % reduction in the risk of contracting influenza in population (Hobson et al. 1972) and it is assumed that high titers of anti-HA antibodies ($\geq$1:40) provide protection against influenza infection. Furthermore, this value of titer, after vaccination, is required by European Agency for the Evaluation of Medicinal Products for assessment of vaccines (Committee for Proprietary Medicinal Products 1997). On the other hand, the anti-NA antibodies, even at high titers, only support the resistance because they do not prevent influenza infection themselves. However, it has been shown that the anti-NA antibodies block the replication process, alleviate the severity of infection, and reduce the incidence of disease (Brydak 2008).

Serological methods for detection of anti-influenza antibodies include, among others, hemagglutination inhibition (HAI) test and neuraminidase inhibition (NI) test. Serological tests are used not only for diagnostic purposes, but also to evaluate the resistance of the population resulting from both, natural infection and vaccination against influenza. Most laboratories use the hemagglutination inhibition test for serological diagnosis. The method is based on the ability of anti-HA antibodies to inhibit virus-induced agglutination of erythrocytes (WHO – Global Influenza Surveillance Network 2011). It is a simple, inexpensive, and rapid test, during which a small amount of reagents is used. Due to the high cost and labor-consumption, not many laboratories determines the level of antibodies with neuraminidase inhibition test (Brydak 2008).

The recorded number of cases of influenza and influenza-like illnesses depends on reporting by health care workers. In Poland, this is done by Local Sanitary Epidemiological Stations. Thereafter, these data in the form of weekly reports are sent to the National Institute of Public Health – National Institute of Hygiene by Voivodeship Sanitary Epidemiological Stations. There are two types of influenza vaccines, which are currently available in Poland: inactivated

Table 1 Influenza virus strains used in hemagglutination inhibition tests (HAI) (WHO 2013)

Epidemic season 2013/2014	Influenza virus strains		
	A/H1N1/	A/H3N2/	B
	A/California/7/2009 (H1N1) pdm09 – like virus	A/Victoria/361/2011 (H3N2) – like virus	B/Massachusetts/2/2012 – like virus

split virion and subunit. Split type vaccine contains inactivated split virus, whereas subunit type contains only viral glycoproteins, hemagglutinin and neuraminidase. It should be noted that already for many influenza seasons, numerous local governments offer free influenza vaccinations for people over 50 years of age, who often belong to high-risk groups of health. Despite this, the proportion of people vaccinated is diminishing season by season. In the epidemic season 2013/2014 only 3.75 % of the population was vaccinated against influenza (Brydak 2014).

The epidemic season 2013/2014 was mild in intensity in comparison to the previous season. A total number of 2,036,215 cases of influenza and influenza-like illness were registered in Poland in this season and the incidence was 5,284 (per 100,000 population), causing 15 deaths as a result of complications from influenza (GIS 2014). Our paper describes determination of the level of antibodies against hemagglutinin of influenza viruses present in the sera of people in different age groups, during the epidemic season 2013/2014.

2 Methods

The serum samples of people in age groups: 0–3, 4–7, 8–14, 15–25, 26–44, 45–64, and ≥65 years of age were collected by departments of epidemiology and virological laboratories of Voivodeship Sanitary Epidemiological Stations in Poland. The collected samples were stored at −80 °C until tests were done. Among the 1,050 serum samples, 150 sera were tested in each age group. Antibody status was determined by hemagglutination inhibition reaction by using eight hemagglutinin units of the virus. Prior to the HAI test, sera were inactivated according to WHO standards (Tyrell and Horsfall 1952; WHO – Global Influenza Surveillance Network 2011).

The following parameters were used for analysis of the results:

- Geometric mean of anti-hemagglutinin antibodies in tested sera (GMT).
- Protection rate, i. e. the proportion of subjects showing anti-hemagglutinin antibody titres ≥40.

The antigens listed in Table 1 were used in hemagglutination inhibition (HAI) tests, in agreement with the WHO recommendations (WHO 2013). All antigens were prepared in National Influenza Center, Department of Influenza Research, National Institute of Public Health – National Institute of Hygiene. Preparation and dilution of antigens for HAI tests were performed in accordance to WHO protocols (WHO – Global Influenza Surveillance Network 2011).

3 Results and Discussion

The geometric mean titers of anti-HA antibodies in sera collected from people in different age groups in the epidemic season 2013/2014 are shown in Fig. 1. The highest level of antibodies against hemagglutinin H1 was found in the age group 4–7 (GMT-27.51). The GMT of anti-H1 antibodies was slightly lower in the age group 8–14 (15.60) and low in the age groups 0–3, 15–25, 26–44 and 45–64, where there was almost the same level of antibodies (12.09-13.44). The lowest level of anti-H1 antibodies was observed in the age group ≥65 (GMT-10.05). In the case of the H3 hemagglutinin, the highest geometric mean titers were in the 4–7 (21.14) and 8–14 (20.37) years old groups. In other age groups, these values were at similar levels from 11.97 to 15.58. For type B hemagglutinin the observed level of antibodies was the highest in group of 15–25 years (GMT-24.85), lower in group of 8–14 years (GMT-12.14), and the lowest in

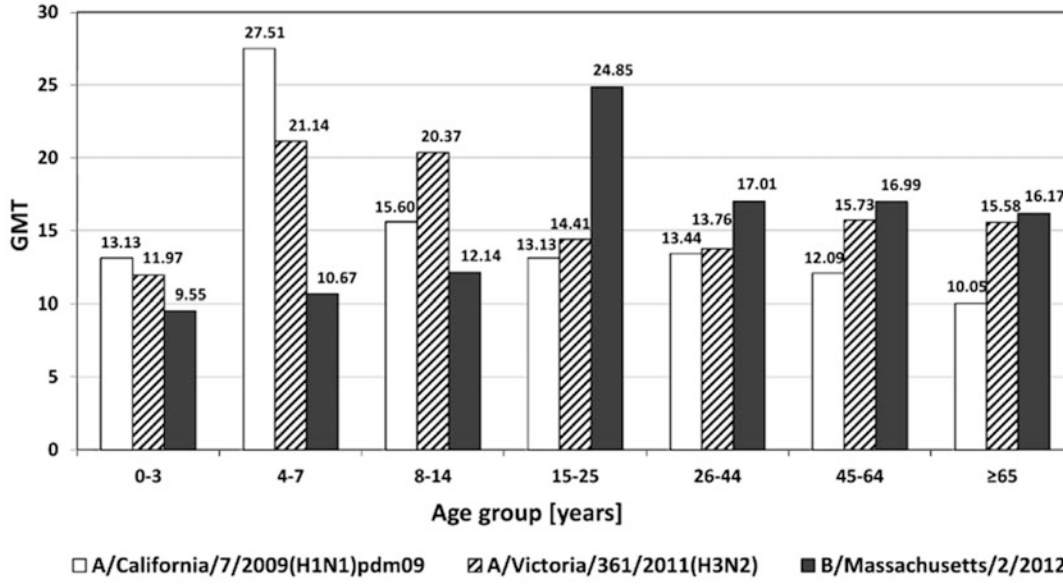

Fig. 1 **Geometric mean titers of anti-HA antibodies** in sera of people in different age groups in the epidemic season 2013/2014

groups of 0–3 and 4–7 years (GMT: 9.55 and 10.67, respectively). Almost identical geometric mean titers were observed in the age groups 26–44 (17.01), 45–64 (16.99), and ≥65 (16.17). In conclusion, the highest GMT titers were observed in the age groups: 4–7, 8–14, and 15–25, and a different type of anti-HA antibody had dominated in each of these groups: H1, H3 and B, respectively. The lowest geometric mean titer of antibody directed against the HA was observed in children aged 0–3 years.

The protection rate is the percentage of people with the protective titer of anti-HA antibodies of at least 40 after vaccination and, depending on age, should achieve different values: ≥70 % for people aged 18–60 years and ≥60 % for people over 60 years of age (Brydak 2008). However, the protection rate did not exceed 60 % in any age group (Fig. 2). The highest level of protection rate was observed for the hemagglutinin H1 of A/California/7/2009pdm09 strain in the age group 4–7 (53.33 %). An analysis of the protection rate with respect to the age group, including all three types of hemagglutinin, revealed that in the 0–3 years old group the level of protection exhibited similar values: 16.00 % (B), 20.67 % (H3), and 26.67 % (H1) without the domination of particular hemagglutinin type. In the age groups 4–7 and 8–14, the protection rate for H1 and H3 hemagglutinins was much higher than for hemagglutinin type B. However, in 15–25, 26–44 and 45–64 years old groups significantly higher protection rates were observed for type B hemagglutinin compared to the other two types.

In the last three epidemic seasons: 2011/2012, 2012/2013 and 2013/2014, the values of protection rate were different in particular age groups (Table 2). The comparison of protection rates for hemagglutinin H1 of A/California/7/2009pdm09 strain is especially interesting. This type of A influenza virus has been circulating in the population since 2009. In the age groups of 4–7, 26–44, 45–64, and ≥65 years there has been a significant increase in the protection rate compared to the previous two seasons. In the youngest age group this value is higher in comparison to the last season, but lower than in the 2011/2012 season. However, in groups of 8–14 and 15–25 years old the protection rates remain at a similar level in all analyzed seasons. In the case

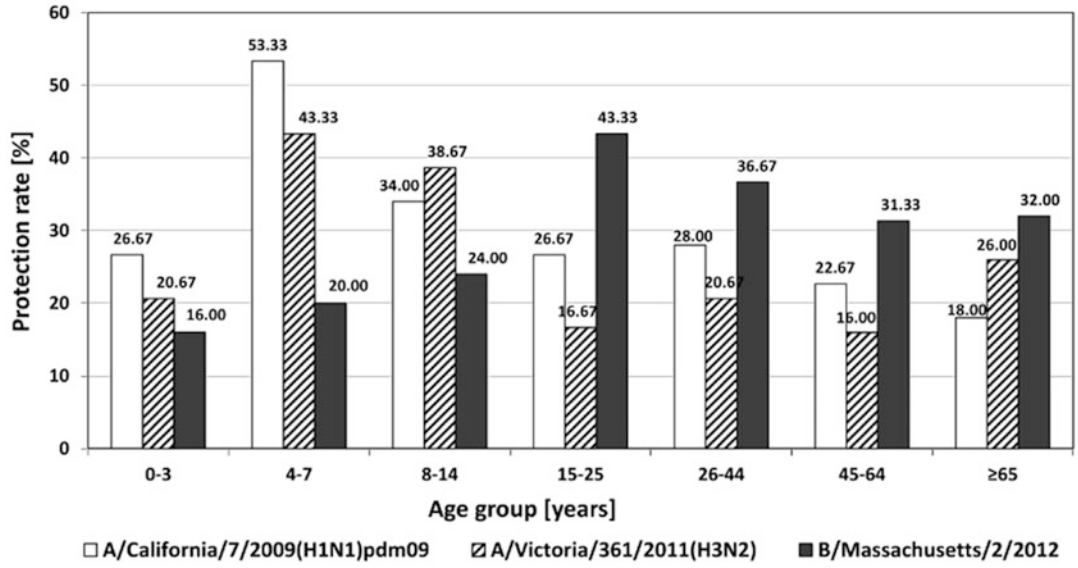

Fig. 2 **The percentage of people with protective level of anti-HA antibodies** with titers of at least 40 in the epidemic season 2013/2014

Table 2 Protection rate values (%) in the epidemic seasons 2011/2012, 2012/2013 and 2013/2014 (Bednarska et al. 2014)

	Age groups (years)							
Antigen	0–3	4–7	8–14	15–25	26–44	45–64	≥65	Epidemic season
A/H1	39.59	48.52	30.95	24.76	22.85	15.75	12.30	2011/2012
	19.43	35.10	31.54	26.49	22.97	12.28	8.57	2012/2013
	26.67	53.33	34.00	26.67	28.00	22.67	18.00	2013/2014
A/H3	14.20	25.98	33.38	40.47	28.09	25.23	26.19	2011/2012
	38.86	37.75	35.57	9.93	29.73	5.26	6.43	2012/2013
	20.67	43.33	38.67	16.67	20.67	16.00	26.00	2013/2014
B	9.64	14.70	22.85	9.04	12.38	30.47	36.66	2011/2012
	8.00	3.97	8.72	14.57	21.62	8.19	17.14	2012/2013
	16.00	20.00	24.00	43.33	36.67	31.33	32.00	2013/2014

of H3 hemagglutinin of A/Victoria/361/2011 strain the protection rate increased in the age group 4–7, decreased in the age group 26–44, and did not change significantly in the group of 8–14 years, compared to the previous two epidemiological seasons. There has been a significant increase in the values of this factor in groups 15–25, 45–64, and ≥65 years old as compared to the epidemic season 2012/2013. With regard to the last season, value of protection rate substantially increased in the youngest group. A significant increase in protection rates was observed in four age groups: 0–3, 4–7, 15–25, and 26–44 for influenza B virus strain B/Massachusetts/2/2012 in epidemic season 2013/2014. In other age groups these values were similar to 2011/2012 season and lower than in previous season.

Despite the apparent increase of protection rate in some age groups, this value did not achieve 60 %. These results are truly alarming as it shows that the value of protection rate against influenza in the Polish population is very low. The complications of influenza may be hazardous to health and life-threatening to persons who are not vaccinated, especially in the very young, the

elderly, and those with other serious medical conditions. Influenza vaccination is the most effective way to prevent infection, but this fact is ignored by many people. It can be concluded, on the basis of serological screening of sera from people at different age groups in the epidemic season 2013/2014, that:

1. our results confirm the circulation of three antigenically different influenza strains: two subtypes of influenza A virus – A/California/7/2009(H1N1)pdm09 and A/Victoria/361/2011(H3N2) – and type B, B/Massachusetts/2/2012;
2. protection rate did not exceed 60 % in any age group, which demonstrates that the proportion of people vaccinated against influenza in Poland is very low. This situation can be changed when more people will vaccinate against influenza.

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

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Neutrophils: The Role of Oxidative and Nitrosative Stress in Health and Disease

Aneta Manda-Handzlik and Urszula Demkow

Abstract

Neutrophils constitute the first line of the innate immunity in humans. They employ several strategies to trap and kill microorganisms, such as phagocytosis, degranulation, and the formation of extracellular traps (NETs). It has been well documented, that generation of reactive oxygen and nitrogen species (ROS and RNS) is crucial in the life cycle of a polymorphonuclear phagocyte. These compounds due to high reactivity act as powerful antimicrobial factors in the process of pathogens clearance and can also modulate immunological response. On the other hand, excessive amount of free radicals may have detrimental effect on host tissues and markers of oxidative and nitrosative stress are detectable in many diseases. It is necessary to maintain the balance between ROS/RNS formation and removal. The review highlights our current understanding of the role of ROS and RNS produced by neutrophils in health and disease.

Keywords

Cell signaling • Nitrosative stress • Neutrophils • Oxidative stress • Pathology

1 Introduction

Neutrophils represent the first line of defense against pathogens and they are recruited as firsts to sites of infection and tissue damage. This most abundant subpopulation of leukocytes is released to venous sinuses of the bone marrow and the marrow reserve is estimated at 6×10^{11} cells. Neutrophils are released into the blood stream, where they constitute the circulating and marginating pools of granulocytes, both of approximately equal size. Neutrophils have

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short circulating half-life (6–8 h) and they quickly migrate to tissues where they perform immune functions (Brinkmann et al. 2004; Summers et al. 2010).

Neutrophils react with pathogens in a non-specific way and they employ several strategies to combat invading microorganisms. Mature neutrophils can phagocyte microbes. Whereas macrophages efficiently engulf pathogens in body fluids and on tissue surfaces, polymorphonuclear leukocytes engulf only surface-associated and not fluid-borne bacteria (Colucci-Guyon et al. 2011). The repertoire of neutrophil mechanisms to fight infections includes degranulation and the generation of reactive oxygen species in the process called oxidative burst. During degranulation, a neutrophil releases potent antimicrobial factors normally stored within intracellular granules, such as elastase or gelatinase (Simard et al. 2010). In 2004 a novel mechanism employed by neutrophils was described, consisting of releasing structures resembling nets. These structures are called neutrophil extracellular traps (NET) and are formed by nuclear DNA, histones, and granular and cytoplasmic antimicrobial proteins (Brinkmann et al. 2004; Urban et al. 2009). NETs physically trap bacteria, prevent them from spreading, and provide a high local concentration of antimicrobial factors which can kill pathogens or downgrade their virulence (Brinkmann et al. 2004). Nonetheless, it has been pointed out that the formation of NETs may have not only beneficial but also detrimental consequences as they participate in the pathogenesis of several inflammatory and autoimmune diseases (Brill et al. 2012; Cools-Lartigue et al. 2013; Gupta et al. 2007; Papayannopoulos et al. 2011). It seems that generation of reactive oxygen species (ROS) plays a crucial role in the formation of NETs. Thus, ROS's involvement in NETosis has been an area of extensive study of late.

Among many substances produced by polymorphonuclear granulocytes, a special role is attributed to ROS and reactive nitrogen species (RNS), released during oxidative burst, being not only antimicrobial factors but also important regulators of immune response. Reactive species have ideal characteristics to fulfill this function, as their small molecules can easily diffuse through cell membranes. Additionally, they are rapidly produced and degraded (Tonks 2005). It has been shown that these molecules can regulate immune response on a very basic, molecular level. On the other hand, ROS and RNS produced by neutrophils were shown to play a crucial role in the pathogenesis of several diseases. Further studies are warranted to understand the link between ROS and RNS and immunity, to develop new therapies targeting these specific cell signaling pathways.

2 Oxidative Burst

Respiratory or oxidative burst is the mechanism in which activated neutrophils (or other cell types, as these mechanisms is not limited to these phagocytes) generate and release reactive oxygen intermediates. Reactive oxygen species can be both radicals, like superoxide ($O2^{\bullet -}$), hydroxyl (•OH) radical, and nonradicals, like hydrogen peroxide or hypochlorous acid. ROS formation is a critical step in phagocytosis, as these highly reactive compounds can kill pathogens within phagolysosomes.

A key enzyme in this process is the nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase, NOX) (Fig. 1). It consists of six subunits, which are separated under physiological conditions to prevent ROS generation and consequent cell damage. Before the complex is activated and produces large amounts of oxidative agents, the subunits have to assemble to form multicomponent enzyme. Two of these proteins, $p22^{phox}$ and $p91^{phox}$, are located within the cell membrane and together build cytochrome b558. Cytochrome b558 is the catalytic core of the enzyme, as electron transfer occurs through $gp91^{phox}$. After neutrophil activation, three regulatory subunits ($p67^{phox}$, $p47^{phox}$, and $p40^{phox}$) are phosphorylated and along with GTPase Rac2 are dislocated from cytosol to phagolysosome membrane, where active NADPH oxidase

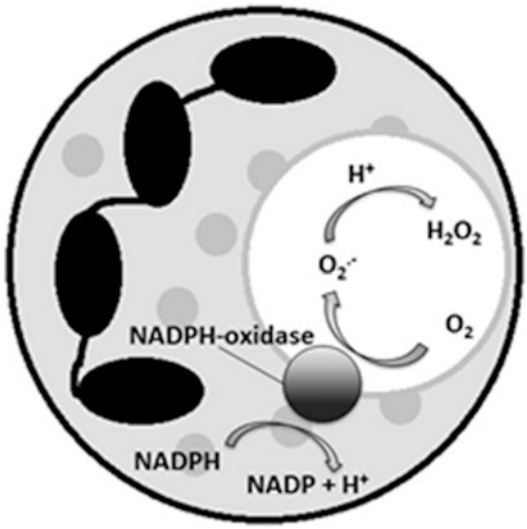

Fig. 1 Function of NADPH oxidase in oxidative burst of neutrophils

is formed (Knaus et al. 1991). NOX is capable of transferring electrons from NADPH to O_2 to form superoxide. Patients bearing mutations in genes for NOX subunits suffer from recurrent infections due to inability of neutrophils to generate oxidative stress during immunological response. The most common mutation in patients with this condition, chronic granulomatous disease (CGD), is a mutation in the gene encoding the protein $p91^{phox}$, observed in two thirds of all the cases (Patino et al. 1999).

Superoxide is not the only reactive oxygen species formed during oxidative burst, as further cascade of chemical reactions leads to the generation of other oxidative agents. Superoxide may dismutate in the reaction either catalyzed by superoxide dismutase (SOD) or spontaneously into hydrogen peroxide. It is possible to generate hydroxyl radical from hydrogen peroxide in the Fenton reaction, catalyzed by iron, although this mechanism is deficient in neutrophils as they lack ferrous ions (Britigan et al. 1990). After neutrophils' activation azurophilic granules release myeloperoxidase (MPO) into the phagosome, where MPO catalyzes the synthesis of hypochlorous acid from hydrogen peroxide as a substrate. HOCl then reacts with amino acids, giving chloramines. Furthermore, superoxide may react with hypochlorous acid, which results in the formation of hydroxyl radical (Ramos et al. 1992). In the reaction between hydrogen peroxide and hypochlorous acid singlet oxygen is formed. Another source of ROS is the reaction between hydrogen peroxide and superoxide, but low amounts of synthesized singlet oxygen and hydroxyl radical seem not to be of high importance in biological systems (MacManus-Spencer and McNeill 2005).

Generation of ROS is supported by the production of RNS, which are also involved in the clearance of pathogens. Enhanced synthesis of nitric oxide (NO) is associated with the process of inflammation, when mRNA for inducible form of nitric oxide synthase (iNOS) is being transcribed. The substrates for NO synthesis are L-arginine, NADPH, and oxygen, with the tetrahydrobiopterin as a cofactor. NO diffuses easily across cellular membranes and its antimicrobial properties are significantly enhanced when it reacts with superoxide, forming peroxynitrite, a potent pro-inflammatory and cytotoxic molecule (Cuzzocrea et al. 2001). Other RNS that might be formed during reactions of endogenous NO with oxidants are nitrogen dioxide and dinitrogen trioxide. Both ROS and RNS are strong oxidants and these compounds can react with many biomolecules including DNA, proteins, lipids, and thiols. Reactions of oxidation and nitration/nitrosylation result in the formation of toxic products and, in turn, they facilitate clearance of pathogens (Eiserich et al. 1998).

3 ROS and RNS in Chemotaxis, Neutrophil Adhesion, Rolling, and Phagocytosis

It has been observed that ROS are crucial regulators of neutrophil chemotaxis. In the process of chemotaxis, a neutrophil forms pseudopods. The well-directed pseudopods are maintained, whereas pseudopods facing the wrong direction are destroyed enabling migration toward chemoattractants. It has been proposed that disruption of misoriented

pseudopods may be, in large part, mediated by ROS production (Hattori et al. 2010).

As mentioned above, compounds released during oxidative burst are able to move across cell membranes. Therefore, even if produced within cell, they may affect processes occurring extracellularly. Neutrophils migrating to tissues release ROS, which, in turn, activate endothelial cells (Fialkow et al. 2007). It creates a specific loop, as ROS induce the prolonged expression of the glycoprotein ligand for polymorphonuclear adhesion GMP140 on endothelial cell surface. ROS are able to regulate the expression of endothelial cell adhesion molecules (CAM) by a direct activation of CAMs on the surface and also by a transcription-dependent mechanism which involves transcription factors sensitive to oxidation (Patel et al. 1991).

Not only reactive oxygen species but also nitrogen species may regulate endothelium – neutrophil adhesion. Depletion of NO causes increased oxidative stress within endothelium and mast cells. It results in a promotion of neutrophil adhesion to the blood vessel wall (Niu et al. 1996). Other studies revealed that treatment of neutrophils with NOS inhibitors enhanced carrageenan-induced rolling/adhesion of neutrophils to endothelial cells. Additionally, NOS inhibitors block the apoptosis process in migrating neutrophils (Dal Secco et al. 2003). Moreover, NO can modulate migration of neutrophils by inducing neutrophil-derived microparticles (Nolan et al. 2008).

On the surface of neutrophils there are two types of Fc receptors: FcγRIIa and FcγRIIIb. They are responsible for phagocytosis and killing of opsonized pathogens within the cell. Activation of FcγRIIa may be induced by crosslinking of FcγRIIIb, which, in turn, leads to neutrophil degranulation and generation of reactive oxygen intermediates (Salmon et al. 1995). Under physiological conditions, respiratory burst is involved in amplification of Fc receptor-mediated phagocytic function and in patients suffering from chronic granulomatous disease Fc receptor-mediated phagocytosis is abrogated. This pathway requires hydrogen peroxide, lactoferrin, and superoxide anion, but not hydrogen peroxide-MPO-halide system, which underscores the pivotal role of ROS in the amplification of phagocytosis (Gresham et al. 1988). Interestingly, it has been recently shown that inducible nitric oxide synthase interacts with Rac2 and is translocated to phagosomes after phagocytosis. As a consequence, ROS and RNS are produced to kill microbes within this cellular compartment (Jyoti et al. 2014).

4 Role of Oxidative Stress in NETs Formation

Convincing evidence on the role of ROS in NETosis has come from studies on neutrophils isolated from CGD-patients. Lack of NADPH-oxidase in cell membrane impairs the production and release of NETs in response to different stimuli (Fuchs et al. 2007). In line with those findings, murine model of CGD confirmed this observations as neutrophils isolated from gp91 −/− mice do not release extracellular traps (Ermert et al. 2009). In the same study, NET formation interrelated with the amount of ROS produced. It has been shown that the mechanism underlying ROS-induced NETosis is based upon translocation of neutrophil elastase (NE) to the nucleus, where it degrades histones and facilitates chromatin decondensation. Myeloperoxidase acts synergistically with NE in this process. Further support for the role of ROS in NETosis comes from the fact that phorbol myristate acetate (PMA) – a potent stimulant of NETosis becomes inactive when exposed to diphenyleneiodonium chloride (DPI), an NADPH oxidase inhibitor (Papayannopoulos et al. 2010). Some studies were undertaken to establish the clear role of different oxidants in NETosis. Due to difficulties with targeting appropriate cell compartment using oxidant scavengers and enzymes inhibitors, and multiple reactions involved in the generation of ROS, there are no apparent conclusions (Parker and Winterbourn 2012). Yet, it has been shown that mitochondrial ROS are not involved in NETosis (Kirchner et al. 2012).

Notably, in some cases of NETs formation, ROS are not involved. For instance, in cystic fibrosis airway inflammation traps may be released upon activation of G protein-coupled receptor CXCR2 and this process is independent of NADPH oxidase (Marcos et al. 2010). Furthermore, early formation of NETs observed after exposure to *S. aureus* is also independent of ROS produced by NADPH oxidase (Pilsczek et al. 2010). Accordingly, it is possible that ROS might account for most but not all NETs.

To date, the role of NO and nitrosative stress in NETs formation has not been largely studied. However, it has been recently discovered that NO is an inducer of NETosis and acts *via* augmenting free radical generation. It has been suggested that oxidative stress and iNOS induction may act in concert during inflammation to promote NETosis (Patel et al. 2010).

5 Reactive Oxygen and Nitrogen Species in Cell Signaling Pathways

Nuclear factor kappa B (NF-κB) is a transcription factor that once activated regulates the process of transcription of numerous genes. Their products (e.g., cytokines, protein p53, growth factors, and cell adhesion molecules) are involved in cell cycle regulation, inflammation, and immune response (Celec 2004). The influence of ROS on NF-κB regulation varies in different compartment of a cell. ROS in the cytoplasm stimulate the signal transduction pathways for NF-κB activation and translocation into the nucleus. On the other hand, ROS inhibit the activity of this transcription factor in the nucleus. Oxidative and nitrosative stress may regulate NF-κB pathway in many ways. The sulphydryl group of Cys62 in NF-κB molecule is a determinant of DNA recognition by the p50 subunit and it is especially sensitive to oxidation (Matthews et al. 1993). It cannot be excluded that oxidation is followed by the S-glutathiolation, as changes in the GSH/GSSG ratio are responsible for inhibition of binding of p50 subunit to DNA (Klatt and Lamas 2000). It has also been shown that NF-κB/RelA Ser(276) phosphorylation induced by tumor necrosis factor-α (TNF-α), a modification critical for NF-κB transcriptional activity, is mediated by the ROS signaling pathway (Jamaluddin et al. 2007).

Several studies highlighted that ROS affect the phosphorylation of I-κBα and independently induce NF-κB activation and translocation to the nucleus (Schoonbroodt et al. 2000; Takada et al. 2003). Contradictory findings on the influence of ROS on IKK have been reported. Some studies show that ROS activate IKK kinase complex, the core element of the NF-κB cascade, while others found that ROS may have an inhibitory effect on this kinase (Byun et al. 2002; Herscovitch et al. 2008). Moreover, catalytically active subunit of IKK is a site for S-nitrosylation by nitric oxide. Following inhibition of NF-κB provides an explanation for anti-inflammatory effect of NO (Reynaert et al. 2004). As mentioned above, release of NO down-regulates neutrophil migration, as it decreases rolling and adhesion of neutrophils to endothelium and induces apoptosis (Dal Secco et al. 2003).

Oxidants may also modulate cell signaling pathway involving the mitogen-activated protein kinase (MAPK) family. They are activated to induce the production of cytokines, like Il-6, and inflammatory mediators (Craig et al. 2000; Lang et al. 2006). Exogenous oxidants induce tyrosine phosphorylation and activation of ERK, which is a member of MAPK. This effect is enhanced by the inhibition of CD45, which also is a result of oxidative influence. Oxidized CD45 cannot efficiently dephosphorylate and inactivate MAP kinase (Fialkow et al. 1994). It has been recently demonstrated that PMA activates both extracellular signal-regulated kinases (ERK) and p38 MAPK and the process of phosphorylation of these two kinases is ROS-dependent (Keshari et al. 2013).

ROS are capable of regulating the activity of protein tyrosine kinases and phosphatases. The balance between these two activities is responsible for the level of tyrosine phosphorylation. Signaling pathways involving these enzymes are of great importance for neutrophil inflammatory response and its components, such

as adherence, chemotaxis, and phagocytosis (Kobayashi et al. 1995). Invariant cysteine residue in protein tyrosine phosphatase superfamily (PTP) functions as a nucleophile during catalysis. The active site Cys may be oxidized, which abrogates its nucleophilic properties and inhibits PTP activity. When oxidation by ROS occurs upon activation, it leads to inactivation of PTP and facilitates the response to the activating stimulus (Rhee et al. 2003). It has also been found that oxidative burst can inhibit expression of CD45, a tyrosine phosphatase commonly found on the surface of leukocytes. This inhibition is, at least partially, mediated by NOX (Fialkow et al. 1997). Cysteine residue is also a target for oxidants in protein kinase C (PKC). This family of proteins is responsible for regulation of cell growth, differentiation, apoptosis, transformation, and tumorigenesis. Cysteine is located within the zinc-binding domain of autoinhibitory function and when oxidized it leads to the activation of PKC (Cosentino-Gomes et al. 2012).

6 Reactive Oxygen/Nitrogen Species and Neutrophils in Pathology

It is known that patients with human immunodeficiency virus (HIV) suffer from systemic oxidative stress. It leads to the activation of NF-κB and, as a consequence, increases the HIV replication (Nakamura et al. 2002). Increased ROS production is due to changes in the expression of Bcl-2 and thioredoxin, antiapoptotic and antioxidant molecules. Spontaneously increased production of ROS by phagocytes (both macrophages and neutrophils generate high amounts of H_2O_2) can participate in the oxidative injury in the course of HIV infection. Despite the production of hydrogen peroxide by neutrophils, these cells show decreased release of ROS after the specific stimulation when exposed to oxidative burst inducers such as TNF-α, Il-8, or bacterial N-formyl peptides. That may contribute to the enhanced susceptibility to bacterial infections in HIV-infected patients (Elbim et al. 2001).

In patients suffering from acute respiratory distress syndrome, bronchoalveolar lavage contains a high number of neutrophils, which are a source of inflammatory mediators and superoxide anion. Peroxynitrite generated from NO and superoxide, nitrates, and oxidized amino acids inhibit function of proteins, such as surfactant protein A. Improper balance between antioxidants and reactive oxygen and nitrogen species aggravates the disease (Lang et al. 2002). Oxidant/antioxidant imbalance is observed also in patients with chronic obstructive pulmonary disease (COPD). Numerous neutrophils in the airways of these patients produce oxidants and the amount of free radicals is additionally increased in patients smoking cigarettes. Evidence for enhanced oxidative stress in smoking patients are also supported by the presence of nitrated proteins: ceruloplasmin, plasminogen, fibrinogen and transferrin, as well as elevated levels of lipid peroxidation products in blood (Morrison et al. 1999; Pignatelli et al. 2001). Oxidants can activate NF-kB, which, in turn, causes transcription of inflammation-related mediators. That results in increased sputum concentration of TNF-α, nitric oxide, and IL-8. Oxidation also silences α-1-antitrypsin, disturbing the balance between proteases and antiproteases, which causes tissue injury (Dalle-Donne et al. 2005). It has been shown that during acute exacerbation of COPD the production of superoxide by neutrophils is increased (Rahman et al. 1997).

In rheumatoid arthritis, neutrophils present in inflamed joints produce both ROS and RNS, which can exacerbate the disease. Nitrotyrosine, which is a marker of peroxynitrate production, is elevated in serum and synovial fluid collected from patients with the disease and the level of 3-nitrotyrosine correlates directly with disease activity (Kaur and Halliwell 1994; Khan and Siddiqui 2006). The level of S-nitrosoproteins in synovial fluid is especially high when compared with plasma concentrations (Hilliquin et al. 1997). This supports a notion of NO being formed by inflammatory cells within the joint. Superoxide and hydroxyl radicals are also significantly

raised in peripheral blood and synovial infiltrate collected from rheumatoid arthritis patients. ROS can serve as an indirect measure of the intensity of inflammation (Kundu et al. 2012). Similarly, superoxide dismutase activity is higher in the joint fluid of these patients than in healthy subjects and its activity may serve as a valid index of articular destruction and repair (Sumii et al. 1996).

Patients with ulcerative colitis have an elevated neutrophil count when compared with healthy subjects (Hanai et al. 2004). Moreover, polymorphonuclear granulocytes accumulate in the epithelial crypts of the intestinal mucosa in inflamed bowel. Increased production of ROS by neutrophils leads to tissue destruction (Ramonaite et al. 2013). It has been suggested that oxidative stress damages DNA in a damage-regeneration cycle and enhances the risk of carcinogenesis in ulcerative colitis patients (Seril et al. 2003). In patients with Crohn's disease, neutrophils respond to stimulation less than in healthy subjects. Superoxide and lysozyme release by neutrophils, a possible mechanism limiting the extent of inflammation in the intestinal wall, is significantly reduced in patients with active Crohn's disease (Maor et al. 2008).

ROS may also participate in the pathogenesis of preeclampsia, where oxidative stress contributes to endothelial dysfunction. It is suggested that generation of ROS and RNS is caused by reduced blood flow through the placenta. Neutrophils, in line with macrophages, can be activated by oxidative stress, inflammatory agents, or hypoxic conditions during their passage through placental blood vessels. Once activated, polymorphonuclear granulocytes are a source of ROS (Dalle-Donne et al. 2005). Neutrophils are more intensively adhering to the endothelium and infiltrating the intimal space of systemic blood vessels in preeclamptic compared to normal pregnant and non-pregnant women (Cadden and Walsh 2008). It has been shown that supplementation with vitamins C and E in women at increased risk of preeclampsia may be of benefit (Chappell et al. 1999).

7 Conclusions

The role of reactive oxygen and nitrogen species in the physiology of neutrophils is complex. These compounds are potent antimicrobial factors killing bacteria *via* oxidation, nitration, and nitrosylation. It is also evident that these cells regulate the immune response and the intercellular communication. Nonetheless, production of oxidants by polymorphonuclear phagocytes has been shown to exacerbate several diseases. Thus, further investigation is warranted to discern pathologies that dependent on nitrosative and oxidative insults.

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

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The Incidence of Respiratory Tract Infections in Vertically HIV-Infected Children in Lower Silesia in Poland and the Approach to Infection Prevention

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Abstract

Human immunodeficiency virus (HIV) is a risk factor associated with respiratory tract infections. However little is known about the prevalence of these infections in HIV-infected children in Poland. We investigated the incidence of respiratory tract infections in 26 HIV-infected children (aged 4–18, mean 10.3 years, including 17 girls) treated in our center and compared it with the age-matched control group of 70 children. The prevalence of chronic diseases and other factors such as cigarette smoking by household members or attending educational institutions were also taken into consideration. Among the HIV-infected children, 48 respiratory infections were observed, including 4 cases of pneumonia and 44 other respiratory infections for 312 - person-month observations *vs.* 256 infections including 13 cases of pneumonia and 243 other respiratory infections for 840 person-month observations in the control group. Thus, incidence of respiratory infections per month was lower in HIV-infected children (14 %) compared with the control group (29 %), i.e., 0.14 95 % CI (0.10–0.18) infections per month *vs.* 0.29 95 % CI (0.26–0.32). There was no difference in the incidence of pneumonia. The lower incidence of respiratory infections in HIV-infected children may be explained by their avoiding sick people, taking influenza vaccination on the annual basis, and possibly antiviral medication. We conclude that the influence of modifiable environmental factors that reduce the risk of respiratory tract infections is more significant than the HIV infection itself.

Keywords

Human immunodeficiency virus • Hygiene • Immunization • Immunodeficiency • Prophylaxis

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1 Introduction

Respiratory tract infections are some of the most common health problems suffered by children in developed countries. In the US, common cold is the third most common diagnosis made during ambulatory care visits by patients of all ages (Hsiao et al. 2010). Human immunodeficiency virus (HIV)-infected individuals are generally at a higher risk of infectious diseases including respiratory tract infections due to associated immunodeficiency, but in patients on antiretroviral therapy respiratory tract infections are similar in type to those present in the general population (Capocci and Lipman 2013).

Up to the year 2000, respiratory disease had been the main cause of mortality among children with the human immunodeficiency virus (Abrams 2000; Graham et al. 2001; Zar et al. 2001). It has been shown that HIV-positive children were more likely to develop bacterial pneumonia (Graham and Gibb 2002). They are also at increased risk of severe viral lower respiratory tract infections (Madhi et al. 2000b; Mendoza Sanchez et al. 2006). The use of combined antiretroviral therapy and routine *P. jiroveci* prophylaxis in patients with a low CD4 count has improved the outcome of respiratory infections in HIV-positive children and increased survival rates (Graham and Gibb 2002). The majority of previous studies have focused on opportunistic infections and bacterial pneumonia and have not analyzed the prevalence of milder upper respiratory tract infections, e.g., common colds or bronchitis in HIV-infected children (Mendoza Sanchez et al. 2006). Prophylactic measures such as combining good hand hygiene with facemasks or vaccinations, including the annual influenza vaccination, have been shown to be effective tools to protect against influenza in the healthy population (Lee and Shah 2012; Suess et al. 2012; Wong et al. 2014). Little is known about the incidence of acute respiratory tract infections in HIV-infected children in Poland and the efficacy of hygienic precautions and vaccinations in this group of patients. The objective of the present study was, therefore, to assess the general incidence of upper and lower respiratory tract infections among HIV-positive children Trochę brzmi tak, jakbyśmy wszystkie dzieci z HIV w Polsce włączyli do badania in Poland and the efficacy of preventive measures currently used such as good hygiene practices and annual influenza vaccination.

2 Methods

The study was approved by a local Ethics Committee. Over a 1-year period, we observed 26 - HIV-infected children (aged 4–18, mean age of 10.3 years; F/M – 17/9) treated in the Department of Pediatrics and Infectious Diseases in Wroclaw Medical University, in the region of Lower Silesia in Poland. HIV incidence in this region is relatively high amounting to over 100 new cases reported each year in the population of 2.9 million. The age-matched control group consisted of 70 children (aged 3–18, mean age 9.8 years; F/M – 33/37) representing the general population. All HIV-infected children regularly attended educational institutions: nine pre-school children attended kindergarten, ten children attended primary school, and seven children attended secondary school. All patients were vertically infected. A HIV infection was confirmed in all children with a culture or polymerase chain reaction (PCR)-confirmed and was diagnosed, on average, at 3 years of age. All patients received combined antiretroviral therapy (azidothymidine (AZT), lamivudine (3TC), and lopinavir/ritonavir or AZT, 3TC, and nevirapine). They had a very low or undetectable viral load in regular PCR testing repeated every 3 months and CD4 within normal limits. With respect to clinical classification at the time of diagnosis of HIV infection, three children were asymptomatic (N category), seven children had category A, eight children category B, and another eight had category C. Six children suffered from an underlying chronic disease and condition (hepatitis C, tuberculosis, pollinosis and allergy, dilated cardiomyopathy, hypoplasia of the corpus callosum, and left-sided hemiparesis due to previous toxoplasma encephalitis).

We counted episodes of respiratory tract infections including colds, sore throat, tonsillitis,

otitis media, sinusitis, laryngitis, tracheitis, bronchitis, and pneumonia, according to the incidence reported by parents. All children were vaccinated against pneumococcal infections with the conjugated pneumococcal vaccine (PCV13), followed by the polysaccharide pneumococcal vaccine (PPSV23) and annually against influenza with one or two doses of trivalent inactivated flu vaccine (Vaxigrip®). The patients and their parents were given instructions concerning avoiding people with respiratory symptoms, proper hand hygiene and use of facemasks, while the control group did not receive these instructions and were simply observed by a primary care physician working with our team. The incidence of chronic diseases and other factors such as cigarette smoking by household members, social status, the presence of chronic diseases and the number of siblings were examined by means of a 24-item questionnaire covering all demographic data as well as the attendance of educational institutions. The children had facilitated access to healthcare (24 h a day/7 days a week free of charge). The majority of HIV-infected children (24 out of the 26) were treated for respiratory tract infections by primary care physicians in the places of residence. The patients and their parents were interviewed every 3 months.

The incidence rate of respiratory tract infections in HIV-infected children was calculated in absolute numbers per year. For comparison with the control group, the person-time calculation was used; the number of events divided by the number of children times months of observations. In addition, 95 % confidence intervals were calculated. Statistica software ver. 10.0 was used for data elaboration.

3 Results

The incidence of respiratory tract infections in HIV-infected children ranged from 0 to 12 episodes per year. In the group of six children suffering from an underlying chronic diseases, only one teenager with tuberculosis had more than 10 infections, i.e., 12 infections per year. There was a single case of bronchitis and four cases of radiologically confirmed pneumonia (including one patient admitted due to tuberculosis with culture-confirmed diagnosis). The average number of respiratory tract infections (excluding pneumonia) was 1.7 episodes per year (95 % CI: 0.9–2.5) with 3.2 episodes in the preschool children (95 % CI: 1.5–4.9), 3.8 episodes in the primary school children (95 % CI: 1.8–5.8), and 1.4 episodes in the secondary school children (95 % CI: 0.5–2.2). The preschool children usually had 2–3 episodes annually, the primary school children had 4–10 episodes, while the secondary school children usually had only one episode per year. More details are provided in Table 1.

In the control non-HIV-infected children, the average number of respiratory tract infections

Table 1 The incidence of respiratory tract infections (RTI) in different age-groups of vertically infected children with human immunodeficiency virus (HIV) *vs.* controls during 1-year observation period

No. of RTI per year, excluding pneumonia	No. of preschool children		No. of primary school children		No. of secondary school children		All patients	
	HIV *(n = 9)*	Non-HIV *(n = 16)*	HIV *(n = 10)*	Non-HIV *(n = 30)*	HIV *(n = 7)*	Non-HIV *(n = 24)*	HIV *(n = 26)*	Non-HIV *(n = 70)*
0	0	0	1	2	0	12	1	14
1	2	0	2	4	5	4	9	8
2–3	5	2	4	11	2	6	11	19
4–10	2	10	2	13	0	2	4	25
>10	0	4	1	0	0	0	1	4
No. of cases of pneumonia	0	6	4	7	0	0	4	13

Preschool children, under 6 years old; *Primary school children,* 6–12 years old; *Secondary school children*, 13–18 years old

Table 2 The incidence of respiratory tract infections (RTI) in vertically infected children with human immunodeficiency virus (HIV) compared with the control group

No. of RTI per person-month of observation	HIV-infected children (95 % CI)	Control group (95 % CI)	P-value
No. of respiratory infections, excluding pneumonia	44/312; 14 % (0.10–0.18)	243/840; 29 % (0.26–0.32)	<0.0001
No. of pneumonia	4/312; 1.3 % (0.00–0.03)	13/840; 1.5 % (0.01–0.03)	NS

CI confidence interval, *NS* non-significant

(excluding pneumonia) was 3.5 episodes per year (95 % CI: 2.7–4.3) with 6.7 episodes in the preschool children (95 % CI: 4.8–8.6), 3.6 episodes in the primary school children (95 % CI: 2.6–4.5), and 1.2 episodes in the secondary school children (95 % CI: 0.5–1.9). The preschool children had a higher incidence of respiratory tract infections than the primary school children and both these groups had a higher incidence of respiratory tract infections than the secondary school children. The preschool and primary school children usually had 4–10 episodes annually, while the secondary school children had 2–3 episodes annually (Table 1). The incidence of respiratory tract infections (excluding pneumonia) per person-month was significantly lower in HIV-infected children compared with the control group (14 % *vs.* 29 %, respectively; $p < 0.0001$), while the incidence of pneumonia was similar (Table 2).

Caregivers of HIV-infected children rated their social conditions as average (12/26; 46.2 %), good (10/26; 38.5 %), or poor (4/26; 15.3 %). The majority of HIV-infected children (17/26; 65.4 %) were exposed to tobacco smoke at home, usually over 20 cigarettes per day. Five out of the 26 children were hospitalized. The primary care physician was informed of the HIV infection in 18/26 children. Four children were routinely treated with antibiotics for each infection; on average, three times per year.

4 Discussion

Respiratory tract infections are a major public health concern and represent a common problem both in healthy and immunocompromised children, particularly in HIV-positive individuals (Mendoza Sanchez et al. 2006). It has been estimated that in the US an adult person suffers from 2 to 4 colds per year, whereas a schoolchild suffers from 6 to 10 colds (Spector 1995; Johnston and Holgate 1996; Winther et al. 1998; Eccles 2005). According to a study conducted in the general child population in Germany, up to eight episodes of respiratory infections per year at pre-school age and four episodes per year at school age should be regarded as normal and does not indicate any kind of immunodeficiency (Grüber et al. 2008). Therefore, respiratory tract infections represent a significant clinical and therapeutic problem and an economic burden (Wat 2004).

Interestingly, the overall incidence of respiratory tract infections, excluding pneumonia, in the present study was lower in HIV-infected children than in the control group. We attribute these results to the effectiveness of preventive strategies implemented by patients and their caregivers. They were aware of the routes of transmission of respiratory infections, avoided sick people, and were advised on good hygiene practices, notably hand hygiene and use of facemasks. They also benefited from an extensive immunization program including the conjugated pneumococcal vaccination and annual influenza vaccinations as well as the appropriate treatment of infections. With widespread combined antiretroviral therapy, the pattern of HIV-associated pulmonary disease has been changing. Whereas opportunistic infections such as pneumocystis pneumonia still occur in people not using antiretroviral therapy, respiratory infections in successfully treated HIV-infected individuals have a similar course

to those present in the general population (Capocci and Lipman 2013). In Poland, and in our center, HIV-infected children receive a high standard of care, including free access to medical care and combined antiretroviral therapy, ensuring the regular dosing of antiretroviral medications. As a result, all the patients of the present study had an undetectable viral load in PCR testing and CD4 within normal limits. Similar to the general population, mild upper respiratory tract infections (common colds) were the most frequent infection in HIV-positive children. The incidence of pneumonia in HIV-infected children was similar to that in the control group, i.e., about 15 % of HIV-infected children developed pneumonia. In contrast, the majority of previous studies have reported a higher prevalence of pneumonia in HIV-positive than in non-HIV positive children (Spira and Lepage 1999; Madhi et al. 2000a; Taha and Graham 2000). That was, however, before the era of highly active antiretroviral therapy. At that time, bacterial pneumonia was 5–10 times more common in HIV-infected children in the US and Europe (Dankner and Lindsey 2001). The availability of HAART and prophylaxis against *Pneumocystis jiroveci* has favorably changed the pattern of respiratory infections in HIV-infected individuals. Nonetheless, it has been shown that bacterial pneumonia also occurs in children with a relatively high CD4 cell count (Graham and Gibb 2002).

It is possible that our results show further progress in the care of HIV-infected children or the observation period was too short to enable recognition of differences with respect to the control group. The present study underscores the importance of widespread, up-to-date immunization of HIV-infected children, despite that the precise benefit of immunization remains unclear. This conclusion is in agreement with that expressed a recent review of respiratory tract infections in HIV-infected population (Capocci and Lipman 2013).

There are several limitations of this study. Part of the data come from questionnaires, and thus may be biased. The study included a relatively small group of HIV-infected children, and the 1-year observation period may also be short. Further, there is lack of details of respiratory infections, e.g., onset of infection and microbiological tests or cultures confirming the etiology. The diagnosis of respiratory tract infection was based on clinical presentation rather than on laboratory test, such white blood cell count, C-reactive protein level, or chest X-ray. We believe, however, that the study has strongly pointed attention to the prophylactic value of hygienic precautions and annual influenza and pneumococcal vaccinations in common respiratory tract infections in HIV-infected children.

5 Conclusions

Modifiable environmental factors such as improved hygiene and influenza vaccination reduce the risk of respiratory tract infections, with the exception of pneumonia, in HIV-infected children. All HIV-infected children and they caregivers should be trained in hand hygiene, the use of facemasks and avoiding sick individuals as well as being immunized against influenza. These preventive measures are effective in controlling respiratory tract infections in HIV-infected children.

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

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Implementation of Hospital's Antibiotic Policy Decreases Antimicrobial Use in the General Pediatric Ward

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Abstract

Hospitalized children are often treated with antibiotics. However, 30–75 % of antibiotic treatment in pediatric hospitals is administrated incorrectly or unreasonably. Implementation of Hospital's Antibiotic Policy (HAP) should improve antibiotic consumption patterns in pediatric wards. The objective of this study was to determine the effectiveness of HAP by assessing antibiotic consumption in the General Pediatric Ward of an academic hospital in the city of Warsaw, Poland before and after this policy was introduced in the years 2012 and 2013, respectively. Antibiotic use was calculated in daily-defined doses (DDDs) per 100 patient-days and DDDs per 100 admissions. Antibiotics were ranked by the volume of DDDs and the number of antibiotics which accounted for 90 % and 100 % of the total volume: DU90% and DU100% (where DU stands for drug use). The total antibiotic consumption and significantly decreased after the implementation of HAP; DDDs were 2,177.5 before and 1,335.4 after implementation of HAP. The number of DDDs/100 patient-days was also lower; 36.3 *vs.* 24.9 before and after HAP, respectively. After implementation of HAP a decreased use of ceftriaxone and cefuroxime was observed. The most commonly used antibiotic was amoxicillin with clavulanic acid. The DU100% rates remained the same (8 antibiotics) and DU90% increased (from 3 in 2012 to 5 in 2013). We conclude that implementation of HAP resulted a decreased consumption of antibiotics in the General Pediatric Ward, despite the hardly changed number of children treated with antibiotics.

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Keywords
Antimicrobials • Antibiotics • Drug utilization • Policy • Stewardships

Abbreviations

AAP	American Academy of Pediatrics
ATC	Anatomical Therapeutic Chemical Classification
CAP	Community-acquired pneumonia
CDC	Centers for Disease Control and Prevention
DDDs	Daily-defined doses
DU90%	90 % drug utilization
DU100%	100 % drug utilization
IDSA	Infectious Diseases Society of America
HAP	Hospital Antibiotic Policy
NPAP	National Program of Antibiotic Protection
PIDS	Pediatric Infectious Disease Society
SHEA	Society of Healthcare Epidemiology of America
WHO	World Health Organization

1 Introduction

Antibiotics are among the drugs most commonly prescribed for children. Although the vast majority of antibiotics are consumed in primary care, the pressure to opt for antimicrobial drugs in hospitals appears to be even higher than in outpatient care. It has been estimated that 36–49 % of hospitalized children receive antibiotics (Potocki et al. 2003; van Houten et al. 1998). It should also be highlighted that 15–45 % of antibiotic treatment regimens for pediatric patients may be inappropriate (Principi et al. 1981; Schollenberg and Albritton 1980). The frequent use of antibiotics is considered to be the main reason for the high prevalence of antimicrobial resistance, which may also result in side effects and excessive costs (Berild et al. 2008; Shehab et al. 2008; de Man et al. 2000). Therefore, monitoring of antibiotic use in hospitals is important for identifying prescribing trends, linking results with antimicrobial resistance data and identifying areas for improvement (Atti et al. 2008). In 2007, the Infectious Diseases Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) published guidelines for developing institutional antimicrobial stewardship programs. Initial efforts were focused on the adult patient population but efforts for the widespread implementation of formal pediatrics programs have occurred in recent years. In 2010, the Pediatric Infectious Disease Society (PIDS) and the American Academy of Pediatrics (AAP) recommended the implementation of the Hospital Antibiotic Policy (HAP) for healthcare establishments providing inpatient and outpatient pediatric care (Hyun et al. 2013). To address the growing problem of antibiotic overuse and resistance, the Polish Ministry of Health has also urged all hospitals, including pediatric establishments, to develop systems to monitor antibiotic use and have encouraged physicians to reduce inappropriate use (Hryniewicz and Ozorowski 2011). According to the Centers for Disease Control and Prevention (CDC), antibiotic stewardship strategies should include one or more of the core principles: timely management of antimicrobial therapy, appropriate selection of antimicrobials, appropriate administration and de-escalation of antimicrobial therapy, use of available expertise and resources at the point of care and transparent monitoring of antibiotic use data (CDC 2012). As two of the goals mentioned have already been achieved in our hospital (formation of a multidisciplinary antibiotic stewardship committee and auditing and monitoring of antibiotic use), we decided to evaluate the results of HAP introduction in the General Pediatric Ward in Warsaw, Poland. According to our best knowledge this is the first such analysis in

Table 1 Age distribution of patients admitted to the General Pediatric Ward

Year	Number of hospitalized patients	Number and proportion (%) of infants	Number and proportion (%) of children aged 1–3 years	Number and proportion (%) of children aged 4–6 years	Number and proportion (%) of children aged >6 years
2012	1,083	545 (50 %)	346 (32 %)	144 (13 %)	48 (5 %)
2013	1,111	559 (50 %)	358 (32 %)	147 (13 %)	47 (5 %)

Poland, while the amount of research in this field by central and eastern European countries is also very limited.

2 Methods

Approval from the local Ethics Committee was obtained prior to the study. A retrospective analysis of antibiotic consumption in the General Pediatric Ward before and after implementation of HAP was conducted. The period analyzed was the year 2012 (a year before HAP's implementation) and the year 2013 (a year after HAP's implementation). The General Pediatric Ward is a 21-bed primary level unit providing care for children aged 0–18 years in Warsaw, the capital of Poland. The total number of admissions at the General Pediatric Ward was 1083 in 2012 and 1,111 in 2013. The number of patient-days was 5,592 in 2012 and 5,349 in 2013, while the mean length of hospitalization was 5.5 days in 2012 and 4.48 in 2013. The majority of patients hospitalized were infants and children under 3 years of age (Table 1). In 2013, an extensive antibiotic policy program created by the members of the hospital's infection control team (ICT) was rolled out at the hospital. The HAP was defined as written guidelines for prescribing antibiotics. Antibiotics used in the hospital were divided into three groups: first-line antibiotics (e.g., penicillin, 1st generation cephalosporins), second-line antibiotics (e.g., macrolides, 2nd and 3rd generation cephalosporins, amoxicillin with clavulanic acid, piperacillin with tazobactam), and third-line antibiotics (restricted antibiotics) only available after pre-authorization (or additional authorization) from a physician from the infection control team (e.g. glycopeptides, carbapenems). Data on the quantitative and qualitative use of antibiotics in 2012 and 2013 were reported by the hospital's pharmacy. The data represented the dispensing of antibiotics from the hospital pharmacy to the General Pediatric Ward. The annual report on the total volume of antibiotics prescribed was analyzed and antibiotic use was calculated in daily-defined doses (DDDs) per 100 patient-days and DDDs per 100 admissions according to the Anatomical Therapeutic Chemical Classification (ATC) System, from the WHO, version 2009. Furthermore, antibiotics were ranked by volume of DDDs and the number of antibiotics, which accounted for 90 % and 100 % of the total volume (DU90% and DU100%, respectively, where DU stands for drug use). For statistical analysis, the chi-square test was used for categorical variables. The medical statistical calculator, available on www.medcalc3000.com, was used.

3 Results

The majority of patients were referred to the hospital by general practitioners or primary care pediatricians due to respiratory tract infections (RTI) (Table 2). Among RTI cases, the most common diagnoses were: pneumonia (82 %), bronchitis (10 %), bronchiolitis (2 %), tonsillitis (1 %), laryngitis (2 %), sinusitis (2 %), and otitis media (1 %). The second biggest cause of admission to the hospital was gastrointestinal infection: gastroenterocolitis of unknown etiology (51 %), gastroenterocolitis of rotavirus or adenovirus etiology (36 %), and gastroenterocolitis of bacterial etiology (13 %). After the implementation of HAP, the proportion of children with RTI treated with antibiotics significantly decreased (91 % *vs.* 86 %, $p < 0.05$). The proportions of patients receiving antibiotics for the treatment of other infections (e.g., urinary tract or gastrointestinal infections) remained unchanged ($p > 0.05$). The total proportion of patients treated with

Table 2 Reasons for hospitalization and proportions of patients receiving antibiotic therapy at the General Pediatric Ward

Year	2012	2013	2012–2013	2012	2013	2012–2013
Diagnosis	No (%) of patients receiving diagnosis			No (%) of patients receiving antibiotics		
Respiratory tract infections	603	607	1,210	552	523	1,075
	(56 %)	(55 %)	(55 %)	(91 %)*	(86 %)*	(89 %)
Gastrointestinal infections	327	378	705	93	102	195
	(30 %)	(34 %)	(32 %)	(28 %)	(27 %)	(28 %)
Urinary tract infections	99	76	175	99	76	175
	(9 %)	(7 %)	(8 %)	(100 %)	(100 %)	(100 %)
Skin infections	18	15	33	7	6	12
	(2 %)	(1 %)	(2 %)	(38 %)	(40 %)	(36 %)
Sepsis	15	4	19	15	4	19
	(1 %)	(0.4 %)	(1 %)	(100 %)	(100 %)	(100 %)
Other infections	12	14	26	8	9	18
	(1 %)	(1 %)	(1 %)	(67 %)	(64 %)	(69 %)
Other reasons	9	17	26	3	6	9
	(1 %)	(2 %)	(1 %)	(33 %)	(35 %)	(35 %)
Total	1,083	1,111	2,194	777	726	1,503
	(100 %)	(100 %)	(100 %)	(72 %)*	(65 %)*	(68 %)

*statistically significant ($p < 0.05$)

Table 3 Antibiotic consumption patterns before and after HAP's implementation

Antibiotics for systemic use	DDDs		DDDs/100 Patient-days		DDDs/100 admissions		% of DDDs	
Year	2012	2013	2012	2013	2012	2013	2012	2013
Penicillin:								
Ampicillin	1.3	0.0	0.0	0.0	0.1	0.0	0.1	0.0
Penicillin with beta-lactamase inhibitors:								
Amoxicillin & clavulanic acid	739.6	650.6	12.3	12.2	68.3	58.6 %	34.0	48.7
Aminoglycoside:								
Amikacin	103.8	92.3	1.7	1.7	9.6	8.3	4.8	6.9
2nd generation cephalosporins								
Cefuroxime	667.5	295.0	11.1	5.5	661.6	26.8	30.6	16.5
3rd generation cephalosporins:								
Cefotaxime	0.0	75.0	0.0	1.4	0.0	6.8	0.0	5.6
Ceftazidime	7.5	10.0	0.1	0.2	0.7	0.8	0.4	0.7
Ceftriaxone	540.0	65.0	9.0	4.9	49.8	5.9	24.7	4.9
Macrolides:								
Clarithromycin	85.0	160.0	1.4	3.0	7.8.4	14.5	3.9	12.0
Lincosamides:								
Clindamycin	32.8	62.5	0.5	1.2	3.0	5.7	1.5	4.7
Total	2177.5*	1335.4*	36.3*	24.9*	201.0*	121.4*	100 %	100 %

DDD daily defined doses
*Statistically significant differences ($p < 0.05$)

Table 4 Drug use rates (DU90%, DU100%) before and after HAP's implementation

Year	DU100%	DU90%
2012	8:	3:
	Ampicillin	Amoxicillin & clavulanic acid
	Amoxicillin & clavulanic acid	Cefuroxime
	Amikacin	Ceftriaxone
	Cefuroxime	
	Ceftazidime	
	Ceftriaxone	
	Clarithromycin	
	Clindamycin	
2013	8:	5:
	Amoxicillin & clavulanic acid	Ampicillin & clavulanic acid
	Amikacin	Cefuroxime
	Cefuroxime	Clarithromycin
	Ceftazidime	Amikacin
	Cefotaxime	Ceftazidime
	Ceftriaxone	
	Clarithromycin	
	Clindamycin	

DU90% drug use accounting for 90 % of the total volume, *DU100%* drug use accounting for 90$ of the total volume

antibiotics was significantly reduced after the implementation of HAP (72 % *vs*. 65 %, $p < 0.05$) (Table 2).

None of the restricted antibiotics (including glycopeptides or monobactams) were used either before or after HAP's implementation (Table 3). After implementation of HAP, a decreased use of ceftriaxone and cefuroxime was observed. The most commonly used antibiotic was amoxicillin with clavulanic acid (Table 3). The DU100% remained the same before and after implementation of HAP (8 antibiotics), while DU90% increased after implementation of HAP (5 *vs*. 3 antibiotics) (Table 4).

4 Discussion

An important issue identified in our results is a high proportion of hospitalized children who were treated with antibiotics (65–72 %). This proportion is higher than observed previously in the eastern European countries (36–45 %) (Ang et al. 2008; Potocki et al. 2003; van Houten et al. 1998). However, some researchers recently found even a higher proportion of hospitalized children receiving antimicrobials (57 %) (Levy et al. 2012). Our data are similar to those obtained in pediatric intensive care units (70.8 %) (Blinova et al. 2013; Grohskopf et al. 2005). However, the present study was conducted in the general pediatric ward. The results indicate that Poland is a country with a high antimicrobial usage rate, which has also been reported by others (Rossignoli et al. 2007; Stichele et al. 2006).

We observed that the majority of patients were admitted due to respiratory tract infections. It is well known that a high proportion of pediatric respiratory tract infections are of a viral origin and do not require antibiotic therapy. We presume that antibiotics were overused in our group of patients. Several studies have demonstrated the overuse of antibiotics in clinical situations where antimicrobials are not indicated, including exacerbation of asthma, pharyngitis, viral respiratory infections, and bronchiolitis secondary to syncytial virus infection (de Boeck et al. 2011; Gaur et al. 2005; Stallworth et al. 2005; Benin et al. 2003). To decrease the risk of inappropriate use of antimicrobials, they should be prescribed less often empirically and more often after microbiological examination (Atti et al. 2008).

We reported that just 1 year after the implementation of the HAP, the total antibiotic consumption in the general pediatric ward measured in DDDs decreased. It can be explained by a shorter inpatient treatment and less often use of the combined therapy, i.e., two or three antibiotics in the same patient. This indicates that benefits from the HAP's implementation can be expected very quickly, improving the quality of care for hospitalized children, preventing the emergence of resistance and lowering drug-related costs (Ohl and Luther 2011; di Pentima et al. 2011). It is also worth pointing out that the proportion of children with respiratory tract infection treated with antimicrobials decreased after HAP's implementation, which is

in agreement with other findings (Papaevangelou et al. 2012). The most commonly used antibiotic in the present study was amoxicillin with clavulanic acid followed by cefuroxime. It has been previously described that these antibiotics are overused in Polish hospitals (Bruce et al. 2009; Stichele et al. 2006; Cars et al. 2001). However, these are first-line antimicrobials in the treatment of community-acquired pneumonia (CAP) in young children according to the Polish recommendations (NPAP 2011). Amoxicillin with clavulanic acid was also found to be the most commonly used antibiotic in other pediatric wards in Europe (Svestina and Mozgis 2014; Atti et al. 2008). High pharmacological effectiveness against most local and systemic infections, a low incidence of side effects, and availability in many suitable dosage forms are the likely reasons behind the preferential prescribing of amoxicillin with clavulanic acid (Katakam et al. 2012). According to the Pediatric Infectious Disease Society of America (PIDS), the first-line antibiotics in the hospital treatment of uncomplicated CAP in fully-immunized children without any underlying medical conditions are ampicillin, macrolides, and beta-lactams when atypical pneumonia is suspected (Bradley et al. 2011). These differences in recommendations may be explained by different national immunization schedules (for example, in Poland the pneumococcal vaccination is not a universal one). After implementation of HAP in the general pediatric ward we observed a decreasing trend in the use of third generation cephalosporins which is in agreement with the national recommendations and the findings of others (di Pentima et al. 2011).

It should be noted that the number of antibiotics in the DU100% segment remained the same before and after HAP's implementation (8 antibiotics). Interestingly, the number of antibiotics in the DU100% (8) and in the DU90% (3–5) was relatively low and comparable with Dutch data (Liem et al. 2010), but lower than reported in China (between 16 and 20) (Zhang et al. 2008), Russia (8, out of a total of 22 antibiotics used) (Hajdu et al. 2007), and Croatia (11, out of a total of 35) (Palcevski et al. 2004). The DU90% rate increased after the implementation of HAP (5 vs 3 antibiotics). The DU90% has been proven to be an important tool for assessing the quality of drug prescription. In addition to the number of drugs in the DU90% segment, the presence of treatment guidelines and adherence to them may serve as general quality indicators (Bergman et al. 1998). Our findings clearly suggest that the implementation of HAP in the general pediatric ward quickly resulted in a decrease in the total use of antimicrobials, with good adherence to the national recommendations.

The introduction of HAP in the General Pediatric Ward described in the present study demonstrated not only a positive change in the antibiotic consumption pattern but also a general decrease in the antibiotic use. These results are thus more advantageous than those described previously for the Special Care Neonatal Unit (SCNU) (Nitsch-Osuch et al. 2015), where just the consumption pattern of antibiotics was improved. The difference may be explained by several factors. Newborns hospitalized in SCNU are treated mainly for congenital intrauterine infections, so that antibiotics should be effective against Gram negative bacteria. On the other side, children hospitalized in the General Pediatric Ward are diagnosed with community acquired infections, so antimicrobials should be active mainly against Gram positive bacteria. Our results raise attention to the necessity of comparing the antibiotic consumption rates and patterns between similar hospital wards to make the comparison meaningful.

Our study has some limitations. Firstly, it was conducted in just one hospital and the results cannot be representative of the entire country. Secondly, we did not evaluate the appropriateness of the antibiotic prescriptions. Thirdly, antibiotic consumption was estimated with DDDs, while they are targeted at the average maintenance dose per day for a specific antimicrobial drug per adult population and do not take into consideration differences in dose-range and weight-based dosing. However, the alternative metrics, including days of therapy and doses administered, have not been fully evaluated yet (di Pentima et al. 2011). That is the reason why DDDs are still used for the

assessment of antibiotic consumption in a pediatric population (Kuster et al. 2008). Additionally, we only compared two consecutive years and some epidemiologic variations in diseases patterns, e.g., influenza incidence, may have influenced antibiotic consumption.

5 Conclusions

The consumption of antibiotics in the General Pediatric Ward decreased after the implementation of the Hospital Antibiotic Policy, especially among children with respiratory tract infections. This intervention was effective in bringing about desirable positive changes in antibiotic consumption patterns. This successful experience in our General Pediatric Ward should encourage other pediatric centers to pursue similar programs.

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

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Detection of *Chlamydophila Pneumoniae* and Typical Bacteria in Patients with Chronic Cough

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Abstract

The aim of research was to analyze the results of microbiological tests for typical and atypical bacteria in patients with symptoms of chronic cough. A total of 214 outpatients aged from 2 to 94 years (110 women, 64 men, and 40 children) with chronic cough were studied. Four hundred twenty eight throat swabs were examined for atypical bacteria antigen (*Chlamydophila pneumoniae*) (n = 214) and typical pathogens (n = 214). *Chl. pneumoniae* detection was performed using indirect immunofluorescence test. Classical microbiological culture was used for typical bacteria detection. *Chl. pneumoniae* antigen was detected in 55/214 (26.0 %) patients with chronic cough (in 31 (28.2 %) women, 14 (21.9 %) men, and 10 (25.0 %) children). Positive culture for typical pathogens was observed in 30 (27.3 %) women, 22 (34.4 %) men, and 21 (52.5 %) children. Simultaneous occurrence of *Chl. pneumoniae* and typical pathogens (*Staphylococcus aureus* strain MSSA, *Streptococcus pyogenes*, or *Moraxella catarrhalis*) was found in 16 (7.5 %) patients. The findings show that in patients with chronic cough *Chl. pneumoniae* infection, although less than that with typical pathogens, is rather frequent. Further, the performance of test for *Chl. pneumoniae* in throat swabs from patients with chronic cough is good and provides an efficient way to diagnose the infection and implement appropriate therapy.

Keywords

Atypical bacteria • Co-infection • Immunofluorescence • Respiratory tract • Throat swabs

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1 Introduction

Respiratory tract infections represent a heterogeneous group of common acute infectious problems and consist of various underlying

causes and symptoms which are difficult to distinguish one another. Depending on the location, infections can be divided into upper (URTI) and lower respiratory tract infections (LRTI). The most frequent URTI are common cold, tonsillitis, pharyngitis (sore throat), and sinusitis. LRTI, in turn, are acute bronchitis and pneumonia. Clinical features of different respiratory tract infections largely depend on the affected anatomical structure and on inflammation induced functional alterations.

Respiratory infections are the most common cause of outpatient counseling. They constitute about 50–60 % of all community-acquired infections and are the most common cause of fever in infants and young children (Armstrong and Pinner 1999). Infections occur at different frequencies depending on the interaction of age and risk factors. The main cause of acute respiratory tract infections are viruses; most notably rhinoviruses, followed by adenoviruses, coronaviruses, influenza and parainfluenza viruses, respiratory syncytial viruses, and enteroviruses (Alter et al. 2011; Griffin et al. 2004; Monto 2004).

Likewise, bacterial infections are of highly variable etiology. Community-acquired bacterial infections are most often caused by microorganisms included in the group of typical pathogens, such as *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis,* and *Streptococcus pyogenes.* Identification of typical microorganisms is possible in every microbiological laboratory, and the waiting time for the outcome does not exceed 48 h. Belonging to the group of typical bacteria, although less frequently underlying the etiology of respiratory tract infections, are also gram-negative rods, for instance *Escherichia coli, Klebsiella pneumoniae, Enterobacter* sp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and anaerobic bacteria.

An important role in the development of respiratory tract infections also play atypical bacteria – *Chlamydophila pneumoniae, Mycoplasma pneumoniae,* or *Legionella pneumophila* (Käding et al. 2014). In the diagnosis of atypical bacterial infections, molecular methods, such as polymerase chain reaction (PCR), are increasingly used. Serological studies, which are widely used in this type of infection, are primarily of epidemiological importance (Woodhead et al. 2011). The aim of the present study was to analyze the results of microbiological tests for typical and atypical bacteria in patients with symptoms of chronic cough, the most typical feature of respiratory tract infection.

2 Methods

The investigation was performed in accordance with the Declaration of Helsinki for Human Research and the study protocol was accepted by the Ethics Committee of the Medical University of Wroclaw, Poland.

The study group consisted of 214 outpatients (110 women, 64 men, and 40 children) aged from 2 to 94 years. The main criterion for inclusion in the study was persisting cough and hoarseness due to respiratory tract infection. The study covered the period September 2013 to September 2014. The analyzed material were pharyngeal swabs collected in the morning, after overnight fasting and before performing routine oral hygiene. Pharyngeal swabs were examined for atypical bacteria – *Chl. pneumoniae* antigen and for typical pathogens. *Chl. pneumoniae* antigen was detected using indirect immunofluorescence test (Chlamydia Cel PN-IFT Kit; Cellabs Pty Ltd, Sydney, Australia). Specimens were stained in two sequential steps; the first one using suspension with monoclonal antibodies which bind specifically to *Chl. pneumoniae* antigen and the second using FITC- conjugated goat anti-mouse antibodies to visualize *Chl. pneumoniae* microorganisms. Visualization of 4 or more chlamydial elementary bodies among epithelial cells was taken as the criterion of positive diagnosis.

Typical bacteria were detected using a classical microbiological throat swab culture. All swab specimens were taken before the onset of antibiotic therapy.

3 Results

Table 1 presents the results of pharyngeal swabs examination for *Chl. pneumoniae* and typical

Table 1 Distribution of pathogens found in throat swabs in patients with respiratory tract infection

	No. of patients	*Chl. pneumoniae*	Typical pathogens[a]	Co-infection
Women	110	31 (28.2 %)	30 (27.3 %)	9 (8.2 %)
Men	64	14 (21.9 %)	22 (34.4 %)	3 (4.7 %)
Children	40	10 (25.0 %)	21 (52.5 %)	4 (10.0 %)
Total	214	55 (25.7 %)	73 (34.1 %)	16 (7.5 %)

[a]*Staphylococcus aureus* strain MSSA, *Streptococcus pyogenes*, *Moraxella* sp.

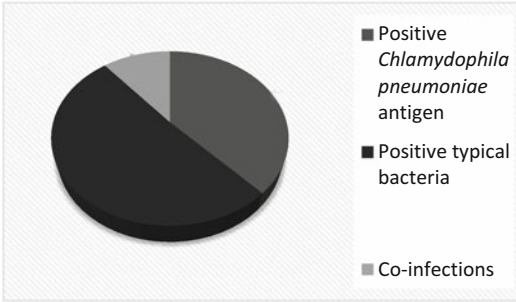

Fig. 1 Distribution of typical and atypical pathogens underlying respiratory tract infections

bacteria in 214 patients with chronic cough and hoarseness. *Chl. pneumoniae* antigen was found in 55/214 (25.7 %) patients; including 31 (28.2 %) women, 14 (21.9 %) men, and 10 (25 %) children. Typical pathogens were detected in 73/214 (34.1 %) patients; including 30/110 (27.3 %) women, 22/64 (34.4 %) men, and 21/40 (52.5 %) children. The most frequently occurring typical pathogen was *Staphylococcus aureus* MSSA, followed by *S. pyogenes* and *Moraxella catarrhalis*. Figure 1 presents the distribution of typical and atypical bacteria pathogens underlying respiratory tract infections manifesting with cough and hoarseness as well as the prevalence of co-infections with both types of pathogens. Co-infections occurred in 11 % of the patients in whom positive test results were found.

4 Discussion

The most common etiological agents of bacterial community-acquired respiratory tract infections include *Streptococcus pneumoniae*, *Streptococcus pyogenes, Haemophilus influenzae*, *Moraxella catarrhalis*, and atypical bacteria such as *Chlamydophila pneumoniae*, *Mycoplasma pneumonia,* and *Legionella pneumophila*. Infections caused by atypical pathogens are not characterized by specific clinical course; therefore they are difficult to diagnose empirically. In addition, recommendations do not point to a single standardized diagnostic test which could distinguish between typical and atypical pathogens. A high percentage of specific antibodies to *Chl. pneumoniae* in the general population indicate the appreciable prevalence of infections with this microorganism. In a study of Miyashita et al. (2001), the occurrence of antibodies against *Chl. pneumoniae*, indicative of previous infection, was 58.8 % in men and 39.6 % in women, although the acute signs of infection were manifest in just 10.6 % of these cases. Acute phase of infection often leads to cough and hoarseness lasting many weeks, whereas chronic phase of infection is generally asymptomatic.

Epidemiological data on the incidence of infection caused by *Chl. pneumoniae* are variable, depending on the diagnostic methods used. Direct methods, such as PCR or cell culture, demonstrate that *Chl. pneumoniae* infection also affects young children (She et al. 2010; Michelow et al. 2004). Modern molecular techniques allow detecting the presence of pathogens in infants, where the widely used serologic tests usually fail to confirm the occurrence of infection caused by this pathogens (Verkooyen et al. 1998). Interestingly, publications of many authors point to large differences regarding the prevalence of *Chl. pneumoniae* in respiratory tract infections in children; ranging from about 10 % in hospitalized patients and 21–43 % in outpatients. However, the prevalence of *Chl. pneumoniae* may be underestimated due to a relatively low rate of acute infection requiring outright medical intervention (Choroszy-Krol et al. 2010, 2014;

Schmidt et al. 2003; Normann et al. 1998). Concerning the etiological factors of respiratory tract infections, it is important to consider co-infections, which are quite common. In the course of *Chl. Pneumoniae*-induced acute pharyngitis in children, this pathogen was found in 3 % of patients, while other co-infecting bacterial pathogens were isolated in 13 % of cases (Esposito et al. 2004). Schmidt et al. (2003) have demonstrated a much higher proportion of infections caused by *Chl. pneumoniae*, amounting to 28 %, in which the antigen was found in 25 %. The present results are generally in line with those findings, although we found a lower co-infection rate with other bacterial pathogens, which may be related to a different patient population studied or different diagnostic methods.

We conclude that prevalence of infection with *Chl. pneumoniae* is high, as it nears about two thirds of infections with typical pathogens, *Chl. pneumoniae* infection occurs frequently in children, and is often associated with co-infections.

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

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Compliance with Vaccination Against Influenza Among the Elderly

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Abstract

Protective vaccination against influenza is one of the most beneficial methods of preventing this viral disease. The use of vaccines brings not only the health benefits but also has positive implications related to diminishing the costs of treatment, prolonged hospitalization or post-influenza complications. Promoting vaccinations against influenza among the elderly is especially important. The article concerns the perception of these vaccinations and evaluation of the general knowledge on influenza among listeners of a university of the third age in Warsaw, Poland. It aims also at assessing the potential to change opinions and decisions regarding vaccinations against influenza and widely understood influenza prevention among this target group. The research tool, apart from the scientific lecture-like presentation rich in examples, was a self-reported questionnaire designed by the authors specifically for this study purpose. This paper presents the results of survey conducted with the questionnaire completed by 29 persons over 60 years of age. We found that the recent vaccination rate against influenza was just was about 20 % in the studied sample of the elderly. The study demonstrates that educational training through a professional lecture presentation facilitates the promotion of health and vaccination coverage against influenza in the elderly.

Keywords

Elderly • Influenza • Survey • Third age university • Vaccination

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1 Introduction

Seniors constitute a growing social group in developed countries. Currently, in Poland people over 65 years of age account for over 14 % of the population. Research carried out by the Central

Statistical Office (CSO) shows that this group includes merely 3.5 % of professionally active individuals (CSO 2013). Various organizations are created for this social group to prevent different types of social exclusions of older people. Universities of the third age (UTA) are one of those forms. These universities promote education among the elderly, exchange of knowledge and experiences between different countries, and research on adult education.

Old age, as a phase of life and a biological and social phenomenon, has become the common experience of humanity in the face of the rapidly accelerating demographic aging. The WHO data show that annually 5–25 % of the world population suffer from influenza virus infection and flu-like diseases, while 0.5–1 million people die because of multiorgan complications (Brydak 2008). A directive on the prevention and control of seasonal influenza pandemic and epidemic, adopted by WHO (2003), contains recommendations to increase the vaccination coverage against influenza, especially in high-risk groups.

In the 2012/2013 epidemic season there were around three million cases of influenza recorded in Poland and more than 12,000 hospitalizations associated with it (Czarkowski et al. 2014; Reports of influenza in Poland 2012). Most cases of influenza complications, including deaths, refer to older people, regardless of their general health status. According to WHO data, total mortality from influenza and pneumonia among seniors is in the fifth place. Among people over 60 years old, mortality due to influenza is estimated at 95 % (Material of Conference Woman and Man 'Healthy Aging' 2012). Given a low level of vaccination coverage against influenza (3.7 % of the population in the 2012/2013 season), it seems necessary to disseminate knowledge, especially among older people, about the dangers of flu, its complications as well as the economic and social effects in this population group. In Poland influenza vaccinations are recommended since 1994.

The aim of the present study was to investigate the level of basic knowledge, opinions, and declared behavior related to influenza and influenza vaccination among listeners of the Mokotowski University of the Third Age (MUTA) in Warsaw, Poland, in 2013. The study, in particular, evaluated the opinion of people over 60 years of age concerning the decision to vaccinate themselves and their relations against influenza after receiving a professional consult on the vaccine effectiveness in influenza prevention and potential benefits regarding the development of life threatening side effects if influenza has been caught.

2 Methods

The study consisted of self-reported survey. Respondents gave informed consent to the survey procedure which was conducted in accord with the guidelines of the Helsinki Declaration for Human Research. Participation in the survey was anonymous, voluntary, and unpaid. The survey study was conducted among 29 seniors (F/M – 21/8) of the average age of 69 years in the 2012/2013 influenza season. Nineteen persons had college education, while the remaining had high school or vocational education. An original diagnostic survey questionnaire was used among the audience of the MUTA in Warsaw, Poland. The survey included the information on the scope of the study, personal data (age, sex, place of residence, and education) and 15 research questions. The survey included 13 multiple-choice questions, while two additional questions, apart from the possibility to select a given response, allowed the respondent to express his own opinion. Respondents answered questions concerning both the general knowledge about the flu and its complications as well as those regarding factors that may influence a decision to be or not to be vaccinated. Seniors completed the survey twice, before and after the professional lecture-type presentation with slides entitled 'Influenza in Questions and Answers'. The aim of the survey was to determine how a richly illustrated medical presentation would influence the perception of the elderly on the influenza issue.

The results were elaborated with the use of an Excel spreadsheet by specifying the percentage of responses to a given item concerning the knowledge, opinions, and behavior on influenza and influenza vaccination.

3 Results and Discussion

The majority of respondents were women (72.4 %) living in cities with the inhabitants of more than 100,000 persons, which caused that the determination of the influence of gender or the place of residence on the opinion about vaccination against influenza could not be analyzed. The respondents' answers concerned three thematic blocks:

- Basic information about the knowledge and the sources of knowledge regarding influenza virus;
- Opinions on influenza and influenza vaccination;
- Motivation of seniors to become vaccinated against influenza or the lack thereof.

The survey focused on such aspects of influenza as the source of information on the disease, the clarity of communication, the best time of the year to be vaccinated, the content of vaccines, complications of influenza, as well as the average yearly number of deaths worldwide due to influenza and influenza related complications. Table 1 presents the effects of the prevention lecture on seniors' awareness of the influenza issue.

The results demonstrate the effectiveness of disseminating professional medical information on seniors' attitude toward the influenza issue. Respondents indicated the mass media and scientific publications available *via* the Internet, followed by medical personnel, as the most notable sources of information about influenza and vaccination. The mean rating of the knowledge on vaccination and influenza vaccine was also high. The comprehensive understanding of medical issues linked to influenza clearly increased after the lecture presentation as the positive response to the item 'Sufficiently communicated by the media' more than doubled; from 24.1 % before to 51.7 % after the lecture (Table 1). The lecture presentation enhanced the knowledge of the elderly listeners on the composition of the vaccine, its most components, health complications of influenza, including possible fatality, and when and who to vaccinate.

Table 1 Effects of prevention lecture on seniors' awareness and knowledge on influenza

	Before lecture	After lecture
	% of answers	% answers
Information given by the mass media on vaccination against influenza is:		
Sufficiently communicated	24.1	51.7
Insufficiently communicated	55.2	41.4
No opinion	10.3	3.4
No answer	10.3	3.4
Sources of information on vaccination against influenza:		
Mass media	31.0	48.3
Scientific publications *via* Internet	37.9	27.6
Family	6.9	10.3
Medical staff	37.9	24.1
Others	10.3	10.3
Influenza complications:		
Known	93.1	93.1
Unknown	6.9	6.9
Optimal timing of vaccination against influenza:		
Beginning of autumn	69.0	69.0
Winter	0	3.4
Spring	0	0
It does not matter	6.9	10.3
I do not know	24.1	17.2
Influenza vaccine component content:		
Whole virion	6.9	10.3
Glycoproteins: hemagglutinin and neuraminidase	6.9	17.2
RNA of the virus	13.8	37.9
I do not know	72.4	34.5
Number of people who die worldwide each year from influenza and its complications:		
10,000 cases	6.9	0
100,000 cases	13.8	6.9
1,000,000 cases	6.9	51.7
I do not know	72.4	41.4

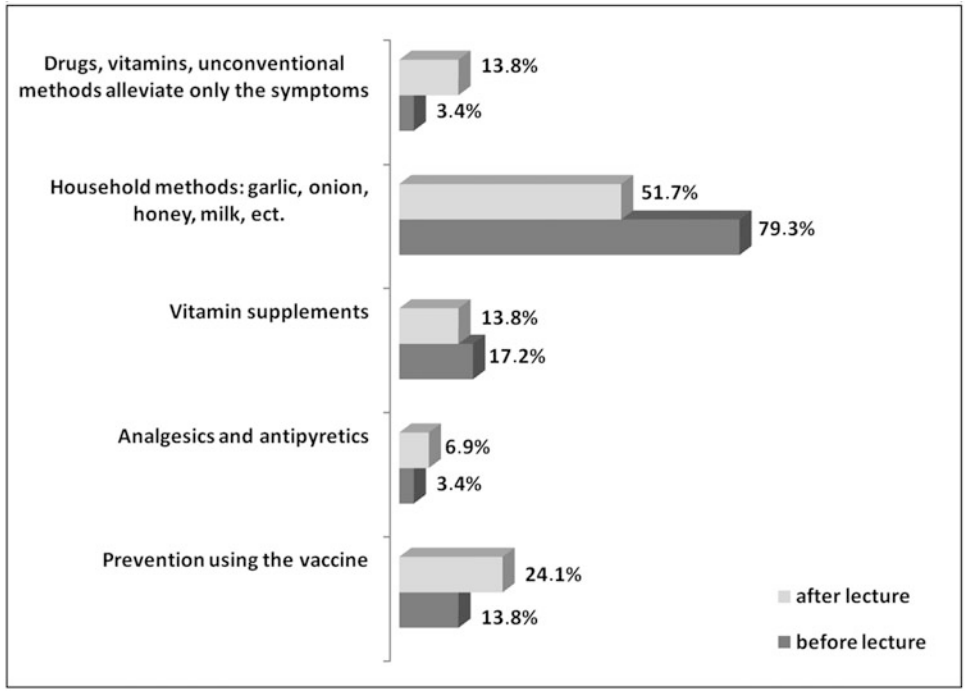

Fig. 1 Methods of protecting against influenza in elderly's opinion

The second thematic block concerned the opinion of the elderly on influenza and influenza vaccination. Respondents were asked about the best ways to prevent the flu, the merit of vaccination in pregnant women and in children over 6 months of age. The percentage distribution of responses to the surveys is shown in Figs. 1 and 2.

The analysis demonstrates that the lecture presentation, among others, positively shifted the elderly's frame of mind on the protective value of influenza vaccine, as more than 10 % of participants changed their attitude toward vaccination, marking that vaccination is the best preventive method (Fig. 1). However, the long-lived untoward habits made the elderly choose household remedies as the most popular method of protection against influenza infection; although also in this case the percentage of people who selected this method significantly decreased after the lecture. It is likely that the persisting attitude toward household protective methods reflects the view and knowledge of the elderly at large, a sector of the society that particularly shuns flue vaccination despite being clearly vulnerable to infection.

In Poland, vaccination calendar has recommended influenza vaccination since 1994. The cyclical nature of influenza and periodic epidemic or pandemic associated with significant mortality resulted in the creation of guidelines and recommendations for the prevention and treatment of influenza in both general population and high-risk groups, which include, among others, seniors, pregnant women, and children over 6 months of age. The present survey demonstrates that the enhancement of knowledge about this subject and overthrowing of the myths concerning danger of influenza vaccination in these groups increased the percentage of the elderly who answered that vaccination is a good way to protect both mother and baby against the flu (Fig. 2). Annual influenza vaccination not only protects against the viral infection, but also stimulates the body immunity to operate more efficiently, which spurs the ability to protect against influenza complications, reduces the number of hospitalizations and mortality. The

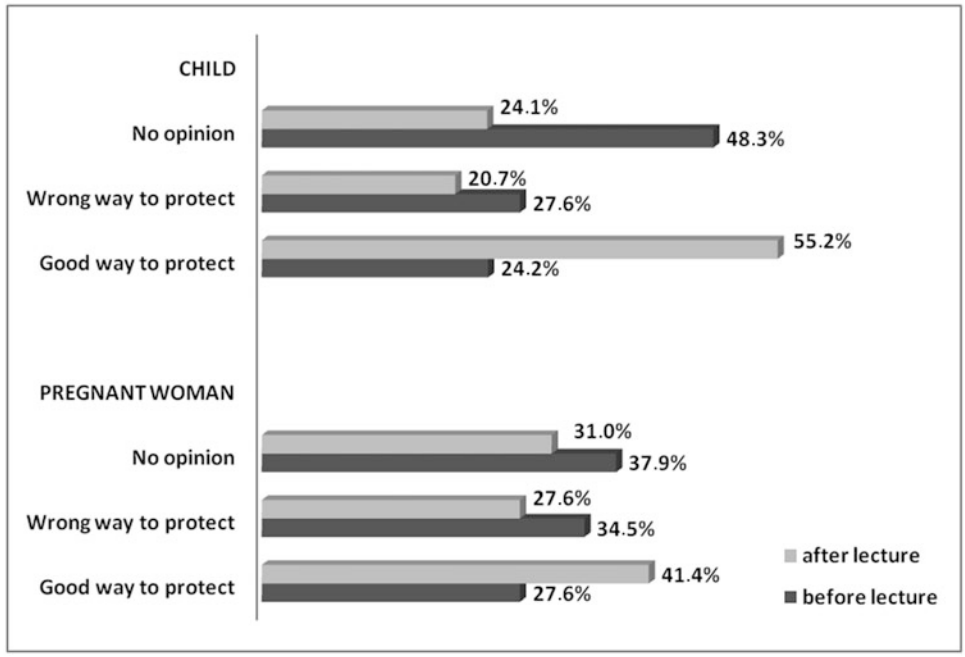

Fig. 2 Elderly's opinions regarding influenza vaccination in young children and pregnant women

shift in attitude and opinions of the elderly underscores the importance of professional educational efforts in disseminating the updated medical knowledge.

Motivation of the elderly to become or not vaccinated against influenza was investigated by survey items concerning vaccination in the past five consecutive seasons, reasons for vaccination rejection, an interest in free of charge vaccination, a tendency to encourage family members and friends to get vaccinated, and the like. The results of this analysis are shown in Table 2.

Overall, the present study shows that vaccination rate against influenza is unreasonably low in the elderly population. Less than 1/5th of the elderly surveyed were vaccinated during the last epidemic season. These results are in line with some other European studies (Sobkowiak et al. 2008; Blank et al. 2008; Holm et al. 2007). Although with age, the number of people receiving the flu vaccine appreciably increases, popularity of vaccinations in this group is still unsatisfactory. The vast majority of respondents in the present survey stated that during the preceding five influenza seasons they were not vaccinated against the flu, and about 20 % of respondents declared that they get vaccinated irregularly or sporadically. The low vaccination coverage level is determined by various factors. Unjustified fear of developing the disease after vaccination or belief in vaccine ineffectiveness is a common cause of giving up vaccination. Quite often, a decision to resign from being vaccinated is influenced by the lack of sufficient knowledge about influenza, its complications, and the ways to prevent the infection; the factors clearly amenable to correction. Nowalk et al. (2004) has demonstrated that older people are more likely to be vaccinated when they know that their family doctor, family members, or friends also underwent the procedure. Recommendations received from a close person counts importantly in the acceptance of vaccination. However, the present survey shows that about half of respondents did not bother to recommend to their relations the idea of obtaining vaccination. The lack of awareness among the elderly of possible health and social costs associated with influenza outbreaks

Table 2 Beliefs of the elderly on advantages of vaccination against influenza

	Before lecture % of answers	After lecture % of answers
Information on vaccination against influenza given to respondents over 5 last seasons:		
Regularly, every season	10.3	17.2
Not regularly	13.8	13.8
Occasionally	17.2	6.9
Not at all	58.6	62.1
Coverage of influence vaccination during the epidemic season 2012/2013		
Number of vaccinated people	17.2	20.7
Number of unvaccinated people	82.8	79.3
Reasons not to get vaccinated:		
Lack of faith in vaccination efficacy	20.7	17.2
No sense of vaccination	3.5	10.3
Fear of side effects	20.7	20.7
Financial aspects	6.9	6.9
Vaccination seen as manipulation of pharmaceutical industry	6.9	0
Lack of knowledge of vaccine's protective value	6.9	6.9
Others	17.2	24.1
No answer	17.2	13.8
Would vaccination free of charge encourage you to get vaccinated?		
Yes	34.5	34.5
No	34.5	31.0
I do not know	17.3	24.1
No answer	13.8	10.3
Have you encouraged family/friends get vaccinated?		
Yes	44.8	41.4
No	41.4	48.3
No answer	13.8	10.3
Optimal time for vaccination against influenza:		
As soon as vaccine becomes available	27.6	27.6
Vaccination concerns only high-risk groups	27.6	24.1
No opinion	27.6	27.6
No answer	17.2	20.7

weakens their motivation for vaccination. However, as indicated by economic analyses, the costs of influenza treatment are several times higher than those spent on vaccination (The National Programme for Combating Influenza 2013; Rothberg and Rose 2005). In this respect, the introduction of free vaccination for the elderly would be the best solution (Marcinkowski et al. 2013).

4 Conclusions

We found that the vaccination rate against influenza was unsatisfactorily low in the elderly population studied, as less than 20 % of the persons surveyed were vaccinated during the epidemic season. Nonetheless, the number of people receiving the flu vaccine clearly increases with person's age, as the 20 % figure starkly contrasts the 3.7 % of the general Polish public immunized against influenza in the season 2012/2013. The present study demonstrates that educational training through a professional lecture presentation facilitates the promotion of health and vaccination coverage against influenza in the elderly. The preventive value of popularization of influenza vaccine in the older age-groups has also been shown in other studies (Sobkowiak et al. 2008; Holm et al. 2007; Dymek-Skoczyńska et al. 2012). Educational efforts are also of importance to the perception of universities of third age by state institutions dealing with health care programs, such as seasonal vaccination campaign for the elderly (Governmental Program for the Social Activity of the Elderly for the years 2012). The importance of health issue to seniors has been confirmed by the adoption of a declaration of the European Council making the year 2012 as the year of active aging and intergenerational solidarity in Europe (Blank et al. 2008; Nowalk et al. 2004), which can help reverse the stereotypes about age-related diseases and marginalization of pensioners in society. In response to the risks arising from influenza infection and its complications, and to underscore the role of

medical staff in reducing the transmission of influenza viruses in the population, the American Advisory Committee on Immunization Practices (ACIP) has presented a list of recommendations aimed at raising the vaccination coverage (ACIP 2015). The ACIP also draws attention to the methods of promotion of vaccinations against influenza among the elderly. Educational expert campaigns on influenza should be a key element of such methods in the elderly (The National Programme for Combating Influenza 2013).

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