[1]Katarzyna Pancer, [1]Agnieszka Ciąćka, [1]Włodzimierz Gut, [2]Bożena Lipka, [3]Justyna Mierzejewska, [2]Bogumiła Milewska-Bobula, [2]Anna Smorczewska-Kiljan, [3]Karina Jahnz-Różyk, [1]Bogumiła Litwińska

DETECTION OF hMPV ANTIGEN BY EIA IN CLINICAL SPECIMENS

WYKRYWANIE METODĄ EIA ANTYGENÓW hMPV W MATERIALE KLINICZNYM

[1] Department of Virology, National Institute of Public Health –NIH , Warsaw, Poland
[2] The Children's Memorial Health Institute, Warsaw, Poland
[3.]Department of Immunology and Clinical Allergology,
Military Institute of Medicine, Warsaw, Poland

STRESZCZENIE

Metapneumowirus człowieka (hMPV) został wykryty dopiero w ostatnim dziesięcioleciu i zakwalifikowany do rodziny *Paramyxoviridae*. Wirus ten, po RSV, jest drugim co do częstości wirusem wywołującym zakażenia dróg oddechowych u dzieci, a szczególnie u dzieci poniżej 5 roku życia. Wykazano, że od 5% do 25% zakażeń układu oddechowego u dzieci jest spowodowanych hMPV. U osób dorosłych, powtarzające się zakażenia hMPV są na ogół ograniczone do górnych dróg oddechowych. Celem obecnej pracy było ustalenie możliwości stosowania testu ELISA wykrywającego antygeny hMPV do diagnostyki zakażeń hMPV oraz przeprowadzenie analizy tych zakażeń w odniesieniu do diagnozy klinicznej. Materiał/Metody. Pobrano 273 wymazy z jamy nosowo gardłowej od dzieci (189 wymazów) i od dorosłych (84 wymazy) w okresie od października 2008 r do marca 2010 r. Ponieważ sezony epidemiczne zakażeń hMPV i RSV występują w podobnym okresie czasu, w 120 próbkach równolegle do badań antygenu hMPV wykonano oznaczenia obecności antygenu wirusa RS. Wyniki: antygeny hMPV wykryto w 24,2% badanych próbek (n=67): 0,0% w 2008 r, 29% w 2009 r i 36,8% w pierwszym kwartale 2010 r. Najwięcej zakażeń hMPV obserwowano w okresie od lata 2009 do końca marca 2010 (VIII-IX 2009 – 62,5%; X – XII 2009 – 41,1% i I – III 2010 – 36,8%). Zakażenie hMPV stwierdzono u 26,5% dzieci i u 24,0% dorosłych z rozpoznanym zapaleniem płuc, a u osób z zapaleniem oskrzeli odpowiednio u 28,4% i 17,6% pacjentów. Zapalenie oskrzelików zdiagnozowano u dwojga dzieci zakażonych hMPV. Równoczesne zakażenie wirusami hMPV i RSV potwierdzono w 15 na 120 przebadanych próbek. Wykluczono reakcje krzyżowe wykonując test ELISA wykrywający antygen hMPV z zawiesiną wirusa RS oraz przeprowadzając analizę statystyczną wyników. Równoczesne zakażenie hMPV i RSV wykryto w 8% przypadków zapaleń płuc, w 11,0% zapaleń oskrzeli i w 24,2% innych zdiagnozowanych przypadków

ABSTRACT

Human Metapneumovirus (hMPV) is one of the latest discovered viruses. It has been classified to *Paramyxoviridae* family. It is the second viral etiological agent, after RSV, which causes respiratory tract infections (RTI) in children, especially children below 5 years old. It is estimated that 5-25 % of RTI in children is due to hMPV. In adults hMPV reinfections are bounded to upper respiratory tract infections. The aim of the study was to establish usefulness of ELISA test in detecting hMPV antigen and to analyze hMPV infection in connection to clinical diagnosis. *Material/Methods*. 273 nasopharyngeal swabs from children (189 swabs) and adults (84 swabs) with respiratory tract infections collected from 2008 to 2010 were examined. Due to similarity of hMPV and RSV viruses and overlapping of their epidemic season rapid immunochromatographic test for RSV antigen detection was also performed in case of 120 samples. hMPV antigen was detected in 24.5% of all swabs (n=67): in 0.0% probes in 2008, 29.0 % in 2009 and 36.8% in first quarter of 2010. The highest rate of hMPV infection was detected from summer of 2009 till the end of March 2010 (VIII-IX 2009 – 62.5%, X-XII 2009 – 44.1% and I-III 2010 – 36.8%). We analyzed respiratory tract diseases reported in patients with hMPV infection. Infection due to hMPV was found in 26.5% of children and 24.0% of adults with recognized pneumonia, respectively in 28.4 and 17.6 % of patients with bronchitis. Bronchiolitis was diagnosed in two children with hMPV. RSV and hMPV coinfections were confirmed in 15 out of 120 examined probes. Cross reaction pattern was excluded thanks to ELISA hMPV antigen test which was performed with suspension of RSV and thanks to statistical analysis. Coinfections were confirmed in 8% of pneumonia, 11% of bronchitis and 24.2% of the rest concomitant diagnoses. *Conclusions*: we found hMPV infection as the significant agent of pneumonia not only in children but also in adults. ELISA hMPV antigen test can be used

zakażeń oddechowych. Wnioski: wykazano, że metapneumowirus człowieka jest istotnym czynnikiem wywołującym zapalenia płuc nie tylko u dzieci, ale również u dorosłych. Test ELISA wykrywający antygeny hMPV może być stosowany w prowadzeniu diagnostyki zakażeń oddechowych zarówno u dzieci, u dorosłych, jak i w przypadkach zakażeń mieszanych.

Słowa kluczowe*: metapneumowirus człowieka, antygen, zakażenia u dzieci i u dorosłych, diagnostyka*

in diagnosis of etiological agent of respiratory infections in children and adults and in coinfections as well.

Key words: *human metapneumovirus, antigen, infection in children and adults, diagnosis*

INTRODUCTION

Respiratory tract diseases, according to WHO, are the second-leading cause of death worldwide in young children (below 5 years old). In developed countries this proportion of death caused by respiratory diseases is lower but still significant (1). The etiological agents of respiratory diseases are identified in a half of infections (bacterial, viral, parasites, mixed). One of the new described by van den Hoogen and co-workers in 2001y. (2) virus connected with respiratory tract infections was *human Metapneumovirus* (hMPV). That virus is genetically similar to avian pneumovirus and was classified in family *Paramyxoviridae*, subfamily *Pneumovirinae*, genus *Metapneumovirus* (3,4). According to epidemiological data presented by other authors – *human Metapneumovirus* is spread worldwide and affects many of subpopulations similarly to RSV: children below 5 years old, compromised patients and elderly people. The clinical symptoms are also similar to those caused by RSV (bronchiolitis, pneumonia, upper respiratory tract infections) but with less severity. Reinfections have been observed in all age groups (1,3,5,6,7,8,9). The epidemic season of hMPV infections is also similar to RSV epidemic season (1,8,10).

The laboratory diagnostics of hMPV infection can be performed by detection of viral antigens, amplification of RNA genome or virus isolation in cell cultures. However, the method of choice for diagnostics of hMPV infection was the detection of viral antigens due to very high level of variety of genetic sequence of hMPV. Mostly common antigen tests are based on direct immunofluorescent technique (DIF) (3). This method needs huge experience on laboratory staff side in DIF. Detection of hMPV antigens by ELISA test seems to be more objective method.

The aim of this study was evaluation of usefulness of ELISA test for detection of hMPV antigens and to analyze hMPV infections in connection to clinical diagnosis. The second aim was to determine frequency of hMPV infections in two selected groups: the youngest children and adults with chronic respiratory diseases.

MATERIAL AND METHODS

Clinical specimens: the nasopharyngeal swabs were collected from 273 patients with respiratory tract infection. 189 samples were collected from children and 84 swabs from adults. Samples were collected in the period from October 2008 to March 2010 and stored in -70^{o}C.

Age of 189 examined children ranged from 1 day of life to 55 months. However, the majority of young patients (90,7%) were below 1 y. old. All of the young patients were hospitalized in The Children's Memorial Health Institute, 91% of them were admitted to the hospital because of acute respiratory infection.

Age of adults ranged from 25 y. to 87 y., but 52,4% of them were in age above 59 y. They were the outpatients of The Immunology and Clinical Allergology Department of Military Institute of Medicine because of chronic respiratory diseases. The patients visited their doctors in case of acute respiratory infection.

Detection of hMPV antigens. Antigens were detected by Biotrin hMPV Antigen EIA (Ireland) according to manufacturer instruction in all 273 collected samples.

Detection of RSV antigens. Due to very similar epidemic seasons of infections caused by hMPV and RSV described in scientific literature 120 out of 273 samples were chosen for RSV antigen tests. There were 54 samples collected from children, and 66 samples collected from adults. The swabs were tested for RSV antigen by Biotrin RSV Solo (rapid immunochromatographic test).

Statistical analysis were done by Statgraphic Centurion v.XV. The correlation analysis of data: age, sex of patients, date of onset, main clinical diagnosis were performed.

RESULTS

Generally, hMPV antigens were found in 67/273 swabs (24.5%). The frequency of hMPV positive samples differs in particular years: none out of 31 samples tested in 2008 year; 29.0% of samples tested in 2009 year and 36.8% during the first three-months period of 2010 (Fig.1).

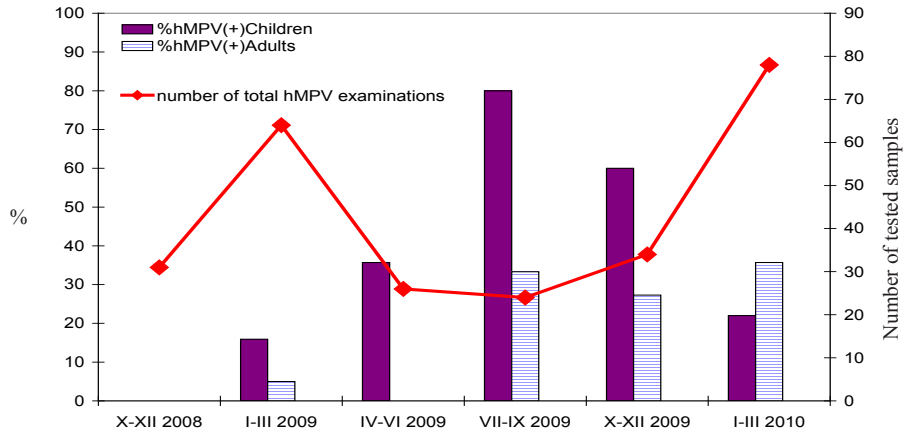

Fig. 1. Percentage of hMPV-positive samples among specimens collected from children and adults by quarter/year
Ryc.1. Odsetek próbek pobranych od dzieci i dorosłych, w których wykryto antygeny hMPV w rozbiciu na kwartały/lata

High frequency of hMPV positive samples was found from summer to winter months of 2009-2010 years, the highest – during the period July-September 2009 year (62.5% of positive samples). In the next quarters, the frequency was 44.1% (X-XII.2009) and 36.8% (I-III.2010). However, there were some slight differences in frequency of hMPV-positive samples among children and adults but generally, the percentage of hMPV-positive samples in that populations was similar, in children - 25.0%; in adults - 20.2%.

The highest frequency of hMPV-positive swabs collected from children was observed in July – September 2009 (80.0%) followed by the quarters X-XII 2009 (60.0%) and IV-VI 2009 (35.7%). Among adults the highest frequency was during the first three-months period of 2010 (35.7%) followed by the quarters VII-IX 2009 (33.3%) and X-XII 2009 (27.3%). The difference of frequency of hMPV infections among children and adults was observed at the beginning of 2009 year, especially in period IV-VI. The number of samples collected in that period among children and adults was similar but 5 out of 14 examined children were positive and none out of 12 examined adults.

Cross-reactions between antigens of RSV and hMPV in ELISA hMPV antigen test were excluded. Dilutions of RSV were tested in ELISA hMPV antigen test and next, by statistical analysis of obtained results was performed. In our study, the lack of correlation between the results received from hMPV antigen EIA and Biotrin RSV Solo tests was found (Po=0.8419).

Generally, antigens of RSV or hMPV viruses were found in 65% of tested 120 samples (78 swabs), among them 29 were hMPV-positive (24% of all tested samples), 64/120 were RSV-positive (53% of all tested swabs). Both antigens were found in 15 samples (12.5%): 9 specimens collected from children and 6 ones from adults (Fig.2).

In the second part of this study the demographic parameters and main clinical recognition were analyzed. The largest examined age group was the youngest children (from 1 day of life to 1 y.) group and the hMPV antigens were found in 25.9% of tested swabs (Fig. 3). Age groups among young children (from 1.01 years old to 4.6 years old) and adults were not so numerous and results of analysis may be uncertain. However, it should be indicated that hMPV infections were found in patients belonging to all examined age groups, except one – the oldest people group.

The lack of correlation (Po=0.9386) between result of hMPV antigen detection and sex of examined children was found. However such correlation (rather week correlation) was found in the adult patients group

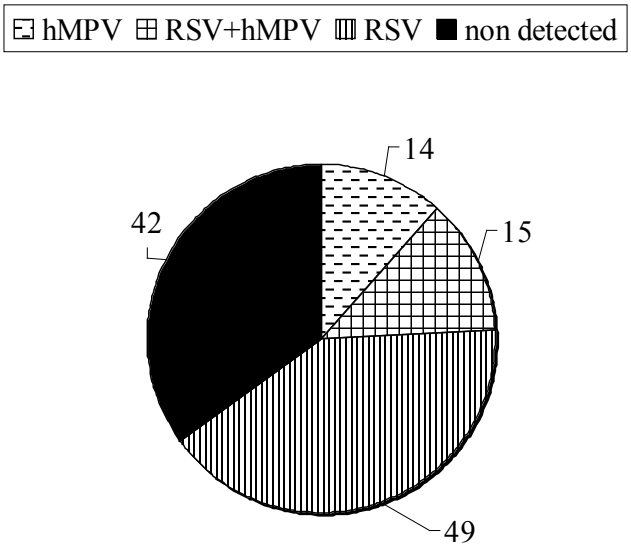

Fig.2. Occurrence of hMPV and RSV coinfections – detected antigens of viruses in tested 120 samples
Ryc.2. Występowanie zakażeń mieszanych wywoływanych przez hMPV i RSV – oznaczanie antygenów w/w wirusów w badanych 120 próbkach

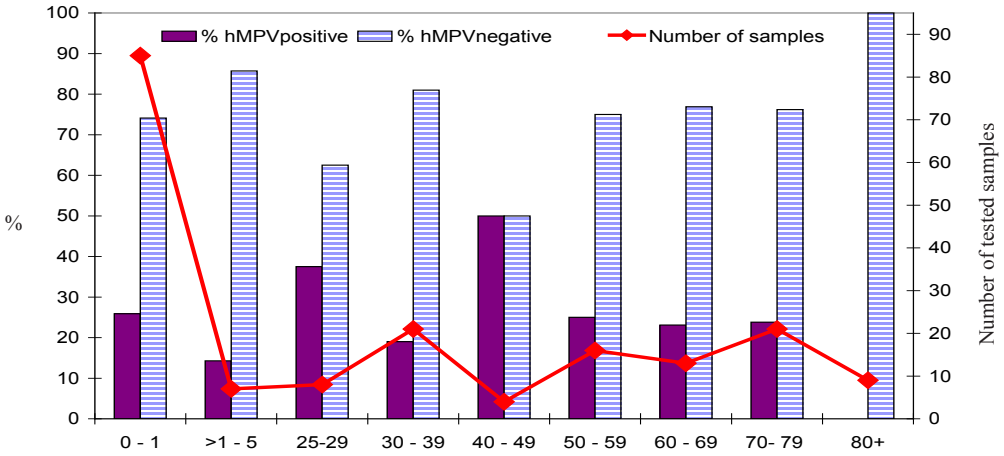

Fig. 3. Percentage of hMPV antigen - positive and negative samples by age group and number of examined samples
Ryc. 3. Odsetek próbek dodatnich i ujemnych dla antygenów hMPV z uwzględnieniem wieku pacjentów i liczby badanych próbek

(Po=0.0445). Antigen hMPV was found more rarely among men (10.8%) than women (28.9%) (table I).

Table I. Detection of hMPV antigen in clinical samples (by EIA) by age group (adults/children) and sex of patients
Tabela I. Obecność antygenu hMPV w badanych próbkach (wykrywanych testem EIA) z uwzględnieniem wieku (dorośli/dzieci) i płci pacjentów

Examined patients	Antigen hMPV (-)	Antigen hMPV (+)	Total number
Boys	82	30 (26,8%)	112 (100%)
Girls	59	20 (25.3%)	79 (100%)
Men	33	4 (10.8%)	37 (100%)
Women	32	13 (28.9%)	45 (100%)
Totally	206	67 (24,5%)	273 (100%)

The data of main clinical diagnosis was available for 232 cases (155 children and 77 adults). According to it, hMPV antigen was detected in 26% of patients with pneumonia, 12.5% patients with bronchiolitis and 25.4% with bronchitis. Other upper respiratory tract symptoms were observed in 25.0% of hMPV positive cases. The frequency of detection of hMPV antigen among patients with pneumonia was similar among adults and children (respectively: 24.0%; 26.5%). Higher frequency of hMPV-positive samples was among children with bronchiolitis (none of adults had that symptom) or with bronchitis (28.5% vs. 17.6%). The hMPV and RSV co-infections were found in 8% of patients with pneumonia, 11% - with bronchitis and 24.2% with other upper respiratory symptoms.

DISCUSSION

According to our knowledge, this is the first research (not case report) in Poland when hMPV was detected as the cause of respiratory tract infections. One study was performed among children with laryngitis but no hMPV positive specimen was found (11) .The results of our studies indicated that respiratory tract infections due to hMPV occur among children and adults in Poland.

Generally, the suspicion of hMPV infection and the laboratory confirmation have been still limited to scientific research rather than to standard diagnosis. The differences in frequency of hMPV positive samples observed in publications may be caused by: examined populations, chosen laboratory methods, season and the number of examined samples. In Canada, according to surveillance system data, 23559 examinations for detection of hMPV infection were done in period from 2nd January 2010 to 12 June 2010 (12). Generally, 2077 samples were positive (8.8%), but data analyzed by months showed the diversity in frequency of hMPV positive tests in that period: from 0.4% (June 2010) to 13.7% (March 2010). In our study the influence of the number of examined samples on results was also found but the season (P_o=0.0000) seemed to be more important factor. In period from October 2008 to December 2008 no sample was hMPV- positive however, the number of examined specimens was 1,3-times higher than number of samples collected in period July 2009-September 2009 (frequency was 62.5%). Similar difference in prevalence of hMPV in different years was observed among adults (healthy and high-risk patients) in the USA (3,5).

Generally, frequency of hMPV infection determined by hMPV antigen test turned out to be really high (24.2%) in our studies. However, the cross-reactions with relative RSV were excluded by both experimental tests and statistical analysis. In that study two high risk groups of patients were examined: hospitalized young children and adults with chronic respiratory diseases. In Germany, the frequency of hMPV RT-PCR positive in young children was determined generally <1% (3162

outpatients and inpatients together) but among patients admitted to intensive care unit – 18%. Among those patients 60% got RSV coinfection (13). In our study, the similar frequency of RSV coinfections in hMPV positive patients (15/29) was also found (51.7%). In Greece, the genome of hMPV was found in 6.05% of examined samples in three influenza seasons 2005-2008. The majority of the hMPV-infected patients was below 10 years old (69.5%) but hMPV infections were observed in all age groups (8). In West of Scotland infections caused by hMPV were also found in all age groups, with the highest frequency in the youngest children group (below 1 year old) (9). The results of our study confirm that observation.

The analysis of clinical findings in cases of hMPV infections among children showed relatively high contribution in bronchiolitis: in our study -12.5%, in Taiwan – 21.0% (14), in Greece -16.1% (8) as well as in pneumonia (respectively: 26.5%; 45.0%; nd). It should be pointed out that the similar occurrence of hMPV infections among children and adults with pneumonia was found in our study.

CONCLUSIONS

In Poland the infections due to hMPV among children and adults have been confirmed. The predominance of young children infected by hMPV was found but respiratory tract infections caused by this virus among elder adults were also observed. Based on the obtained results the important contribution of hMPV in lower respiratory tract infections in children (bronchiolitis and pneumonia) and adults (pneumonia) is suggested.

The majority of hMPV infections was found in late summer-winter season in both examined groups but more studies should be conducted to describe the thoroughly epidemic seasons of hMPV infections in Poland.

ELISA hMPV antigen test can be used in diagnosis of etiological agent of respiratory infections in children and adults and in coinfections as well.

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Adress for correspondence:
Dr Katarzyna Pancer
Zakład Wirusologii NIZP-PZH
Ul. Chocimska 24, 00-791 Warszawa
e-mail: kpancer@pzh.gov.pl