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Problem of immunoglobulin M co-detection in serological response to bacterial and viral respiratory pathogens among children suspected of legionellosis

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Abstract

The objective of this research was an analysis of the serological response to respiratory bacterial and viral pathogens, in 156 children admitted to hospital in Warsaw with a suspicion of legionellosis. Levels of immunoglobulin (Ig) M to Bordetella pertussis, Mycoplasma pneumoniae, respiratory syncytial virus (RSV), adenoviruses, human parainfluenza virus (HPIV) t. 1-4 and influenza t. A + B viruses were determined retrospectively by ELISAs. In the prospective examinations (only Legionella pneumophila sg1), a positive level of IgM was found in 35 patients, but in 59 children together with retrospective tests. There were positive results for B. pertussis (21 children), followed by HPIV (10 children), M. pneumoniae (5 patients), RSV (4 persons), adenoviruses (3 children), and influenza A + B virus (3 persons). Positive results for > 1 agent were found in 16 children. The most often co-detected IgM were to L. pneumophila sg1 and B. pertussis (9 children) and L. pneumophila sg1 and M. pneumoniae (5 patients). The distribution of IgM to L. pneumophila sg1, B. pertussis and HPIV among children ≤ 4 years differed significantly from IgM specific to other pathogens. A high number of HPIV infections, mainly single, was found among infants. Positive results of IgM to L. pneumophila sg1 were mainly found in children aged 4-5 years. and the oldest children (over 10 years.). However, among the oldest children, anti-L. pneumophila sg1 antibodies were often found together with IgM to B. pertussis. Infections due to more than 2 pathogens were only observed among patients with pneumonia, especially due to L. pneumophila sg1 and/or B. pertussis. Conversely, co-detection of IgM ELISA for L. pneumophila and M. pneumoniae were mainly detected among patients hospitalized without pneumonia.

Key words: *legionellosis, co-detection of IgM, HPIV, pertussis.*

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Introduction

Legionellosis, a disease caused by the bacteria *Legionella* spp., is an infection of the respiratory tracts without characteristic clinical symptoms. It can be manifested from severe pneumonia (Legionnaires' disease – LD) to a flu-like infection (e.g. Pontiac fever) [1]. Symptoms such as persistent and heavy cough, fever, and fatigue might be observed as a result of infection due to *Legionella* spp. (mainly *Legionella pneumophila* sg1), but also to *Bordetella pertussis*, *Mycoplasma pneumoniae* and some viruses [parainfluenza viruses, influenza viruses, respiratory syncytial virus (RSV), adenoviruses and others) [1-4]. The co-detection of immunoglobulin (Ig) M in tests directed at different pathogens might be caused by co-infection, or be a result of prior infection or even cross-reactions of IgM [4-8]. All of the possibilities should be considered as possible trouble-makers in serological diagnostic procedures.

The aim of this study was to evaluate the frequency of detection of IgM to different bacterial and viral pathogens among children suspected of legionellosis.

Material and methods

One hundred and eighty-five serum samples were collected from 156 children aged from 1 month to 17 years of life. They were hospitalized from June 2005 to December 2006 because of a suspected infection due to *Legionella* spp. Serum samples were prospectively tested by IgM anti-*L. pneumophila* sg1 ELISA tests according to the manufacturer's instructions (Euroimmun, Medizinische Labordiagnostika AG, Lübeck, Germany) [9]. In total, positive

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Table 1. The results of immunoglobulin M (IgM) anti-*L. pneumophila* sg1 antibodies determination by age of patients

Age of children (years)	Results of ELISA IgM for *L. pneumophila* sg 1 (number of patients)			
	positive	borderline	negative	total
< 2	0	2	25	27
2-3	7	2	24	33
4-5	11	5	12	28
6-9	6	3	19	28
≥ 10	11	2	27	40
total	35	14	107	156

results for IgM were found in 35 children (22.4%). The ages of examined children and the results of ELISA IgM tests for *L. pneumophila* sg 1 are presented in Table 1.

ELISA. A retrospective study was carried out to determine the level of IgM antibodies specific to selected pathogens, using commercial tests. These were tests for detection of IgM specific to the bacterial surface of *B. pertussis* (Novatec, Immunodiagnostica GmbH) and *M. pneumoniae* (Novatec, Immunodiagnostica GmbH). There were also tests for viral agents such as RSV (Virotech, Germany), parainfluenza viruses t. 1-4 (Euroimmun, Germany), influenza A and B viruses (Euroimmun, Germany) and adenoviruses (Novatec, Immunodiagnostica GmbH). However, because of the very high prevalence of IgG antibodies against such viral agents in older children or adults, the ELISA tests for parainfluenza, influenza and adenoviruses were only done on younger children (under 5 years, 74 patients). The results were calculated and interpreted according to manufacturers' instructions; however, for the comparative analysis, all ELISA IgM test results were presented in one way – as a ratio of the OD value of the sample to the OD value of the calibrator. Such result was defined as the value of the ELISA test (VE). Interpretation of results: positive VE ≥ 1.1, negative VE < 0.9; borderline ≥ 0.9 – < 1.1.

Statistical analysis

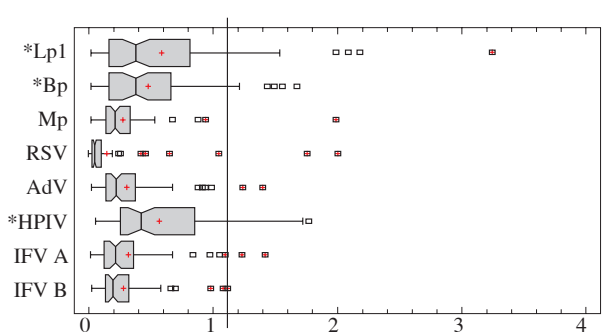
Statistical analysis was done using Statgraphics for Windows, Centurion, v.XV. StatPoint Tech. Inc. USA. For qualitative/categorical data cross-tabulation, tests of independence (χ^2 or Fisher's exact tests), the degree of association between rows and columns (contingency coefficient, Lambda test and Pearson's correlation), odds ratios and relative risk (if possible) were done. For a significant relation, we considered results where $P_o < 0.05$.

Results

Among 156 children with acute respiratory infection and suspected legionellosis, positive results of IgM serological test to other than *Legionella* pathogens were found in 24 children. In total, in 59 (37.2%) patients, a positive result of IgM to *L. pneumophila* sg1 or to other respiratory pathogens were detected. There were mainly positive results for *L. pneumophila* sg1 (35 patients), *B. pertussis* (21 children), followed by parainfluenza viruses type 1-4 (10 children), *M. pneumoniae* (5 patients), RSV (4 persons), adenoviruses (3 children), influenza A virus (2 persons), and influenza B virus (1 child). At least one IgM positive test was found among 43.2% of children ≤ 4 years and 33% of older children.

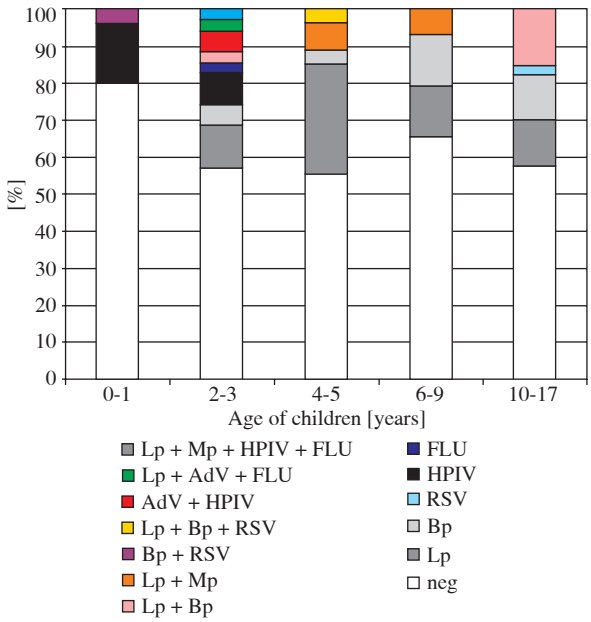
Significant differences in multivariate analysis of the distribution of IgM levels for 8 pathogens were found in examined samples from children under 5 years ($P_o = 0.0000$). The distribution of IgM antibodies level to *L. pneumophila* sg1, *B. pertussis* and human parainfluenza virus (HPIV) type 1-4 varied from the response to other pathogens (Fig. 1). In older children, no significant statistical differences in median or mean of distribution of IgM antibodies to bacterial agents was found; however, the standard deviations varied significantly ($P_o = 0.0000$).

Generally, there was a significant relation between the age of the patients and the IgM response to bacterial patho-



Multivariable analysis of IgM results to bacterial (Lp1 – *L. pneumophila* sg1; Bp – *B. pertussis*; Mp – *M. pneumoniae*) and viral pathogens (RSV – respiratory syncytial virus; AdV – adenoviruses; HPIV – human parainfluenza virus; IFV A – influenza virus type A; IFV B – influenza virus type B); cut-off – 1.1 (line)
*significant difference

Fig. 1. Distribution of immunoglobulin M level determined by ELISA tests for 8 pathogens in sera of children under 5 yrs



Lp – *L. pneumophila* sg1; Bp – *B. pertussis*; Mp – *M. pneumoniae*; AdV – adenoviruses; HPIV – human parainfluenza viruses type 1-4; RSV – respiratory syncytial virus; FLU – influenza A virus or influenza B virus

Fig. 2. Immunoglobulin M positive results in serum samples collected from 156 children – by age group and pathogens

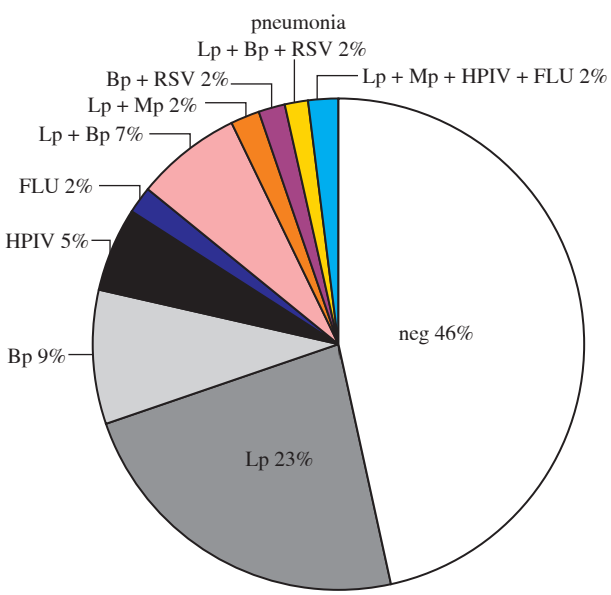


Fig. 3. Results of immunoglobulin M by main clinical symptoms: 57 patients with pneumonia, and 29 patients with ARTI without pneumonia

majority of single IgM positive results to pathogens other than *L. pneumophila* sg1 pathogen were anti-*B. pertussis* (11 patients) and anti-HPIV type 1-4 (7 children, mainly infants) and these correlations were significant ($P_o < 0.05$). gens ($P_o = 0.0099$), especially for *B. pertussis* ($P_o = 0.0160$) and *L. pneumophila* sg1 ($P_o = 0.0375$). No child below 2 years was positive for *L. pneumophila* sg1 but positive results were mainly found in children aged 4-5 years and the oldest children (over 10 years). No correlation between age and IgM was found for viral pathogens, although some trends were found. A high number of HPIV infections, mainly single viral infections, was found among children under 4 years, especially infants (Fig. 2). The co-detection of a positive IgM for *L. pneumophila* sg1 and other bacterial or viral agents was also analyzed. Significant levels of IgM for one etiological agent of infection was found in 43 children, and in 16 (27% of positive) for more than one infectious agent in children. Among them, there were mainly positive ELISAs for two pathogens (13 patients). There was no correlation between the age of patients and co-detection of IgM ($P_o > 0.5$), but the highest variation of co-detection of IgM to different pathogens was observed among children aged 2-3 years Among children older than 9 years anti-*L. pneumophila* sg1 IgM antibodies were often found together with anti-*B. pertussis* IgM (Fig. 2).

Analysis of the detected IgM and main clinical symptoms (data obtained for 86 children) was also done. There was no significant difference in the prevalence of IgM to specific pathogens among 57 children with pneumonia, or in 29 patients hospitalized because of acute respiratory tract infection but without pneumonia (ARTI). However, infections due to 3 or 4 pathogens were only observed among patients with pneumonia. Moreover, a trend that bacterial and viral co-infections were only in this group of patients, was observed. This relation was not significant but there was a low number of cases. Moreover, positive results of IgM to *L. pneumophila* sg 1 and *B. pertussis* in multi-infection were found mainly in patients with pneumonia. Conversely, co-detection of IgM to *L. pneumophila* and *M. pneumoniae* were more often found in patients without pneumonia (Fig. 3).

In further studies, the co-detection of IgM to *L. pneumophila* sg1 and other pathogens were analyzed. The most frequent co-detection of positive levels of IgM were found for *L. pneumophila* sg1 and *B. pertussis* (9 children) and *L. pneumophila* sg1 and *M. pneumoniae* (5 patients) (Table 2). All patients with positive IgM result for *M. pneumoniae*, were also positive for *L. pneumophila* sg1. There was no simultaneous occurrence of a positive result for *B. pertussis* and *M. pneumoniae*; however, in two children with a positive result of IgM for *L. pneumophila* sg1 and *M. pneumoniae*, we also determined the borderline concentration of IgM for *B. pertussis*. Against this, among 121 patients with a negative result of anti-*L.pneumophila* sg1 IgM, in 22 children a single positive IgM result to other pathogens was found, and in two patients for 2 pathogens. Together, a pathogen other than the *L. pneumophila* sg1 pathogen was identified as the causative agent in 24 patients, among them 10 children with pneumonia. The

Table 2. Co-detection of anti-*L. pneumophila* sg 1 immunoglobulin M (IgM) and IgM for other pathogens

Results of IgM for *L. pneumophila* sg1	Positive results of IgM ELISA for the tested pathogen						
	Bp	Mp	AdV	HPIV t. 1-4	RSV	FLU A	FLU B
Lp sg1 IgM positive	9	5	1	1	1	1	1
Lp sg1 IgM negative	12*	0	2	9*	3	2	0
Total	21	5	3	10	4	3	1

Lp sg1 – L. pneumophila sg1; Bp – B. pertussis; Mp – M. pneumoniae; AdV – adenoviruses; HPIV t.1-4 – human parainfluenza viruses type 1-4; RSV – respiratory syncytial virus; FLU A – influenza A virus; FLU B – influenza B virus
**significant correlation (details in text)*

Table 3. Change of immunoglobulin M (IgM) level to *L. pneumophila* sg1 (Lp1), *B. pertussis* (Bp), *M. pneumonia* (MP), respiratory syncytial virus (RSV), human parainfluenza viruses type 1-4 (HPIV t. 1-4), adenoviruses (AdV) and influenza viruses type A and B (FLU) in consecutive serum samples collected from 14 children by characteristics of patients, main symptoms and season

Pneumonia	Patient's age (years)/ gender	Change in IgM level determined in paired serum samples to different pathogens (1st serum /2nd serum])	Month of sample collection
P	0.4/F*	**HPIV [+/–]**	XII/XII
P	1.5/F	**Bp [–/+]**; **RSV [–/+]**	I/II
P	1.5/F	Lp [–/(+/–)]	I/I
P	2/M	**Lp1 [+/+ ↑]**; HPIV [–/(+/–)]; **AdV [(+/–)/+]**; **FLU [–/+]**	VI/VII
P	4/F	**Lp1 [+/(+/–)]**; **HPIV [–/+]**; FLU [–/(+/–)]	II/III
P	4/F	Lp1 [(+/–)/–]; **Bp [+/(+/–)]**; HPIV [(+/–)/+]	X/X
P	4/M	**Bp [+/(+/–)]**	VI/VII
P	4.8/M	**Lp1 [+/+ ↑]**; Bp [–/(+/–)]; **Mp [–/+]**	IX/X
P	9.5/F	**Lp1 [–/+]**	I/II
P	13/F	Bp [(+/–)/+]	III/III
N	5.5/M	Lp1 [+/+]; Mp [+/+]	XII/XII
N	11/F	**Bp [+/–]**	II/III
nd	5/F	**Lp1 [+/–]**	IV/VI
nd	16/F	Bp [(+/–)/–]; **RSV [+/–]**	V/VI

(+) – positive, (–) – negative, (+/–) – equivocal (borderline); ↑ – increase in IgM level, bold – significant change of IgM level; P – pneumonia, N – respiratory tract infection without pneumonia, nd – no data; M – male, F – female
**LD confirmed by the urinary antigen test*

Next, the results obtained in paired serum samples collected from the same child were analyzed. In total, more than one serum sample were collected from 19 children. In the paired sera of 5 children, negative levels of IgM to all tested pathogens were determined. There were patients hospitalized in January (3 children) or in summer time (June-August).

Significant changes of the IgM level, from negative to positive results in the consecutive serum or at least a double increase in the level in ELISA assay, were analysed in 14 paired samples which were IgM positive. The significant changes were found for *L. pneumophila* sg1 (in 5 patients), *B. pertussis* (4 children), *M. pneumoniae* (1 child), RSV (2 persons), HPIV t. 1-4 (2 children), adenoviruses (1 patient) and influenza (1 child). Also a high variation of co-detection was found. In some children a simultaneous increase in IgM to two pathogens was observed; in some patients – an increase in IgM for one agent was observed together with a decrease in IgM for the second one so the possibility of following co-infection might be considered. Also, there was no correlation between the month of onset/ sample collection and determined levels of IgM (Table 3).

Discussion

The importance of serological tests for detection of acute viral respiratory infection agents in children is very limited, because of the delayed production of IgM in comparison to the short period of incubation before symptoms, and a lack of criteria for evaluation of the results in this age group. Moreover, the level of IgG increase and IgM response decrease significantly with the age of children

as a result of recurrent infections, due to tested viruses in the first 5 years of life. Serological tests for causative viral agents are mainly used for diagnosis of chronic infection; for identification of agents of acute viral respiratory infections (by RSV etc.), other methods are used, mainly PCR or immunofluorescence assay [7, 10, 11]. Interpretation of results obtained in serological tests is one of the most difficult parts of laboratory diagnosis of infectious diseases, but in practice the tests are widely used in the identification of bacterial infections [1, 2, 5, 6, 9, 12, 13]. Despite the delay in the diagnostic process, serological tests are the basis for diagnosis of most cases of infection caused by bacteria *L. pneumophila*, *M. pneumoniae* and *B. pertussis* in Poland [6, 9, 14, 16].

A strong long-lasting cough, fatigue, fever > 38°C, headaches and muscle aches are common clinical symptoms of many bacterial and viral infections, also so-called "flu-like infections". Acute respiratory infections in children might be presented as bronchitis, laryngitis, tracheitis and pneumonia. The clinical picture of infections caused by *L. pneumophila* or *B. pertussis*, *M. pneumoniae* or HPIV might be similar [3, 7, 8]. Usually, symptoms of respiratory viral infections resolve after 5-10 days, but not of HPIV infection. Symptoms of infections due to *Legionella*, *M. pneumoniae* or *B. pertussis* also persist for longer than 10-14 days. This is enough time to develop an immunological response and use serological tests for diagnostics. This indicates that the diagnostic procedures should be supplemented by the use of ELISA IgM tests for some bacterial and viral pathogens which might be a cause of acute respiratory infection in children. In China, the most common pathogens of acute respiratory infection were *M. pneumoniae*, influenza viruses, RSV and HPIV out of 9 tested by immunofluorescence assay [15]. In our study, an infection due to *Legionella* spp. was suspected, and moreover some of the patients were previously examined for *M. pneumoniae*, and these patients were not suspected of legionellosis. This is probably why a high level of anti-*L. pneumophila* sg1 IgM and a low level of *M. pneumoniae* IgM positive results were observed, in opposition to other studies [8, 15]. Moreover, selection of the best diagnostic test with a balance of sensitivity and specificity is the crucial point in the diagnosis of infection. As has been shown by the Sobieszczańska team [16], a high prevalence (47%) of false-positive *M. pneumoniae* (IgM and/or IgG) results among screening (agglutination) tests was observed. Even ELISAs are more specific than screening agglutination tests, but the possibility of infection due to one or more other aetiological agents should be considered [4, 7]. Unfortunately, in routine practice, a positive IgM result to one pathogen was the basis for deciding to finish the diagnostic procedure, and the possibility of IgM co-detection or even IgM cross-reaction was neither studied nor considered. In Finland, the main aetiological agent of community-acquired pneumonia in children was *Streptococcus pneumonia* and *Haemophilus influenzae*. At the same time, bacterial and viral co-infections were found in 66% of children [11]. In the presented study, the probable causative agent of 24 cases, including 10 cases of pneumonia, was identified by the use of an extended panel of ELISAs. These were mainly cases due to *B. pertussis* and HPIV.

Co-detection of IgM indicated the possibility of many events, among others (and the most important for physicians) the possibility of co-infection, which might exacerbate the disease, prolong hospitalization, and result in treatment failure. In Portugal, among determined cases of pertussis, co-infection was found in 21% of cases. These were mainly viral infections (adenoviruses and RSV) but legionellosis or mycoplasma infections were not examined [7, 8, 17].

The determined co-detection of a high level of IgM to more than one pathogen might be an effect of other phenomena. In the presented work, we also considered the possibility of following infections. Bacterial infection due to *L. pneumophila* sg1 or *B. pertussis* was probably the first of these, followed by viral infection due to HPIV, RSV, or adenoviruses. The most interesting case was a 5-month-old infant with a high level of IgM to parainfluenza viruses only. However, the urinary test for *L. pneumophila* sg1 was also positive, and confirmed legionellosis. It indicated the possibility of *L. pneumophila* sg1 infection which followed HPIV infection. Unfortunately, because of the limited number of paired sera and data regarding the course of infection or treatment, more detailed analyses of this and other cases were not done.

Conclusions

An extended panel of ELISA tests should be used for identification of the causative agent of acute respiratory tract infection in hospitalized children, because the clinical manifestation of infection due to atypical bacterial and some viral pathogens might be very similar. The possibility of parainfluenza should be considered, especially among younger children.

Simultaneous detection of IgM antibodies to various pathogens may be a result of many factors, and additional tests, performed on at least 2 serum samples, are necessary to determine the dynamics of antibodies and the sequence of events.

The author declares no conflict of interest.

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