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Cross-reactions in IgM ELISA tests to *Legionella pneumophila* sg1 and *Bordetella pertussis* among children suspected of legionellosis; potential impact of vaccination against pertussis?

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Abstract

The objective of this study was preliminary evaluation of IgM cross-reaction in sera collected from children hospitalized because of suspected legionellosis. Sera with positive IgM results to L. pneumophila sgs1-7, B. pertussis or with simultaneous detection of IgM antibodies to L. pneumophila sgs1-7 and B. pertussis, or IgM to L. pneumophila sgs1-7 and M. pneumoniae in routine tests, were selected. In total, an adapted pre-absorption test was used for the serological confirmation of legionellosis in the sera of 19 children suspected of legionellosis, and also in 3 adult persons with confirmed Legionnaires' disease. Sera were pre-absorbed with antigens of L. pneumophila sg1, B. pertussis or both, and tested by ELISA tests. The reduction of IgM antibody level by pre-absorption with antigen/antigens was determined. Reduction of anti-Lpsgs1-7 IgM by pre-absorption with L.pneumophila sg1 antigen ranged from 1.5 to 80, and reduction of anti-Bp IgM by pre-absorption with B. pertussis ranged from 2.0 to 23.8. Reduction by both antigens varied depending on the age of the patients: among children <4 yrs.old, the reduction of anti-B. pertussis IgM by both antigens was higher than for B. pertussis antigen alone. Based on the high difference (≥ 2 times) between reduction by L.pneumophila sg1 and by B. pertussis antigen, legionellosis was confirmed in 8/19 children. The majority of them also indicated IgM positive/borderline results for B. pertussis or M.pneumoniae in routine ELISA tests. As a preliminary, we posed a hypothesis of a potential impact of an anti-pertussis vaccination on the results obtained in anti-L. pneumophila ELISA IgM tests among young children.

Key words: *legionellosis, pertussis, atypical pneumonia, serum pre-absorption, false positive results.*

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Introduction

The phenomenon of cross-reactions is observed in serological tests. It refers particularly to tests in which bacterial surface antigens are used [1-3]. This phenomenon may influence the results of serological tests and their interpretation, so it may have a very big impact on the diagnosis and treatment of infections [1-6]. Interpretation of serological test results is one of the most difficult tasks in the diagnosis of bacterial or viral infections, because of the insufficient specificity and limitations of the tests. A positive result of specific IgM determination is possible only in a particular, often 3-5 weeks, period of infection. The dynamics of the formation of specific antibodies and their disappearance depends on the type of antibody, the individual properties of the host (age, immunocompetence etc.), the interval between onset and sampling, and also the type of antigen or technique used [1-3, 7].

Infection caused by bacteria *Legionella* spp. might be manifested as an infection of the respiratory tract without characteristic clinical symptoms or a flu-like fever (eg. Pontiac fever), or as an atypical pneumonia (Legionnaires' disease, LD). Symptoms such as persistent and heavy cough, fever, and fatigue might be observed as a result of infection due to *Legionella* spp., but also *Bordetella pertussis*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae* and respiratory viruses [4, 6, 8-10]. Bacteria *Legionella* spp., *M. pneumoniae* and *C. pneumoniae* need to grow inside eukaryotic cells, including human, for their life-cycle. Intracellular growth of these bacteria and

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non-specific clinical symptoms are the main reason for the delay in diagnosis and the use of a serological test. In Poland, the majority of reported cases of *L. pneumophila* infection were diagnosed based on serological diagnostics tests. Serological tests are also widely used in diagnosis of the infection caused by *B. pertussis* [11, 12].

In a previous study, a high prevalence of co-detection of IgM for *L. pneumophila* sg1 and *B. pertussis*, also *L. pneumophila* sg1 and *M. pneumonia,* was observed in children, especially if older than 4 yrs. The possibility of a cross-reaction in a serological test might be the cause of false diagnosis. However, co-detection of IgM in tests directed at different pathogens might be caused by co-infection, or be a result of prior infection or even vaccination [4-6, 8, 13, 14]. All of these possibilities should be considered.

The high proportion of serum samples with co-detection of IgM to selected bacteria indicated that additional tests to confirm legionellosis should be carried out. For this purpose, the adapted test of pre-absorption of serum samples was used [15]. Sera collected from adult patients with confirmed legionellosis (by seroconversion and PCR) were also examined using this test.

The aim of this study was to evaluate the influence of IgM cross-reaction in serological tests to *L.pneumophila* sg1 and *B. pertussis* on diagnostic procedures in children suspected of legionellosis. Futhermore, the potential impact of vacination against pertussis on IgM cross-reaction was considered.

Material and methods

Material

Sera for the pre-absorption test were selected on the basis of results of routine ELISA tests for *L. pneumophila* sgs1-7, *B. pertussis* and *M. pneumoniae*. In total, 19 serum samples from children hospitalized in 2006-2007 were chosen. In 15 out of 19 sera positive IgM results for at least two pathogens were previously determined by ELISA tests. Moreover, serum samples collected from 3 adults with confirmed Legionnaires' disease hospitalized in 2007 were also used in this study.

Methods

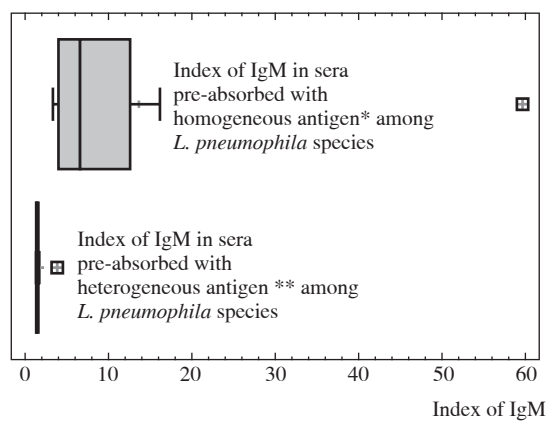
Test of serum pre-absorption

A test of sera pre-absorption was done for the confirmation or exclusion of the infectious agent. A modified method for pre-absorption was developed for confirmation of legionnellosis in case when serum sample was only available specimen [15]. For the purpose of determination of possible cross-reactions among serogroups belonging to species *L. pneumophila*, validation tests were done using sera collected from 10 patients with confirmed legionellosis by culture, serological tests or by urinary antigen *L.pneumophila* sg1 assay. There were examined sera from patients infected with bacteria belonging to 6 different subgroups among serogroup 1 of *L. pneumophila* or cases due to *L. pneumophila* sg3. The sera were kindly provaded by dr Pernille Elverdal from Statens Serum Institut, Denmark. It was found that the pre-absorption test might be useful tool for differentiation of aetiological agent of infection, even among species *L. pneumophila* (Fig. 1).

In this work, the reduction of cross-reaction and increase in the specificity of reaction in pre-absorbed sera was determined by using antigens of *B. pertussis* (strain 3628/97) and *L. pneumophila* sg1 (ATCC 33152). Briefly, each serum sample was divided into 4 parts. To each part, the same volume of one of the 4 prepared solutions was added:

- to the first part – antigen *L. pneumophila* sg1 (Lp sg1);
- to the second part – antigen *B. pertussis* (Bp);
- to the third part – mix of *L. pneumophila* sg1 and *B. pertussis* antigens (in equal volume);
- to the fourth part – PBS only.

All samples were incubated for 18 hours at 4°C. After incubation, all samples were centrifuged and supernatants tested by ELISA IgM tests for *L. pneumophila* sgs1-7 (Euroimmun, Medizinische Labordiagnostika AG, Lübeck, Germany), *B. pertussis* (used purified lysate antigens) and *M. pneumoniae* (both Novatec, Immunodiagnostica Gmbh).



* Homogeneous antigen – sera from patients with confirmed by culture infection due to *L. pneumophila* sg 1, and pre-absorbed with antigen prepared from reference strain *L. pneumophila* sg 1 subgroup Philadelphia (ATCC 33152) or sera from patients with confirmed
L. pneumophila sg 3 infections and pre-absorbed with *L. pneumophila* sg 3 antigen (reference strain ATCC 33155)
**Heterogeneous antigen – sera from patients with legionnellosis caused by *L. pneumophila* sg 1 and pre-absorbed with antigen Lp3 or sera of patients with infection due to *L. pneumophila* sg 3 pre-absorbed with *L. pneumophila* sg1 antigen.

Fig. 1. Significant differences in reduction of IgM level by pre-absorption (determined values of IgM Index) by used heterogenous or homogeneous antigen of *L. pneumophila* (P_o = 0,00437498 in Kolmogorov-Smirnov test)

Results were calculated and interpreted according to manufacturers' instructions. However, for the comparative analysis all the results of ELISA IgM tests were presented in one way – as a ratio of the OD value of the sample to the OD value of the calibrator. This result was called the ELISA test value, VE. Interpretation of the results showed positive VE ≥ 1.1, negative VE < 0.9; borderline ≥ 0.9 – <1.1.

- The reduction of IgM concentration was presented as an IgM Index , the ratio of the ELISA result in serum non-absorbed (VENA, with PBS only) to the result in serum pre-absorbed with one or two antigens (VENA/VEAbs = Index).

Statistical analyses

Statistical analyses were done using Statgraphics for Windows, Centurion, v.XV. StatPointTech.Inc.USA. The multiple variables analysis method was used for quantitative data. For data without a normal probability distribution, the comparison of medians-Mann-Whitney (Wilcoxon), W test and Kolmogorov-Smirnov tests were used. The multiple variable comparison included ANOVA, Multiple Range tests (based on 95% LSD), Kruskal-Wallis test and Mood's Median test. 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 coeff., lambda test and Pearson's correlation), odds ratios and relative risk (if possible) were performed. The significant relation was considered the result of $P_o < 0.05$. For the statistical analysis, all results of ELISA IgM tests were presented in one way – as the ratio of the OD value of the sample to the OD value of the calibrator.

Results

Significant differences in the reduction of IgM level by pre-absorption of sera with specific antigens, so-called homogeneous antigens for ELISA test, in relation to the pre-absorption with non-specific antigens, so-called heterogeneous, was found (Table 1). Overall, the highest reduction and diversification of the results was obtained when sera were pre-absorbed with the homogeneous antigen of *L. pneumophila* sg1. The reduction in serum pre-absorbed with *L. pneumophila* sg1 antigen and tested by the anti-*L. pneumophila* sgs1-7 ELISA IgM assay was as high as 80 times. As a control of the specificity of the pre-absorption test with antigen *L. pneumophila,* a test of pre-absorption of selected sera with *L. pneumophila* sg 12 antigen was also done. The results of reduction (not presented) were always lower than the reduction by antigen *L. pneumophila* sg1, but in same cases – similar. Generally, the lowest values and the differentiation were found in a study of sera pre-absorbed with heterogeneous antigen and these results were similar, regardless of the configuration used.

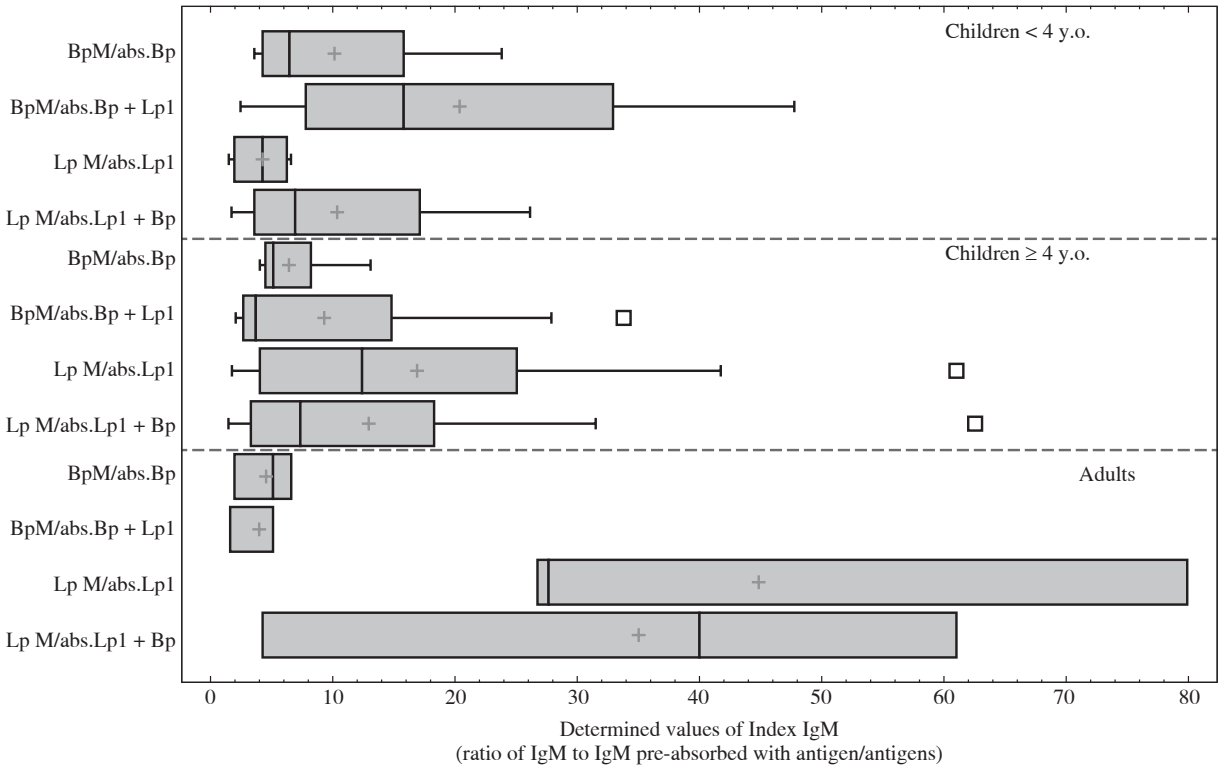
The group of 22 patients from whom sera were pre-absorbed, was divided into 3 subgroups based on the patients' age: < 4 yrs., 4-17 yrs. and adults (> 18 yrs.). It is worthwhile to emphasize that statistically significant differences in the values of the IgM index for each antigen, depending on the age of the patients, were identified. There were significant differences in the reduction of IgM level in tests of sera pre-absorbed with homogeneous, or both antigens, between the groups of patients ($P_o = 0.0018$) (Fig. 2). Index values for tests of sera pre-absorbed with heterogeneous antigen were similar and the lowest in all 3 age groups.

Generally, the highest reduction of anti-*B. pertussis* IgM as a result of pre-absorption with *B. pertussis* (homogeneous antigen) or both antigens was observed among children, whereas a low level of the reduction was found within the group of adults. Conversely, the highest reduction of anti-*L. pneumophila* sgs1-7 IgM in sera pre-absorbed with homogeneous or both antigens was observed

Table 1. Determined values of IgM Index (ratio of IgM in non-absorbed serum (NA) to IgM in pre-absorbed serum)

Absorbed with	Test IgM ELISA/ sera pre-absorbed with antigens Bp or Lp1 or both	Index of IgM tests (NA/pre-absorbed) In all 22 serum samples		
		Min.-Max.	Average	Coeff. of variation
Homogenous antigen	BpM/abs.Bp	2.0-23.8	6.71	66.66%
	LpM/abs.Lp1	1.5-80.0	18.30	113.89%
Heterogeneous antigen	BpM/abs.Lp1	1.2-2.7	1.86	22.94%
	LpM/abs.Bp	1.3-3.0	1.96	20.57%
	MpM/abs.Lp1	0.8-3.75	1.60	45.43%
Both antigens	BpM/abs.Bp+Lp1	1.8-47.7	10.55	115.14%
	LpM/abs.Bp+Lp1	1.4-62.5	15.47	118.74%

LpM/abs.Lp1 – sera pre-absorbed with antigen L. pneumophila sg1 tested by anti-L. pneumophila sgs1-7 ELISA IgM assay; BpM/abs.Bp – sera pre-absorbed with antigen B. pertussis tested by anti-B. pertussis ELISA IgM assay; BpM/abs.Bp+Lp1– sera pre-absorbed with both antigens L. pneumophila sg1 and B. pertussis tested by B. pertussis ELISA IgM assay; LpM/abs.Lp1+Bp = sera pre-absorbed with both antigens L. pneumophila sg1 and B. pertussis tested by anti-L. pneumophila sgs1-7 ELISA IgM assay



LpM/abs.Lp1 – sera pre-absorbed with antigen *L. pneumophila* sg1 tested by anti-*L. pneumophila* sgs1-7 ELISA IgM assay;
BpM/abs.Bp – sera pre-absorbed with antigen *B. pertussis* tested by anti-*B. pertussis* ELISA IgM assay;
BpM/abs.Bp+Lp1 – sera pre-absorbed with both antigens *L. pneumophila* sg1 and *B. pertussis* tested by *B. pertussis* ELISA IgM assay;
LpM/abs.Lp1+Bp – sera pre-absorbed with both antigens *L. pneumophila* sg1 and *B. pertussis* tested by anti-*L.pneumophila* sgs1-7 ELISA IgM assay

Fig. 2. Reduction of IgM level by pre-absorption (determined values of IgM Index)

among adults with diagnosed *Legionella* pneumonia (80 and 61 times, respectively). However, among children aged 4-17 yrs., the values of the IgM Index were also high, especially in two patients (4.5 yrs. and 9.5 yrs.) with diagnosed Legionnaires' disease (reduction of IgM: 61.0 and 31.5 times, respectively).

Further analysis indicated greater serological variation among children, depending on the age. Among younger children (< 4 yrs.), levels of reduction of anti-*B. pertussis* IgM were found, in particular in tests of sera pre-absorbed with two antigens together. These were significantly higher than in older children. Moreover, in this group we observed the highest reduction of anti-*B. pertussis* IgM in sera pre-absorbed with homogeneous antigen (Index BpM/Bp = 23.8). Conversely, levels of reduction of anti-*L.pneumophila* sgs1-7 IgM after pre-absorption with homogenous antigen were significantly increased in older children in comparison with younger children.

Differences in IgM reduction by homogeneous antigens in individual patients were also analyzed. For this purpose, the ratio between two values of the determined index was evaluated (the ratio of index LpM/abs.Lp1 to index BpM/abs.Bp). Among all children, this ratio ranged from 0.13 to 15.25. A value of 0.13 means that a reduction of anti-*B. pertussis* IgM in serum pre-absorbed with *B. pertussis* was 7.7 times higher than the reduction of anti-*L. pneumophila* sgs1-7 IgM in the same serum pre-absorbed with *L. pneumophila* sg1. This was observed in a 13 year-old child with the results of routine ELISA IgM tests: Bp (+); Lp sg1 (+/–), Mp (–). The value of 15.3 means that in another child, the reduction of anti-*B. pertussis* IgM in serum pre-absorbed with *B. pertussis* antigen was 15.3 times lower than the reduction of anti-*L. pneumophila* sgs1-7 IgM in the same serum but pre-absorbed with *L. pneumophila* sg1 antigen. This was the 4.5 year-old child with routine ELISA IgM results: Bp (+); Lp sg1 (+), Mp (+). In adults, this reduction of anti-*L. pneumophila* sgs1-7 IgM after pre-absorption with homogeneous antigen was at least 5-fold higher than in tests for *B. pertussis* (Table 2).

A two-fold difference was determined as significant between values of IgM Lp1M/abs.Lp1 index and IgM BpM/abs.Bp index (P_o = 0.0151). Based on this result, there was

Table 2. Results of IgM ELISA tests, level of reduction after pre-absorption with homologous antigens, ratio of indexes of IgM and interpretation based on these tests in all 22 patients by age group

Age of patients	Pneumonia/ ARTI	Results of IgM ELISA to			index LpM/ abs.Lp1	index BpM/abs. Bp	Ratio of index LpM/ abs.Lp1 to index BpM/abs.Bp	Interpretation based on the pre-absorption tests*	Results of other tests used for identification of legionellosis in the selected patients
		Lp	Bp	Mp					
Children < 4 yrs.	P	–	+	–	1.2	4.8	0.25	Bp	ELISA IgG(+) and IgA(+), MAT** Lp4(+)
	P	+	–	+	1.5	4.62	0.54	??	PCR(–) in serum; IgG(+), IgA(–); MAT Lp12(+)@
	A	+	+	–	5.8	23.8	0.24	Bp	*L. pneumophila* sg1 urinary Ag (–); IgG(–), IgA(–)
	P	+	+/ –	+	6	7.9	0.76	??	ELISA IgA(–) and IgG(–); MAT (–)
Children 4-6 yrs.	P	+	+	–	2.6	4.8	0.54	??	Lp1 urinary Ag (–); IgG(–), IgA(–); MAT *L. bozemanii* (+/–)
	P	+	+	+	61	4	15.25	Lp	PCR(+/–); IgG(–), IgA(+); MAT Lp1(+), Lp6(+), Lp12(+)
	P	+	+/ –	–	4	7.5	0.53	??	Lp1 urinary Ag (–); IgG(–) and IgA(–),
	A	+	+	+	12.2	4.7	2.6	Lp	nt
	P	+	+	+	4.2	4.6	0.91	??	ELISA IgA(–) and IgG(–); MAT Lp6 (+)
Children 7-9 yrs.	A	+	+/ –	–	2.2	4.2	0.53	??	Lp1 urinary Ag (–); IgG(–), IgA(–); MAT *L. bozemanii* (+)
	P	+	+	+	16.2	4.7	3.45	Lp	Lp1 urinary Ag (+); IgA(–)
	P	+	–	–	41.7	5.5	7.6	Lp	PCR(+); IgG(–), IgA(–); MAT Lp1(+), Lp12(+)@
	P	+/ –	+	–	5.4	8.4	0.64	??	PCR(–); ELISA IgA(–) and IgG(–);
	A	+	+/ –	+	14	4.2	3.33	Lp	PCR(+/–); IgG(–), IgA(–);
Children 10-17 yrs.	P	+	+	+/ –	16.7	8.3	2.01	Lp	IgG(–), IgA(–); MAT Lp12(+)@
	A	–	+	–	7	6.9	1.01	??	PCR(–); IgA (–), IgG (–)
	P	–	+	–	1.7	13	0.13	Bp	PCR(–); IgA (–), IgG(–)
	A	+	+	–	25	8.2	3.05	Lp	PCR(+/–); IgA(–),IgG(–); MAT *L. micdadei* (+), Lp1 (+/–)
	A	+	+	–	38	4.5	8.4	Lp	PCR(+/–); IgA(–),IgG(–); MAT *L. bozemanii* (+), Lp1 (+/–)
Adults > 50 yrs.	P	+	–	–	26.7	5	5.34	Lp	PCR(+); seroconversion
	P	+	–	–	27.5	2	13.75	Lp	seroconversion
	P	+	–	–	80	6.6	12.12	Lp	seroconversion

*Lp – Result of IgM ELISA to L. pneumophila sgs 1-7; Bp – result of IgM ELISA to B.pertussis; Mp – result of IgM ELISA to M. pneumoniae; P – pneumonia; A – acute respiratory tract infection without pneumonia; *interpretation based on results of pre-absorption tests with homogeneous antigen: pertussis (Bp) – if ratio of index LpM/abs.Lp1 to index BpM /abs.Bp ≤ 0.5; legionellosis (Lp) – if the ratio ≥ 2; not determined (??) – if the ratio 0.51 – 1.99; MAT – microagglutination test used for detection of IgG and IgM antibodies to 20 antigens (separately) prepared in-house from reference strains of L. pneumophila sg 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15, L. micdadei, L. bozemanii, L. jordanis, L. longbeacheae and L. anisa. MAT Lp12(+)@ – similar level of reduction of IgM by pre-absorption with antigen L. pneumophila sg1 as with antigen L. pneumophila sg12 (data not presented)*

a possibility of determining the presence of specific antibodies to one pathogen, and the probable aetiological agent of these acute respiratory tract infections. If the LpM/ absLp1 index was ≥ 2times higher than the BpM /abs.Bp index, then infection was probably caused by *L. pneumophila* sg1. Such results were observed in 8 children (> 4 yrs.) and 3 adults. If the ratio of the Index LpM/ absLp1 to Index BpM / abs.Bp was ≤ 0.5, then detected IgM antibodies indicated specificity to *B. pertussis* antigen it was found in 2 younger and 1 older child. However, there were 8 children with results between these limits, and thus a strong probability of cross-reaction in the routine ELISA IgM tests was found. These were mainly children younger than 7 years, with one exception, an 11 year-old girl. Moreover, all children with unclear results of pre-absorption were girls (P_o = 0.0222), although there was no correlation between gender and the results of diagnostic IgM ELISA.

Moreover, significant differences in the ratio of Indexes depending on age, and a significant correlation between age of patients and interpretation of the pre-absorption test (P_o = 0.0328) were found (Fig. 3). Specific antibodies to *L.pneumophila* sgs1-7 were found in children older than 4yrs., mainly from 9yrs., and adults. However, unclear results were found only in children, mainly in children below 7 yrs. of age.

An analysis of the relation between the results of pre-absorption tests and routine ELISA IgM tests was also done (Table 2). The majority of children (3/4) with positive IgM results in 3 tests (*B. pertussis, L. pneumophila* sgs1-7 and *M. pneumoniae*) were interpreted as having *Legionella* infection – based on the results of pre-absorption tests. In 3/5 of children, with positive IgM results for *L. pneumophila* sgs1-7 and *B. pertussis*, bacteria *L. pneumophila* sg1 were also identified as a causative agent. However, among 8 children with a very similar level of IgM reduction after pre-absorption by *B. pertussis* or *L. pneumophila* sg1 antigens, in 4 cases only one routine ELISA IgM test was positive (2 for *L. pneumophila* sg 1 and 2 for *B. pertussis*). Moreover, in 2/3 sera with routine postive IgM to *L. pneumophila* sgs1-7 and *M. pneumoniae,* results were unclear in pre-absorption by *L. pneumophila* sg1 or *B. pertussis* antigen.

A relation between results based on the pre-absorption test and pneumonia symptoms was not found (P_o > 0.05). Among 12 children with pneumonia, in 4 cases - antibodies to *L. pneumophila* sgs1-7 were found, in 2 – IgM to *B. pertussis* and in 6 – inconclusive results were determined. However, in 50% of pneumonia cases we found inconclusive results (Fig.4). We found a high predominance of inconclusive results among children under 6 yrs. with atypical pneumonia (5 out of 7). In 3 of such patients a positive result of IgM to *M. pneumoniae* was also found.

Generally, in routine examinations we were able to find the positive correlation between the positive level of IgM and PCR for *L. pneumophila* done in obtained serum

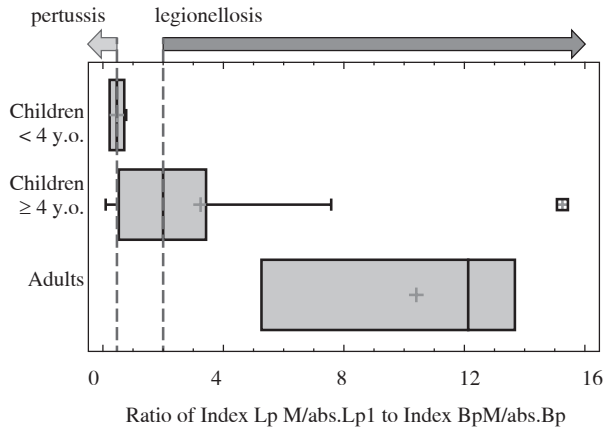


Fig. 3. Interpretation of results of pre-absorption tests with homogenous antigen – by the age of patients

samples. In this work, significant correlation between results based on the pre-absorption test and PCR in serum samples was also found (P_o = 0.04). Also, the relation between high level of IgM reduction by pre-absorption with antigen *L. pneumophila* sg12 (not presented) and positive results by microagglutination test with antigen of Lp12 was shown (Table 2).

Discussion

Interpretation of the results obtained in serological tests is one of the most difficult parts of laboratory diagnostics for infectious diseases, but in practice the tests are widely used in identification of bacterial infections, especially atypical pneumonia [1-5, 7-12, 16]. Despite the delay in the diagnostic process, serological tests are the basis for

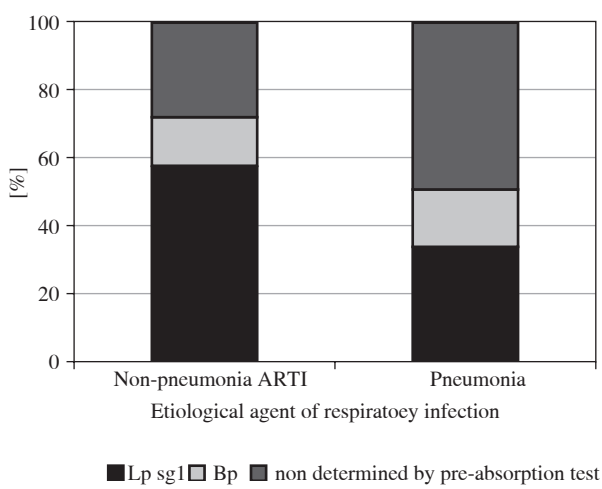


Fig. 4. Determined results by pre-absorption test in 22 patients by main clinical recognition

diagnosis of most cases of infection caused by *L. pneumophila*, *M. pneumoniae* and *B. pertussis* bacteria in Poland [7, 11, 12].

One of the the most important problems seems to be the interpretation of ELISA test results for detection of anti-*B. pertussis*-specific antibodies, especially IgM ELISA. The problem of standardization of ELISA has been considered by the CDC and EU expert groups. According to the EU Pertstrain group (the EU expert group for *B. pertussis* infection), the EIA should use purified non-detoxified PT (pertussis toxin) as an antigen for detection of IgG-anti-PT antibodies [6]. The use of IgM or IgG ELISA tests based on cellular antigen of *B.pertussis* for the diagnosis of infection in children might be the cause of false postive results because of previous vaccination against pertussis.

The majority of children (> 90%) in Poland are vaccinated against pertussis according to Polish regulations. Thus, the main purpose of carrying out this research work was to find out if a similar problem of IgM cross-reactions in laboratory routine serological diagnostics of legionellosis among children is also observed. The problem of a high level of cross-reactivity observed in ELISA tests for *B. pertussis* ELISA and other pathogens, as well as *L. pneumophila* sg1, has been observed by different teams [5], and also in our studies. We found that 43% of patients with positive IgM levels for *B. pertussis* were also positive for IgM *L. pneumophila* sg1. However, this proportion of IgM co-detection among children with a positive IgM result to *L. pneumophila* sg1 was as low as only 25% of patients. The phenomenon of co-detection of IgM to *L. pneumophila* sg1 (the most often identified agent of legionellosis in Europe), and to *B. pertussis,* and the problem of which bacteria were an aetiological agent of infection has been discussed with some paediatricians.

Three commercial ELISAs for IgM detection of anti-bacterial cellular antigens were used: for *L. pneumophila* sg1, *B. pertussis* and also for *M. pneumoniae* – a commonly – applied diagnostic test in atypical respiratory tract infections in Polish children. In the presented study, young patients were hospitalized because of acute respiratory tract infections and legionellosis was suspected. Some of the patients were previously examined for *M. pneumoniae* (as the first diagnostic option), this being probably why a low level of *M. pneumoniae* IgM positive results was observed in the study. For this reason, and also because of a lack of sufficient quantities of the samples for more detailed pre-absorption tests, the phenomenon of co-detection of IgM anti-*L. pneumophila* and anti-*M. pneumoniae* was only preliminarily examined.

A very interesting phenomenon was observed in the youngest children. A strong reduction of the level of IgM for *B. pertussis* after pre-absorption with two antigens was found. This reduction was even higher than in the case of reduction by homogeneous antigen. Conversely, the ratio of reduction of IgM by pre-absorption with two antigens in tests for *L. pneumophila* sg1 was similar to or lower than for homogenous antigen. Perhaps this phenomenon might be connected to mandatory vaccination against pertussis. Currently, up to 2 years of age, the child will be vaccinated four times (2 – 4 – 6 – 18 months of age), and a booster dose is given at 6 years [12]. For the first 4 vaccinations the cellular antigens of *B. pertussis* are recommended, and a non-cellular formulation used only for the booster vaccine. In these studies, inconclusive results were obtained mainly in children under the age of 7, which might indicate a link between vaccination and the co-detection of IgM or cross-reactions in the used tests. The problem of interpretation of serological tests, and of IgG, because of the presence of antibodies following vaccination was also discussed by the EU Pertstrain group. They recommended that diagnostic IgG-anti-PT serology cannot be validly interpreted for one year after vaccination with acellular pertussis vaccines [6].

In contrast, there was no relation between the age of patients and reduction of IgM antibodies for *L. pneumophila* sg1. Results indicating actual *L. pneumophila* sg1 infection were observed in 8 children older than 4 yrs., and the reduction of IgM observed in these children was similar to the level of reduction in 3 adults with diagnosed Legionnaires' disease. However, in 7 out of 8 of these children a positive IgM level for another pathogen was found. In adults, positive results observed in ELISA IgM were only for *L. pneumophila* sg1. This indicated a difference between children and adults, and might be a reason why so few cases of legionellosis were diagnosed in European countries; but in Poland the frequency was higher [7, 10, 13, 16]. Antibodies specific to *L.pneumophila* sg1 were found in 4 children hospitalized with pneumionia, and 4 with ARTI, but without pneumonia. It might be an actual infection or an exacerbation of respiratory infection due to another pathogen (bacterial or viral). Unfortunately, because of the dynamics of IgM production and delay in the serological results, we are unable to determine if there was consecutive or co-infection. However, in some bacterial or viral infection, a temporary decrease of immunocompetence and the opportunity for infection by other pathogens was observed.

Several projects directed at the determination of legionellosis among children in Poland were conducted [7, 11]. Unfortunately, it is rarely possible to analyze more than one positive result of the IgM ELISA test for various pathogens causing respiratory tract infections. Usually, a first IgM positive ELISA result, directed at a pathogen more common than *L. pneumophila*, may end further diagnosis. Moreover, macrolide antibiotics used in treatment of young patients with *L. pneumophila* infections, are also generally effective against *M. pneumoniae* and *B. pertussis*.

However, the appropriate diagnosis of acute atypical respiratory infections is not only a clinical problem, but also an epidemiological problem. Bacteria *M. pneumoniae*

are spread from human to human – in the majority of cases, this occurs in the winter season. Infection due to *L. pneumophila* or *Legionella* spp. is spread by contaminated aerosols, and mortality in LD depends on different factors: the ratio of contamination of water, exposure time, type of *L. pneumophila* strain and other host properties (eg.immunodeficiency). In some special conditions, the mortality was as high as 50-75% [10, 11]. A real, correct diagnosis of *L. pneumophila* infection will allow the conducting of an epidemiological investigation, and will enable identification of sources and ways in which the contaminated aerosol is spread. In this way, the potential risk to the health of many people might be recognized, and reduced or eliminated.

Conclusions

1. The high prevalence of anti-*L. pneumophila* sgs 1-7 IgM in children hospitalized because of ARTI but without pneumonia, indicated the importance of this pathogen as an etiological agent of disease with its middle course in children.
2. The possibility of impact of an anti-pertussis vaccination on the results obtained in anti-*L. pneumophila* ELISA IgM tests among young children was preliminarily evaluated in this work. To avoid any cross-reactions with anti-*B. pertussis* IgM, routine laboratory procedure/s for the diagnosis of *Legionella* infection among young children should be based on other than serological examinations (like PCR).
3. The problem of IgM cross-reactivity in the tests for *L. pneumophila* sgs1-7 and *M. pneumoniae* is still not recognized. This is why the diagnostic system of acute atypical respiratory infections in children should definitely include other techniques (like: culture of bacteria, PCR), and not only serological tests. If only serological tests are available, paired sera should be tested and assays for different pathogens should be done simultaneously.
4. The obtained results suggest the need for further research into the relation between vaccination and the results of serological examinations of young children.
5. The pre-absorption test might be useful supplementary tool for confirmation of legionellosis case, if only serum samples is available.

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References

1. Jørgensen CS, Uldum SA, Elverdal PL (2014): Application of a lateral flow test as an additional serological tool for diagnosis of Legionella infections. J Microbiol Methods 96: 12-15. http://dx.doi.org/10.1016/j.mimet.2013.10.021.
2. Elverdal PL, Jørgensen CS, Krogfelt KA, et al. (2013): Two years' performance of an in-house ELISA for diagnosis of Legionnaires' disease: Detection of specific IgM and IgG antibodies against Legionella pneumophila serogroup 1, 3 and 6 in human serum. J Microbiol Methods 94: 94-97.
3. Yzerman EPF, den Boer JW, Lettinga KD, et al. (2006: Sensitivity of three serum antibody tests in a large outbreak of Legionnaires' disease in the Netherlands. J Med Microbiol 55: 561-566.
4. Lai C-H, Chang L-L, Lin J-N, Chen W-F, Kuo L-L, et al. (2013): High Seroprevalence of Mycoplasma pneumoniae IgM in Acute Q Fever by Enzyme-Linked Immunosorbent Assay (ELISA). PLoS ONE 8: e77640.
5. Benson RF, Thacker WL, Plikaytis BB, et al. (1987): Cross-reactions in Legionella antisera with Bordetella pertussis Strains. J Clin Microbiol 1987: 594-596.
6. Guiso N, Berbers G, Fry NK, et al. (2011): What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis 30: 307-312.
7. Pancer K (2014): Patient's age and the dynamics of IgM for L. pneumophila sg1. Przegl Epidemiol 68: 21-26.
8. Cunha BA (2009). Severe adenovirus community-acquired pneumonia mimicking Legionella. Eur J Clin Microbiol Infect Dis 28: 313-315.
9. Kusnetsov J, Neuvonen L-K, Korpio T, et al. (2010): Two Legionnaires' disease cases associated with industrial waste water treatment plants: a case report. BMC Infect Dis 10: 343.
10. Beaute J, Zucs P, de Jong B, on behalf of the European Legionnaires' Disease Surveillance Network (2013). Legionnaires' disease in Europe, 2009-2010. Euro Surveill 18(10):pii = 20417. http://www.eurosurveillance.org/ViewArticle.aspx-?ArticleId =20417.
11. Pancer K, Napiórkowska A, Gut W, et al. (2011): Demographic characteristics of reported cases of legionellosis in years 2005-2009 in Poland in comparison to EWGLI data. Przegl Epidemiol 65: 433-440.
12. Paradowska-Stankiewicz I, Rudowska J (2013): Pertussis in Poland in 2011. Przegl Epidemiol 67: 199-201.
13. Reinton N, Manley L, Tjade T, Moghaddam A (2013): Respiratory tract infections during the 2011 Mycoplasma pneumonia epidemic. Eur J Clin Microbiol Infect Dis 32: 835-840.
14. Mina MJ, Burke RM, Klugman KP (2014): Estimating the prevalence of co-infection with influenza virus and the atypical bacteria Bordetella pertussis, Chlamydophila pneumoniae, and Mycoplasma pneumoniae. Eur J Clin Microbiol Infect Dis 33: 1585-1589.
15. Pancer K, Jahnz-Różyk K, Kucharczyk A, et al. (2012). Sequence based typing and pre-absorption test in retrospective analysis of a pseudo-outbreak of Legionella infections differentiates true cases of legionellosis. AAEM 19: 437-443.
16. Javed S, Chaudhry R, Passi K, et al. (2010). Serodiagnosis of Legionella infection in community acquired pneumonia. Indian J Med Res 131: 92-96.