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PATIENTS' AGE AND THE DYNAMICS OF IgM FOR *L. PNEUMOPHILA* sg1

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ABSTRACT

MATERIAL AND METHODS. The results of IgM *L. pneumophila* sg1 test in 304 adults and 270 children performed at NIPH-NIH in 2004-2007 were analyzed to determine the effects of patients' age and the interval between collected sera on the results and the interpretation.

RESULTS. Significant difference in the level of IgM, depending on the age of the patients (P_o = 0.0084) was found. Positive results (in total 20.4% of patients) were the most frequently observed in patients aged 19-29 years (42.5%), and the least - in patients 60 y.o. and <2 y.o. (7%). Average and median levels of IgM in these two groups (+60 y.o. and <2 y.o.) were similar and significantly different from the results in the other groups. From 44 adults and 33 children ≥ 2 sera were collected. There was a significant difference in the interval between collecting the first and second serum sample in adults (mainly 3-5 weeks) and children (mainly 2-4 weeks). Significant increase of IgM levels was observed in children when the interval between 1 and 2 sample didn't exceed 4 weeks, while in adults this change was also observed at > 5 weeks (25% of patients). No significant differences in the analysis of the IgM ratio in children (1.25-14) and adults (1.5-26) was found, but longer persistence of IgM in adults than in children was observed.

CONCLUSIONS. Demonstrated trend of faster decline in the level of IgM among children than in adults indicated that in suspected case of legionellosis in children, the serum sample should be taken up to 4-5 weeks after the onset, and at intervals of 1-2 weeks maximum.

Key words: *Legionella pneumophila, legionellosis, serological tests, dynamics of IgM, seroconversion*

INTRODUCTION

Determination of specific antibodies level (class M and/or G) for bacterial or viral aetiological agent of infection is one of the frequently used diagnostic methods. For confirmation of infection the demonstration of seroconversion or a significant decrease/ increase of specific IgM or IgG in paired serum samples taken at the appropriate time interval should be determined. Dynamics of increase or decrease of specific antibody concentration depends on the class of antibodies, the properties of pathogen or the individual host. Also, the type of used serological method (ELISA, IFA or other), as well as the technique of reading (automatic or visual, using labeled mono-antibodies or other) and the kind of used antigen influence on the results of serological examination. Moreover, age of patient should be also considered as one of the many factors influenced on the result of serological test (1,2,3).

Legionellosis is a disease with various symptoms, caused by bacteria *Legionella* spp., mostly *L. pneumophila* sg1. In Poland, serological examinations are the most often used diagnostic method for diagnosis of *Legionella* infection (4,5,6).

The aim of this study was to analyze the impact of patient age on the concentration and dynamics of IgM antibodies specific to *L. pneumophila* sg1 .

MATERIAL AND METHODS

The results of routine diagnostic tests (IgM and/ or IgG) for infection due to *Legionella pneumophila* sg1 (serogroup 1), performed at the National Institute of Public Health - National Institute of Health (NIPH -NIH) in years 2004-2007 were analyzed. In total, there were results of examination of 658 serum samples collected from 574 patients, including 304 adults (aged ≥18 y.o.) and 270 children. The determination of IgM or IgG specific to *L. pneumophila* sg1 was performed by the commercial ELISA kits (Euroimmun Poland). Interpretation of the result was according to the manufacturer.

Information about the age of the patient was available for 251 children and 235 adults. More than one serum sample were collected from 51 adults and 33 children.

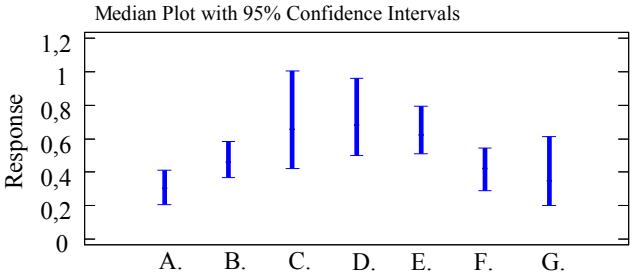
Statistical analysis were done using Statgraphics for Windows, Centurion, v.XV. StatPointTech.Inc.USA. As the significant relation was considered the result of $P_o < 0.05$. The analysis of median values was performed by the Mood Median test. Moreover, the mean values of the median IgG and IgM, depending on the age of the patients was determined by Kruskal - Wallis test - due to the different than normal distribution of values in the group.

RESULTS

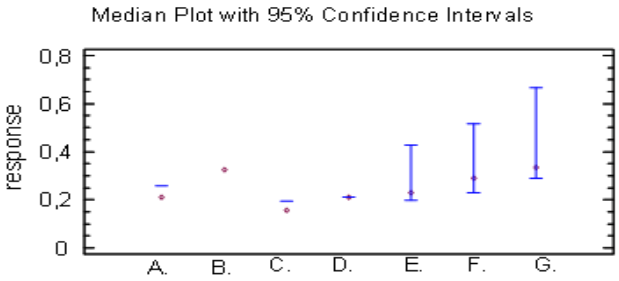
Totally, positive results of IgM were found in 117 patients (20.4%), and borderline value - in 48 patients (8.4%). In contrast, positive IgG results were found in 34 out of 397 examined patients (8.6%), and borderline - in 19 patients (4.8 %).

There were found statistically significant differences in the level of IgM, depending on the age of the patients ($P_o = 0.0084$). The highest percentage of positive IgM was found in young adults (32.5%), especially those aged from 18 to 29 years - 42.5 %. The high (>30%) percentage of IgM positive results were also found in children aged > 4 years. The lowest percentage of IgM positive results was found among the youngest patients (7.1%) (Table I).

Analysis of the median and the mean of median of IgM in particular groups of patients indicated significant differences. The highest diversity of the IgM results was observed in children aged 5-9 years and 10-17 years old. In contrast, the highest values of IgM was observed among adults (40-59 y.o. and 18-39 y.o.) and children in the age group 5-9 y.o. The average level of IgM in the young children was similar to that indicated in the elderly, and definitely different from the results in children 5-9 years of age, young adults and older children (Table I, Fig.1).



IgM - Mood's Median Test: Test statistic = 29.1889; P_o=0.000056028 (the medians of the IgM in the groups are significantly different at the 95% confidence level)



IgG - Mood's Median Test: Test statistic = 6.24943; P_o=0.395837 (there is not a statistically significant difference between the groups of the 7 variables at the 95,0% confidence level).

Fig. 1. Analysis of determined values of median IgM and IgG by age group of patients: A =<2 y.o.; B = 2-4 y.o.; C = 5-9 y.o.; D = 10-17 y.o.; E = 18-39 y.o., F = 40-59 y.o.; G = 60+ y.o.

Lack of statistically significant differences between IgG values depending on the age were found, neither in the individual, distinguished groups ($P_o = 0.18$), or in the analysis of median values or means of median. However, there were the differences in the results obtained in children and adults. Among children, the positive IgG results are few, while among adults significantly increased the mean and the most often determined level of IgG and noticeable was the significant variation of that level. The highest value of IgG occurred mainly in adults (Table I, Fig.1).

Next, the IgM results obtained in the paired serum samples were analyzed. There were included results

Table I. Comparison of determined results of IgM and IgG antibodies specific for *L. pneumophila* sg1 in patients' sera examined in NIPH-NIH, by age group

Age group (years)	IgM – %positive, maximum and minimum of arithmetic average (Av) and median (M)						IgG – % positive, maximum and minimum of arithmetic average (Av) and median (M)					
	Tested	% pos.	Min.	Max.	Av.	M	Tested	% pos.	Min	Max	Av.	M
<2	42	7,1	0,05	1,85	0,45	0,31	21	0	0,08	0,85	0,31	0,21
2 - 4	73	24,7	0,02	3,50	0,73	0,46	34	8,8	0,04	1,96	0,37	0,33
5 - 9	70	30,0	0,05	**8,84**	1,05	0,66	40	5,0	0,01	2,14	0,33	0,16
10- 17	67	31,3	0,06	3,79	0,97	0,68	41	4,9	0,04	1,62	0,31	0,21
18- 39	80	32,5	0,01	**7,03**	1,06	0,62	67	5,9	0,00	**8,5**	0,52	0,23
40- 59	99	13,3	0,04	**14,16**	0,85	0,42	83	12,2	0,02	**3,5**	0,49	0,29
60 +	60	18,3	0,01	3,71	0,59	0,45	49	12,2	0,01	**5,42**	0,70	0,34
In total	491	22,9	0,01	14,16	0,84	0,49	335	8,0	0,00	8,5	0,46	0,23

obtained in the sera from the same patient collected in the interval not exceeded 12 weeks (between the first and the second serum). Sera collected from 7 adults were excluded from the analysis, because of the interval (43 - 76 weeks), which might indicate another infection.

Overall, there were analyzed the data of 77 patients from whom pairs of sera were collected: 44 adults, of which the second serum sample was taken at intervals of from 0.5 to 9 weeks (mean distance was 4.38 weeks, median = 4.0); and 33 children, from whom the second sample was taken at a distance of from 0.5 to 10 weeks (mean = 2.782; median 2.0). Statistically significant difference in the time interval between taking the first and the second serum samples in adults and children was found (median - $P_o = 0.0004$; mean - $P_o = 0.0016$). The majority of paired serum samples in children was collected in the range of 1.5 to 4 weeks, while in adults - from 3 to 5 weeks (Fig. 2).

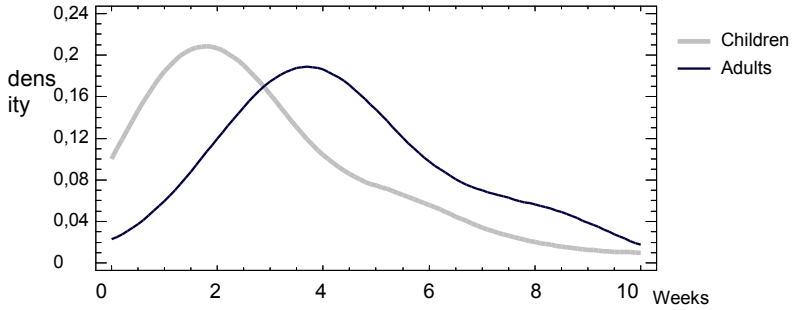


Fig. 2. Comparison of interval between collection the first and the second serum samples among children and adults

Analysis of obtained values of IgM in the paired samples of a total of 77 patients were done. Positive result in both samples were found in 14 out of 77 (18.2%) patients, while negative in 29 (37.7%) patients. Then the increase/decrease of the IgM concentration in paired sera were further examined (excluding negative paired sera). Paired sera, in which the change in IgM level was less than 20% were also excluded from further analyzes. It was considered that the variation in the IgM value of < =20% may be caused by other external factors not necessarily due to actual changes in the level of antibodies. There were mainly pair of sera, in which both results were positive.

In total, the change of more than 20% of IgM concentration was observed in 33 patients, including 17 children. Seroconversion was found in a total of 12 people, both adults and children. The decrease or increase in IgM level, when both samples were positive was found in 8 patients. Also, the change >20% of IgM level from positive to equivocal result (or opposite) in 7 patients was observed. The changes of IgM level from negative values to bordeline level were mainly observed among children (80%). The highest difference of IgM level in the paired sera were found when the change was from negative to positive result, both in adults (IgM VE difference in the range of -1.02 to 8.73) and children (IgM VE difference in the range of -1.13 to 8.21) (P_o>0.05). However, the differences of IgM value in the paired sera were also significant when IgM results were positive in both samples (the difference VE from -1.12 to 4.63) or positive/borderline (from -3 to 2.39).

Then the change in the IgM level as a function of time was also analyzed. It was found that among children the significant increase/decrease in IgM levels occurred when the interval between samples did not exceed four weeks. Among the remaining children, the seroconversion was not indicated - both serum samples were negative. The results of IgM level (VE) determined in 4 consecutive serum samples collected from 9.5 year old girl with LD in a total distance of 35 days (5 weeks) were presented on the figure 3 as an example of period when IgM might be detected.

In contrast, a significant change of the IgM level among adults was detected at different time intervals, also above five weeks, although in only 25% of patients.

We analyzed the rate of growth of IgM (ratio IgM1/IgM2), which defined how many times the concentration of IgM in the second serum samples had increased /decreased (with the exception of the pairs of sera where the both results were negative). It was found that the ratio of increase of IgM among children was in the range 1.25-14 (mean:3.56 and median:3.2), whereas among adults - in the range of 1.5-26 (mean:4.17; median:1.73) and this difference was not significant (Po> 0.05). Overall, at least two-fold increase of IgM level was found in 18 patients: 12 children and 6 adults. In this group of patients the change in result was mainly in the range: from negative to positive (56%) or from bordeline to positive (28%). In contrast, if determined ratio of IgM was lower than 2, the change of results have been in the range of positive values of IgM in both serum samples (7 patients, 47%) mainly. Also there were pairs of sera with indicated change from negative to positive results (2 patients, seroconversion), from positive to equivocal (2 patients), and from negative to bordeline (4).

It should be pointed that the majority of patients, with change of results from negative to equivocal level, were children <5 years of age (4 out of 6 persons). However, lack of significant relationship between age and the rate of growth of IgM was found.

The increase of IgM levels among children and adults was described by the same formula: lnY= a + bX (where X and Y means IgM levels in subsequent samples), however, there were significant differences in the values of the coefficients a and b in both groups. In the group of children the coefficients were: a = 1.4 + / -0.43 (Po = 0.0054), b = -1.12 + / -0.34 (Po = 0.0051), and in the group of adults: a = 0.70 + / -0.30 (Po = 0.036), and b = -0.74 + / - 0.17 (Po = 0.0008). Differences in the coefficients a and b indicated a different characteristics of the IgM growth course and response to an infection in both groups. Significant relation (Po = 0.0243) between the ratio of increase of IgM level and the interval between samplings were observed in the adults groups, while in the children group this correlation was not significant (Po = 0.5).

DISCUSSION

Many authors emphasize that only the complex laboratory diagnosis of Legionnaires' disease, examination of various clinical specimens and use a variety of techniques allow to obtain high (>95%) diagnostic ability (2,7). Serological examinations done in paired serum samples allow to demonstrate the immune response to *Legionella* spp. infection, in cases of extrapulmonary infection also. The dependence of the specific antibodies level on the phase of the disease cause that the sampling time is one of the most important condition which affects the possibility to interpret the results. The negative result of a serological test of a single serum sample does not exclude the Legionnaires' disease because it may be caused by too early or too late collecting a sample. Such possibility was illustrated in this studies also. There were presented results of examinations of 4 sera collected from 9.5 y.o. girl with confirmed LD (fig.3). If we analyzed the results of the first and the forth serum samples only, then we would not be able to detect the positive level of IgM. Moreover, positive or borderline level of IgM in a single sample might indicate a probable infection due to *L. pneumophila* or be the result of other factors (eg. non-specific or cross- reactions). In such a situation the diagnosis should be complemented by demonstration of the dynamics of IgM / IgG or other test using another technic (eg. PCR).

The definition of a significant increase/decrease of the specific antibody level depends on an used method. The EWGLI definition (at least 4 - fold increase /decrease in antibody titers, if at least titer was> 1:128 in one sample), was developed based on micro-agglutination test (MAT), in which the double dilutions of serum were examined. In contrast, the EIA test consists in the OD measurement and it is dependent on the logarithm of the antibody concentration. Fourfold increase in the value of ELISA test can correspond to greater values actually. The presented analysis of the serological tests done in the NIPH -NIH confirms the suggestion of Elverdal et al. (8), who decided that 2- fold increase of the IgM in ELISA was significant for confirmation of *Legionella* infection. In our studies at least 2- fold increase in IgM was found in 18 patients (including 12 children). Among them, in 10 persons, the change from negative result to positive (seroconversion) was found. Lower increment of IgM (<2) was found in 15 patients, mostly adults. Among these patients, in 2 individuals the seroconversion have also been determined, which indicates the importance of the used definition and the problem of the interpretation of such results.

Moreover, it was found that in 55% of patients with IgM index ≥2, the interval between the first and the second serum ranged from 0.5 to 2 weeks. Significant differences in the intervals in the groups of children and adults was probably related to the different diagnostic process and treatment in these groups. Most of the children were hospitalized because of respiratory tract infections or the infection was acquired in a hospital,

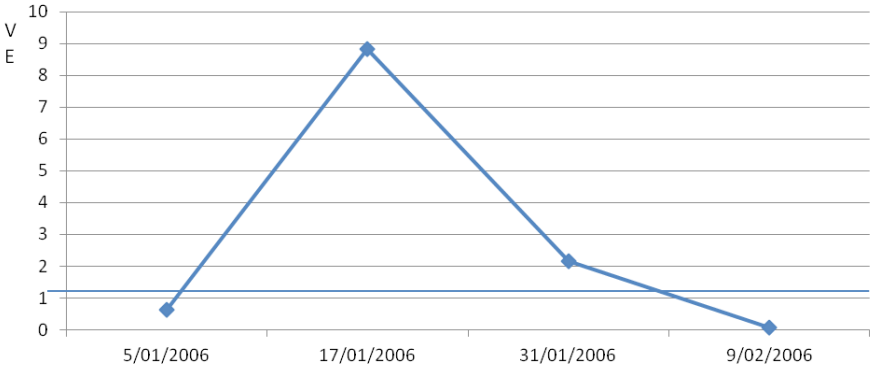


Fig. 3. Determined IgM value (VE) in 4 serum samples collected from the same patient with diagnosed Legionnaire's disease - by the date of the serum collection (positive results VE ≥1,1)

so the samples were collected as only the suspicion was done and in short intervals. In contrast, adults are often treated at their home, so the problems with collecting second serum samples might occurred (delayed or lack of control visit).

Among children the significant change in IgM concentration was observed when the interval between serum samples was no longer that 4 weeks, among adults – also in the interval longer than 4 weeks. It suggests a faster decrease of IgM antibody response during the *Legionella* infection in children than in adults. This thesis has significant practical importance, as the collection of samples in period of 2-5 weeks, or delayed collection of the first sample may cause that seroconversion / significant increase in the level of IgM antibodies will be not determined. This result is also important because of the data in the literature often suggest that seroconversion in cases of Legionnaires' disease can be delayed in some patients and occur even after 6 weeks (1,9). This thesis is confirmed in this studies but only in the adults group - by the demonstration of the relation of the interval between the samples and the increase in IgM. However, it should be pointed, that the data in the literature regarding seroconversion in *Legionella* infection were based on the researches done mainly among adults patients. The occurrence of *Legionella* spp. infections in immunocompetent children was considered as a very rare and exceptional for many years (10). Nowadays, it is believed that the disease caused by *Legionella* spp. are also observed in previously health children (6,11) and this work also confirmed this thesis.

The scale of exposure to infection by *Legionella* species may be indicated as the level of antibodies in the group of people - as it was demonstrated by Wedege et al. (12). Among the most exposed individuals the medians of IgG and IgM were significantly higher than in those less exposed. That study was conducted after the occurrence of a large outbreak of LD (103 patients) and it was found not only immune response to antigens of *L. pneumophila* sg 1, which caused the outbreak, but also to *L. pneumophila* sg 4. This result suggests that the immune response may indicate a number of different, also previous *Legionella* infections. Some of these infections might occur in the form of a self-limiting or influenza-like illness as well as an asymptomatic form.

Repeated exposure to infection with *Legionella* spp. may be one of the reasons of higher values of median IgG, IgM as well as the average of these medians in adults than in children – what was presented in this work. This thesis indicate the necessity of diversification of procedures for serum sampling in children and adults. In children, further samples should be taken at an interval of 1-2 weeks, starting with the first signs of illness, while in adults these period might be longer (2-4 weeks). Undoubtedly, the first serum samples should be taken as soon as possible.

The presented results confirm the data obtained by other authors regarding the significant decrease of the IgM level produced in response to *Legionella* infection in the elderly compared to the level determined in the younger adults (12). The similar level of immune response and IgM among the youngest and oldest patients was also demonstrated in the present study.

CONCLUSIONS

1. Demonstrated trend of faster the IgM level decline in children than in adults has important practical significance, because in every suspicious cases of Legionnaires' disease in children, there should be very carefully determined of serum samples collections, and the serum sample should be taken up to 4-5 weeks from the onset, and at intervals of 1-2 weeks maximum.
2. Sampling in the appropriate stage of the disease and at intervals along with taking into account the differences arising from the age of the patients, are necessary for proper assessment of the epidemic situation of Legionnaires' disease and legionellosis in Poland.

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