BREIF COMMUNICATION
Iran J Allergy Asthma Immunol
October 2018; 17(5):490-496.

Increased Total Serum Immunoglobulin E in Children Developing *Mycoplasma pneumoniae*-related Extra-pulmonary Diseases

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Received: 10 October 2017; Received in revised form: 14 December 2017; Accepted: 25 December 2017

ABSTRACT

Mycoplasma pneumoniae has been recognized to be involved in several extra-pulmonary diseases, but the underlying immunologic mechanisms are still largely unknown. Recently, we observed a significant elevation of serum Immunoglobulin E (IgE) in a small group of these children. Here, we assessed total serum IgE levels in children affected with *Mycoplasma pneumoniae*-related extra-pulmonary diseases.

We prospectively collected the data of 162 children admitted to the hospital (because of respiratory infections or extra-pulmonary diseases) who were evaluated for *Mycoplasma pneumoniae* serology and total serum IgE levels, concomitantly. Based upon clinical and serology aspects, 3 groups of children were identified: I) with non-mycoplasma respiratory disease; II) with mycoplasma-related respiratory diseases; III) with extra-pulmonary diseases related to concomitant/recent *Mycoplasma pneumoniae* infection. Interestingly, children with *Mycoplasma pneumoniae*-related extra-pulmonary diseases showed a significant elevation of total serum IgE.

In particular, patients developing *Mycoplasma pneumoniae*-related extra-pulmonary diseases (group III) showed significantly higher level of IgE than both previous groups ($p<0.001$ vs. group I; $p<0.01$ vs. group II).

In conclusion, hospitalized children diagnosed with *Mycoplasma pneumoniae*-related extra-pulmonary diseases resulted to have significantly increased serum IgE compared to children developing respiratory illnesses only.

Keywords: Children; Extra-pulmonary diseases; Immunoglobulin E; Mycoplasma pneumoniae; Post-infectious immune-mediated diseases

INTRODUCTION

Mycoplasma pneumoniae (*M. pneumoniae*) has emerged as a common respiratory pathogen in children in last decades. Indeed, *M. pneumoniae* is a well known cause of upper respiratory tract infections, bronchiolitis, tracheobronchitis, bronchitis and community-acquired pneumonia. Moreover,

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Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

M. pneumoniae respiratory infections have been associated to asthma exacerbations.[1]

Interestingly, *M. pneumoniae* infection has been related to the onset of several extra-respiratory diseases, too. Actually, non-respiratory disorders sometimes represent the most evident clinical manifestations of *M. pneumoniae* infection: indeed, it is often limited to the upper respiratory tract (leading to mild symptoms only) and, therefore, can remain completely unnoticed.[2]

Most clinical descriptions of extra-pulmonary manifestations due to *M. pneumoniae* are skin (urticarial rashes, multiform erythema, Stevens-Johnson syndrome, nodular erythema, etc.) and joints (reactive arthritis/arthralgia) diseases. However, several clinical disorders, affecting nervous, heart, gastro-intestinal, hematological systems, have been related to *M. pneumoniae* infection, as well.[3]

Unfortunately, despite the growing number of clinical reports describing *M. pneumonia*-related extra-pulmonary diseases, the pathogenesis is still elusive. Probably, several pathologic changes in the respiratory tract induced by *M. pneumoniae* are mediated by the innate and adaptive immune response, in addition to a direct pathogenic effect of this infectious agent. Similarly, extra-pulmonary diseases have been supposed to be triggered by *M. pneumoniae* through immune-mediated mechanisms in predisposed individuals, maybe because of their genetic background and/or concomitant environmental factors.[3-7]

Recently, by observing a small group of children diagnosed with different *M. pneumoniae*-related extra-pulmonary diseases, we noticed a common and simple immunological alteration, namely a significant elevation of total serum immunoglobulin E (IgE).[8]

Based upon this finding, we decided to analyze serum IgE levels in children admitted to the hospital because of clinical syndromes being consistent with *M. pneumoniae* infection, in order to confirm the aforementioned data in a larger cohort of patients.

MATERIALS AND METHODS

Patients and Study Design

Pediatric patients (aged 1 to 16 years) admitted to the Pediatric Department of Melegnano Hospital (Milano, Italy) in the period from June 1st 2015 and July 31st 2016, were collected prospectively, according to the presence of respiratory and extra-pulmonary diseases being potentially consistent with *M. pneumoniae* infection. Then, after completing the data collection, the consistent data were retrospectively analyzed, as explained later. The medical decision of admitting each patient to the hospital ward was exclusively based upon the clinical condition and was independent from the probability to find the involvement of *M. pneumoniae*. The present study is in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No identifying details of any participant have been reported anywhere in the manuscript and a privacy form obtained from the guardians, authorizing the use of clinical data for research purposes only. All the required serological analysis (including, total serum IgE measurement) was performed without any further diagnostic procedures in addition to all those needed to complete the usual diagnostic work-up.

As the aim of this analysis was studying the relationship between serum IgE levels, namely atopy, and different clinical forms of *M. pneumoniae* infections, total serum IgE measurement was evaluated and analyzed in all children receiving an evaluation of *M. pneumoniae* specific serology. Children with evidence of other concomitant infections (e.g. group A *Streptococcus pyogenes*, *Streptococcus pneumoniae*, Epstein-Barr Virus, Cytomegalovirus, Respiratory Syncytial Virus, etc.) were excluded from this study, as well as children affected with genetic or immunological/hematological diseases (e.g. cystic fibrosis, hypogammaglobulinemia, neoplastic and hematological diseases, etc.).

Serology

M. pneumoniae infection was diagnosed as being acute or recent by the detection of specific IgM anti-*M. pneumoniae*, which were measured by a chemiluminescent immunoassay (CLIA) performed through a Liason XL analyzer [DiaSorin S.p.a, Saluggia (VC), Italy].[9] A level of specific IgM>10 AU/mL was consistent with acute/recent *M. pneumoniae* infection. In children showing increased levels of specific IgM anti-*M. pneumoniae*, absent or low level of specific IgG were demonstrated. Total serum level of IgE was measured by an enzimatic immunoassay (Immunocap) and was expresses as UI/mL.

Statistics

Statistical analysis among three groups was performed through Kruskal-Wallis test; the comparison among pairs of selected groups was completed through Dunn's multiple comparison tests. The statistical analysis between two groups was performed through two-tailed Mann-Whithney test. The statistical elaboration was made by Prism software (GraphPad Software). Statistical variables in the text and in the Figures are expressed as average±standard error. A *p*-value of < 0.05 was considered as significant.

RESULTS

Prevalence of *M. Pneumoniae* Infections and Related Diseases

Among 162 children (aged 1 to 16 years) with respiratory or extra-respiratory symptoms, being potentially consistent with *M. pneumoniae* infection, 4 were excluded for the presence of a pre-existing comorbidity (cystic fibrosis [n=1], hypogammaglobulinemia [n=2], histiocytosis [n=1]). Moreover, 23 children were excluded because of the evidence of other concomitant infections, regardless of the positive or negative result of *M. pneumoniae* serology and the clinical picture (Group A *Streptococcus pyogenes* [n=7], *Streptococcus pneumoniae* [n=2], Epstein-Barr virus [n=8], Cytomegalovirus [n=1], Parvovirus B19 [n=1], Respiratory Syncytial Virus [n=2], Varicella-Zoster Virus [n=1], type 6 Human Herpes Virus [n=1]).

Thus, the remaining children (n=135, male: female [M:F]=64:71) were analyzed in three groups, after the completion of the data collection: I) children with respiratory disease showing negative serology for acute/recent *M. pneumoniae* infection (n=61, M:F=30:31); II) children with respiratory disease showing serology consistent with *M. pneumoniae* infection (n=56; M:F=32:24); III) children with extra-pulmonary disease associated to acute/recent *M. pneumoniae* infection (n=18, M:F=9:9). Unfortunately, the measurement of total serum IgE was not available for all recruited patients and, thus, the remaining number of each group was reduced, as follows: 43 children (M:F=20:23) in group I, 42 (M:F=23:19) in group II and 15 (M:F=8:7) in group III. Precisely, *M. pneumoniae*-related extra-pulmonary diseases included in the group III were: vasculitis-related urticarial rashes (n=4), multiform erythema (n=4), myocarditis/pericarditis (n=2), myositis (n=2) nephritis (n=1), reactive arthritis (n=1), meningitis (n=1).

Age difference according to the Etiology

The age of children admitted to the hospital for *M. pneumonia*-related respiratory infections resulted to be significantly different compared to children affected with respiratory infections caused by other pathogens (Group I: 32±3.3 months vs. Group II: 56.8±5.7 months, $p<0.01$). Patients developing *M. pneumoniae*-related extra-pulmonary diseases (Group III) had a mean age of 76.2 months (±11.3), but they were not significantly older than children affected with mycoplasma respiratory infections, as showed in Figure 1.

Only 11 children reported a previous diagnosis of environmental allergy (made by skin prick tests) at the admission: among those, 6 were in group I, 4 in group II and only 1 in group III. Moreover, also the presence of recurrent respiratory infections (RRI) was investigated at the admission: interestingly, 12 children with RRI were in group I and other 9 were in group II, but none was reported in group III.

Increased total Serum Immunoglobulin E in Children with *M. Pneumoniae*-related Extra-pulmonary Diseases

Serum IgE levels have been calculated and expressed as mean value ± standard deviation [M±SD] for each group of patients. There was no significant difference of total serum IgE between children with *M. pneumoniae* respiratory infections and those with airways infections caused by non-mycoplasma infectious agents (253.52±56.8 UI/mL vs. 164.37±48.3 UI/mL, respectively). On the contrary, patients developing *M. pneumoniae*-related extra-pulmonary diseases showed significantly higher level of IgE than both previous groups (402.15±94.7 UI/mL; $p<0.001$ vs. group I; $p<0.01$ vs. group II), as showed in Figure 2.

The difference of mean age between group II and group III (that was not significant statistically, anyway) may have an analytical effect on the difference of total serum IgE among the study groups. Therefore, we tried reducing the potential effect of patients' age on serum IgE levels, by comparing the absolute value of total serum IgE in each patient with the correspondent upper limit of reference values for age. In Figure 3, the relative increase of total serum IgE was expressed as percentage of the upper limit of the reference values

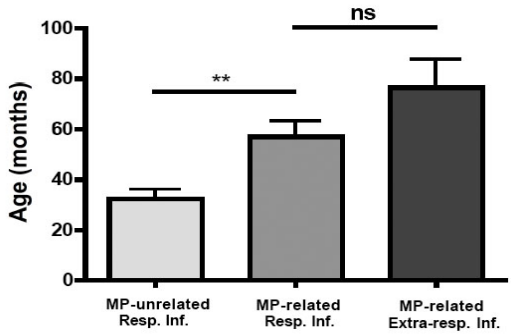

Figure 1. Age distribution of study groups (ns: not significant; **: $p<0.01$): *M. pneumoniae* -unrelated respiratory infections [first column]; MP-related respiratory infections [second column]; MP-related extra-respiratory infections [third column].

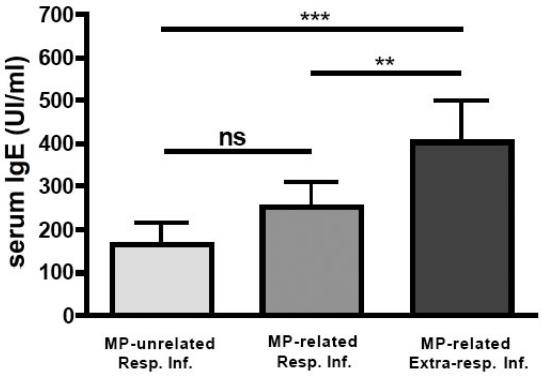

Figure 2 Total serum IgE levels (expressed as absolute value) in all study groups (ns: not significant; **: $p<0.01$; ***: $p<0.001$): *M. pneumoniae* (MP)-unrelated respiratory infections [first column]; MP-related respiratory infections [second column]; MP-related extra-respiratory infections [third column].

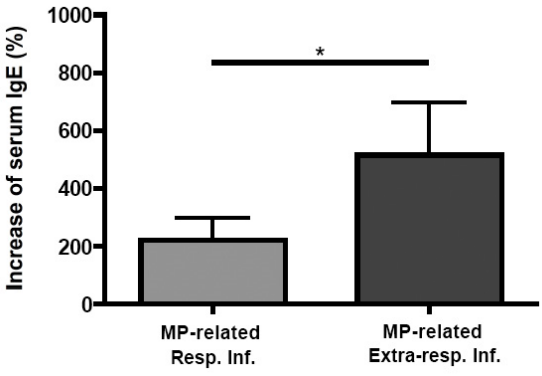

Figure 3. Relative increase of total serum IgE (expressed as percentage above the upper limit of the reference value for age) in children developing *Mycoplasma pneumoniae* (MP)-related diseases (*: $p<0.05$): *M. pneumoniae* (MP)-related respiratory infections [first column]; MP-related extra-respiratory infections [second column].

(single patient's IgE absolute value/upper limit of IgE reference value for age x 100). Based upon this calculation, group III showed a 516±261% (M±SD) increase of total serum IgE compared to normal value for age, which resulted to be significantly higher than group II (M±SD: 223±69%, $p<0.05$).

DISCUSSION

M. pneumoniae is primarily an extracellular pathogen, needing tight cell contact for survival. Although in vitro studies showed that some species, including *M. pneumoniae*, are able to penetrate cell membranes and invade culture cells, the cytotoxic effect can be simply mediated by its adherence to host cells, (through the release of enzymatic and lytic metabolites directly on the cell surface).[10-12]

However, the most relevant pathologic damages to humans are probably mediated by the host immune response, leading to the production of inflammatory mediators both in respiratory and extra-respiratory tissues, as suggested by several in vivo and in vitro studies. [13-16] Indeed, many interleukins (IL) have been found to be altered in serum of patients affected with *M. pneumoniae* infections. In particular, extra-pulmonary diseases have been supposed to be reactive disorders arising in immunologically predisposed individuals.[12,17]

Several studies analyzed the immunologic response in respiratory infections caused by *M. pneumoniae* and also its potential role in pediatric asthma.[18,19] *M. pneumoniae* has been showed to promote airway inflammation, by eliciting the production of several cytokines (such as interferon [IFN]-α, IL-1β, IL-2, IL-6 and tumor necrosis factor [TNF]-α) and suppressing others. Therefore, the balance among multiple cytokines during *M. pneumoniae* infection may play a significant role in the development of respiratory complications, such as severe pneumonia and/or asthma.[20-22]

The prevalent generation of Th2 immune responses and inflammation may promote pediatric asthma during *M. pneumoniae* infection.[23] Total and specific IgE responses have been described during *M. pneumoniae* respiratory infections, as well as in several viral and bacterial airways infections.[24,25] However, despite atopy is considered a risk factor to develop asthma in children, actually there is no evidence supporting a prominent specific IgE response during respiratory infections due to *M. pneumoniae* or in the complicated clinical forms.[26,27]

In this regard, several interesting findings emerged from our clinical study. First, *M. pneumoniae*-related and clinically relevant diseases resulted to affect older children than those developing respiratory infections caused by other infectious agents. That observation is in agreement with previous clinical data from the medical literature: although *M. pneumoniae* resulted to be a common respiratory pathogen in children <5 years, actually lower airways infections, pneumonia and complications were reported to be more frequent in school-aged children (from 5 to 15 years of age).[28,29] Recently, a study by Sun H et al. supported the concept that younger infants with *M. pneumoniae* infection usually have a milder clinical course than older children.[30] Indeed, in our case series, the median age of children with *M. pneumoniae*-unrelated respiratory infections was <3 years and, thus, significantly younger than the age of children requiring hospital admission for relevant or complicated respiratory infections due to *M. pneumoniae*. Moreover, such a result was valid for both pulmonary and extra-pulmonary diseases caused by *M. pneumoniae.* According to these findings, we might suppose that the age of clinically relevant *M. pneumoniae* diseases is older because previous contacts with this agent might be required, providing a kind of immunological imprinting to predisposed patients.

Probably, the main result of this study is that hospitalized children diagnosed with different *M. pneumoniae*-related extra-pulmonary diseases showed significantly increased serum IgE, compared to children developing respiratory illnesses only.

As mentioned above, a recent study found significantly higher circulating levels of several cytokines (such IL-6, IL-10 and IFN-γ), IgA, and percentages of $CD8^+$ cells, in patients with refractory *M. pneumoniae*-related pulmonary infections.[31] Moreover, some studies indicated that several cytokines, including IL-8, IL-9, IL-10, IL-17, IL-18 and IFN-γ, could modulate pulmonary lesions during *M. pneumoniae* respiratory infections.[16,22]

On the contrary, pathological and immunologic mechanisms of extra-pulmonary diseases triggered by this infectious agent are still unveiled and our findings might be a starting point to improve this knowledge.

Very recently, we described a case series of 5

children with different extra-respiratory complications of *M. pneumoniae* infection. Interestingly, all showed an elevation of total serum IgE, as a common immunologic feature.[8]

In this study, we confirmed this observation in a wider cohort of children, as in the group with extra-pulmonary diseases total serum IgE levels resulted to be significantly higher than in children with classical respiratory infections due to *M. pneumoniae*. Moreover, such a difference was maintained also by evaluating this biological parameter in a relative manner, namely as a percentage increase compared to the upper reference limit specific for age.

Of course, our study has several limitations due to the loss of some statistical units, the limited number and the absence of randomization, as being an observational study. However, it might prompt further experimental validation in experimental and more controlled study settings.

In conclusion, we might speculate that atopy might be a biological marker of predisposition to develop extra-pulmonary complications during *M. pneumoniae* infection. According to some recent observations, atopy may be related to some potential and concomitant immune mechanisms in autoimmune diseases, such as basophil-dependent (self-reactive) IgE and IL-17 production.[32-34]

Probably, IgE plays no direct role in the pathogenesis of these clinical manifestations, but the immune imprinting to produce IgE (and, particularly, the cellular processes and cytokine environment underlying the individual atopic constitution), might trigger a number of different mechanisms, leading to the heterogeneous spectrum of clinical disorders described in association to *M. pneumoniae* infection.

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