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ORIGINAL ARTICLE

Retrospective Study

Can the detection of IgA anti-*Mycoplasma pneumoniae* added to IgM increase diagnostic accuracy in patients with infections of the lower respiratory airways?

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Abstract

AIM

To evaluate the increase in diagnostic yield, by using IgA in addition to IgM, instead of IgM alone, in relation to the age of the patients.

METHODS

The study considered 1067 blood samples from patients with clinical signs of lower respiratory tract infections, tested for anti-Mycoplasma IgG, IgM and IgA antibody.

RESULTS

The increase in diagnostic yield with IgA, compared to IgM detection alone was of 3.5% with statistically significant differences between age groups (0.8% for those equal/under 50 years of age and 4.3% for those over 50).

CONCLUSION

Our findings demonstrate that IgA detection lead to a twofold increase in the number of diagnoses among the older age groups, but it did not result in relevant increase among the younger age groups.

Key words: Community-acquired infections; Diagnostic yield; Elderly patients; IgA; Mycoplasma pneumoniae

Core tip: Diagnosis of Mycoplasma pneumoniae infection relies on IgM detection but also IgA can be searched. There are few data on the range of increase of diagnosis adding the search for the IgA. Detection of IgA (without IgM) increases diagnosis of 3.5% compared to the detection of IgM alone. The greater increase is for the patients older than 50 years. Detection of IgA antibodies could be included in laboratory routine only in older patients.

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INTRODUCTION

Mycoplasma pneumoniae (*M. pneumoniae*) is one of the main causative agents for community-acquired infections of the lower and upper respiratory tract, especially during the first two decades of life[1-4]. Because symptoms can be commonly confused with those caused by other pathogens, diagnosis must rely on specific tests such as immunological assays[4-7]. Specific IgMs rapidly increase after the onset of the disease, reaching peak levels between 1-4 wk and then disappearing within a few months[8,9]. Compared to IgGs, which increase at slower rates and persist longer at high levels in the serum, the detection of IgM allows to diagnose acute infection[10-12].

However, while IgM values markedly increase in children and young patients[8,13-16] adult and elderly patients who might have repeatedly been exposed to the infection may have a less vigorous immune response, or no response at all[2,8,9,17-20]. In the event of reinfection, IgMs are produced less frequently and negative assay findings cannot exclude an ongoing infection especially in patients above the age of 45[14,18,21].

Because IgAs develop in a more predictable way and a more rapid rate compared to IgMs, and rapidly decrease during the second month from onset of the disease, these antibodies are considered reliable markers of infection[9,14,22]. Indeed, some authors emphasize that IgA detection can reveal to be useful in diagnosing infections[23,24] especially in IgM negative patients[9,22]. So far, different tests for IgA have been marketed and used in the laboratory routine[25], however the impact of the introduction of these tests in terms of increase in laboratory diagnosis accuracy must be fully reevaluated.

The aim of the present study was to assess the usefulness of IgA in confirming suspicion of infections by *M. pneumoniae* and the increase in diagnostic yield using IgA in addition to IgM - compared to IgM alone - in both younger and older patient groups.

MATERIALS AND METHODS

The present study was performed at the Microbiology Unit, Hospital of Legnano, which serves both patients hospitalized in the specialist medical and surgical departments, well as patients from the out-clinic. Between January 2012 and December 2014, 1067 samples collected from as many consecutive patients (49 out-patients and 1018 in-patients: 622 males and 445 females; mean age: 62.9 years, range 0.5-100) with clinical manifestations of infections of the lower respiratory airways who had been requested either by the hospital staff or by a GPs specifically for the search of anti-Mycoplasma IgG, IgM and IgA antibodies.

The IgG and IgM detection was performed by means of chemiluminescent assay (LIAISON Mycoplasma pneumoniae IgG and IgM; DiaSorin, Saluggia, Italy), whereas IgA detection was performed by immunoenzymatic assay (SeroMP recombinant IgA; Savyon Diagnostics Ltd, Ashdod, Israel). The tests were performed according to the manufacturers' instruction and the cut off for the three tests is 10 as index value (that is expressed for IgG and IgA as AU/mL or Arbitrary Units/mL and only as index value for IgM).

Statistical analysis

The data were statistically analyzed using the Fisher's exact test and linear regression method by SPSS software (Version 16.0, SPSS Inc. Chicago, IL).

RESULTS

The immunological assays of the 1067 samples yielded 178 (16.7%, 95%CI: 14.46-18.94) IgG positive, 66 (6.2%; 95%CI: 4.75-7.65) IgM positive and 50 (4.7%; 95%CI: 3.43-5.97) IgA positive with no statistical differences between out-patients and in-patients (P = 0.845 for IgG; 0.763 for IgM and 0.724 for IgA). Table 1 shows complete antibody profiles. Specifically, 53 individuals (groups C + E) (5.0%; 95%CI: 3.69-6.31) resulted positive for IgM but not for IgA, 37 (groups D + F) (3.5%; 95%CI: 2.40-4.60) IgA without IgM and 13 (groups G + H) (1.2%; 95%CI: 0.55-1.85) positive for both IgM and IgA. Overall, 103 subjects (groups C + D + E + F + G + H) (9.7%; 95%CI: 7.92-11.48) presented IgM and/or IgA antibodies.

The increase of diagnostic yield achieved by adding IgA investigation to IgM, compared to considering IgM alone resulted to be 3.5% (95%CI: 2.40-4.60).

Table 2 lists the positivity for antibodies classes for age groups and Table 3 shows the percentage increase of diagnosis (for each age group) adding IgA to IgM compared to cases that could be diagnosed considering IgM alone (with or without IgG). The data were analyzed by linear regression method, which pointed out a

Table 1 Serological profile for anti-*Mycoplasma pneumoniae* antibodies in patients with of lower respiratory airway infections

	Anti-*Mycoplasma pneumoniae* antibodies profiles			Patients	
Group	IgG	IgM	IgA	*n* (%)	95%CI
A	Negative	Negative	Negative	821 (76.9%)	74.37-79.43
B	Positive	Negative	Negative	143 (13.4%)	11.36-15.44
C	Negative	Positive	Negative	40 (3.7%)	2.57-4.83
D	Negative	Negative	Positive	21 (2.0%)	1.16-2.84
E	Positive	Positive	Negative	13 (1.2%)	0.55-1.85
F	Positive	Negative	Positive	16 (1.5%)	0.77-2.23
G	Negative	Positive	Positive	7 (0.7%)	0.20-1.20
H	Positive	Positive	Positive	6 (0.6%)	0.14-1.06

Table 2 Presence of serological markers for anti-*Mycoplasma pneumoniae* antibodies, divided per age group in patients with infections of the lower airway tract

	Age (yr)								
Antibodies	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	> 80
n	91	30	25	48	55	91	155	289	283
IgG	12	7	6	13	10	21	29	42	38
Positive	(13.2%)	(23.3%)	(24.0%)	(27.1%)	(18.2%)	(23.1%)	(18.7%)	(14.5%)	(13.4%)
95%CI	6.25-20.15	8.17-38.43	7.26-40.74	14.53-39.67	8.00-28.40	14.44-31.76	12.56-24.84	10.44-18.56	9.43-17.37
IgM	22	8	3	7	2	5	5	6	8
Positive	(24.2%)	(26.7%)	(12.0%)	(14.6%)	(3.6%)	(5.5%)	(3.2%)	(2.1%)	(2.8%)
95%CI	15.40-33.00	10.87-42.53	0.00-24.74	4.61-24.59	0.00-8.52	0.82-10.18	0.43-5.97	0.45-3.75	0.88-4.72
IgA	6	3	1	2	0	5	5	14	14
Positive	(6.6%)	(10.0%)	(4.0%)	(4.2%)	(0%)	(5.5%)	(3.2%)	(4.8%)	(4.9%)
95%CI	1.50-11.70	0.00-20.74	0.00-11.68	0.00-9.87	0.00-0.00	0.82-10.18	0.43-5.97	2.34-7.26	2.38-7.41
IgM and/or IgA	23	8	3	8	2	9	10	18	22
Positive	(25.3%)	(26.7%)	(12.0%)	(16.7%)	(3.6%)	(9.9%)	(6.5%)	(6.2%)	(7.8%)
95%CI	16.37-34.23	10.87-42.53	0.00-24.74	6.15-27.25	0.00-8.52	3.76-16.04	2.62-10.38	3.42-8.98	4.68-1.92

Table 3 Increase of diagnosis divided per age group adding search of IgA to IgM, compared to cases that can be diagnosed considering IgM alone

	Age (yr)								
Antibodies	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	> 80
n	91	30	25	48	55	91	155	289	283
IgM without IgA	17 (18.7%)	5 (16.7%)	2 (8.0%)	6 (12.5%)	2 (3.6%)	4 (4.4%)	5 (3.2%)	4 (1.4%)	8 (2.8%)
95%CI	10.69-26.71	3.35-30.05	0.00-18.63	3.14-21.86	0.00-8.52	0.19-8.61	0.43-5.97	0.05-2.75	0.88-4.72
IgA without IgM	1 (1.1%)	0 (0%)	0 (0%)	1 (2.1%)	0 (0%)	4 (4.4%)	5 (3.2%)	12 (4.1%)	14 (4.9%)
95%CI	0.00-3.24	0.00-0.00	0.00-0.00	0.00-6.16	0.00-0.00	0.19-8.61	0.43-5.97	1.81-6.39	2.38-7.41
IgM plus IgA	5 (5.5%)	3 (10.0%)	1 (4.0%)	1 (2.1%)	0 (0%)	1 (1.1%)	0 (0%)	2 (0.7%)	0 (0%)
95%CI	0.82-10.18	0.00-20.74	0.00-11.68	0.00-6.16	0.00-0.00	0.00-3.24	0.00-0.00	0.00-1.66	0.00-0.00
Increase of diagnosis	1.1%	0%	0%	2.1%	0%	4.4%	3.2%	4.1%	4.9%
95%CI	0.00-3.24	0.00-0.00	0.00-0.00	0.00-6.16	0.00-0.00	0.19-8.61	0.43-5.97	1.81-6.39	2.38-7.41

significant correlation with the patient age for the IgA (P = 0.048) and, stratified subjects according to two age groups: up to 50 years of age and > 50 years of age; a statistical difference was found (P = 0.035). Stratifying by the age groups (below/equal and above 50 years of age), a diagnostic increase of 0.8% was observed for individuals under/equal 50 years (95%CI: 0.00-1.91), and of 4.3% in those over 50 years of age (95%CI: 2.91-5.69) (P = 0.0052).

DISCUSSION

Overall, our results show that 9.7% of patients presenting lower respiratory airway infections were actually infected by *M. pneumoniae*, in agreement with data from literature, documenting it as the causative agent in 5%-30% of cases of community acquired pneumonia[18,26-29]. Our study revealed a higher percentage of infection in younger patients under the age of twenty, among which

Mycoplasma was associated to approximately one fifth of the overall infections.

Based on IgA screening, the detection of these antibodies (without IgM) led to a broad diagnostic increase of 3.5% compared to the detection of IgM alone. However, the greater increase was for the "over 50" group. Indeed, as suggested in literature, older patients may not produce IgM during infection by *M. pneumoniae*[8,13,16-21]; hence its inconsistent absence in this category of patients is a well-acknowledged limitation to *Mycoplasma* serology. In this setting, IgAs appear to be so far the only way to detect infection by this agent. Yet, the presence or absence of specific IgM in presence of specific IgA levels allows to differentiate between primary infection and reinfection, therefore, the estimation of both IgM and IgA is necessary for the maximal detection of an ongoing *M. pneumoniae* infection. Moreover, specific IgG levels in our patient population remained elevated for many weeks and were not useful from a diagnostic point of view.

In general, IgA were detected across all age groups; while these were associated to IgM in the younger age groups, this finding did not translate into an increase in diagnostic yield for such age groups. Nevertheless, IgA doubled the number of diagnoses in absolute values among the older age groups, suggesting that the search for IgA could be helpful whenever more sophisticated techniques, such as those of molecular biology, are not available.

Some authors have indicated the DNA detection by PCR as the gold standard for diagnosis of acute Mycoplasma infection[8,30-33], but other authors have emphasized the limits and have stressed the heterogeneity in sensitivity, the variability of results with regard to the time of collection (detection more frequent in early infection, less frequent during later stages of the disease) and positivity even in some healthy subjects[7,14,16,18,34,35]. Accordingly, the Authors suggest that serology should be combined with PCR, rather than be replaced by it[7,14,18]. Such observation is even more relevant considering that the molecular biology techniques may not be always available in some hospitals especially in countries and regions with limited resources[7].

In conclusion, IgA detection has demonstrated to be useful and reliable in confirming diagnoses of suspected *M. pneumoniae* infections in older patients, yielding higher diagnostic accuracy as compared to detection of IgM alone. This suggests detection of IgA antibodies could be included in laboratory routine in older patients showing clinical signs of lower respiratory tract infections.

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COMMENTS

Background

Mycoplasma pneumoniae (*M. pneumonia*) is one of the main causative agents for community-acquired infections of the lower and upper respiratory tract, especially during the first two decades of life. Because symptoms can be commonly confused with those caused by other pathogens, diagnosis must rely on specific tests such as immunological assays. Diagnosis of *M. pneumoniae* infection relies on detection of anti-Mycoplasma IgM; yet, while IgM values markedly increase in children and young patients, the immune response in adult and elderly patients who might have repeatedly been exposed to the infection may be less vigorous or even absent. Because IgAs develop in a more predictable way and at a more rapid rate compared to IgMs, the search of these antibodies in addition to IgM might add to diagnostic accuracy. However, so far, there is not enough evidence to support IgA as a reliable marker for infection, nor on the impact of its introduction in terms of increase in laboratory diagnosis accuracy so far has not been sufficiently evaluated.

Research frontiers

In the area of laboratory diagnosis, there is much interest in obtaining a higher diagnostic yield when there is a suspicion of infections by *M. pneumoniae*. Currently diagnoses are based on determination of IgMs alone for all age groups, and do not foresee routine determination of IgA in addition to IgM.

Innovations and Breakthroughs

Different tests for IgA have been marketed and used in the laboratory routine, but the impact of the introduction of these tests in terms of increase in laboratory diagnosis accuracy has not been sufficiently evaluated. In the present study, the authors evaluated the increase in diagnostic yield and the utility of this test in relation to the age of the patients.

Applications

IgA is a significantly useful marker in patient age groups > 50 years of age, increasing up to twofold the number of positive diagnoses.

Terminology

Specific IgM anti-Mycoplasma rapidly increase after the onset of the disease, reaching peak levels between 1-4 wk and then disappearing within a few months. Specific IgGs increase at slower rates and persist longer at high levels in the serum. The specific IgAs develop in a more predictable way and a more rapid rate compared to IgMs, and rapidly decrease during the second month from onset of the disease.

Peer-review

It is an interesting paper showing that IgA mycoplasma antibodies contribute to diagnostic yield increase, compared with usage of only IgM mycoplasma antibodies in older patients > 50 years old.

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