

Association between ImmunoCard *Mycoplasma* test and particle agglutination (PA) method in *Mycoplasma pneumonia* diagnosis

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ABSTRACT We examined the association between ImmunoCard *Mycoplasma* test and particle agglutination (PA) method in *Mycoplasma pneumonia* diagnosis. Subjects were 105 pneumonia patients who were positive for ImmunoCard *Mycoplasma* test at initial consultation and followed up by PA method using paired sera. The coincidence rates of positive cases of ImmunoCard *Mycoplasma* test and positive cases of PA method were examined by generation. The results showed that the coincidence rate was 87.5% in aged less than 19 years, 48.8% in aged 20-39 years, 36.4% in aged 40–59 years, 21.1% in aged 60–79 years, and 25.0% in aged 80 or greater, for a total of 44.8% (47 of 105 patients). The results suggested that a positive result for ImmunoCard *Mycoplasma* test may be due to acute infection in patients aged 19 years or less; however, 50% or more of patients aged 20 years or greater were false positive, which may reflect the presence of past infection.

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Key words : *Mycoplasma pneumonia*, ImmunoCard *Mycoplasma* test, Particle agglutination method

INTRODUCTION

Mycoplasma pneumonia is diagnosed when particle agglutination (PA) or complement fixation (CF) tests show a 4-fold or greater increase in antibody titer by paired sera at the time of onset and recovery. ImmunoCard *Mycoplasma* test detects anti-*Mycoplasma* antibody (IgM) in serum using enzyme immunoassay and has been employed extensively in the clinical setting for rapid test without paired sera^{1,2)}. However, doubt has been raised due to the number of false positives for

ImmunoCard *Mycoplasma* test. For this reason we examined the association between the ImmunoCard *Mycoplasma* test and PA method which is considered the established test for diagnosis.

SUBJECTS AND METHODS

Subjects

We examined 105 pneumonia patients who were positive for ImmunoCard *Mycoplasma* test at initial consultation and who were followed up by PA method using paired sera (interval:14.2 ± 2.1 days)

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at Kawasaki Hospital, Kawasaki Medical School during the 5 years from April 1, 2008 to March 31, 2013.

Methods

The coincidence rates between positive cases of ImmunoCard *Mycoplasma* test and positive cases of PA method were examined by generation (~19 years, 20–39 years, 40–59 years, 60–79 years, 80~years).

ImmunoCard *Mycoplasma* test kit from Meridian Diagnostics (Cincinnati, Ohio, USA) is used in this study.

A positive PA method result is defined as a 4-fold or greater increase in PA antibody titer by paired sera.

This study was performed with the approval of the Kawasaki Medical School ethical committee.

RESULTS (Table)

The results showed that coincidence rate was 87.5% in aged less than 19 years, 48.8% in aged 20–39 years, 36.4% in aged 40–59 years, 21.1% in aged 60–79 years, and 25.0% in aged 80 or greater, for a total of 44.8% (47 of 105 patients).

DISCUSSION

Published reports have suggested the existence of a number of false positives for ImmunoCard *Mycoplasma* test. These reports include our report showing that ImmunoCard *Mycoplasma* test positive pneumonia is more frequent in individuals aged 70 years or greater than it is in younger patients, and particularly frequent in patients aged 80 years

or greater³⁾, and Narita's report of 9 (36%) of 25 positive adult patients with healthy serum⁴⁾.

One explanation may be that positive ImmunoCard *Mycoplasma* test continue for an extended period of time, as suggested by Katayose *et al.*⁵⁾ in their report of a patient who was positive for ImmunoCard *Mycoplasma* test after 256 days. We also experienced a patient who showed positive for 72 weeks⁶⁾, and Narita experienced a patient who was positive at 527 days⁴⁾.

In this study, coincidence rate was 87.5% in patients aged 19 years or less. Narita *et al.*⁷⁾ also reported 13 patients (92.9%) showing positive PA method results among 14 patients aged 16 years or less who were positive for ImmunoCard *Mycoplasma* test. Fuse *et al.*⁸⁾ also reported all 5 patients aged 15 years or less positive for ImmunoCard *Mycoplasma* test were positive of PA method (100.0%). Therefore, false positive for ImmunoCard *Mycoplasma* test in younger people is considered to be lower.

However, this study revealed a coincidence rate of 48.8% in patients 20–39 years of age, 36.4% in patients 40–59 years of age, 21.1% in patients 60–79 years of age, and 25.0% in patients 80 years of age or greater, showing that the higher the age was, the lower the coincidence rate. Therefore, it is thought that ImmunoCard *Mycoplasma* test positive suggests acute infection in patients aged 19 years or less, while it frequently reflects past infection in patients aged 20 years or greater.

A study examining the association between ImmunoCard *Mycoplasma* test and CF method⁹⁾

Table Coincidence rate between positive cases of ImmunoCard *Mycoplasma* test and positive cases of PA method

| Age | Positive cases of IC | Positive cases of PA | Coincidence rate(%) |
|-------|----------------------|----------------------|---------------------|
| ~19 | 16 | 14 | 87.5 |
| 20~39 | 43 | 21 | 48.8 |
| 40~59 | 11 | 4 | 36.4 |
| 60~79 | 19 | 4 | 21.1 |
| 80~ | 16 | 4 | 25.0 |
| Total | 105 | 47 | 44.8 |

showed 37 patients (30.3%) positive by CF method among 122 children and adults positive for ImmunoCard *Mycoplasma* test, which is nearly the same as the results in this study: 47 patients (44.8%) with positive PA method results among 105 patients positive for ImmunoCard *Mycoplasma* test.

We doubted false negatives by PA method. According to a study¹⁰⁾ comparing the PCR method using sputum with the PA method in 339 children, the sensitivity at 640-titer or greater for the PA method in single serum was 50.0%, and sensitivity when a 4-fold or greater increase was observed in pair sera was 83.3%. This indicates that, even in children, the PA method returns a 50% false negative in single serum and a 16.7% false negative even in pair sera by PA method. False negatives in elderly patients by PA method may increase due to the fact that antibody production is weak. It is desirable to compare the PCR using sputum and PA methods in adult patients.

It has been argued that the method of differentiating between bacterial pneumonia and atypical pneumonia by the Japanese Respiratory Society scoring system is more useful than the examination for ImmunoCard *Mycoplasma* test¹¹⁾.

Hereafter, if anti-*Mycoplasma* antibody (IgM and IgG) in serum using ELISA method is applied in the clinical setting, the diagnosis of *Mycoplasma pneumoniae* may become ever easier.

As described above, a positive result for ImmunoCard *Mycoplasma* test indicates acute infection of *Mycoplasma pneumoniae* in patients aged 19 years or less, whereas a 50% or more rate of false positive can be expected in patients aged 20 years or greater, which may reflect past infection.

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