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Short Communication

Changes in Serum IgA Antibody Levels against the Glycopeptidolipid Core Antigen during Antibiotic Treatment of *Mycobacterium avium* Complex Lung Disease

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SUMMARY: We evaluated serial changes in the levels of serum immunoglobulin A (IgA) antibody to the glycopeptidolipid (GPL) core antigen during antibiotic treatment in 57 patients with *Mycobacterium avium* complex (MAC) lung disease at baseline (T0) and after 3 months (T3) and 6 months (T6) of treatment. The median patient age was 59 years, and 37 (65%) patients were women. The etiologic organisms included *M. avium* in 32 (56%) patients and *M. intracellulare* in 25 (44%) patients. Seven (12%) patients had the fibrocavitary form of the disease on computed tomography. After 12 months of treatment, 42 (74%) patients had a favorable response to treatment, whereas 15 (26%) patients had an unfavorable response to treatment defined as the absence of sputum culture conversion within 12 months of treatment. The initial median serum anti-GPL IgA levels in the 57 patients was 3.50 U/mL, and the antibody levels at T0 (median 3.50 U/mL), T3 (median 2.71 U/mL), and T6 (median 2.61 U/mL) were significantly decreased following treatment ($P < 0.001$). The results of the multivariate analysis indicated that an initially elevated anti-GPL IgA level (> 3.50 U/mL) was associated with an unfavorable response ($P = 0.049$). Our data suggest that elevated anti-GPL IgA levels may reflect disease activity and may help predict treatment response in patients with MAC lung disease.

The *Mycobacterium avium* complex (MAC) is the most common etiologic agent of lung disease caused by nontuberculous mycobacteria (NTM) (1,2). The current guidelines for managing MAC lung disease recommend long-term antibiotic therapy with a macrolide-based regimen (1). However, the prediction of treatment responses by physicians is often limited primarily because of the lack of evaluable parameters that reflect disease activity.

An enzyme immunoassay (EIA) was recently developed to detect serum immunoglobulin A (IgA) antibodies against the glycopeptidolipid (GPL) core antigen, an important epitope of the MAC cell wall (3), and studies indicate that this EIA is useful for diagnosing MAC lung disease (4-12). However, the changes in serum IgA antibody levels after treatment of MAC lung disease and the association between treatment response and initial IgA antibody levels remain largely unknown to date. Therefore, we evaluated serial changes in the levels of serum IgA antibody to the GPL core antigen during antibiotic treatment in patients with MAC lung disease and investigated whether initially elevated IgA antibody levels were associated with an unfavorable treatment response.

Our study was conducted between August 2011 and December 2014 at the Samsung Medical Center (a 1,979-bed referral hospital in Seoul, South Korea). The patients were enrolled in an institutional review board-approved, prospective, observational cohort study investigating NTM lung disease (ClinicalTrials.gov Identifier: NCT00970801). All patients signed an informed consent and met the diagnostic criteria for NTM lung disease (1).

Serum samples were collected from 57 patients with MAC lung disease treated with a macrolide-based regimen for at least 12 months; the regimen consisted of an oral macrolide (azithromycin or clarithromycin), rifampin, and ethambutol. The serum samples were obtained at the start of treatment (T0) and 3 months (T3) and 6 months (T6) after antibiotic treatment. Serum IgA antibody levels were measured in duplicate using an EIA kit (Tauns Laboratory Inc., Shizuoka, Japan), and serial changes in the antibody levels at T0, T3, and T6 were evaluated using the generalized estimating equation in SAS software version 9.4 (SAS Institute, Cary, NC, USA). Sputum examination for acid-fast bacilli (AFB) was performed at 1, 3, and 6 months after initiation of treatment, and serial changes in AFB culture positivity were evaluated using ordinal logistic regression analysis. The AFB cultures were classified according to the recommended criteria, as follows:

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trace, <50 colonies; 1+, 50–100 colonies; 2+, 100–200 colonies; 3+, 200–500 colonies; and 4+, >500 colonies (13). Sputum conversion was defined as 3 consecutive negative cultures, and a favorable response to treatment was defined as a negative sputum culture conversion at 12 months after treatment; an unfavorable response was defined as the absence of sputum culture conversion within 12 months of initiation of treatment (14,15). Multivariate logistic regression analysis was conducted to evaluate the factors associated with unfavorable response to treatment using PASW software version 18.0 (SPSS Inc., Chicago, IL, USA).

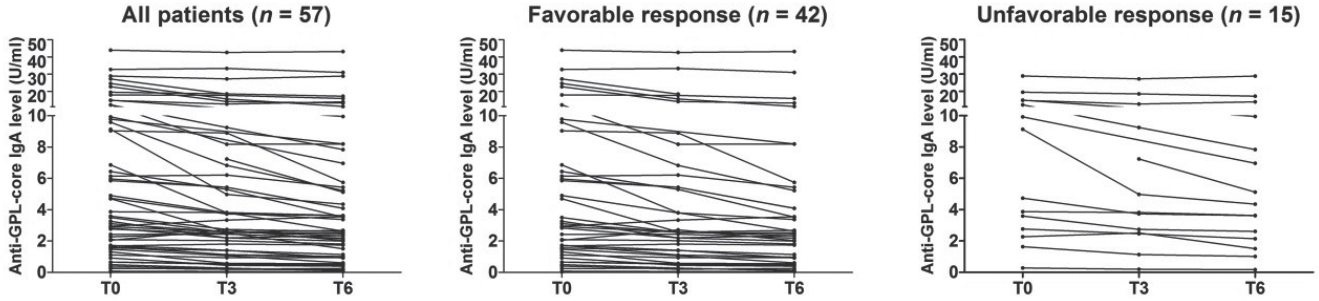
The median patient age was 59 years [interquartile range (IQR), 50–66 years]; 37 (65%) patients were women, and the median body mass index was 20.8 kg/m^2 (IQR, 19.2–22.7 kg/m^2). Twenty-five (44%) patients had a previous history of pulmonary tuberculosis, and 3 (5%) had diabetes mellitus. The etiologic agents of MAC lung disease included *M. avium* in 32 (56%) patients and *M. intracellulare* in 25 (44%) patients. Sputum AFB smears were positive in 30 (53%) patients. The results of chest radiography and high-resolution computed tomography, which were available for all patients, showed that 50 (88%) patients had the nodular bronchiectatic form of MAC lung disease and 7 (12%) had the fibrocavitary form. Cavitary lesions were found in all patients with the fibrocavitary form and in 11 (22%) patients with the nodular bronchiectatic form. After 12 months of antibiotic treatment, 42 (74%) patients achieved a favorable response to treatment, whereas 15 (26%) patients had an unfavorable response (Table 1).

The initial median serum anti-GPL IgA level in the 57 patients was 3.50 U/mL (IQR, 1.60–9.85 U/mL) (Fig. 1). Based on a recommended IgA cutoff value of 0.7 U/mL (4,5), 50 (88%) of these patients were seropositive for MAC lung disease and 7 (12%) were seronegative for this disease. The serum anti-GPL IgA levels at T0 (median 3.50 U/mL), T3 (median 2.71 U/mL), and T6 (median 2.61 U/mL) were significantly decreased in the 57 patients following antibiotic treatment ($P < 0.001$), and

Table 1. Baseline characteristics of study patients (n = 57)

Characteristic	Value
Age (year)	59 (50 - 66)
Gender (female)	37 (65)
Never-smoker	43 (75)
Body mass index (kg/m^2)	20.8 (19.2 - 22.7)
Previous history of pulmonary tuberculosis	25 (44)
Diabetes mellitus	3 (5)
Chronic heart disease	2 (4)
Chronic obstructive lung disease	1 (2)
Etiologic organism	
M. avium	32 (56)
M. intracellulare	25 (44)
Erythrocyte sedimentation rate (mm/h)	26 (17 - 40)
C-reactive protein (mg/dL)	0.14 (0.06 - 0.37)
Positive sputum AFB smear	30 (53)
Radiologic type	
Nodular bronchiectatic form	50 (88)
With cavity	11
Without cavity	39
Fibrocavitary form	7 (12)
Initial anti-GPL IgA antibody level (U/mL)	3.50 (1.60 - 9.85)

Data are presented as number (%) or median (interquartile range). AFB, acid-fast bacilli; GPL, glycopeptidolipid; IgA, immunoglobulin A.



patient	Serum anti-GPL IgA level(U/mL)[1]			*P*-value
	T0	T3	T6	
Favorable response (n = 42)	2.93 (1.26-7.40)	2.56 (0.85-6.37)	2.34 (0.59-5.27)	< 0.001
Unfavorable response (n = 15)	9.13 (2.76-14.76)	4.98 (2.51-10.77)	4.35 (2.14-9.96)	< 0.001
All patients (n = 57)	3.50 (1.60-9.85)	2.71 (1.12-8.54)	2.61 (0.96-6.35)	< 0.001
	AFB culture positivity[2]			
	T0	T3	T6	
4+	2 (4)	–	–	
3+	5 (9)	–	–	
2+	9 (16)	1 (2)	1 (2)	
1+	14 (24)	3 (5)	4 (7)	
Trace	27 (47)	6 (11)	5 (9)	
Negative	0 (0)	47 (82)	47 (82)	

Data are presented as median (interquartile range) or number (%). AFB, acid fast bacillus; GPL, glycopeptidolipid; IgA, immunoglobulin A; T0, at the start of treatment; T3, 3 months after treatment; T6, 6 months after treatment. [1] T0 versus T3 ($P < 0.001$), T3 versus T6 ($P < 0.001$), T0 versus T6 ($P < 0.001$) for IgA[1]; [2] T0 versus T3 ($P < 0.001$), T3 versus T6 ($P = 0.929$), T0 versus T6 ($P < 0.001$) for AFB culture positivity in all 57 patients.

Fig 1. Serial changes in anti-GPL IgA antibody levels and sputum AFB culture positivity during antibiotic treatment.

Table 2. Univariate and multivariable analysis of factors associated with unfavorable response

Variable	Univariate analysis		Multivariable analysis	
	OR (95% CI)	*P*-value	Adjusted OR (95% CI)	*P*-value
Age > 60 years (*n* = 26)	0.50 (0.15-1.71)	0.270	–	–
Male (*n* = 20)	1.33 (0.40-4.50)	0.643	–	–
Body mass index < 18.5 kg/m^2 (*n* = 11)	3.00 (0.76-11.90)	0.118	–	–
Mycobacterium intracellulare (*n* = 25)	2.44 (0.73-8.14)	0.148	–	–
Fibrocavitary form of MAC lung disease (*n* = 7)	4.73 (0.92-24.36)	0.063	–	–
Initial anti-GPL IgA antibody level (> 3.5 U/mL) (*n* = 29)	3.67 (1.01-13.42)	0.049	3.67 (1.01-13.42)	0.049

Data are presented as median (interquartile range). GPL, glycopeptidolipid; IgA, immunoglobulin A; OR, odds ratio; CI, confidence interval.

the differences in the IgA levels between measurement periods were also significant (all $P < 0.001$ for T0 versus T3, T3 versus T6, and T0 versus T6). For patients with either a favorable ($n = 42$) or unfavorable ($n = 15$) response, the serum anti-GPL IgA levels were significantly decreased during treatment (both $P < 0.001$). With regard to AFB culture positivity, 2 (4%) patients were scored at 4+, 5 (9%) patients were scored at 3+, 9 (16%) patients at 2+, and 14 (24%) patients at 1+ at the start of treatment, and the positivity tended to decrease during treatment, particularly at T0 versus T3 and T0 versus T6 (both $P < 0.001$). The median IgA levels in the nodular bronchiectatic form and fibrocavitary form were 3.2 U/mL (IQR 1.4–9.8 U/mL) and 1.6 U/mL (IQR 1.7–10.1 U/mL), respectively; however, there was no significant difference between the two groups ($P = 0.527$). Multivariate analysis was conducted using the median initial anti-GPL IgA level (3.50 U/mL) and previously known risk factors for treatment response, including age, sex, body mass index, etiologic organism, and fibrocavitary form, and our results indicated that only an initially elevated anti-GPL IgA level (> 3.50 U/mL) was significantly associated with an unfavorable treatment response ($P = 0.049$) (Table 2).

The most important findings of our study were that the IgA antibody levels against the GPL core antigen decreased during antibiotic treatment of patients with MAC lung disease and that this decrease was correlated with a decrease in AFB culture positivity, suggesting that the IgA antibody levels may reflect disease activity. Because AFB culture positivity may reflect disease burden in MAC lung disease (16), our results indicate the importance of IgA levels in monitoring treatment responses. In support of our results, several studies have consistently suggested the relevance of the relationship between anti-GPL IgA levels and disease activity in patients with MAC lung disease. In a study of 485 Japanese patients, anti-GPL IgA levels were higher in AFB smear-positive patients with MAC lung disease than in smear-negative patients (9), and the anti-GPL IgA levels in Taiwanese patients with high smear-positivity were higher than in those with low smear-positivity or a negative smear (8). Moreover, in a study conducted in the United States involving 100 patients with MAC lung disease, a significant positive correlation was observed between anti-GPL IgA levels and disease extent based on chest computed tomography (5). However, no correlation was found between anti-GPL IgA levels and the radiographic extent of the disease in other studies (7,17).

The results of the multivariate analysis indicated that higher anti-GPL IgA levels were associated with an unfavorable response to treatment. To date, several clinical, radiological, and microbiological factors have been found to be associated with unfavorable response to treatment of MAC lung disease, including old age, male sex, low body mass index, infection with *M. intracellulare* rather than *M. avium*, and presence of the fibrocavitary form (14,18). However, data on whether the initial levels of IgA antibody to the GPL core antigen are correlated with treatment response are limited. A previous study involving 34 patients with MAC lung disease did not find significant differences in the initial IgA levels between the culture conversion, recurrence, and non-conversion group (17). Several factors may explain the association between serum IgA levels and disease activity in patients with MAC lung disease, including an immune response to abundant antigens and/or the strong immune response of the host to even a small number of antigens. However, more studies are needed to address these issues.

In our study, 7 (12%) patients were seronegative for MAC lung disease based on a recommended IgA cutoff value of 0.7 U/mL. The fact that these seronegative patients met the standard criteria for MAC lung disease indicates limitations in the use of anti-GPL antibody for diagnosis and monitoring of the response to treatment of MAC lung disease.

In conclusion, the levels of serum IgA antibody to the GPL core antigen were decreased after antibiotic treatment, suggesting that IgA levels might reflect disease activity. Our findings may help to predict treatment response in patients with MAC lung disease.

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Conflict of interest None to declare.

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