ERJ Express. **Published on January 24, 2007 as doi: 10.1183/09031936.00061806**

Serological test and chest computed tomography findings in patients with MAC lung disease

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Short title: SEROLOGICAL TEST AND CCT FINDINGS

ABSTRACT: We previously reported the usefulness of a serodiagnostic test to detect serum glycopeptidolipid (GPL) core antibody in diagnosing *Mycobacterium avium* complex (MAC) lung disease in immunocompetent patients. The aim of the present study was to investigate correlations between the levels of antibody against GPL core and chest computed tomography (CCT) findings in patients with MAC lung disease.

Forty-seven patients with MAC-positive culture from their sputum and who had radiographic abnormality were investigated. 33 patients met the American Thoracic society criteria for MAC disease, 14 did not. All patients underwent both CCT examination and the serodiagnostic test for MAC at the same time.

Small nodular shadows were seen in all patients and bronchiectasis shadows were seen in 39 of them on CCT (83.0%). There was a significant positive correlation between the extent of the disease and the level of GPL core IgA antibody ($r=0.514$, $p<0.001$). The levels of GPL core IgA antibody were significantly elevated in patients who had nodular shadow (10-30 mm) compared to patients who had small nodular shadow (<10 mm)

These results document that the levels of IgA antibody against GPL core do correlate with the CCT findings of MAC lung disease.

Key words:
early stage
mycobacteria others than tuberculosis
enzyme immunoassay
glycopeptidolipid

INTRODUCTION

It has long been recognized that *Mycobacteria avium* complex (MAC) is an important pathogen causing chronic pulmonary infection in immunocompetent individuals (1) and that the incidence of the disease has been increasing recently in Japan (2) and other countries (1, 3). The diagnosis and management of MAC lung disease is therefore becoming a matter of increasing concern among respiratory physicians.

We previously reported the usefulness of a serological test for diagnosing MAC lung disease with a glycopeptidolipid (GPL) core antigen that was used for enzyme immunoassay (4). GPL core is a common structure of GPL which is a major cell surface antigen in MAC and which is not present in the cell wall of either *Mycobacterium tuberculosis* complex or *M.kansasii* (5, 6). We examined the usefulness of the GPL serodiagnostic test in immunocompetent patients with lung disease, and found that MAC lung disease could be clearly differentiated from colonization with MAC and from lung diseases caused by *M. tuberculosis* or *M. kansasii*. The sensitivity and specificity of the test for diagnosing MAC lung disease was 92.5% and 95.1%, respectively, for IgA. Combining this serodiagnostic test with the criteria advocated by the American Thoracic Society (ATS) for nontuberculous mycobacterial respiratory disease in 1997 (7), facilitate easier and more rapid definitive diagnosis of MAC lung disease.

Moreover, the levels of GPL core antibodies reflected disease activity, because they decreased in MAC patients responding to chemotherapy (4). However,

correlations between levels of GPL core antibody and radiographic findings have not been evaluated thus far. Therefore, here we assess the levels of GPL core antibody in relation to chest computed tomography (CCT) findings in patients with MAC culture-positive sputum whose radiographic findings were infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules.

MATERIAL AND METHODS

Study subjects

Forty-seven patients, who 1) had MAC-positive cultures from sputum, 2) had abnormal shadow that were infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules on their chest radiographs and 3) did not have predisposing lung disease, were enrolled at the NHO National Toneyama Hospital between September, 2001 and May, 2004. Patients were divided into two groups (the MAC disease group and the MAC culture-positive group) based on the guidelines advocated by the ATS (Table 1) (7). The individuals, who had a single and small amount of culture positive MAC but did not have clinical symptoms and had no abnormal lesions on CCT findings, were excluded from this study as a contamination of respiratory specimens. These cases did not have evidence of active disease.

Of 47 patients with MAC-positive cultures, 30 met the ATS criteria at enrollment. Patients who did not meet these criteria were followed up for 12 months with monthly radiographic and sputum examination with Ziehl-Neelsen stains and

cultures on Ogawa egg medium. Three patients met the criteria and 14 patients still had not over the 12 months' follow-up period after enrollments. Based on these observations, the subjects were divided into the MAC disease group, which was composed, of 33 patients met the ATS criteria, and the MAC culture-positive group, which was composed of 14 patients who did not. All patients underwent CCT examination and a serodiagnostic test at the same time. These took place when the diagnosis of MAC lung disease was made in the MAC disease group, or when the follow-up period ended in the MAC culture-positive group. The clinical data were collected on each patient at the time of CT, including sex, age, body mass index, smoking history, drinking history, complications, past history, and laboratory data including erythrocyte sedimentation rate (ESR), GPL core immunoglobulin (Ig) G, IgA and IgM antibody. We investigated whether there was a correlation between GPL core antibody level and CCT findings. Fifteen patients in the MAC disease group had previously received combination chemotherapy for mycobacterial diseases recommended by the ATS guideline before enrollment, but they had positive cultures of MAC at enrollment. All patients were seronegative for human immunodeficiency virus type 1 and type 2. Informed consent was obtained from all patients. This study was approved by the NHO National Toneyama Hospital institutional review board for experimentation on human subjects and complies with international guidelines for studies involving humans.

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CCT findings

All patients underwent conventional CT examination. CCT scans were obtained using a Toshiba Asteion TSZ-021A CT scanner (Toshiba, Tokyo, Japan). We categorized the CCT findings into small nodular shadow (<10mm), nodular shadow (10 - 30 mm), large nodular shadow (>30mm) or infiltrate, bronchiectasis, cavity and atelectasis. CCT findings were assessed by two individual respiratory physicians with consensus reading, without prior knowledge of the clinical or laboratory data. To assess the extent of disease, we divided the lung into 18 segments on conventional CCT according to the anatomical segment as follows: right upper lobe (RUL) apical segment, RUL posterior segment, RUL anterior segment, middle lobe (ML) lateral segment, ML medial segment, right lower lobe (RLL) superior segment, RLL medial basal segment, RLL anterior basal segment, RLL lateral basal segment, RLL posterior basal segment, left upper lobe (LUL) apicoposterior segment, LUL anterior segment, lingular superior segment, lingular inferior segment, left lower lobe (LLL) superior segment, LLL anterior medial basal segment, LLL lateral basal segment, and LLL posterior basal segment. The extent of the lesions was expressed as the numbers of involved CCT segments in which MAC lesions were present.

GPL core antibody

GPL core antibody was measured as described previously (4). Briefly, microtiter plates (Nunc Products, Roskilde, Denmark) were coated with 0.5 μg/well of GPL core of *M. avium* serotype 4, which had been prepared by the previously described method (4). Serum samples were diluted 40-fold with phosphate-buffered saline containing 1% bovine serum albumin. Diluted serum samples were added, followed by incubation for 1 hr at 37°C. Plates were washed, then peroxidase-conjugated F (ab') 2 of goat antibody against human IgG, IgA, or IgM (Sigma, St. Louis, MO) was added, and plates were incubated for 2 hr at 37°C. Unbound labeled antibody was removed by washing and the substrate, o-phenylenediamine dihydrochloride (Sigma), was added. Following color development, the optical densities (OD) of the wells on the plates were read for absorbance at 492 nm (model 550, BIO-Rad Laboratories, Tokyo, Japan).

Statistical analysis

All data were analyzed using the statistical analysis software package Stat View 5.0 (SAS Institute, Cary, NC). All values were means ± standard deviation. The Mann Whitney U test was used to compare the differences between groups. The chi-squared test was used to compare the difference of CCT findings between groups. Correlation coefficients were calculated using Spearman's rank method. Probability values < 0.05 were considered significant.

RESULTS

Clinical background and laboratory data

The clinical background and laboratory data are shown in Table 2. 33 patients met the ATS criteria (MAC disease group) and 14 patients did not (MAC culture-positive group). 46 of the patients were women, who tended to be thin; there was only one smoker, and none with alcohol abuse or severe systemic complications. The main symptoms were coughing (19 patients), sputum (21 patients), bloody sputum (9 patients), chest pain (4 patients), and dyspnea (3 patients). 40.4% of the subjects had past histories of major surgery that included myomectomy (7 patients), appendectomy (5), mastectomy (3), cholecystectomy (2), gastrectomy (2), and oophorectomy (1). There were no statistically significant differences in clinical characteristics between the two groups.

Levels of GPL core antibody

IgG, IgA, and IgM antibodies specific for GPL core antigen were measured, as shown in Fig 1. The levels of IgG against GPL core antigen were 0.219 ± 0.292 OD for MAC disease and 0.268 ± 0.372 OD for the MAC culture-positive group. These values for IgA were 0.547 ± 0.438 OD and 0.452 ± 0.345 OD, respectively, and for IgM 0.628 ± 0.362 OD and 0.535 ± 0.213 OD, respectively. Applying the cut-off value 0.064 OD for IgG, 0.072 OD for IgA, and 0.312 OD for IgM in our previous study (4), the positive rate was 66.7% for IgG, 81.8% for IgA, and 78.1% for IgM respectively in the MAC disease group, and 71.4% for IgG, 100% for IgA and 84.6% for IgM in the MAC culture-positive

group. There were no statistically significant differences between the MAC disease and MAC culture-positive groups for any Ig isotype.

CCT findings

CCT findings are summarized in Table 3. Abnormal CCT were also similar in the MAC disease and MAC culture-positive groups, except for findings related to large nodule or infiltrate, which were more frequent in the former ($p<0.05$). Small nodules <10 mm in diameter were seen in all patients. Analysis of the distribution of the lesions showed that MAC frequently involved the middle lobe lateral segment (33 of 47 patients, 74.5%), middle lobe medial segment (33 patients, 70.2%) and lingular inferior segment (30 patients, 63.8%). The mean numbers of involved segments in each finding were similar regardless of large nodules or infiltrate. The total numbers of involved segments were 6.7 ± 4.2 and 5.0 ± 4.3 in the MAC disease group and the MAC culture-positive group, respectively. From these results of clinical characteristics, serodiagnosis using GPL core antibody and CCT findings, it could be considered that the patients of the MAC culture-positive group had an active MAC lung disease.

Correlation between CCT findings and level of GPL core antibody

Table 4 showed the correlation coefficients between the numbers of involved CCT segments, representing the extent of disease, and the level of GPL core antibody in the MAC disease group and the MAC culture-positive group. There is a significant positive

correlation between the extent of disease and the level of GPL core IgA antibody in both groups (Fig. 2). Next, we compared the level of GPL core antibody to each CCT finding, including small nodular shadow (<10mm), nodular shadow (10-30mm), large nodular shadow (>30mm) or infiltrate, bronchiectasis and atelectasis. The levels of GPL core IgA antibody were significantly elevated in patients who had nodular lesion(s) (≥ 10 mm) compared to patients who had small nodular lesion(s) (< 10 mm) in both groups (Fig 3). There were no differences in GPL core antibody levels correlating with other findings. These results document that a higher level of GPL core IgA indicated a wider extent of MAC disease and larger nodule formation on CCT.

DISCUSSION

This is the first study to assess a correlation between GPL core antibody levels and radiographic findings. Forty-seven patients with MAC-positive culture from sputum and abnormal shadow on chest radiographs were examined. We found that the level of IgA antibody against GPL core antigen was associated with CCT finings: a higher level of GPL core IgA antibody indicated a wider extent of MAC disease and larger nodule formation on CCT. Obviously, in order to establish this new knowledge, further studies with a large number of patients are required because of low value of the correlation coefficients between extent of disease and levels of GPL core IgA ($r=0.514$) and a small number of study subjects.

The ATS criteria published in 1997, consisting of clinical, radiographic, and bacteriologic criteria, are the best guide to diagnosis and treatment of pulmonary disease caused by nontuberculous mycobacteria including MAC (7). All three elements are required for the diagnosis of MAC disease. The bacteriologic criterion requires multiple positive cultures for MAC, or a positive culture from a lung biopsy or histologically-proven lung biopsy positivity. We were unable to carry out lung biopsy or bronchial washings on all patients in the MAC culture-positive group, especially with minimal symptoms or elderly subjects, because we had not obtained their informed consent for the bronchoscopic examination. This procedure is invasive and expensive. In elderly patients, the diagnosis and treatment for MAC lung disease may not be so important in respect to the long-term survivals, as 50% survival of MAC lung disease

patients with progressive radiographic abnormalities was 175 months (8). So, we defined MAC culture-positive patients based on an observation with monthly radiographic and sputum examination for 12 months.

Most of patients in the MAC culture-positive group were elderly, non-smoking, thin women with no severe systemic complications; these clinical features are consistent with those of patients with MAC lung disease with nodular bronchiectasis (9). The combination of multiple small nodules on CCT with bronchiectasis, particularly in the middle lobe and /or lingual should suggest the diagnosis of MAC lung disease (10-12). In this study, the clinical background and laboratory findings including GPL core antibody were similar between the MAC disease group and the MAC culture-positive group. A GPL core IgA antibody was positive in all patients of the MAC culture-positive group. Moreover, all patients in the MAC culture -positive group had findings of small nodules on CCT. Some careful investigation of CCT and histological findings revealed that small nodular lesions were caused by granulomas, formed as a specific response to mycobacterial infection (13, 14). Furthermore, the individuals with MAC colonization, who had a single and small amount of culture positive MAC but did not have clinical symptoms or abnormal lesions on CCT findings, were excluded from this study at enrollment. We previously reported that GPL core antibody was not detectable in cases of MAC colonization (4). We considered from these results that the patients of the MAC culture-positive group had an active MAC lung disease. And it could be argued that patients are suffering from MAC lung disease, when they have a positive

respiratory culture for MAC, the chest radiographic findings of infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules and the positive results of GPL core antibody. When using GPL core antibody, it is not necessary to continue collecting respiratory specimens for acid-fast bacilli (AFB) analysis or to observe chest radiographs and/or CCT over a 12 month period of time.

In the MAC disease group, 8 patients had large nodular shadow (>30 mm) or infiltrate, whereas none in the MAC culture-positive group had these findings. Thus, the MAC culture-positive group might be considered to represent early stage MAC disease, because serial CCT scanning in MAC lung disease has shown that the development of infiltrate is preceded by the appearance of nodules (15). It might be recommended for patients in both groups to be administered immediate multi-drug chemotherapy and/or surgical therapy in the context of patients' general conditions and tolerances to the medication. However, 12 of 33 patients with MAC disease and 9 of 14 patients with MAC culture-positive did not undergo multi-drug chemotherapy including clarithromycin after this study. This was because most of these patients were more than 70 years old and/or didn't have substantial symptoms and/or advanced or progressive radiographic abnormalities. Furthermore, the cost of treatment for MAC lung disease is expensive and has not been covered by the healthcare insurance in Japan. MAC lung disease is also difficult to treat, and recurrence frequently occurs in MAC disease patients even after completing the multi-drug chemotherapy,including clarithromycin. We have also experienced many cases of recurrence, smear or culture

test being positive again, over the 12 months after sputum negative conversion during chemotherapy. This is because the radiographic active lesions, which are bronchiectasis or cavity, have usually remained at the time of sputum negative conversion(8). So, the rapid diagnosis and treatment are required at an early stage before the completion of bronchiectasis or cavity lesions.

The serodiagnostic test used here to detect serum GPL core antibodies could add useful information as a supplementary diagnostic aid (4, 16). We believe that this test may have future diagnostic applications. However, for including this serodiagnostic test in routine clinical practice, a study addressing the correlation between the antibody levels and radiographic findings was needed. Here we report such a study. The positive rates of the serological test were 71.4% for IgG, 100% for IgA and 84.6% for IgM in the MAC culture-positive group. If this serological test is combined with the ATS criteria, we might get a better sensitivity to diagnose MAC lung disease without lung biopsy.

The levels of GPL core antibody were similar in the MAC disease group and the MAC culture-positive group. Fifteen (45.5%) of the MAC disease patients had received combination chemotherapy recommended by the ATS guideline (7). It is possible that this might have affected their antibody levels. However, the effects of treatment might be limited, because they had positive culture of MAC at enrollment, which meant the chemotherapy was not successful in converting the culture result from positive to

negative at the time of serum sample collection. In our previous study, unsuccessful chemotherapy did not affect the level of GPL core antibody (4).

The level of IgA but not IgG or IgM GPL core antibody was significantly associated with the radiographic findings of the disease, but the reasons for this remain unclear. IgA is the predominant immunoglobulin isotype in mucosal tissue and is believed to be involved in the defense against viral and bacterial infection at this site. There are some published reports that are consistent with our findings. Rodriguez and colleagues reported that IgA may play an important role in protection against mycobacterial infection in the respiratory tract by blocking the pathogen's entrance and/or by modulation of pro-inflammatory responses (17). In our own previous study (4), we obtained the best serodiagnostic results on sensitivity and specificity for diagnosing MAC lung disease by measuring IgA. Moreover, Watanabe and colleagues reported that total serum IgA was significantly higher in patients with MAC compared to those with pulmonary tuberculosis (18). These reports indicate that IgA antibody might play an important role in the chronic inflammation of mucous membrane of the respiratory tract in patients with MAC lung disease. The role of GPL core IgA antibody in protection against MAC is not clear, and further studies are needed to address this question.

In summary, we have documented that the level of IgA GPL core antibody was significantly associated with radiographic findings. This finding should encourage the use of the serodiagnostic test for MAC lung disease in clinical practice

ACKNOWLEDGEMENT

The authors thank Ms Kyoko Maekura, Ms Yuko Yamamoto and Ms Masami Kobayashi for measurement of antibody levels.

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Figure Legends

Fig. 1 The distribution of serum levels of GPL core antibody in patients with MAC disease and in the MAC culture-positive group. Circles represent individual data. The mean of each group of values is indicated by a horizontal line. There was no statistically significant difference between the IgG, IgA and IgM groups.

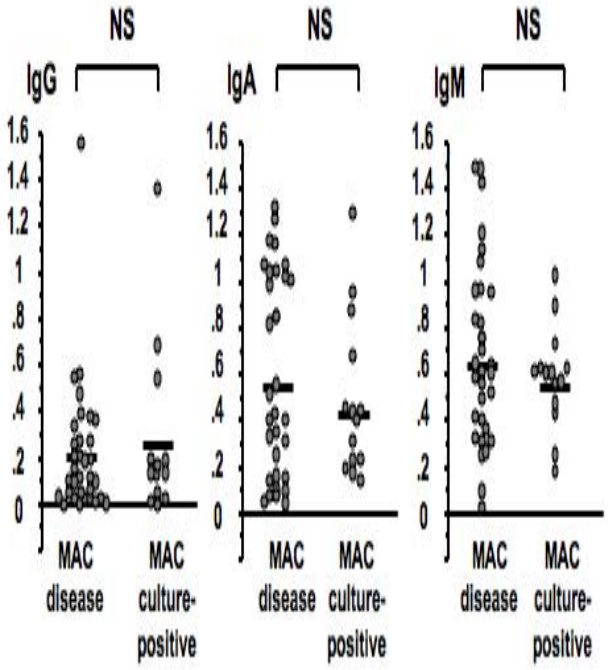

Fig. 2 Correlation between the levels of GPL core-specific IgA antibody and the number of involved CCT segments in patients with MAC lung disease (A) and in the MAC culture-positive group (B). Significant positive correlations were found between the level of GPL core IgA antibody and the number of involved CT segments in the

MAC disease group (r = 0.487, p < 0.01), in the MAC culture-positive group (r = 0.788, p < 0.05), and both of them (r = 0.514, p < 0.001).

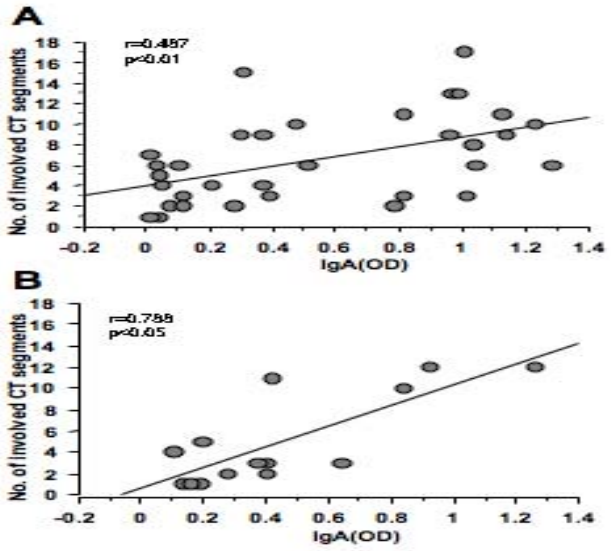

Fig. 3 Serum level of GPL core IgA antibody in patients who had nodular lesions ≥ 10 mm or < 10 mm in diameter assessed by CCT in the MAC disease group and in the MAC culture-positive group. The mean of each group is indicated by a horizontal bar. The levels of GPL core IgA antibody were significantly elevated in patients who had nodular lesions (≥ 10 mm) compared to patients who had small nodular lesions (< 10 mm) in both the MAC disease group (p < 0.05) and in the MAC culture-positive group (p < 0.05), and total of them (p < 0.001).

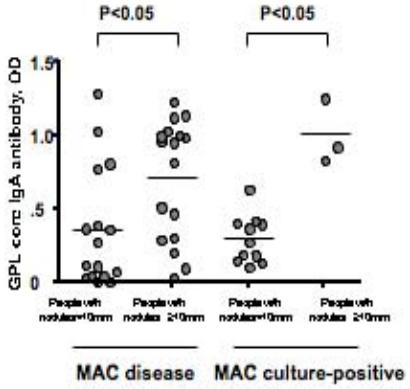
P<0.05
P<0.05
GPL core IgA antibody, OD
1.5
1.0
.5
0
MAC disease
MAC culture-positive

TABLE 1 Criteria for diagnosis of MAC lung disease

Criteria		
1.Clinical criteria	a	Compatible sings and symptoms (coughing, fatigue more common; weight loss, hemoptysis and shortness of breath may be present, particularly in advanced disease) with documented deterioration of the patient's clinical state if a base condition is present and
	b	Reasonable exclusion of other disease (e.g. tuberculosis, cancer, histoplasmosis) to explain condition, or adequate treatment of other condition with increasing sings/symptoms
2. Radiographic criteria	a	Any of the following chest X-ray abnormalities; if baseline films are more than 1 year old, should be evidence of progression
		Infiltrates with or without nodules (persistent ≧2 mo or progressive)
		Cavitation
		Nodules alone (multiple)
	b	Any of these HRCT abnormalities
		Multiple small nodules
		Multifocal bronchiectasis with or without small lung nodules
3. Bacteriologic criteria	a	At least three available sputum/bronchial wash samples within 1 year
		Three positive cultures with negative AFB smears
		or
		Two positive cultures and one positive AFB smear
		or
	b	Single available bronchial wash and inability to obtain sputum samples
		Positive culture with 2+, 3+ or 4+growth
		or
		Positive culture with a 2+, 3+, or 4+ AFB smear
		or
	c	Tissue biopsy
		Any growth bronchoplumonary tissue biopsy
		Granuloma and/or AFB on lung biopsy with one or more positive cultures from sputum/bronchial wash
		Any growth from usually sterile extrapulmonary site

For a diagnosis of pulmonary disease, all three criteria (1-3) must be satisfied.

AFB = acid-fast bacilli

TABLE 2 Clinical data of patients with MAC-positive cultures

	MAC disease	MAC culture-positive
n	33	14
Sex male/female	1/32	0/14
Age, mean year	65.3 ±10.6	71.4 ± 6.0
BMI, mean kg/m^2	19.2 ± 3.0	18.5 ± 2.2
Cigarette smoking	0	1
Alcohol abuse	0	0
Past history of major surgery	15 (45.5)	4 (28.5)
ESR, mean mm/hr	33.2 ± 24.7	26.7 ±17.3
MAC Species		
M. avium	21	12
M. intracellulare	9	2
Both	3	0

Data are presented as n (%) or mean ± SD, MAC = *Mycobacterium avium* complex; BMI = body mass index; ESR = erythrocyte sedimentation rate; CRP = C reactive protein

TABLE 3 Chest Computed Tomography findings

Abnormalities	MAC disease		MAC culture-positive	
	Number of patients with findings (%)	Number of involved segments (range)	Number of patients with findings (%)	Number of involved segments (range)
Small nodule (<10mm)	33 (100)	6.5 ±4.2 (1-17)	14 (100)	4.3± 3.7 (1-12)
Nodule (10-30mm)	17 (51.5)	1.2 ±1.3 (1-4)	3 (21.4)	0.7 ±1.5 (0-4)
Large nodule (>30 mm) or infiltrate[#]	8 (24.2)	0.5± 1.3 (0-5)	0 (0)	0
Bronchiectasis	29 (87.9)	3.1 ±2.5 (0-14)	10 (71.4)	2.7± 3.1(0-10)
Cavity	4 (12.1)	0.3± 1.1 (0-6)	2 (14.3)	0.2 ±0.6 (0-2)
Atelectasis	13 (39.4)	0.5 ±0.7 (0-2)	3 (21.4)	0.2± 0.4 (0-1)
Others	4 (12.1)	0.2 ±0.6 (0-3)	0 (0)	0
Total	33 (100)	6.7±4.2 (1-17)	14 (100)	5.0 ±4.3 (1-12)

Data are presented as n (%), *MAC* = *Mycobacterium avium* complex

[#] Significant difference ($p<0.05$)

TABLE 4 Correlation coefficients between numbers of involved CCT segments versus GPL core antibody level

	Correlation coefficients		
	Total	MAC disease	MAC culture-positive
GPL core IgG antibody, OD	0.150	0.070	0.268
GPL core IgA antibody, OD	0.514[#]	0.487[¶]	0.788[†]
GPL core IgM antibody, OD	0.217	0.306	0.153

[#]P < 0.001 [¶] p < 0.01 [†]p < 0.05

CCT, chest computed tomography; MAC, *Mycobacterium avium* complex; GPL, glycopeptidolipid; OD, optical densities