Eur Respir J 2007; 29: 1217–1223
DOI: 10.1183/09031936.00061806

Serological test and chest computed tomography findings in patients with *Mycobacterium avium* complex lung disease

S. Kitada*, Y. Nishiuchi#, T. Hiraga*, N. Naka*, H. Hashimoto*, K. Yoshimura*, K. Miki*, M. Miki*, M. Motone*, T. Fujikawa*, K. Kobayashi¶, I. Yano+ and R. Maekura*

ABSTRACT: The present authors have 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.

A total of 47 patients with MAC-positive culture from their sputum and who had radiographic abnormalities were investigated. Thirty-three 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 on CCT in all 47 patients and bronchiectasis shadows were seen in 39 (83%) of them. There was a significant positive correlation between the extent of the disease and the level of GPL core immunoglobulin (Ig)A antibody. The levels of GPL core IgA antibody were significantly elevated in patients who had nodular shadows (10–30 mm) compared with patients who had small nodular shadows (<10 mm).

The present results document that the levels of immunoglobulin A antibody against glycopeptidolipid core correlate with the chest computed tomography findings of *Mycobacterium avium* complex lung disease.

KEYWORDS: Early stage, enzyme immunoassay, glycopeptidolipid, mycobacteria

It has long been recognised that *Mycobacterium avium* complex (MAC) is an important pathogen causing chronic pulmonary infection in immunocompetent individuals [1] and that the incidence of the disease has increased 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.

The present authors previously reported the usefulness of a serological test for diagnosis of MAC lung disease with a glycopeptidolipid (GPL) core antigen that was used for enzyme immunoassay [4]. The GPL core is a common structure of the GPL antigen, which is a major cell surface antigen in MAC and which is not present in the cell wall of either *M. tuberculosis* complex or *M. kansasii* [5, 6]. The present authors 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 colonisation with MAC and from lung diseases caused by either *M. tuberculosis* or *M. kansasii*. The sensitivity and specificity of the test for diagnosing MAC lung disease were 92.5 and 95.1%, respectively, for immunoglobulin (Ig)A. Combining this serodiagnostic test with the criteria advocated by the American Thoracic Society (ATS) for nontuberculous mycobacterial respiratory disease in 1997 [7] facilitated 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, the present authors assess herein the levels of GPL core antibody in relation to chest computed tomography (CCT)

AFFILIATIONS
*Dept of Internal Medicine, National Hospital Organization Toneyama National Hospital, and
#Toneyama Institute for Tuberculosis Research, Osaka,
¶Dept of Immunology, National Institute of Infectious Diseases, and
+Japan BCG Laboratory, Tokyo, Japan.

CORRESPONDENCE
S. Kitada
Dept of Internal Medicine
National Hospital Organization
National Toneyama Hospital
5-1-1 Toneyama
Toyonaka-shi
Osaka 560-8552
Japan
Fax: 81 668533127
E-mail: kitadas@toneyama.hosp.go.jp

Received:
May 09 2006
Accepted after revision:
January 09 2007

STATEMENT OF INTEREST
None declared.

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

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

A total of 47 patients were enrolled at the National Hospital Organization (NHO) National Toneyama Hospital (Osaka, Japan) between September 2001 and May 2004. They fulfilled the following criteria: 1) MAC-positive cultures from sputum; 2) abnormal shadows that were infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules on their chest radiographs; and 3) no predisposing lung disease. 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 the present study as contaminated respiratory specimens. These cases did not have evidence of active disease.

Of the 47 patients with MAC-positive cultures, 30 met the ATS criteria at enrolment. 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; 14 patients still had not over the 12-month follow-up period after enrolment. Based on these observations, the subjects were divided into the MAC disease group, which was composed of 33 patients who 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. Clinical data were collected from each patient at the time of computed tomography (CT); these included sex, age, body mass index, smoking history, drinking history, complications, past history and laboratory data, including erythrocyte sedimentation rate, and GPL core IgG, IgA and IgM antibody. The present authors investigated whether there was a correlation between GPL core antibody level and CCT findings. Of the MAC disease group, 15 patients had previously received combination chemotherapy for mycobacterial diseases recommended by the ATS guideline before enrolment, but they had positive cultures of MAC at enrolment. All patients were seronegative for HIV types 1 and 2. Informed consent was obtained from all patients. The present 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.

CCT findings

All patients underwent conventional CT examination. CCT scans were obtained using a Toshiba Asteion TSZ-021A CT scanner (Toshiba, Tokyo, Japan). The CCT findings were categorised into small nodular shadow (<10 mm), nodular shadow (10–30 mm), large nodular shadow (>30 mm) or infiltrate, bronchiectasis, cavity and atelectasis. CCT findings were assessed by a consensus reading performed by two individual respiratory physicians without prior knowledge of the clinical or laboratory data. To assess the extent of disease,

TABLE 1 Criteria for diagnosis of *Mycobacterium avium* complex lung disease

Clinical criteria	1) Compatible signs and symptoms (coughing, fatigue more common; weight loss, haemoptysis 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
	2) Reasonable exclusion of other disease (*e.g.* tuberculosis, cancer, histoplasmosis) to explain condition or adequate treatment of other condition with increasing signs/symptoms
Radiographic criteria	1) Any of the following chest radiographic abnormalities; if baseline films are >1 yr old, should be evidence of progression:
	Infiltrates with or without nodules (persistent for >2 months or progressive)
	Cavitation
	Nodules alone (multiple)
	2) Any of the following high-resolution computed tomography abnormalities:
	Multiple small nodules
	Multifocal bronchiectasis with or without small lung nodules
Bacteriological criteria	1) At least three available sputum/bronchial wash samples within 1 yr, as follows:
	Three positive cultures with negative AFB smears, or
	Two positive cultures and one positive AFB smear; or
	2) A single available bronchial wash and inability to obtain sputum samples, as follows:
	Positive culture with 2+, 3+ or 4+ growth, or
	Positive culture with a 2+, 3+ or 4+ AFB smear; or
	3) Tissue biopsy, as follows:
	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 (clinical, radiographic and bacteriological) must be satisfied. AFB: acid-fast bacilli.

the lung was divided 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 number of involved CCT segments in which MAC lesions were present.

GPL core antibody

GPL core antibody was measured as previously described [4]. Briefly, microtitre plates (Nunc Products, Roskilde, Denmark) were coated with 0.5 μg·well^{-1} of GPL core of *M. avium* serotype 4, which had been prepared according to a previously described method [4]. Serum samples were diluted 40-fold with PBS containing 1% bovine serum albumin. Diluted serum samples were added, followed by incubation for 1 h 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, USA) was added and plates were incubated for 2 h at 37°C. Unbound labelled antibody was removed by washing and the substrate, *o*-phenylenediamine dihydrochloride (Sigma), was added. Following colour 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 analysed and all values are given as mean±SD. The Mann–Whitney U-test was used to compare the differences between groups. The Chi-squared test was used to compare the difference in CCT findings between groups. Correlation coefficients were calculated using Spearman's rank method. A p-value of <0.05 was considered significant.

RESULTS

Clinical background and laboratory data

The clinical background and laboratory data are shown in table 2. A total of 33 patients met the ATS criteria (MAC disease group) and 14 patients did not (MAC-culture positive group). Of these patients, 46 were female, who tended to be thin, there was only one smoker and none were alcohol abusers or had severe systemic complications. The main symptoms were coughing (19 patients), sputum (21 patients), bloody sputum (nine patients), chest pain (four patients) and dyspnoea (three patients). Of the subjects, 40.4% had past histories of major surgery that included myomectomy (seven patients), appendectomy (five patients), mastectomy (three patients), cholecystectomy (two patients), gastrectomy (two patients) and oophorectomy (one patient). 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 (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. The 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 the present authors' previous study [4], the positive rate was 66.7% for IgG, 81.8% for IgA and 78.1% for IgM 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

The CCT findings are summarised in table 3. Abnormal CCT were similar in the MAC disease and MAC-culture positive groups, with the exception of findings related to large nodules 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 ML lateral segment (33 out of 47 patients, 70.2%), ML 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 shows 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, the level of GPL core antibody was compared with each CCT finding, including the occurrence of a small nodular shadow (<10 mm), a nodular shadow (10–30 mm), a large nodular shadow (>30 mm) 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 with 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

The present study is the first to assess a correlation between GPL core antibody levels and radiographic findings. A total of 47 patients with MAC-positive culture from sputum and abnormal shadow on chest radiographs were examined. The present authors found that the level of IgA antibody against GPL core antigen was associated with CCT findings: 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

TABLE 2 Clinical data of patients with *Mycobacterium avium* complex (MAC)-positive cultures

	MAC disease	MAC-culture positive
Subjects n	33	14
Sex male/female	1/32	0/14
Age yrs	65.3±10.6	71.4±6.0
BMI 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 mm·h^{-1}	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. BMI: body mass index; ESR: erythrocyte sedimentation rate.

larger number of patients are required due to the low value of the correlation coefficients between the 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 bacteriological 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 bacteriological criterion requires multiple positive cultures for MAC, or a positive culture from a lung biopsy or histologically proven lung biopsy positivity. In the present study, it was not possible to carry out lung biopsy or bronchial washings on all patients in the MAC-culture positive group, especially on those with minimal symptoms or on elderly subjects, because informed consent for the bronchoscopic examination had not been obtained. Bronchoscopic examination is invasive and expensive. In elderly patients, the diagnosis for MAC lung disease may be less important with respect to the long-term survival because, for MAC lung disease patients with progressive radiographic abnormalities, the 50% survival rate was 175 months [8]. Thus, MAC-culture positive patients were defined based on an observation with monthly radiographic and sputum examination for 12 months.

Most patients in the MAC-culture positive group were elderly, nonsmoking, thin females 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 lobe, should suggest the diagnosis of MAC lung disease [10–12]. In the present 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

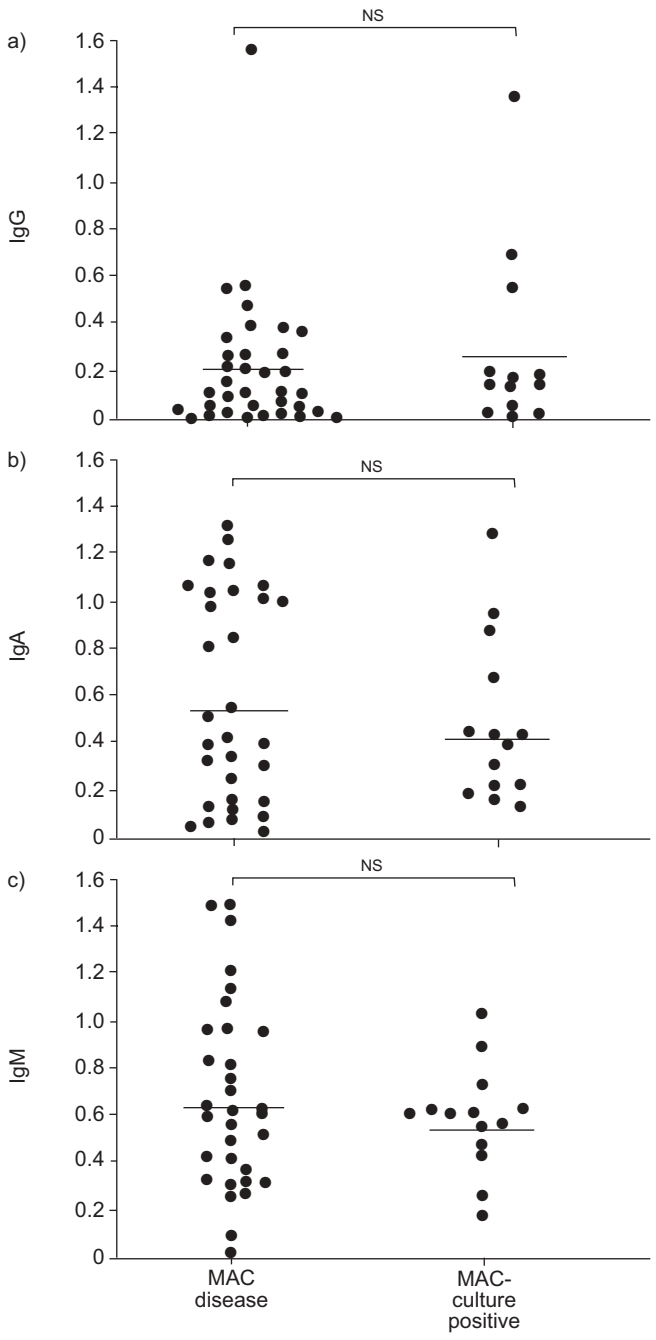

FIGURE 1. The distribution of serum levels of glycopeptidolipid core antibody in patients with *Mycobacterium avium* complex (MAC) disease and in the MAC-culture positive group in the a) immunoglobulin (Ig)G, b) IgA and c) IgM groups. The mean of each group of values is indicated by a horizontal line. There was no statistically significant difference between the IgG, IgA and IgM groups. NS: nonsignificant.

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 colonisation, 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 the present study

TABLE 3 Chest computed tomography findings

Abnormalities	MAC disease		MAC-culture positive	
	Patients with findings	Involved segments	Patients with findings	Involved segments
Small nodule (<10 mm)	33 (100)	6.5±4.2 (1–17)	14 (100)	4.3±3.7 (1–12)
Nodule (10–30 mm)	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)
Other	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 (%) or mean±SD (range). MAC: *Mycobacterium avium* complex. *: $p<0.05$.

at enrolment. The present authors have previously reported that GPL core antibody was not detectable in cases of MAC colonisation [4]. From results, it was considered that the patients of the MAC-culture positive group had an active MAC lung disease. It could be argued that patients are suffering from MAC lung disease when they: 1) exhibit a positive respiratory culture for MAC; 2) have chest radiographic findings of infiltrate, nodular cavitary lesions or bronchiectasis and/or multiple small nodules; and 3) show positive results for GPL core antibody. When using GPL core antibody it is not necessary to continue collecting respiratory specimens for acid-fast bacilli analysis or to observe chest radiographs and/or CCT over a 12-month period of time.

In the MAC disease group, eight patients had a 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 with immediate multidrug chemotherapy and/or surgical therapy in the context of the patient's general condition and

TABLE 4 Correlation coefficients between numbers of involved chest computed tomography segments *versus* glycopeptidolipid (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

MAC: *Mycobacterium avium* complex; Ig: immunoglobulin; OD: optical density. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

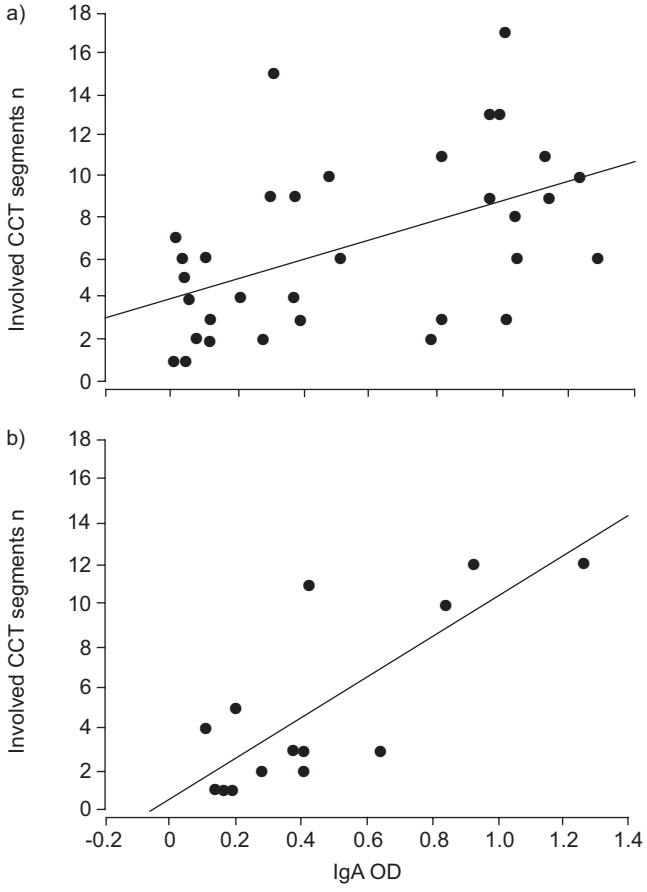

FIGURE 2. Correlation between the levels of glycopeptidolipid (GPL) core-specific immunoglobulin (Ig)A antibody and the number of involved chest computed tomography (CCT) segments in patients with a) *Mycobacterium avium* complex (MAC) lung disease and b) in the MAC-culture positive group. Significant positive correlations were found between the level of GPL core IgA antibody and the number of involved computed tomography 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 in both of them ($r=0.514$, $p<0.001$). OD: optical density.

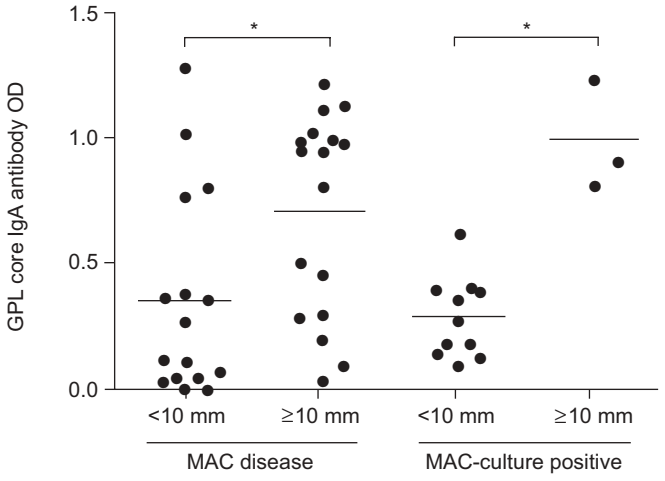

FIGURE 3. Serum level of glycopeptidolipid (GPL) core immunoglobulin (Ig)A antibody in patients who had nodular lesions <10 mm or ⩾10 mm in diameter assessed by chest computed tomography in the *Mycobacterium avium* complex (MAC) disease group and in the MAC-culture positive group. The mean of each group 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 with 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 the total of them ($p<0.001$). OD: optical density. *: $p<0.05$.

tolerance to the medication. However, 12 out of 33 patients with MAC disease and nine out of 14 MAC-culture positive patients did not undergo multidrug chemotherapy, including clarithromycin, following the present study. This was because most of these patients were >70 yrs old and/or did not have substantial symptoms and/or advanced or progressive radiographic abnormalities. Furthermore, treatment for MAC lung disease is expensive and is not covered by healthcare insurance in Japan. MAC lung disease is also difficult to treat and recurrence frequently occurs in MAC disease patients, even after completing multidrug chemotherapy, including clarithromycin. Many cases of recurrence have been experienced by the present authors, with the smear or culture test being positive over the 12 months following sputum-negative conversion during chemotherapy. This is because the radiographic active lesions, which are bronchiectasis or a cavity, have usually remained at the time of the sputum-negative conversion [8]. Thus, rapid diagnosis and treatment are required at an early stage before the completion of bronchiectasis or cavity lesions.

The serodiagnostic test used in the present study to detect serum GPL core antibodies could add useful information as a supplementary diagnostic aid [4, 16] and the present authors believe that this test may have future diagnostic applications. However, to include this serodiagnostic test in routine clinical practice, a study addressing the correlation between the antibody levels and radiographic findings was needed; the present study fulfils this requirement. 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, a better sensitivity to diagnose MAC lung disease without lung biopsy might be obtained.

The levels of GPL core antibody were similar in the MAC disease group and the MAC-culture positive group. Fifteen out of 33 (45.5%) of the MAC disease patients had received combination chemotherapy recommended by the ATS guidelines [7]. It is possible that this might have affected their antibody levels. However, the effects of treatment might be limited because they had a positive culture of MAC at enrolment, which meant the chemotherapy was not successful in converting the culture result from positive to negative at the time of serum sample collection. In the present authors' 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 defence against viral and bacterial infection at this site. There are some published reports that are consistent with the present findings. RODRIGUEZ *et al.* [17] reported that IgA may play an important role in protection against mycobacterial infection in the respiratory tract by blocking the pathogens' entrance and/or by modulation of pro-inflammatory responses. In the present authors' previous study [4], the best serodiagnostic results for sensitivity and specificity for diagnosing MAC lung disease were obtained by measuring IgA. Moreover, WATANABE *et al.* [18] reported that total serum IgA was significantly higher in patients with MAC compared with those with pulmonary tuberculosis. 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, the present article documents that the level of immunoglobulin A glycopeptidolipid core antibody was significantly associated with radiographic findings. This observation should encourage the use of the serodiagnostic tests for *Mycobacterium avium* complex lung disease in clinical practice.

ACKNOWLEDGEMENTS

The authors wish to thank K. Maekura, Y. Yamamoto and M. Kobayashi for measurement of antibody levels.

REFERENCES

1 Field SK, Fisher D, Cowie RL. *Mycobacterium avium* complex pulmonary disease in patients without HIV infection. *Chest* 2004; 126: 566–581.
2 Sakatani M. The non-tuberculous mycobacteriosis. *Kekkaku* 2005; 80: 25–30.
3 Marras TK, Daley CL. Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin Chest Med* 2002; 23: 553–567.
4 Kitada S, Maekura R, Toyoshima N, *et al.* Use of glycopeptidolipid core antigen for serodiagnosis of *Mycobacterium avium* complex pulmonary disease in immunocompetent patients. *Clin Diagn Lab Immunol* 2005; 12: 44–51.

5 Brennan PJ, Nikaido H. The envelope of mycobacteria. *Annu Rev Biochem* 1995; 64: 29–63.
6 Aspinall GO, Chatterjee D, Brennan PJ. The variable surface glycolipids of mycobacteria: structures, synthesis of epitopes, and biological properties. *Adv Carbohydr Chem Biochem* 1995; 51: 169–242.
7 American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. Medical Section of the American Lung Association. *Am J Respir Crit Care Med* 1997; 156: S1–S25.
8 Maekura R, Okuda Y, Hirotani A, *et al.* Clinical and prognostic importance of serotyping *Mycobacterium avium–Mycobacterium intracellulare* complex isolates in human immunodeficiency virus-negative patients. *J Clin Microbiol* 2005; 43: 3150–3158.
9 Prince DS, Peterson DD, Steiner RM, *et al.* Infection with *Mycobacterium avium* complex in patients without predisposing conditions. *N Engl J Med* 1989; 321: 863–868.
10 Swensen SJ, Hartman TE, Williams DE. Computed tomographic diagnosis of *Mycobacterium avium-intracellulare* complex in patients with bronchiectasis. *Chest* 1994; 105: 49–52.
11 Primack SL, Logan PM, Hartman TE, Lee KS, Muller NL. Pulmonary tuberculosis and *Mycobacterium avium-intracellulare*: a comparison of CT findings. *Radiology* 1995; 194: 413–417.
12 Wittram C, Weisbrod GL. *Mycobacterium avium* complex lung disease in immunocompetent patients: radiography–CT correlation. *Br J Radiol* 2002; 75: 340–344.
13 Jeong YJ, Lee KS, Koh WJ, Han J, Kim TS, Kwon OJ. Nontuberculous mycobacterial pulmonary infection in immunocompetent patients: comparison of thin-section CT and histopathologic findings. *Radiology* 2004; 231: 880–886.
14 Fujita J, Ohtsuki Y, Suemitsu I, *et al.* Pathological and radiological changes in resected lung specimens in *Mycobacterium avium intracellulare* complex disease. *Eur Respir J* 1999; 13: 535–540.
15 Obayashi Y, Fujita J, Suemitsu I, Kamei T, Nii M, Takahara J. Successive follow-up of chest computed tomography in patients with *Mycobacterium avium-intracellulare* complex. *Respir Med* 1999; 93: 11–15.
16 Kitada S, Maekura R, Toyoshima N, *et al.* Serodiagnosis of pulmonary disease due to *Mycobacterium avium* complex with an enzyme immunoassay that uses a mixture of glycopeptidolipid antigens. *Clin Infect Dis* 2002; 35: 1328–1335.
17 Rodriguez A, Tjarnlund A, Ivanji J, *et al.* Role of IgA in the defense against respiratory infections IgA deficient mice exhibited increased susceptibility to intranasal infection with *Mycobacterium bovis* BCG. *Vaccine* 2005; 23: 2565–2572.
18 Watanabe K, Fujimura M, Kasahara K, *et al.* Characteristics of pulmonary *Mycobacterium avium-intracellulare* complex (MAC) infection in comparison with those of tuberculosis. *Respir Med* 2003; 97: 654–659.