Serodiagnosis of *Mycobacterium avium* Complex Pulmonary Disease in Rheumatoid Arthritis

Yoshitoshi Komazaki^a Yasunari Miyazaki^a Toshihide Fujie^a
Hiroyuki Sakashita^a Kimitake Tsuchiya^a Meiyo Tamaoka^a Yuki Sumi^a
Yuichiro Maruyama^c Toshihiro Nanki^b Naohiko Inase^a

Departments of ^aIntegrated Pulmonology, and ^bMedicine and Rheumatology, Tokyo Medical and Dental University, Tokyo, and ^cDepartment of Radiology, Komoro Kosei General Hospital, Komoro, Japan

Key Words

Glycopeptidolipid · *Mycobacterium avium* complex
pulmonary disease · Rheumatoid arthritis · Sensitivity ·
Specificity

Abstract

Background: *Mycobacterium avium* complex (MAC) pulmonary disease (PD) is often difficult and complicated to diagnose or to discriminate from follicular bronchitis, bronchiectasis, or other conditions associated with rheumatoid arthritis (RA) lung in the clinical setting. **Objective:** We investigated whether a serologic test for anti-glycopeptidolipid (GPL) antibody was useful for distinguishing MAC-PD from RA lung in diagnosis. **Methods:** Serum IgA antibody to MAC-specific GPL core antigen was measured by an enzyme immunoassay. Antibody levels were measured in sera from 14 RA patients with MAC-PD (RA + MAC), 20 RA patients with bronchial or bronchiolar lesions without MAC-PD (RA w/o MAC), 20 RA patients without pulmonary lesions (RA only), and 25 healthy volunteers (HV). **Results:** The levels of serum anti-GPL antibodies were higher in the RA + MAC group than in the RA w/o MAC, RA-only, and HV groups (2.87 ± 2.83 vs. 0.50 ± 0.45 , 0.31 ± 0.24 , and 0.38 ± 0.10 U/ml, respectively; $p < 0.001$). With the cutoff point in receiver-operating char-

acteristic analysis set at 0.7 U/ml, the serologic test differentiated RA + MAC from RA w/o MAC with a sensitivity of 100% and specificity of 90%. **Conclusions:** This serologic test for anti-GPL antibody is useful for diagnosing MAC-PD in RA.

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Introduction

Recent reports have shown a rising prevalence of disease caused by nontuberculous mycobacteria (NTM) [1–5]. Seventy percent of patients with NTM disease in Japan are diagnosed with *Mycobacterium avium* complex (MAC) [6]. MAC causes chronic and progressive pulmonary disease (PD) in immunosuppressed patients and immunocompetent patients alike. Chest computed tomography (CT) of patients with rheumatoid arthritis (RA) reveals bronchial and/or lung abnormalities along with various other distinguishing features [7]. While only 1–3% of RA patients exhibit bronchiectasis clinically, as many as 30% manifest bronchiectasis in high-resolution CT [8]. MAC-PD is therefore difficult to diagnose or to differentiate from follicular bronchitis, bronchiectasis, or other conditions associated with RA lung in the clinical setting.

The tumor necrosis factor (TNF)- α antagonists infliximab and etanercept are used for the treatment of RA, as well as sarcoidosis and other collagen diseases and inflammatory conditions that interfere with granuloma formation [9]. Infections with intracellular pathogens such as NTM have been exacerbated in patients treated with TNF- α antagonists [10]. Because TNF- α antagonists pose a high risk for NTM-infected patients, they are not indicated for NTM under the guidelines from the American College of Rheumatology 2008. Whether TNF- α antagonists can be administered for RA remains an important issue [10, 11]. For this reason, we normally expect to conduct specific screening tests whenever we plan to administer anti-TNF- α drugs to RA patients.

In this study, we investigated whether a serologic test for anti-glycopeptidolipid (GPL) antibody was useful for distinguishing MAC-PD from RA lung in diagnosis.

Materials and Methods

Subjects

All subjects were enrolled between April 2009 and September 2011. Serum samples were collected from 14 RA patients with MAC-PD (RA + MAC), 20 RA patients with bronchial or bronchiolar lesions without MAC-PD (RA w/o MAC), 20 RA patients without pulmonary lesions (RA only), and 25 healthy volunteers (HV). Blood was collected from 11 of the 14 RA + MAC patients after the start of the MAC-PD treatment. Three of the 14 RA + MAC patients were treated with a 3-drug regimen, 3 received no drugs, and 8 were treated with a 1-drug treatment. Among the 8 RA + MAC patients treated with the 1-drug treatment, they received the single drugs for the following reasons: 4 were diagnosed after blood collection, 2 failed to properly comply with the multi-drug regimen, 1 was elderly, and 1 had been treated with the 3-drug regimen but required dose reduction. The Research and Ethics Committees of the Tokyo Medical and Dental University approved the study as a study on human subjects (identification No. 984), and all of the subjects provided written informed consent.

Criteria

Our study subjects were selected retrospectively from patients who regularly visited our hospital because of RA and/or abnormal chest shadows. First, the RA patients were divided into two groups, namely patients without abnormal shadows on chest X-ray (RA only) and patients with abnormal shadows. Then, in the latter group, the patients with radiologic findings compatible with MAC-PD were divided into two subgroups: those in whom MAC was detected by sputum culture or bronchoscopy (RA + MAC) and those in whom no MAC was detected (RA w/o MAC). All patients with MAC-PD met the diagnostic criteria of the American Thoracic Society (ATS) guideline [1]. Clinical criteria included: (1) pulmonary symptoms, nodular or cavitory opacities on chest radiograph or a high-resolution CT scan manifesting multifocal bronchiectasis with multiple small nodules, and (2) appropriate exclusion of other diagnoses. Microbiologic criteria included: (1)

positive culture results from at least two sputum samples, (2) positive culture results from at least one bronchial wash or lavage, or (3) transbronchial or other lung biopsy with mycobacterial histopathological features. All cases of RA + MAC and RA w/o MAC underwent chest CT, and the findings were compatible with MAC-PD.

Enzyme Immunoassay for Anti-GPL Antibody

All serum samples were measured by an enzyme immunoassay (EIA) kit for anti-GPL antibody (Tauns Laboratories, Inc., Shi-zuoka, Japan). All sera were stored at -20°C until assayed for IgA antibodies to GPL antigen according to the manufacturer's instructions [12]. The interfering substance, rheumatoid factor (RF), was <500 IU/ml, a level too low to affect the EIA, in every sample.

Radiological Analysis

The patients with MAC-PD were classified into two groups, namely fibrocavitary (FC) disease and nodular-bronchiectatic (NBE) disease, based on the chest radiographic findings [1]. FC disease was defined as the presence of cavitory forms in the upper lobes. NBE disease was defined as the presence of bronchiectasis and multiple nodular shadows on chest CT. Disease conforming to neither of these types was considered unclassifiable. To localize the infection, the lungs of each patient were divided into 10 fields (right lung, S^{1+2} , S^3 , S^{4+5} , S^6 , and $S^{7+8+9+10}$, and left lung, S^{1+2} , S^3 , S^{4+5} , S^6 , and S^{8+9+10}) according to Moore's [13] definition. Each field was evaluated with reference to the presence of bronchiectasis, centrilobular nodules, air space disease, cavities, and nodules >10 mm in diameter. The extent of disease was expressed as the number of MAC-involved segments, as described in previous studies [14, 15]. Chest CT findings were assessed by a consensus reading by two respiratory physicians and one radiologist (Y.M., Y.K., and Y.M.).

Statistical Analysis

All statistical analyses were performed using SPSS version 19 (IBM Japan Inc., Tokyo, Japan). Antibody levels in all groups were expressed as means $\pm$ SD. To compare mean values of multiple groups, data were compared using the Kruskal-Wallis test. The Steel-Dwass test, a nonparametric post hoc multiple comparison test, was used to evaluate differences between the groups when appropriate. Spearman's rank correlation coefficient was used for correlation analysis and the χ^2 test was used to assess the degree of compatibility. A probability value of $p < 0.05$ was regarded as significant.

Results

Characteristics of the Study Subjects

Table 1 summarizes the characteristics of the study subjects at blood sampling. None of the patients was seropositive for HIV type 1 or type 2, and none of the patients was suspected of MAC colonization. Among the 14 patients in the RA + MAC-PD group, 1 patient had diabetes mellitus, 2 had sequelae of pulmonary tuberculosis,

and 1 had chronic kidney disease. The HV group was younger than every other group ($p < 0.001$). No significant difference was seen among the groups in patient age at the onset of RA. The RA w/o MAC group used prednisolone (PSL) more frequently than the RA only group ($p < 0.05$). According to the radiological findings of the 14 patients in the RA + MAC-PD group, 2 were determined to have FC disease, 11 were determined to have NBE disease, and 1 was unclassifiable. The radiological findings of the 20 patients in the RA w/o MAC group were similar: 1 was determined to have FC disease, 19 manifested a predominant finding of NBE disease in the baseline CT, and 1 was determined to have FC disease. Figure 1 shows representative CT images of the RA + MAC and RA w/o MAC.

Levels of Anti-GPL Antibodies

The levels of serum anti-GPL antibody in the RA + MAC, RA w/o MAC, RA only, and HV groups were 2.87 ± 2.83 , 0.50 ± 0.45 , 0.31 ± 0.24 , and 0.38 ± 0.10 U/ml, respectively (fig. 2). Serum anti-GPL antibody was significantly higher in the RA + MAC group than in the other three groups ($p < 0.001$).

Sensitivity and Specificity

A receiver-operating characteristic (ROC) curve constructed for RA + MAC and RA w/o MAC had an area under the curve of 0.95 (fig. 3). Fourteen RA + MAC patients and 20 RA w/o MAC patients were included in the ROC analysis. The best cutoff value obtained by measuring the shortest distance between the coordinate point (0, 100) and the respective points on the ROC curve was 0.7 U/ml. A cutoff value of 0.7 U/ml resulted in 90.0% (18/20) specificity and 100% sensitivity (14/14).

Treatments and Levels of Anti-GPL Antibodies

The levels of serum anti-GPL antibodies were compared according to the treatment regimen in the 14 RA + MAC patients. No significant differences in the levels of anti-GPL antibodies were found among these regimens (fig. 4).

Correlations between the Extent of Disease and Levels of Anti-GPL Antibodies

Correlations between the extent of disease and levels of anti-GPL antibodies were investigated in 14 RA + MAC patients who underwent chest CT and serologic tests at the same time. No correlations were found between the extent of disease and the levels of anti-GPL antibodies (fig. 5).

Table 1. Characteristics of the study subjects

Characteristics	RA + MAC	RA w/o MAC	RA only	HV
Patients	14	20	20	25
Age	66.0 ± 9.5^a	66.0 ± 10.2^a	60.9 ± 9.9^a	54.1 ± 6.1
Range	50–87	38–80	35–75	45–67
Sex				
Male/female	2/12	2/18	1/19	4/21
Duration				
MAC disease	2.3 ± 1.7	N/A	N/A	N/A
Age at RA onset	52.0 ± 11.0	56.5 ± 14.7	55.5 ± 12.8	N/A
CT classification				
FC	2	1		
NBE	11	19	N/A	N/A
Unclassifiable	1	0		
Therapy for RA				
DMARDs total	14	19	19	
PSL	2	10*	2	
MTX	8	12	14	N/A
TAC	2	4	1	
Anti-TNF α	0	6	5	
Therapy for MAC-PD				
3 drugs	3			
1 drug	8	N/A	N/A	N/A
No treatment	3			

Data are shown as mean $\pm$ SD years or numbers. N/A = Not available; DMARDs = disease-modifying anti-rheumatic drugs; MTX = methotrexate; TAC = tacrolimus. * $p < 0.05$ vs. RA only, ^a $p < 0.001$ vs. HV.

Discussion

The present study showed that EIA for anti-GPL antibody can be a useful tool for detecting MAC-PD in patients with RA. With the cutoff value set at 0.7 U/ml, the ROC analysis had a sensitivity of 100% and specificity of 90%.

In 2007, a new set of diagnostic guidelines for NTM disease was published by the ATS and Infectious Disease Society of America (IDSA). Not long after, Japanese counterparts published new NTM guidelines with modifications almost identical to those of the ATS and IDSA. The bacteriological criteria are now somewhat simpler than those described under the ATS diagnostic criteria from 1997 [16]. It remains difficult, however, to reach an MAC-PD diagnosis bacteriologically in RA patients who manifest abnormal shadows characteristic of MAC-PD, particularly when bronchoscopy is unfeasible or the culture for bronchial lavage fluid is negative.

Fig. 1. a RA + MAC: axial CT shows cylindrical bronchiectasis and independent nodules in the right middle lobe and a larger nodule in the left upper lobe. **b** RA w/o MAC: axial CT shows cylindrical bronchiectasis in the right middle lobe and centrilobular opacities in the left upper lobe.

Fig. 2. Levels of serum anti-GPL antibody in the RA + MAC, RA w/o MAC, RA-only, and HV groups. All results were expressed as individual data, and the horizontal bars indicate the respective means. *** $p < 0.001$.

Fig. 3. ROC constructed for RA + MAC and RA w/o MAC patients.

Fig. 4. Comparison of antibody levels between 3-drug, 1-drug, and no-treatment regimens. Horizontal bars indicate the means.

Fig. 5. Correlation between antibody levels and radiographic severity by chest CT in 14 RA patients with MAC-PD.

RA patients have pulmonary lesions such as follicular bronchitis and bronchiectasis [7, 8]. Lesions of the respiratory tract on chest CT are reportedly found in 40% of RA patients [17]. While the frequency of NTM infection has yet to be identified in RA, Wickremasinghe et al. [18] reported a 2% rate of NTM infection in bronchiectasis patients in their study. This radiological similarity between MAC-PD and RA lung has often made a differentiation between both diseases difficult. In the present study, we were able to distinguish the levels of serum IgA antibody to GPL core between RA + MAC and bronchitis or bronchiectasis associated with RA.

The most critical tool for MAC-PD diagnosis is generally thought to be smear results and culture of bronchial washing. Among 57 MAC-PD patients tested by Yamazaki et al. [19], sputum acid-fast smears were positive in 11 patients (19.2%) and sputum MAC cultures were positive in 21 (36.8%). In the deteriorated patient group of their study, cultures obtained by fiberoptic bronchoscopy proved to be positive for MAC in 49 patients (85.9%) and negative for MAC in 17% [19]. In serodiagnosis studies by Kitada et al. [20, 21], the EIA for anti-GPL antibody had a sensitivity and specificity of 84.3 and 100%, respectively, in a Japanese population and 87.9 and 94.2%, respectively, in an American population. The sensitivity and specificity in the present study were similar to those reported previously [21, 22].

Two patients manifesting anti-GPL antibodies above our cutoff value of 0.7 were mixed into the RA w/o MAC group in the present study. Though still unable to identify the cause of this discrepancy, RF stands out as a pos-

sible culprit. RF interferes with immunoassay in two ways, namely, in how it reacts with polyethylene glycol in the reagent and in how it recognizes animal antibodies in the reagent as antigens and produces immunoprecipitate in response. RF nonspecifically binds to the Fc region of IgG and IgM-RF immunocomplex in the RF family and can form a bridge structure when it reacts with latex reagent [23]. False-positive results can also result from diseases of other mycobacteria such as *Mycobacterium fortuitum*, *chelonae*, *abscessus*, or *scrofulaceum*, organisms that similarly possess GPLs on their cell wall surfaces [24–26].

False negatives occurred in 15.7% of the patients with MAC-PD in the study by Kitada et al. [21]. HLA genes may govern the immune responses to GPL core, and variation in these responses among individuals may be one cause of false negatives [27]. Another possible cause is the immunosuppressive agents the RA patients may have received. In our study, 14 patients received PSL (2 in RA + MAC, 10 in RA w/o MAC, and 2 in RA only), and 34 (8 in RA + MAC, 12 in RA w/o MAC, and 14 in RA only; table 1) received methotrexate. While most of our patients used >1 drug for RA treatment, a significant number of patients in the RA w/o MAC group received PSL alone. The levels of anti-GPL antibodies did not significantly differ between the patients receiving PSL and patients not receiving PSL (2.40 ± 1.87 vs. 2.95 ± 3.01 in RA + MAC, $p = 0.584$, and 0.633 ± 0.552 vs. 0.386 ± 0.310 in RA w/o MAC, $p = 0.224$, Mann-Whitney U test).

The recommended treatment for NTM-PD since the release of the ATS guideline in 1997 has been a 3-drug regimen of clarithromycin (CAM), ethambutol (EB), and rifabutin (RBT) or rifampicin (RFP) for 12 months for negative smear results. For more severe cases, the recommendation has been a 4-drug regimen (CAM, EB, streptomycin, and RBT or RFP) for 2 months, followed by a switch to a 3-drug regimen for 12 months after the smear results are negative [28]. Sixty to 80% of MAC-PD cases were reported to be smear negative after the first treatment using this standard regimen [29, 30]. In our study, 14 patients with RA w/o MAC were classified into three categories according to the treatment received. There were no significant differences in the levels of GPL antibodies among the three regimens, though the antibodies did tend to be lower in the patients on the 3-drug regimen. Eight of the RA + MAC patients received a 1-drug treatment for MAC infection for the reasons indicated in the Materials and Methods. We have not measured anti-GPL antibodies in our subjects since the end of the study. When Kitada et al. [12] compared serum IgA antibodies

to GPL before and after chemotherapy in both cured (14 MAC patients) and uncured patients (13 MAC patients), they found significantly decreased GPL core antibodies in the cured MAC patients who had responded to the chemotherapy.

Finally, Kitada et al. [14] reported that the levels of GPL core-specific IgA antibodies correlated with the number of involved chest CT segments in patients with MAC lung disease. In our study, we found no such correlations between the extent of the disease and GPL antibody levels (fig. 4). We may have overestimated the lung lesions with MAC in our study because of the RA lesions.

Our study has several limitations. First, the sample size of 74 cases is small. Our sample for RA + MAC, only 14 patients, is especially small, as few cases of RA + MAC presented at our institution between April 2009 and September 2011. Second, the timing for the blood collection varied since our data were retrospective. Among our 14

RA + MAC patients, blood was collected before the MAC diagnosis in 9 patients and after the diagnosis in 5. Finally, this study was a derivation cohort but not a validation cohort.

In conclusion, a serologic test for anti-GPL antibody is useful for the diagnosis of MAC-PD in RA.

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Financial Disclosure and Conflicts of Interest

None of the authors has financial relationships with commercial entities that have interests in the subject of this paper.

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