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MAJOR ARTICLE

Use of Anti-Glycopeptidolipid-Core Antibodies Serology for Diagnosis and Monitoring of *Mycobacterium avium* Complex Pulmonary Disease in the United States

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Background. There is an unmet need for rapid, accurate, and noninvasive assays for diagnosis and monitoring of *Mycobacterium avium* complex pulmonary disease (MAC-PD). We evaluated the diagnostic accuracy of an anti-glycopeptidolipid (GPL)-core immunoglobulin A (IgA) antibody test in a US cohort of MAC patients, and we described serial serology changes during antimicrobial therapy.

Methods. We identified serum samples from MAC patients starting treatment at enrollment and control subjects with or without bronchiectasis within OHSU's NTM Biobank. We conducted diagnostic test accuracy. Changes in mean levels of anti-GPL-core IgA antibodies between 0 and 3, 6, or 12 months after treatment start were assessed using the Student's paired *t* test. Pearson's correlation coefficient was calculated for IgA antibody levels and Student paired *t* test measures.

Results. We included 25 MAC patients and 18 controls. At baseline, IgA antibody concentrations in MAC patients (3.40 ± 6.77 U/mL) were significantly higher than in controls without bronchiectasis (0.14 ± 0.03 U/mL, $P = .02$). Sensitivity and specificity for MAC-PD in this population was 48% and 89% (cutoff point 0.7 U/mL), respectively. Among MAC patients starting antimicrobial therapy, mean IgA levels decreased 0.3202 U/mL ($P = .86$) at month 3, 0.8678 U/mL ($P = .47$) at month 6, and 1.9816 U/mL ($P = .41$) at 1 year. Quality of Life-Bronchiectasis Respiratory Symptom Scale improvement correlated with decreasing IgA titers after 12 months of treatment in MAC patients ($r = -0.50$, $P = .06$).

Conclusions. Anti-GPL-core IgA antibody levels are relatively specific for MAC-PD and decrease with treatment. Larger studies are warranted to evaluate the role of IgA serology in monitoring treatment response or for disease relapse/reinfection.

Keywords. epidemiology; lung diseases; nontuberculous mycobacteria; serodiagnosis.

The global prevalence of nontuberculous mycobacteria (NTM) disease has been steadily increasing over the past 2 decades, with evidence of significant associated morbidity, mortality, quality of life, and healthcare costs [1–5]. Nontuberculous mycobacteria disease trends in the United States are analogous to those worldwide, with increasing prevalence, particularly among females in older age groups, with more than 80% of cases caused by *Mycobacterium avium* complex (MAC) species [2]. *Mycobacterium avium* complex pulmonary disease (MAC-PD) presents diagnostic challenges, requiring composite criteria of pulmonary symptoms, microbiology, and radiographic findings [5]. However, some patients have difficulty expectorating, and isolation of MAC organisms on sputum culture is insufficient to document disease and evaluate microbiologic changes. Chest radiography is used to determine disease burden, but findings are nonspecific and cannot be frequently repeated due to exposure concerns and high cost. Furthermore, MAC is difficult to treat, with frequent drug-related side effects and suboptimal treatment outcomes due to long-term multidrug combination regimens. Refractory and recurrent disease is common even after treatment, leading to lifelong follow-up of patients. Pulmonary MAC can cause chronic cough, fatigue, and other symptoms, such as anxiety and depression, that impact health-related quality-of-life (HRQoL) [5]. Overall, this warrants the need for methods for diagnosis and monitoring of MAC-PD that are simple yet specific.

In Japan, a commercially available anti-glycopeptidolipid (GPL)-core immunoglobulin A (IgA) antibody measurement is approved as a diagnostic tool for pulmonary MAC [6–11]. The kit relies on an enzyme immunoassay that detects serum

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IgA antibodies directed against the MAC-specific GPL-core as the antigen. Several studies in Japan have demonstrated the utility of the kit for the diagnosis of pulmonary MAC and reported its sensitivity to be between 54% and 92% and specificity between 72% and 99% with a 0.7 U/mL cutoff value [9, 11–15]. This tool has received limited evaluation in North America and its potential ability to monitor treatment response is unknown. Accordingly, we sought to evaluate the diagnostic accuracy of this MAC-specific tool and whether it relates to measures of treatment response over time in patients in the United States.

METHODS

Study Design and Population

We conducted a case-control study to evaluate the diagnostic accuracy of the MAC-specific serodiagnostic test that detects anti-GPL-core IgA antibody levels in a United States-based population. We also described changes in the levels of anti-GPL-core IgA during antibiotic therapy among patients starting MAC therapy over time. In addition, we evaluated longitudinal changes and correlation between IgA antibody levels and HRQoL measures in MAC patients.

Oregon Health and Science University's Northwest NTM Biobank (Biobank) was established in 2013 to collect blood samples, clinical data, and patient-reported outcome measures (PROs) from patients with NTM isolation identified through regional laboratory and clinic surveillance. Specimen collection in patients with pulmonary MAC occur at baseline and up to 2 additional time points at 3 months, 6 months, or 12 months. Controls with and without underlying lung disease and no history of positive NTM sputum culture are also enrolled in the Biobank. Laboratory specimens and PROs in the control group populations are collected at baseline only. For the purpose of this study, we selected Biobank patients as cases if they met the following inclusion criteria: (1) they have confirmed MAC disease based on American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) diagnostic guidelines, (2) they initiated antibiotic treatment at the time of enrollment, (3) and they completed follow-up sample collection within 18 months of enrollment. Biobank patients without NTM-PD were enrolled in this study in 2 control cohorts: (1) healthy individuals, defined as individuals without immunosuppression, either by medication or underlying illness, or without any other underlying lung disease, including chronic obstructive pulmonary disease (COPD) and emphysema; (2) and individuals with bronchiectasis.

For patients meeting inclusion criteria, serum samples were taken from the Biobank for evaluation. All samples were previously collected in serum separator tubes, spun, and frozen at −20°C through March 2021. The samples were shipped to the National Institute of Infectious Diseases (NIID), Tokyo, Japan. All samples were deidentified and labeled with a unique sample identifier. Trained laboratory staff of NIID measured the titer of anti-GPL-core IgA antibodies by Capilia MAC Ab ELISA (TAUNS Laboratories, Inc., Shizuoka, Japan) using previously described methods [10].

Statistical Analysis

Baseline data were reported as medians and interquartile ranges (IQRs) for continuous variables and frequencies and percentages for categorical variables. The χ^2 tests and independent sample t tests were used to test demographic and comorbidity relationships between cases and controls. The anti-GPL-core IgA antibody titer data, measured using the Capilia MAC Ab ELISA, were expressed as means and standard deviations. We made group comparisons using 2 independent sample t test and nonparametric analysis, using a statistically significant cutoff point of $P < .05$.

We constructed a receiver operating characteristic (ROC) curve to explore cutoff points differentiating MAC patients and controls. The optimal cutoff point for the IgA concentration threshold in our cohort was determined using the optimality criterion from 3 different methods: (1) Youden index, (2) Distance to (0,1), and (3) Sensitivity, Specificity equality [16–18]. Summary estimates of diagnostic odds ratio, positive likelihood ratio (LR^+) and negative likelihood ratio (LR^-), sensitivity and specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed.

The temporal changes in antibody levels in treated patients were explored by analyzing the mean levels of anti-GPL-core IgA antibodies at different time points (baseline, 3 months, 6 months, and 12 months) after treatment start. We evaluated the mean change between 2 time points (ie, 0 and 3 months, 0 and 6 months, and 0 and 12 months) using the Student's paired t test. Pearson's correlation coefficient was calculated to evaluate the correlation between IgA antibody levels and 2 PRO measures,: the Quality of Life Bronchiectasis Respiratory Symptoms Scale (QOL-B RSS) and the NTM Symptoms Scale (1–100, where 100 equates to best HRQoL) [19].

RESULTS

We identified 25 pulmonary MAC patients within the Biobank meeting our inclusion criteria. Multidrug treatment regimens used by these patients included azithromycin and ethambutol (9 of 25, 36%), azithromycin, ethambutol, and rifampin (10 of 25, 40%), or other triple-drug therapy combinations among the remaining 6 (24%) subjects; however, all included azithromycin. The majority were newly diagnosed and treatment naive before this treatment episode (19, 76%).

Baseline demographic data from 25 patients who began treatment for pulmonary MAC disease versus the control groups are shown in Table 1. Half of the control population were bronchiectasis patients without NTM pulmonary disease

Table 1. Baseline Demographics and IgA Concentrations of 25 Patients With MAC Disease and 18 Healthy or Bronchiectasis Non-NTM Controls, Through March 2021

Characteristics	MAC Cases[a] ($n=25$)	Bronchiectasis Control ($n=9$)	*P* Value[b]	Healthy Control ($n=9$)	*P* Value[c]
Age, median [IQR]	65.6 [58.8– 72.0]	66.1 [59.2–66.8]	.90	72.9 [65.6–72.9]	.35
Female, *n* (%)	19 (76.0)	5 (55.6)	.40	4 (44.4)	.11
Comorbidities					
COPD[d]	8 (33.3)	4 (44.4)	.69	NA	NA
Bronchiectasis	23 (92.0)	9 (100)	1.0	NA	NA
Cavitary disease	5 (20.0)	NA	NA	NA	NA
Treatment History at Enrollment, *n* (%)					
Treatment naive	19 (76.0)	NA	NA	NA	NA
Previously treated	6 (24.0)	NA	NA	NA	NA
IgA concentration, mean (±SD)					
Baseline (Mo 0)	3.40 (6.77)	1.74 (4.15)	.50	0.14 (0.03)	.02

Abbreviations: COPD, chronic obstructive pulmonary disease; IgA, immunoglobulin A; IQR, interquartile range; MAC, *Mycobacterium avium* complex; Mo, month; NA, not applicable; NTM, nontuberculous mycobacteria; SD, standard deviation.

[a]Two subjects had blood draws at baseline, 3 months, and 12 months. Two subjects had blood draws at baseline, 6 months, and 12 months. All remaining subjects are mutually exclusive, total (n) is 25 for cases.

[b]Cases versus bronchiectasis controls.

[c]Cases versus healthy controls.

[d]COPD cases (n) = 24.

($n=9$), and the other half were healthy individuals. The median age of cases was 66 years (IQR, 58.8–72.0), 66 years (IQR, 59.2–66.8) for bronchiectasis controls, and 73 years (IQR, 65.6–72.9) for healthy controls. There were 19 (76%) female cases, and 5 (55.6%) and 4 (44.4%) were female in the bronchiectasis and healthy control groups, respectively. Five (20%) MAC patients had cavitary disease, 8 (33%) had COPD, and 23 (92.0%) had bronchiectasis. The baseline mean value of anti-GPL-core IgA antibodies in MAC patients (3.40 ± 6.77 U/mL) was higher than in controls with bronchiectasis (1.74 ± 4.15 U/mL, $P=.50$) and significantly higher than in healthy controls (0.14 ± 0.03 U/mL, $P=.02$) (Table 1).

Using the Capilia MAC Ab ELISA manufacture-provided cutoff value of 0.7 U/mL, the diagnostic odds ratio was 7.38 (95% confidence interval [CI], 1.40–39.08), whereas LR^+ was 4.32 (95% CI, −1.59 to 10.23) and LR^- was 0.59 (95% CI, .34–.83). Sensitivity and specificity in this population was 48% (95% CI, 28%–68%) and 89% (95% CI, 74%–100%), respectively, with 86% (95% CI, 67%–100%) PPV and 55% (95% CI, 37%–73%) NPV (Table 2). Cutoff levels for discrimination of MAC patients from controls were calculated with a ROC curve. Optimum anti-GPL-core IgA cutoff level of 0.178 U/mL had a sensitivity of 84% (95% CI, 70%–98%) and specificity of 72% (95% CI, 52%–93%) (Figure 1). The diagnostic odds ratio was 13.65 (95% CI, 3.09–60.30), LR^+ was 3.02 (95% CI, .71–5.34), and LR^- was 0.22 (95% CI, .01–.43) (Table 2). Using this optimal cutoff point, the kit correctly classified cases versus controls in our cohort 79.67% of the time (95% CI, .6457–.9386), performing significantly better than chance ($P<.0001$).

Among MAC patients starting antimicrobial therapy, mean IgA levels between baseline and 3 months decreased 0.3202 U/mL ($P=.86$); 6 months decreased 0.8678 U/mL ($P=.47$); and 12 months decreased 1.9816 U/mL ($P=.41$) (Table 3). The QOL-B RSS scores correlated negatively with IgA antibody levels after 12 months of treatment ($P<.06$) (Table 4).

DISCUSSION

In this study, we examined the diagnostic accuracy of the Capilia MAC Ab ELISA kit in a United States-based patient population. The baseline mean anti-GPL-core IgA serum level was higher in MAC cases than in uninfected bronchiectasis and healthy control cohorts. Our findings suggest high specificity (89%) for this IgA diagnostic assay, although sensitivity (48%) was lower than previously reported in Japan for the cutoff point of 0.7 U/mL. The ROC analysis determined the optimal cutoff level of 0.178 U/mL in this population. We are one of

Table 2. Diagnostic Test Evaluation for Capilia MAC Ab ELISA Cutoff Values of 0.7 U/mL and 0.178 U/mL

	Estimate (95% CI)	
Test Accuracy Measure	Cutoff Value 0.7 U/mL	Cutoff Value 0.178 U/mL
Sensitivity	.48 (.28–.68)	.84 (.70–.98)
Specificity	.89 (.74–1.03)	.72 (.52–.93)
Positive predictive value	.86 (.67–1.04)	.81 (.66–.96)
Negative predictive value	.55 (.37–.73)	.76 (.56–.97)
Positive likelihood ratio	4.32 (1.59–10.23)	3.02 (.71–5.34)
Negative likelihood ratio	.59 (.34–.83)	.22 (.01–.43)
Diagnostic odds ratio	7.38 (1.39–39.08)	13.65 (3.09–60.30)

Abbreviations: Ab, antibody; CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; MAC, *Mycobacterium avium* complex.

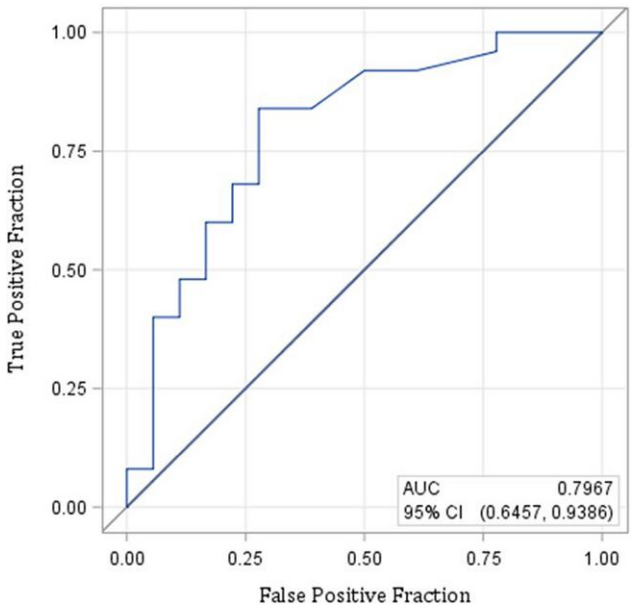

Figure 1. Receiver operating characteristic curve for immunoglobulin A concentration threshold cutoff points between 25 *Mycobacterium avium* complex (MAC) patients and 18 controls. The sensitivities and specificities of the MAC-specific serodiagnostic test under various cutoff points include 100% sensitivity and 22% specificity for 0.11 U/mL; 92% sensitivity and 50% specificity for 0.141 U/mL; 84% sensitivity and 72% specificity for 0.178 U/mL; 68% sensitivity and 77.8% specificity for 0.234 U/mL; 60% sensitivity and 83.3% specificity for 0.294 U/mL; 48% sensitivity and 88.9% specificity for 1.34 U/mL; and 40% sensitivity and 94.4% specificity for 1.821 U/mL. AUC, area under the curve; CI, confidence interval.

few studies to longitudinally evaluate anti-GPL-core IgA in MAC patients and the first to correlate with HRQoL measures over time. We report a decrease in IgA levels over the course of antibiotic treatment, which also corresponded to an increase in QOL-B RSS scores indicating improved HRQoL.

Individuals with bronchiectasis are at increased risk of infection with MAC, which is a likely explanation for the elevated IgA levels in this control group compared to healthy controls. Our criteria for uninfected controls relies on having no history of positive NTM sputum culture, and these may include patients with undetected and undiagnosed MAC infection. Measurable levels of MAC-specific IgA in controls may be indicative of subclinical or prior MAC infection and reflective of the diagnostic sensitivity of serological methods. Longitudinal analysis of IgA levels among high-risk groups, such as those with bronchiectasis, could evaluate the utility of IgA for early detection of MAC-PD and distinguish between infection and disease. Immunological investigations of antigenic responses to airway inflammation in MAC infection in the context of bronchiectasis should also follow to address this observation fully.

Other studies in various countries have also reported satisfactory diagnostic efficacies of the anti-GPL-core IgA antibody enzyme immunoassay [20–25]. Jeong et al [20] reported a sensitivity of 85% and specificity of 100% in Korea, whereas a

Table 3. Mean Change in IgA Antibody Levels Between Longitudinal Time Points in MAC Patients

	Paired Differences			
Visit	IgA Mean (±SD)	95% CI		*P* Value
Mo 0 vs 3	0.32 (1.84)	−1.31	1.95	.86
Mo 0 vs 6	0.87 (2.79)	−1.61	3.35	.47
Mo 0 vs 12	1.98 (6.75)	−2.89	6.86	.41

Abbreviations: CI, confidence interval; IgA, immunoglobulin A; MAC, *Mycobacterium avium* complex; Mo, month; SD, standard deviation.

NOTES: Statistical analysis: Student's paired *t* test. 0-/12-month cohort (*n*) = 16. 0-/3-/6-month cohort (*n*) = 11.

Taiwanese study reported 60% sensitivity and 87% specificity with the same cutoff value [21]. A meta-analysis of 16 studies by Shibata et al [23] reported summary estimates of sensitivity and specificity of 0.70 (95% CI, .62–.76) and 0.91 (95% CI, .84–.95) using the 0.7 U/mL cutoff. A United States study reported a sensitivity of 51.7% and specificity 93.9% with the 0.7 U/mL cutoff, and it determined a best combination of sensitivity and specificity with the cutoff point 0.3 U/mL (70.1% and 93.9%, respectively) [22]. The result of our ROC analysis revealed an optimal cutoff point of 0.178 U/mL with a sensitivity of 84% and specificity 72% (Table 2, Figure 1). These few studies in the United States using the Capilia MAC Ab ELISA reveal that a lower antibody titer cutoff may make this test more useful in this local setting; however, further evidence is needed to determine optimal cutoff points for differing patient populations.

Structurally, GPLs consist of a lipopeptide core and a variable oligosaccharide [24]. The GPL core is found in all subspecies of MAC and shows a common antigenicity, which is the basis of the development of the kit [7, 8]. Although it has been demonstrated that the GPL core is the dominant epitope, it is possible that other components of GPLs possess antigenicity, such as the serotype-specific oligosaccharide. Because MAC is an environmental pathogen distributed widely across the globe [25], it may be appropriate to further test the diagnostic performance of the kit in different geographic locations if different subspecies predominate.

In our patients, the diagnostic odds ratio, the odds of obtaining a positive test result in a diseased individual compared to a nondiseased individual, was 7.38 for the cutoff value of 0.7 U/mL (Table 2). This was lower than what was reported in the pooled analysis by Shibata et al [23] (23.1 [10.7–50.1, $I^2 = 7.2\%$]) for the same cutoff point. The LR^+ and LR^- were 4.32 and 0.58, respectively, suggesting that patients with MAC had a 4-fold higher chance of being positive for IgA, whereas if the patient was negative for IgA, the probability of the patient having MAC was 58%. Overall, the results of the diagnostic odds ratio, LR^+, and LR^- suggest that the kit is diagnostic of only disease and that the negative results should not

Table 4. Correlation of IgA Antibody Levels and HRQL Scores in MAC Patients

	Baseline		Mo 3		Mo 6		Mo 12	
HRQoL Measure	Pearson	*P* Value	Pearson	*P* Value	Pearson	*P* Value	Pearson	*P* Value
QOL-B RSS	0.14	.60	−0.02	.97	−0.58	.23	−0.50	.06
NTM Symptoms Scale	−0.21	.37	0.04	.94	0.31	.55	0.09	.75

Abbreviations: IgA, immunoglobulin A; MAC, *Mycobacterium avium* complex; Mo, month; NTM, nontuberculous mycobacteria; QOL-B RSS, Quality of Life Bronchiectasis Respiratory Symptoms Scale.

NOTE: Statistical analysis: Pearson's correlation coefficient.

be used alone for diagnosis of MAC at the 0.7 U/mL cutoff value. We report a diagnostic odds ratio of 13.65, LR^+ of 3.02, and LR^- of 0.22 with use of the 0.178 cutoff value as determined by our ROC (Table 2), which should be evaluated further in similar patient populations to further assess the kit.

Our study showed MAC-specific IgA levels diminished during antimicrobial therapy (Table 3). One possible explanation is that reductions in anti-GPL-core IgA titers reflect treatment response. Studies have evaluated longitudinal IgA level change with respect to MAC therapy in association with culture positivity and other unfavorable treatment responses, showing that that changes in the antibody levels may reflect disease activity [26, 27]. Anti-GPL-core IgA titer could potentially be used to track relative changes in bacillary burden, if it correlates with quantitative cultures, and used to discern disease activity and progression during the course of infection. Larger, longitudinal studies are needed to further optimize diagnostic criteria and monitoring, especially in comparison to and in combination with symptoms, microbiological, and radiological data.

The QOL-B RSS and NTM Symptoms Scale are both self-administered questionnaires that have been evaluated in patients with NTM. The QOL-B RSS measures respiratory symptoms in addition to other symptoms and functioning domains that capture effects of the disease and treatment on HRQoL. In the current study, we found that anti-GPL-core IgA antibody levels were negatively correlated with QOL-B RSS scores after 12 months of treatment (Table 4). The increase in scores, signifying better HRQoL, and decrease in antibody levels suggest a response to antimicrobial therapy. No studies have examined longitudinal HRQoL outcomes in relation to levels of anti-GPL-core IgA in patients with MAC. Overall, the validation and use of PRO measures in clinical trials has been identified as a top research priority in the field and by patients [28].

Our study was limited in size and in several other ways. We did not have longitudinal data collected during therapy at the same time points for all patients. Some patient had only baseline and then 12-month blood samples, and then others had collection at baseline, 3 months, and 6 months (reflecting a protocol change in our Biobank collecting schema). Although this did not affect the validity of longitudinal assessments for each patient, it would have been ideal to have 12-month measures collected on all patients. Another limitation was the lack of follow-up data for the control group, who only had baseline data available. We were also unable to provide longitudinal acid-fast bacteria culture positivity and culture conversion data to evaluate bacillary burden because this information is not systematically collected in the Biobank.

CONCLUSIONS

Our study suggests that the Capilia MAC Ab ELISA kit has potential to address the unmet need of rapid, accurate, and non-invasive diagnostic systems for MAC disease to be used routinely in the clinical setting in different patient populations. This kit, and similar assays, are of high research priority to advance the care and treatment of patients with MAC disease. Larger, long-term studies of IgA antibodies for MAC diagnosis and monitoring of therapeutic effects are warranted to gain a better understanding of their clinical value and limitations.

Notes

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Potential conflicts of interest. K. L. W. has been a consultant to Insmed, Paratek, RedHill Biopharma, Spero Therapeutics, AN2 Therapeutics, Vast Therapeutics, and Renovion and has received research funding from Insmed, Paratek, RedHill Biopharma, AN2 Therapeutics, and Renovion. E. H. has been a consultant to AN2 and MannKind. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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