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Serological diagnosis of *Mycobacterium avium* complex lung diseases by enzyme immunoassay of IgA antibodies against MAC-specific glycopeptidolipid core antigen

W.O Tam[a,*], C.F Wong[a], S.S.Y. Wong[b], C.L.Y. Kwan[c]

[a] Tuberculosis and Chest Unit, Grantham Hospital, 125 Wong Chuk Hang Road, Hong Kong
[b] Department of Microbiology, The University of Hong Kong, Queen Mary Hospital, 102 Pokfulam Road, Hong Kong
[c] Department of Surgery, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong

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ABSTRACT

Introduction: There is an increasing trend worldwide in the incidence of *Mycobacterium avium* complex pulmonary diseases (MAC-PD) and the diagnosis is sometimes complicated. Recently, an enzyme immunoassay (EIA) kit that detects serum IgA antibody against MAC-specific glycopeptidolipid (GPL) core antigen had been developed and found to be useful in discriminating MAC-PD from other lung diseases. The antibody was subsequently also found to be elevated in patients suffering *Mycobacterium abscessus* pulmonary diseases (MAB-PD). This study is to evaluate this EIA kit in the serological diagnosis of MAC-PD in Hong Kong Chinese patients.
Methods: The study was conducted in Grantham Hospital, Hong Kong between July 2017 and July 2018. Assay of the IgA antibody level using the EIA kit was done on blood samples collected from patients suffering from MAC-PD, MAB-PD, pulmonary tuberculosis and other lung diseases.
Results: There were 100 subjects recruited into the study, among which 11 were excluded. By using the cut-off value 0.7 U/mL provided by the manufacturer, the sensitivity and specificity for diagnosis were 73.7% and 77.6% for MAC-PD; 50% and 77.6% for MAB-PD. By receiver operating characteristic curves analysis, new cut-off for MAC-PD and MAB-PD were calculated as 1.771 U/mL and 0.172 U/m, respectively. The sensitivity and specificity were 68.4% and 86.2% for MAC-PD, whereas 66.7% and 72.4% for MAB-PD.
Conclusions: Our study showed that the enzyme immunoassay of IgA antibodies against MAC-specific glycopeptidolipid core antigen could help to distinguishing MAC and M. abscessus pulmonary diseases from pulmonary tuberculosis and other lung diseases among Hong Kong Chinese patients. Further larger scale studies in our local population for the usefulness of this antibody test in the diagnosis and monitoring of MAC and M. abscessus lung diseases might be warranted.

1. Introduction

There is an increasing trend worldwide in the incidence of non-tuberculous mycobacterial pulmonary diseases (NTM-PD), among which *Mycobacterium avium* complex (MAC) is one of the most common infections. The diagnosis of MAC pulmonary disease (MAC-PD) is sometimes complicated. According to most published guidelines [1,2], one needs to consider the clinical and radiological features and bacteriological results before one could make a diagnosis of lung disease and to distinguish those from sporadic or transient colonization. IgG ELISA may be effective for diagnosis [3] but is not commercially available. Recently, an enzyme immunoassay (EIA) kit that detects serum IgA antibody against MAC-specific glycopeptidolipid (GPL) core antigen has been developed by Kitada et al. [4], and studies [5–8] in Japan and the USA have found it useful in the diagnosis of MAC-PD and discriminating it from other lung diseases. However, it may not differentiate MAC-PD with some other NTM-PD. In the case of a lung infection with *M. abscessus* complex (MAB) which has GPL, a positive antibody response might be found in patients with such NTM-PD. There have been reports on the usefulness of this test in Taiwan Chinese subjects [9] but there has not been any local data on the clinical efficacy of this kit in Hong Kong. The objective of this study is to evaluate this EIA kit in the serological diagnosis of MAC-PD in Hong Kong Chinese patients.

* Corresponding author.
E-mail address: tamwo@ha.org.hk (W.O. Tam).

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2. Method

2.1. Study patients and collection of serum

The study was conducted at Grantham Hospital, Hong Kong between July 2017 and July 2018 with prior approval of the study by the Hong Kong West Cluster Institutional Review Board and written informed consent obtained from all participating subjects. Patients who were younger than 18 years old, pregnant, mentally incapacitated or unable to understand the consent were excluded. Four mL of clotted blood were collected from study subjects who were divided into 4 groups:

1. Patients with MAC lung diseases;
2. Patients with *M. abscessus* lung diseases;
3. Patients with pulmonary tuberculosis (TB, with classical radiographic changes and sputum positive for *M. tuberculosis* on microscopy and culture);
4. Patients with other pulmonary disease (with no radiographic suggestions of NTM lung diseases and sputum for mycobacterial culture being negative on more than one occasion).

Total 100 patients were consented for the study. All patients in group 1 and 2 were consecutive patients within the study period and were unequivocal cases of NTM-PD meeting the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) clinical case definition of NTM-PD with compatible symptoms, radiological appearance and deterioration on serial chest X-rays, and multiple respiratory specimens showing the presence of MAC or MAB on mycobacterial culture. Group 3 and 4 patients were randomly chosen. The blood specimens were sent to the laboratory where serum was stored at −20 °C before testing. The laboratory staff performing the serological assay was blinded to the diagnoses of the study subjects.

2.2. Enzyme immunoassay of IgA antibodies against MAC-specific glycopeptidolipid core antigen

Serum IgA antibodies against the GPL core were measured using the EIA kit (Tauns Laboratory Inc., Shizuoka, Japan) according to the manufacturer's instructions. This test measures the anti-GPL core IgA antibodies in serum by the sandwich EIA method using microplates coated with a solid-phased antigen. An antibody titre of 0.7 U/mL or above is considered to be positive according to the recommendation of the manufacturer.

2.3. Statistical analysis

All statistical analyses were performed using Statistical Product and Service Solutions (SPSS) software. Patient groups were compared by Mann-Whitney test for the median and the student's T-test for the mean. Receiver operating characteristic (ROC) curves were drawn and the best cut-off points were determined by Youden's index for MAC-PD and MAB-PD.

3. Results

One hundred subjects were recruited into the study, among which 11 were excluded due to duplication or missing specimens. Eight-nine study subjects were assigned into the 4 groups accordingly. All participants were of Chinese ethnicity. Baseline characteristic and the antibody levels of the four groups of subjects were shown the Table 1 and Fig. 1. The mean antibody levels of the four groups were: 6.05 U/mL for MAC-PD, 6.09 U/mL for MAB-PD, 0.76 U/mL for TB and 2.3 U/mL for other pulmonary disease. A few of the subjects in the last two groups gave a positive antibody level making the mean levels of these two groups greater than the cut-off value as recommended by the manufacturer, their median antibody level was 0 U/mL. The TB group and the other lung diseases group were put together as control group for comparison with the antibody levels of the MAC group and the MAB group (Table 1 and Table 2). There were statistically significant differences in the mean and median antibody levels in the MAC group when compared to the control. For the MAB group, both mean and median of antibody levels were higher than the control group but only the difference in the median values reached statistical difference.

If one adopts the recommended cut-off value for positivity (0.7 U/mL) by the manufacturer, the sensitivity and specificity for diagnosis were about 73.7% and 77.6% for MAC-PD; 50% and 77.6% for MAB-PD. Receiver operating characteristic (ROC) curves (Fig. 2) were drawn with 75.6% and 70.4% area under curve for MAC-PD and MAB-PD respectively. By Youden's index, the best cut-off for MAC-PD and MAB-PD were 1.771 U/mL and 0.172 U/mL respectively (Table 3), and corresponding sensitivity and specificity were 68.4% and 86.2% for MAC-PD, while 66.7% and 72.4% for MAB-PD.

4. Discussion

The efficacy of enzyme immunoassay of IgA antibodies in diagnosis of MAC-PD was well demonstrated by studies on patients in Japan, the USA, and Taiwan [5–9]. With the cut-off value of 0.7 U/mL, the sensitivity and the specificity of the test was 69.6% and 90.6% respectively according to a meta-analysis by Shibata et al. in 2016 [10]. However, there is no study so far to evaluate its clinical efficacy in Hong Kong. Moreover, whether 0.7 U/mL is the optimal cut-off point for the local patient population remains unknown.

Most of the previous studies used normal subjects as the control group. With the purpose of simulating the real-life daily clinical scenario, patients with confirmed pulmonary tuberculosis and other lung diseases with no suggestions of NTM-PD were chosen as control subjects so as to demonstrate whether the test can differentiate true MAC-PD with other respiratory conditions. Our study showed that patients with MAC-PD have a significantly higher antibody level when compared to the controls and helped to differentiate MAC-PD with pulmonary TB and other lung diseases. The best cut-off for MAC lung disease as derived from our results was 1.771 U/mL, which is much higher than the recommended value. It was probably due to higher mean level of MAC antibodies found in a few patients with other lung diseases. The reason for that remained obscured. We conjectured that there might be a small number of patients in the control group having been exposed to MAC or having indolent MAC-PD and gave rise to a positive antibody response.

In our study, an elevated IgA antibody levels was also found in MAB-PD patients. A previous study by Jeong et al. [11] had demonstrated this finding and our findings largely collaborate with their results in that this EIA kit cannot differentiate MAC-PD from MAB-PD. Therefore, one needs to be cautious in the interpretation of a positive antibody result in our locality as MAB is also a very common NTM-PD in Hong Kong. Our data suggested that this test might help in differentiate MAB-PD from other lung diseases and further study is needed to show its clinical efficacy.

Recent studies [12,13] revealed that changes in the antibody levels may reflect disease activity and serial measurements of antibody levels may assist in the objective monitoring of the disease activity in individual MAC-PD patients. Further studies to compare the IgA antibody level before, during or after treatment might be indicated to explore its role in the monitoring of the course of the disease.

5. Conclusion

Our study showed that the enzyme immunoassay of IgA antibodies against MAC-specific glycopeptidolipid core antigen could help to distinguishing MAC and M. abscessus pulmonary diseases from pulmonary tuberculosis and other lung diseases among Hong Kong Chinese patients. Further larger scale studies in our local population for the

Table 1
The characteristics of the study subjects and the antibody level.

	All		MAC		MAB		TB		Others	
N, %	89	100	19	21	12	13	31	35	27	30
Gender, (M:F)			0.6:1		0.2:1		2.1:1		1.1:1	
Male	44	49	7	37	2	17	21	68	14	52
Female	45	51	12	63	10	83	10	32	13	48
Age	89		19		12		31		27	
Median (range)		65 (23–96)		62 (46–95)		64 (55–74)		71 (23–95)		60 (25–96)
Mean (SD)		64.6 (15.8)		62.26 (11.95)		64.75 (7.33)		67.77 (17.8)		62.52 (18.36)
Antibody	89		19		12		31		27	
Median (range)		0 (0–26.643)		3.456 (0–26.643)		2.1625 (0–23.058)		0 (0–9.841)		0 (0–21.702)
Mean (SD)		3.07 (5.79)		6.05 (7)		6.09 (8.19)		0.76 (2.42)		2.3 (5.16)
Test done	33	37	14	74	6	50	4	13	9	33

usefulness of this antibody test in the diagnosis and monitoring of MAC and *M. abscessus* lung diseases might be warranted.

Author contributions

All authors made substantial contributions to the concept or design of the study, acquisition of data, analysis or interpretation of data, drafting of the manuscript, and critical revision for important intellectual content.

7. Declaration

All authors have disclosed no conflicts of interest. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

CRediT authorship contribution statement

W.O Tam: Conceptualization, Methodology, Writing - original draft, Project administration, Writing - review & editing, Funding acquisition. **C.F Wong:** Conceptualization, Methodology, Supervision, Writing - review & editing. **S.S.Y. Wong:** Conceptualization, Methodology, Resources, Investigation, Writing - review & editing. **C.L.Y. Kwan:** Formal analysis, Visualization, Writing - review & editing.

Table 2
Comparison between groups (p-value shown).

	MAC vs MAB	MAC vs TB + Others	MAB vs TB + Others
Gender, M vs F	0.418	0.074	0.011
Age			
Mann-Whitney *U* test for median	0.372	0.237	0.703
T-test for mean	0.523	0.403	0.857
Antibody			
Mann Whitney-*U* test for median	0.774	< 0.001	0.012
T-test for mean	0.989	0.013	0.081
Test done, done vs not done	0.255	< 0.001	0.03

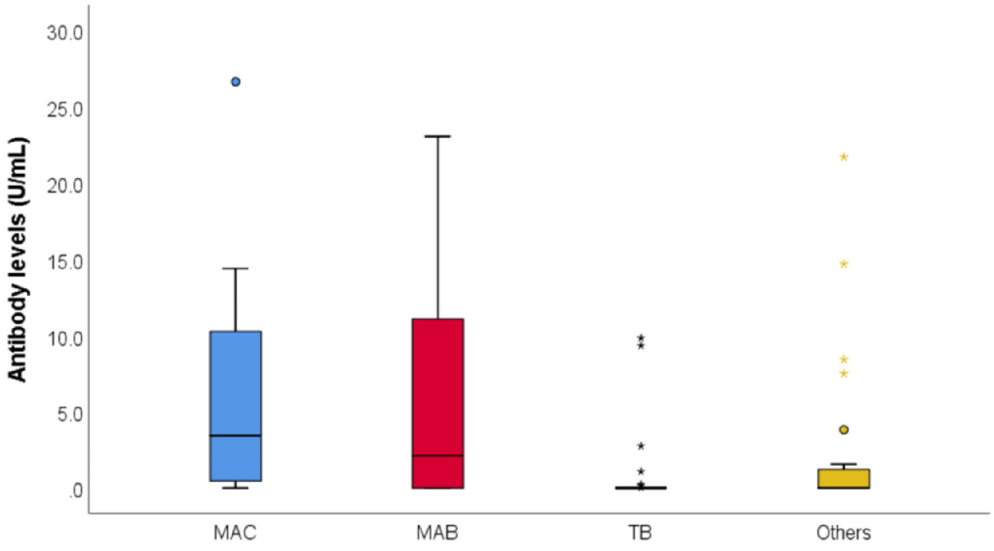

Fig. 1. Antibody levels of different group of patients.

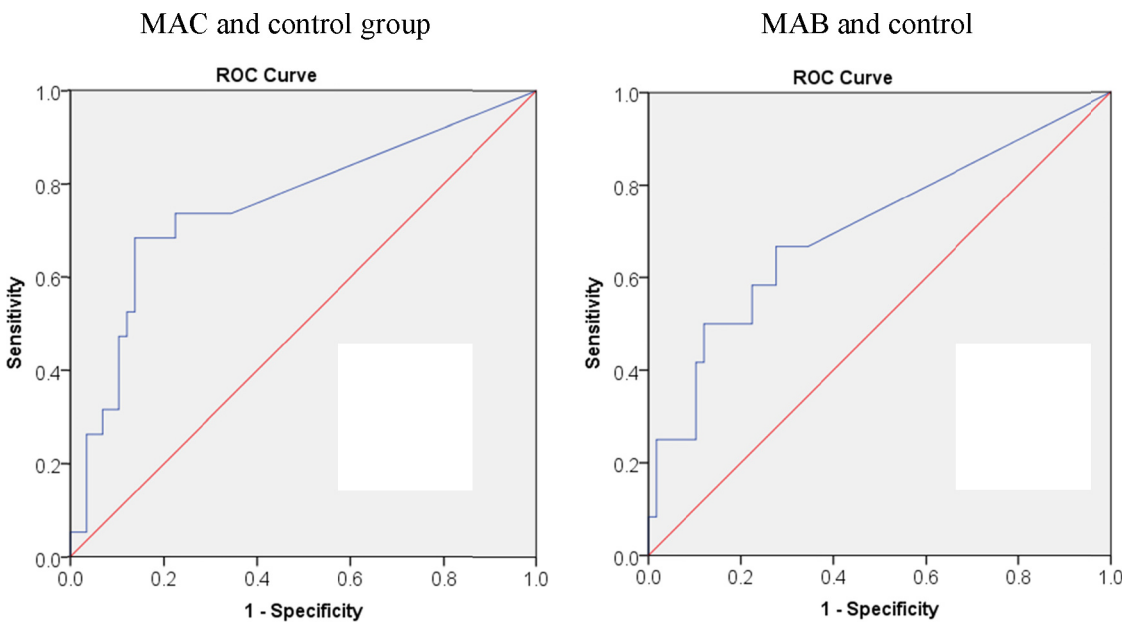

Fig. 2. Finding cutoff of antibody, discriminated by treatment–control group.

Table 3
Finding cutoff of antibody, discriminated by treatment–control group.

	Area under ROC	SE	95% Confidence interval	Best cutoff (by Youden's index)	P-value
MAC	75.6%	0.07	61.8–89.4%	1.771	0.001
MAB	70.4%	0.091	52.5–88.3%	0.172	0.027

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Ethics approval

The study was approved by Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKU/HAH KWI RB) (IRB Reference Number: UW 16-182).

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