Hong Kong Tuberculosis, Chest and Heart Diseases Association
Scientific Committee on Lung Health
Final report – Completion form

Important: Please submit the original copy and an electronic version of the Final Report and any attachments. Complete all sections with sufficient detail to allow review of the findings and effectiveness of the project. Incomplete or insufficiently detailed reports will be returned for revision and resubmission. The principal applicant is required to sign the Completion form.

1. **Project No.:** TB Project 3/2017

2. **Grant Period:** Commencement Date August 2017 End Date January 2020

3. **Title of Project:**
Serological diagnosis and monitoring of Mycobacterium avium complex lung diseases by Enzyme Immunoassay of IgA antibodies against MAC-specific glycopeptidolipid core antigen

4. **Principal Applicant:**
TAM Wai On

5. **Administering Institution:**
TB and Chest Unit, Grantham Hospital

6. **Aims/ Objectives of the research:** List the main objectives as stated in the approved proposal.

Approved aim/ objective	**Objectives / indicators achieved**				
	Low				High
	1	2	3	4	(5)
To evaluate an enzyme immunoassay EIA kit that detects serum IgA antibody against MAC-specific glycopeptidolipid (GPL) core antigen in the diagnosis of the Mycobacterium avium complex pulmonary diseases (MAC-PD) in Hong Kong Chinese patients. We plan to recruited patients with and without the disease and see whether this test can help to differentiate them.					

7. **Timetable of Work:** Document the study progress according to the proposed timetable.

Aug 2017	Start to recruit patient and collect blood sample
July 2018	Complete recruitment of patient
	Start the process of procurement of the test kit from Japan
Nov 2018	Present the interim report in the Meeting of Scientific Committee on Lung Health of HKTBA
	Test of the blood specimen completed.
Dec 2018	Start to analyze the data and write up the report
May 2019	Submit the paper to Hong Kong Medical Journal (rejected)
Jul 2019	Submit the paper to Journal of Clinical Tuberculosis and Mycobacterial Diseases

8. **Summary of the project and its results:**

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 to 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 the cut-off value set 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, the best cut-off for MAC-PD was 1.771 U/mL and for MAB-PD was 0.172 U/mL. Using this best cut-off, the sensitivity and specificity was 68.4% and 86.2% for MAC-PD; 66.7% and 72.4% for MAB-PD respectively.
Conclusions: This study showed that measurement of IgA antibodies against MAC-specific glycopeptidolipid core antigen may be useful in the diagnosis of MAC-PD among Hong Kong Chinese patients and helped to differentiate MAB-PD from other lung diseases.

9. **Final report (Please refer to the attached report)**

10. **Budget & expenditure (Please refer to the attached report)**

Signature of principal applicant	Tam Wai On **Name**	18 October, 2019 **Date**

Project Title

Serological diagnosis of Mycobacterium avium complex lung diseases by Enzyme Immunoassay of IgA antibodies against MAC-specific glycopeptidolipid core antigen

Reference No.

TB Project 3/2017

Investigators

Dr. W O Tam, Tuberculosis and chest unit, Grantham Hospital
Dr. C F Wong, Tuberculosis and chest unit, Grantham Hospital
Dr. Samson SY Wong, Department of Microbiology, The University of Hong Kong
Miss KWAN Crystal Lok Yan, Department of Surgery, The University of Hong Kong

Administering Institution

TB and Chest Unit, Grantham Hospital

Date of Submission

October 2019

Summary

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.

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Results: There were 100 subjects recruited into the study, among which 11 were excluded. By the cut-off value set 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, the best cut-off for MAC-PD was 1.771 U/mL and for MAB-PD was 0.172 U/mL. Using this best cut-off, the sensitivity and specificity was 68.4% and 86.2% for MAC-PD; 66.7% and 72.4% for MAB-PD respectively.

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Main Body of the Report

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 commonest infection. The diagnosis of MAC pulmonary disease (MAC-PD) is sometimes complicated. According to the most published guidelines [1,2], one needs to consider the clinical, radiological features and bacteriological results before one can make a diagnosis of lung diseases and to distinguish those from sporadic or transient colonization. Recently, an enzyme immunoassay (EIA) kit that detects serum IgA antibody against MAC-specific glycopeptidolipid (GPL) core antigen was developed by Kitada et al [3], and studies [4-7] in Japan and USA have found it useful in the diagnosis of MAC-PD, discriminating it from other lung diseases. However, it may not differentiate MAC-PD with all other NTM-PD. In the case of a lung infection with Mycobacterium Abscessus complex (MAB) which has GPL, a positive result may be obtained. There is study concerning its use on Chinese patients [8] but we do not have 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 diagnosis of the MAC-PD in Hong Kong Chinese patients.

Method

Study Patients and collection of serum

The study was conducted at Grantham Hospital, Hong Kong between July 2017 to July 2018. The hospital's research ethics committee approved the study and all participants provided written informed consent. Patients, who were less than 18 years old, pregnant, mentally incapacitated or unable to understand the consent were excluded. Serum was collected from 4 groups of patients after consent form were signed. The patients groups were: 1) MAC lung disease patients who met the ATS criteria; 2) Mycobacterium Abscessus lung disease patients who met the ATS criteria; 3) pulmonary

control to demonstrate whether the test can differentiate true MAC-PD with other respiratory condition. Our study showed that patient with MAC-PD has a higher level of antibody which reached statistical significance as compare to the control group. It showed that it can help to differentiate MAC-PD with pulmonary TB and other lung diseases. The best cut-off for MAC lung disease in our study was 1.771 U/mL which is much higher than the recommended level. It is probably due to higher mean level of MAC antibodies seen in patients with other lung diseases. The reason for that remained obscured. We postulated that there may be small number of undiagnosed MAC lung disease among this group which contributed to the increased mean level of antibody.

We found that level of serum IgA antibody was higher in both MAB and MAC patients. Previous study by Jeong [10] demonstrated similar findings and our study confirmed using this EIA kit cannot differentiate MAC-PD from MAB-PD. Therefore the interpretation of any positive result in our locality is needed to be cautious as MAB is one of the common causes of NTM-PD in Hong Kong. Our study suggested this test may help in differentiate MAB-PD with other lung diseases and further study is needed to show its clinical efficacy.

Recent studies [11, 12] revealed that the antibodies levels may reflect disease activity and serial measurements of antibody levels may allow objective monitoring of disease activity in individual MAC-PD patients. Further studies to compare the antibody level in different treatment phase could be helpful to assess the role of monitoring of the IgA antibodies.

Conclusion

Our results suggest Enzyme Immunoassay of IgA antibodies against MAC-specific glycopeptidolipid core antigen may be useful in making the diagnosis of MAC pulmonary disease among Hong Kong Chinese patients. It may also have a role in differentiating M abscessus lung disease with tuberculosis and other lung diseases. Further larger scale of study in our population for studying the role of test is warranted in future.

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										
Median (range)	89	65 (23-96)	19	62 (46-95)	12	64 (55-74)	31	71 (23-95)	27	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										
Median (range)	89	0 (0-26.643)	19	3.456 (0-26.643)	12	2.1625 (0-23.058)	31	0 (0-9.841)	27	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

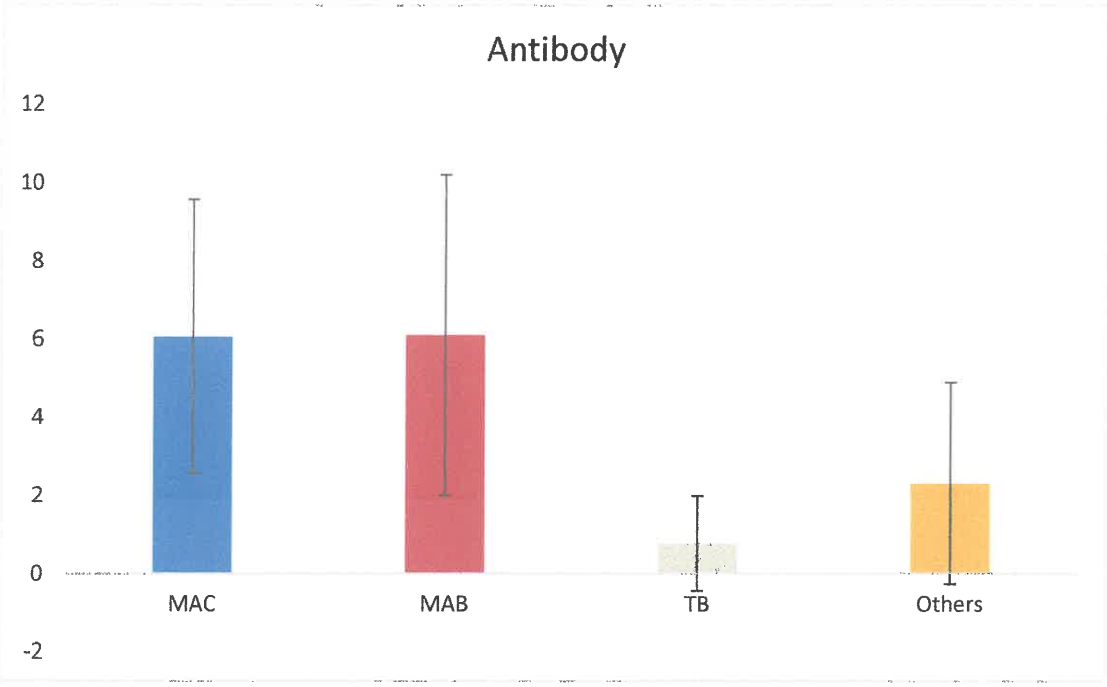

Figure 1 Antibody levels of different group of patients

Table 2 Comparison of antibody level of MAC patients with the control group

	Total		MAC		TB and Others		P-value
N, %	77	100	19	25	58	75	
Gender, M:F	1.2:1		0.6:1		1.5:1		0.074
Male	42	55	7	37	35	60	
Female	35	45	12	63	23	40	
Age							
Median (range)	77	65 (23-96)	19	62 (46-95)	58	66.5 (23-96)	0.237
Mean, SD		64.57, 16.77		62.26, 11.95		65.33, 18.1	0.403
Antibody							
Median (range)	77	0 (0-26.643)	19	3.456 (0-26.643)	58	0 (0-21.702)	<0.001
Mean, SD		2.6, 5.24		6.05, 7		1.48, 3.98	0.013
Test done	27	35	14	74	13	22	<0.001

Table 3 Comparison of antibody level of MAB patients with the control group

	Total		MAB		TB and Others		P-value
N, %	70	100	12	17	58	83	
Gender, M:F	1.1:1		0.2:1		1.5:1		0.011
Male	37	53	2	17	35	60	
Female	33	47	10	83	23	40	
Age							
Median (range)	70	66 (23-96)	12	64 (55-74)	58	66.5 (23-96)	0.703
Mean, SD		65.23, 16.71		64.75, 7.33		65.33, 18.1	0.857
Antibody							
Median (range)	70	0 (0-23.058)	12	2.1625 (0-23.058)	58	0 (0-21.702)	0.012
Mean, SD		2.27, 5.18		6.09, 8.19		1.48, 3.98	0.081
Test done	19	27	6	50	13	22	0.03

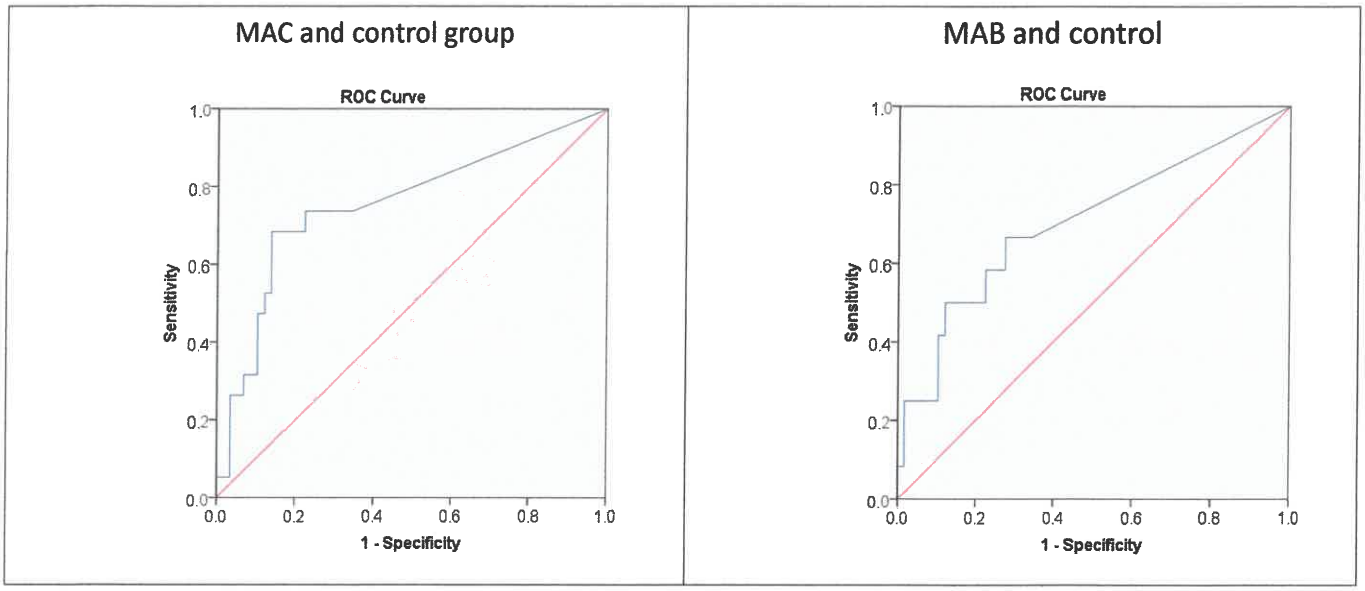

Figure 2 Finding cutoff of antibody, discriminated by treatment-control group

Table 4 Finding cutoff of antibody, discriminated by treatment-control group

	Area under ROC	SE	Confidence interval		Best cutoff (by Youden's index)	P-value
MAC	75.6%	0.07	0.618	0.894	1.771	0.001
MAB	70.4%	0.091	0.525	0.883	0.172	0.027

Reference

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