Original article:

Serum immunoglobulins and anti-pneumococcal antibody levels in patients with bronchiectasis of unknown aetiology

Mustafa Norhazlin[1], Asrul Abdul Wahab[2], Mohd. Faisal Abdul Hamid[3], Hamzaini Abdul Hamid[4], Husyairi Harunarashid[5]

Abstract

Background: Bronchiectasis is a chronic condition which can result in significant physical and social morbidity. The exact prevalence in Malaysia is unknown although several studies have shown a higher prevalence in the Asian population. Several causative factors have been identified but there are many patients with unknown aetiologies. This study looks into the level of serum immunoglobulins and anti-penumococcal antibody in bronchiectasis patients where they were not part of prior routine investigations. ***Methodology:*** Four hundred fifteen bronchiectasis patients were screened and 26 patients who fulfilled the inclusion and exclusion criteria were enrolled for this study. The serum immunoglobulins (IgG, IgA and IgM) concentrations were measured using nephelometry and interpreted according to age-matched reference range. The integrity of antibody production against specific antibody to capsular polysaccharides of *Streptococcus pneumoniae* were assessed using ELISA method and the level of ≥ 10mg/L is considered as reactive. ***Results****:* The twenty six bronchiectasis patients have the mean age of 62 years and a predilection of female gender. Majority of patients presented with typical bronchiectasis symptoms which were further supported by radiological findings. One of 26 patients (4%) had low total serum IgG level. The vaccinated group has higher anti-pneumococcal capsular polysaccharide antibody level (median: 224.2 mg/L) compared to the unvaccinated group (median: 100.4 mg/L). However there is no statistical difference between the anti-PCP levels of both groups (p> 0.05). All of the selected patients had reactive specific antibody to capsular polysaccharides of *Streptococcus pneumoniae* regardless of the vaccination status, which may reflect the natural acquisition of anti-pneumococcal immunity. ***Conclusion:*** Although immunoglobulin deficiency is an uncommon aetiological cause of bronchiectasis, the immunoglobulin parameters can be helpful in selecting patients who should receive the appropriate treatment of immunoglobulin therapy for the prevention of subsequent complications and better quality of life.

Keywords: anti-pneumococcal capsular polysaccharides antibody; bronchiectasis; humoral immunity; immunoglobulin level

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Introduction

Bronchiectasis is defined as an abnormal and irreversible dilatation of bronchi. It is a chronic condition, which can result in significant physical and social morbidity. Pathologically it is characterized by permanent dilatation of bronchi and bronchioles caused by destruction of the muscle and elastic tissue, resulting from or associated with chronic

1. Mustafa Norhazlin, Institute Medical Research, Kuala Lumpur, Malaysia
2. Asrul Abdul Wahab, Department of Medical Microbiology and Immunology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia
3. Mohd. Faisal Abdul Hamid, Department of Internal Medicine, UniversitiKebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia
4. Hamzaini Abdul Hamid, Department of Radiology, UniversitiKebangsaan Malaysia Medical Centre, Kuala Lumpur Malaysia
5. Husyairi Harunarashid, Clinical Epidemiology Unit, UniversitiKebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia

Correspondence to: Dr. Asrul Abdul Wahab, Department of Medical Microbiology & Immunology, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Cheras 56000 Kuala Lumpur, Malaysia. Tel: +603-91459530E-mail: saw@ppukm.ukm.edu.my

necrotizing infection[1]. This disease has the potential to cause devastating illness including chronic productive cough, airway obstruction, shortness of breath and recurrent infections. The prevalence of bronchiectasis is not well-defined. Some studies have shown that bronchiectasis was highest in the Asian population. A study in Korea has shown that about 9.1% of 1409 study subjects who were screened for respiratory disease using CT scan for a year eventually developed bronchiectasis[2].This disease can be further classified into cystic fibrosis (CF) bronchiectasis and non-cystic related. Bronchiectasis in adults are generally referring to non-CF forms. While there are many idiopathic causes, the common aetiologies include post infectious causes, genetic diseases, immune deficiencies and rheumatoid arthritis. Symptoms include chronic cough, mucopurulent sputum production, haemoptysis, breathlessness, and tiredness. Diagnosis of bronchiectasis is confirmed by high resolution computed tomography (HRCT) which is both sensitive and specific in detecting pathologically dilated bronchi[2].

Humoral immunity is a part of adaptive immunity and is highly specific for a particular pathogen or antigen. The immunoglobulin levels and integrity of antibody production are included play important roles in pulmonary host defense from bacterial or viral infections[3]. Humoral immunodeficiency was detected in 11% of adult patients with bronchiectasis, in whom most of the known causes had been ruled out[4,5].This study was done with main objectives of determining serum immunoglobulin and anti-pneumococcal antibody levels in adult patients with underlying bronchiectasis of unknown etiology. At the same time, the clinical and microbiological characteristics were described.

Material and Methods

Study population

Twenty six out of 415 patients, in whom the diagnosis of bronchiectasis were confirmed and fulfilled the inclusion and exclusion criteria were recruited by the respiratory physician. The exclusion criteria were patients who had been known to have congenital causes of bronchiectasis, pregnant ladies, patients with known underlying immunodeficiency and patients who are on immunosuppressive therapy or immunoglobulin therapy. All of these patients were either outpatients of the respiratory clinic or inpatients in Hospital Canselor Tuanku Muhriz Universiti Kebangsaan Malaysia Medical Centre (HCTM UKMMC) over one and half year period (March 2015 to September 2016).

Diagnosis

A radiological diagnosis of bronchiectasis was made on the HRCT if any of the following features were present:a lack of normal bronchial tapering in cuts parallel to the direction of travel on sequential slices, or bronchi those having an internal diameter greater than the diameter of the accompanying pulmonary artery (signet ring sign), or dilated bronchi visible adjacent to the mediastinal pleura[6,7].In all 26 patients, HRCT scans were performed by the radiologists in HCTM/ UKMMC prior to the recruitment in the study.

Demographical, clinical, radiological and microbiological data

The demographic data, clinical presentation at the onset of illness and radiological findings were gathered from the data collection sheets. The sputum microbiology for each patient were retrospectively collected from laboratory information systems since the patients started their follow-up in this hospital.

Assessment of humoral immunity

The humoral immunity was determined by measurement of immunoglobulin levels and the integrity of antibody production against certain antigen. For this study, the immunoglobulin (Ig) isotypes (IgG, IgA and IgM) concentrations were measured in all patients using nephlometry (Behring Nephlometer, Behringwerke, Marburg, Germany). Subsequently, the results of IgG, IgA and IgM were interpreted based on the age matched with the normalreferral range of each of the antibodies. To detect IgG anti-pneumococcal capsular polysaccharides (anti-PCP) antibody level, commercially available test kit (The Binding Site, Birmingham, United Kingdom) with pre-coated micro titer plates were used according to manufacturer's instructions.The antigen used in the assay for pneumococcus specific antibodies was composed of a mixture of capsular polysaccharide serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F and 33F which had the same composition as that of the licensed 23-valent vaccines. The level of ≥ 10mg/L was considered as reactive[8].

Statistical Analysis

Statistical analysis via SPSS version 23 (IBM Corporation, USA) was used throughout the study. Conventional descriptive measures of the

mean, median and spread (e.g. standard deviation, interquartile range) were derived for the continuous variable. The categorical variables were shown as percentages. Differences between means of groups were analysed by independent samples T-test and accepted as significant at $p < 0.05$. For anti-PCP antibody, the Mann Whitney U test was used as the data were not normally distributed.

Ethical approval

The study was approved by the research and ethical review boards of HCTM/ UKMMC with the code number of FF-2015-104.

Results

Patient demographic data, clinical, radiology and sputum microbiology

The gender, age at onset of the symptoms and smoking status are shown in Table 1. Female patients outnumbered male patient with the ratio of 3:1. The mean age is 62 and the age of the onset of the symptoms is 59 but both showed no significant gender difference. Majority of the patients are non-smokers. All female patients are non-smokers and 2 out of 7 male patients were ex-smokers.

Table 1: Patient data: Sex, age, age of onset of symptoms, smoking history.

	Patients			P value (male/ female)
	All (n=26)	Male (n=7)	Female (n=19)	
Mean age(±SD)	61.7(±11.5)	59.9(±13.6)	62.4(±11.0)	0.54
Range	37 – 78	39-76	37-78	
Mean age at onset of symptoms (±SD)	58.6 (±11.6)	56.7(±12.9)	59.3(±11.3)	0.69
Range	37-78	37-71	42 -78	
Smoking history				
Number of lifetime non-smokers/ total number of patients (%)	24/26 (92.3)	5/7 (71.4)	19/19 (100)	
Number of ex-smokers/ total number of patients (%)	2/26 (7.7)	2/7 (28.6)	0	

All but 4 of 26 patients had history of productive cough. More than half of the patients had dyspnoea and almost half of them had daily sputum production. Wheezing was noted in 31% of the patients and none of the patients had rhino sinusitis. The clinical symptoms and signs of the patients were summarized in Table 2.

Table 2: Clinical findings in 26 bronchiectasis patients at time of diagnosis.

Symptoms	N (%)
Chronic cough	24 (92)
Dyspnoea	15 (58)
Daily productive sputum	11 (42)
Reduced effort tolerance	11 (42)
Wheezing	8 (31)
Haemoptysis	4 (15)
Fever	3 (12)
Pleuritic chest pain	2 (8)
Fatigue	1 (4)
Rhino sinusitis	0 (0)
Signs	**N (%)**
Crackles	22 (85)
Rhonchi	6 (27)

A summary of radiology findings of the patients is shown in Table 3. Majority of the patients have both lower lobes involvement based on the findings of high resolution computed tomography and the right lower lobe was more predominant compared to the left lower lobe. More than two thirds of the patients had at least 2 lobes involvement with 3 of them having changes to all of the lungs' lobes.

Table 3: Radiology (High Resolution Computed tomography) findings of 26 bronchiectasis patients.

Involvement of lobe	N (%)
Right upper lobe	12 (46)
Right middle lobe	12 (46)
Right lower lobe	18 (69)
Left upper lobe	13 (50)
Left lower lobe	15 (58)
Number of involved lobes	**N (%)**
1	5 (19)
2	10 (39)
3	3 (12)
4	5 (19)
5	3 (12)

Sputum culture results are as shown in Table 4. *Pseudomonas aeruginosa* was the most frequently isolated organism, followed by *Klebsiella* species and *Haemophilus influenzae*. *Acinetobacter* species and *Pseudomonas* species had also been isolated in 2 patients each. One patient had *Streptococcus pneumonia* isolated from the sputum and another patient had *Staphylococcus aureus* isolated. Majority of the patients had normal mouth flora isolated at least once in their sputum samples.

Table 4: Sputum microbiology.

Organisms of significance isolate	Number of patients (%)
Pseudomonas aeruginosa	6 (25)
*Klebsiella*species	5 (20.8)
Haemophilus influenzae	3 (12.5)
*Acinetobacter*species	2 (8.3)
Pseudomonas species	2 (8.3)
Streptococcal pneumoniae	1 (4.2)
Staphylococcus aureus	1 (4.2)
Other	
Normal mouth flora	21 (87.5)
No organism isolated	2 (8.3)

Assessment of humoral immunity

Immunoglobulin level of the 26 patients is shown in Table 5 and Figure 1. No patients with panhypogammaglobulinaemia were identified. One patient was found to have low level of serum IgG, whereas her IgA and IgM level were within the low side of the normal referral range. The rest of the patients had the immunoglobulin levels within the referral range or slightly higher. The specific antibody titre to the pneumococcal capsular polysaccharide (anti-PCP) according to pneumococcal vaccination status was shown in Table 6 and Figure 2. In summary,the vaccinated patients have higher anti-PCP level than unvaccinated patients. However there is no statistical difference between the anti-PCP levels of both groups.

Table 5: Immunoglobulin isotypes level and immunity status (N=26).

Immmunoglobulin level	Median (mg/dL) (IQR)
IgG	1530 (1332.5, 2012.5)
IgA	367 (289.8, 442.8)
IgM	103.6 (72.5, 131)
Immunity status	**n(%)**
Panhypogammaglobulinaemia	0
IgG deficiency	1/26 (4)
IgA deficiency	0
IgM deficiency	0
Normal immunoglobulins	25/26 (96)

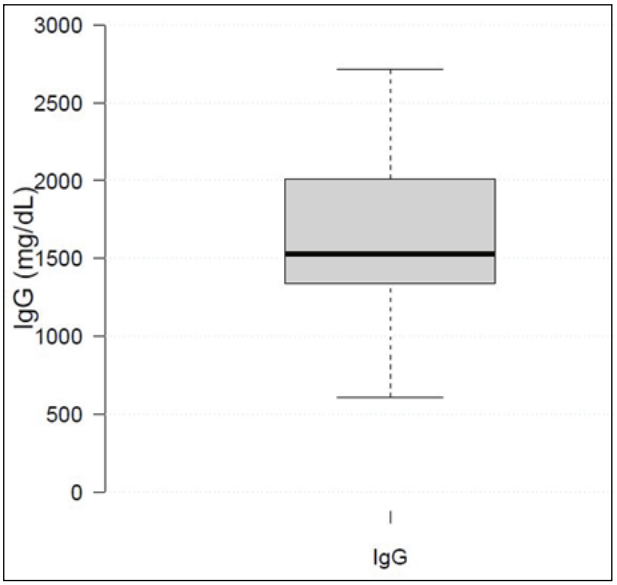

Figure : 1-A

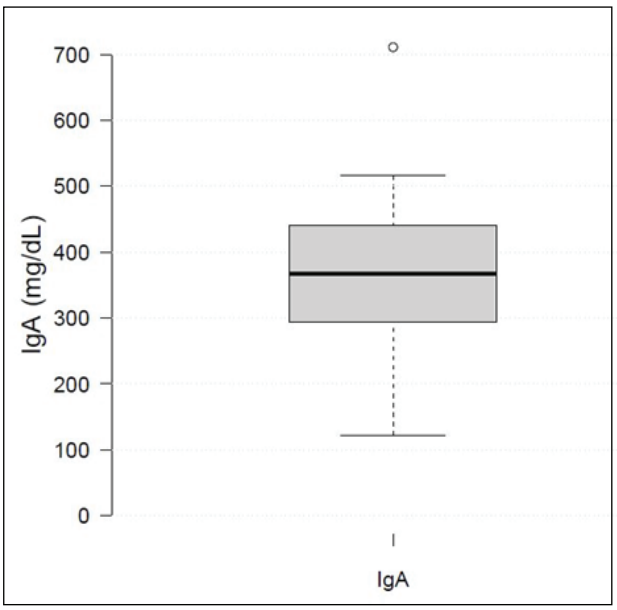

Figure : 1-B

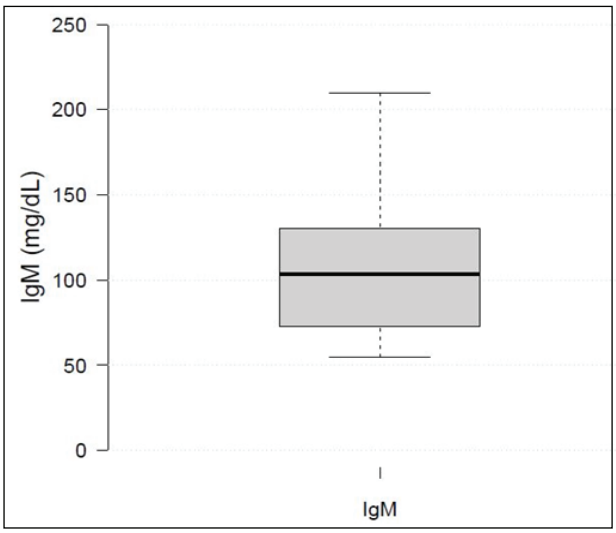

Figure : 1-C

Figure 1A: Level of IgG antibody; normal reference range: 751 -1560 mg/dL.Figure1B: Level of IgA antibody; normal reference range: 82 -453 mg/dL. Figure 1C: Level of IgM antibody; normal reference range: 46 -304 mg/dL.

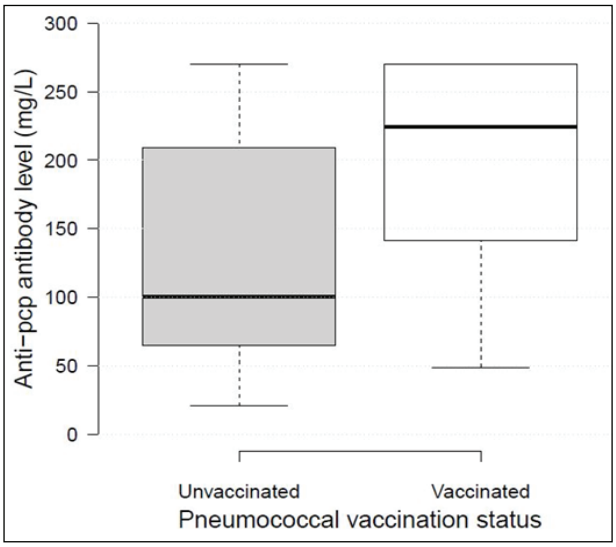

Figure 2: Anti-PCP levels according to pneumococcal vaccination status.

Table 6: Anti-PCP* antibody levels according to pneumococcal vaccination status.

	Patients		p value (vaccinated/ unvaccinated)
	Vaccinated (n=9)	Unvaccinated (n=17)	
Median anti-PCP antibody (IQR) mg/L	224.2 (141.2 – 270) mg/L	100.4 (65.2 – 209) mg/L	0.119

PCP = pneumococcal capsular polysaccharide

Discussion

We assessed 26 patients who fulfilled the exclusions and inclusions criteria and therefore, the study does not provide data on the prevalence of bronchiectasis. In this study, most of the patients were female, with the ratio of 3:1, which is similar to other previous findings[2,3,9]. Women have increased predisposition to develop bronchiectasis. Nevertheless, the reason of sex difference remains elusive. There is similarity on the age distribution of male and female which indicates that the lung damage process happens at the same rate of both sexes[3]. Majority of the patients are lifetime non-smokers in line with several studies. However, we were unable to exclude the secondary smoking exposure among these patients.

The clinical characteristics at presentation of the patients of bronchiectasis in our study are consistent with bronchiectasis presentation. Symptoms include chronic productive cough, wheeze, dyspnoea and repeated respiratory infections[10].Indeed, this disease should be suspected in any non-smoking patient who presented with all of these symptoms[7]. A number of patients had complained of pleuritic chest painwhich may reflect the presence of distended peripheral airways or distal pneumonitis adjacent to a visceral pleural surface[11].In contrast with other studies, none of our patients have the rhinosinusitis symptoms. There is possibility that these symptoms were overlooked by the patients as part of the flu symptoms which were considered not related with their other respiratory symptoms. Majority of our patients had crackles, which was the main finding in the physical examination of bronchiectasis patients. This abnormal respiratory sound are commonly bilateral and bibasal[12].

The HRCT scan findings in the present study showed lesions occurred more on the bilateral lower lobes.A study by Karadag et al, have shown that bronchiectatic lesions were most commonly found in the lower lobes, as the mucociliary clearance is facilitated by gravity in the upper lobes[13]. Idiopathic bronchiectasis also has been reported to be predominantly basal in distribution[6]. Whereas, in hypogammaglobulinaemia, bronchiectasis mainly affects the lower lobes, middle lobes and lingula with none of the upper lobes involved. A study done in Taiwan had shown that the both upper lobes and right middle lobes were affected in majority of their patients who had been diagnosed with pulmonary tuberculosis[2]. Whether the distribution of bronchiectasis might be sufficiently characteristic for a specific cause to be diagnosed

remains controversial. Li et al, have reported that the distribution ofbronchiectasis overlapped greatly among the various causesand no association between the modalities could be established[6].

The sputum microbiology revealed spectrum of organisms that are commonly found in bronchiectasis. The most common significant isolates among our patients were *Pseudomonas aeruginosa* (23.8%), followed by *Klebsiella* species (19%) and *Haemophilus influenzae (*14.3%). *Pseudomonas aeruginosa* and *Haemophilus influenzae* are found commonly in bronchiectasis and patients who have poor lung functions[1,12]. Of note, *Haemophilus influenzae* has been reported in 14 (52%) and *Pseudomonas aeruginosa* in 12 (43%) of patients with non-CF bronchiectasis[14]. These pathogens can develop the means to facilitatetheir own survival, overcoming host defense mechanismsand antimicrobial actions through biofilm production andother bacterial resistance mechanisms[15]. In contrast with other studies, *Klebsiella* species was the second most common isolated in our patients. The possible explanation may be due to this bacterium also known to cause necrotizing infections of the lung, especially in patients who have impaired respiratory host defences[16].

In this study only one patient (4%) was found to have low serum IgG levels with low-normal serum IgA and IgM. The identification of one case of reduced IgG can lead to the treatment of immunoglobulin replacement therapy. The patient also needs to be investigated for any underlying immunodeficiency such as common variable immunodeficiency (CVID) or IgG subclass deficiency. CVID is the most common adult primary immunodeficiency, with an estimated prevalence of 1 in 25,000 in Caucasians[17]. It is defined clinically and immunologically by low serum immunoglobulin concentrations of one or more isotypes, defective specific antibody responses and clinically increased susceptibility to bacterial infections[18]. Previous studies so far have revealed varying percentages of patients with bronchiectasis havingsome kind of abnormal antibody disorder, ranging from 4% to 50%.The difference in the percentage of antibody deficiency may be due to the type of population studied, the different selection criteria and the utilized methods**,** thephysicians' awareness and the quality of health system[19].

Almost half of the patients had IgG level above the 50th percentile of the study population. This finding is partly expected as some of the patients were inpatients who had been admitted for recurrent chest infections or exacerbation of their illnesses. Possibility of the increased level of IgG due to autoantibodies should be interpreted cautiously. It is crucial to correlate with the history as well as clinical presentation. Although the association between bronchiectasis and autoimmune disease such as rheumatoid arthritis is well recognised, autoantibodies should not be routinely tested for during the investigation of a patient with bronchiectasis; they should only be tested for if there are particular clinical features raising autoimmunity as a possible association[20]. A number of bronchiectasis patients may have presented with normal immunoglobulin level but have reductions in certain subclasses, which are not determined in this study. The relationship between serum IgA and IgG with sputum IgA and IgG; as well as the IgG subclasses concentrations, are important and would determine whether local immunoglobulin deficiency, rather than systemic deficiency, can be implicated in the disease process[21].

All of the subjects regardless of the vaccination history have developed anti-PCP antibody. The findings may reflect the natural acquisition of anti-pneumococcal immunity. However the value in the normal range does not necessarily indicate effective protection against every given strain of *Streptococcus pneumoniae*. To date 90 serotypes have been identified throughout the world[22]. In addition, the response to the pneumococcal serotypes is heterogeneous between individuals even of the same age group[8]. The vaccinated group have higher anti-PCP level as compared to the unvaccinated group. It is important to these patients to get their vaccinations accordingly despite of the reactive antibody measured. The latest recommendation from the Advisory Committee Immunization Practices (ACIP) is to give a dose of Pneumococcal conjugate vaccine (PCV13) followed by a dose of Pneumococcal polysaccharide vaccine (PPSV23) at least 1 year later for all adults 65 years of age, including those who had been diagnosed with chronic lung diseases[23].

There are some limitations to the present study. First, the sample size was small as the study population involved the bronchiectasis patients with unknown aetiology. Moreover, the exact prevalence of local adult bronchiectasis patients is unknown. Secondly, the level of anti-PCP antibody were measured at a single time point and no longitudinal findings could be used for comparison.The assessment of specific antibody production (anti-PCP antibody) is best

evaluated pre and post immunization to look for the differences in the response towards the immunization and subsequently to diagnose an antibody production deficiency. The control group is needed to establish the normal total antibody response and the isotypes to the vaccine. The reference value of the anti–PCP was based on a study done by Schauer et al in Germany[8]. It is noted that at the present time, there are no universal criteria for adequate antibody response to polysaccharides and it is suggested that each laboratory to establish its own[24]. All of these limitations must be considered when extrapolating the results to other groups and further study is suggested for a proper evaluation. In this study, we described the clinical features at presentation and the humoral immune status of adult patient with bronchiectasis of unknown aetiology in one of tertiary centre in Malaysia.Although the findings have shown that the humoral immunity deficiency is uncommon, the identification of any immunoglobulin deficiency is crucial as it has a significant impact on management and prognosis. A large scale patient enrolment involving more patients from other centres may help to better investigate the humoral immunity deficiency.

Conclusion

It is proposed that all patients with bronchiectasis of unknown aetiology should undergo thorough immunological evaluation in order to identify the presence of any underlying immunological defect. By determining the levels of all immunoglobulin isotypes and the specific antibody, it may assist the attending physician in adopting a more aggressive treatment in patients very susceptible to infection and lung disease. In fact, the diagnosis could lead to the appropriate treatment, prevention of the subsequent complications and eventually a better quality of life.

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Conflict of Interest

The authors declared no conflict of interest.

Author's contribution

Data gathering and idea owner of this study: Norhazlin M, Wahab AA

Study design: Norhazlin M, Wahab AA, Harunarashid H, Hamid MFA, Hamid HA

Data gathering: Norhazlin M, Hamid MFA, Hamid HA

Writing and submitting manuscript: Norhazlin M, Wahab AA

Editing and approval of final draft: Wahab AA

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