Role of thymus and activation-regulated chemokine in diagnosis of allergic bronchopulmonary aspergillosis

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Background
Allergic bronch opulmonary aspergillosis (ABPA) is a pulmonary disorder, occurring mostly in asthmatic patients and patients with cystic fibrosis, caused by an abnormal T-helper 2 lymphocyte response of the host to *Aspergillus fumigatus* antigens.

Objective
To assess the value of thymus and activation-regulated chemokine (TARC) in the diagnosis of ABPA.

Patients and methods
A total of 75 consecutive patients, comprising 21 males and 54 females, with age range from 20 to 70 years, with mean age of 48 years, complaining of bronchial asthma and suspected to have ABPA with measurements of serum total immunoglobulin E (IgE), specific *A. fumigatus* IgE, and TARC, were included.

Results
Compared with other serological markers of ABPA, it was revealed that TARC was superior to the other markers for diagnosis of ABPA. The sensitivity, specificity, and diagnostic accuracy for diagnosis of ABPA were as follows: 93.3, 100, and 91.7%, respectively, for TARC; 67.1, 93.3, and 63%, respectively, for total IgE; and 60, 100, and 62.4%, respectively, for specific *A. fumigatus* IgE.

Conclusion
TARC marker has high sensitivity and specificity and can help in early diagnosis of ABPA.

Keywords:
allergic bronchopulmonary aspergillosis, bronchial asthma, diagnostic value, immunoglobulin E, thymus and activation-regulated chemokine

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Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a pulmonary disorder caused by hypersensitivity to *Aspergillus fumigatus* that complicates the course of patients with asthma and cystic fibrosis (CF). It presents with varied clinical and radiological manifestations, usually with uncontrolled asthma and recurrent pulmonary infiltrates with or without bronchiectasis [1]. The disease remains underdiagnosed in many countries, and as many as one-third are misdiagnosed as pulmonary tuberculosis in developing countries [2]. The study of the role of different immunological mediators in the formation of chronic allergic inflammation in patients with ABPA is necessary for early diagnosis of the disease and identification of potential targets for therapeutic intervention. One of these different immunological mediators is thymus and activation-regulated chemokine (TARC) marker (also known as CCL17) [3]. Serum levels of CCL17 (also known as TARC) are elevated in patients with CF with ABPA. CCL17 is a chemokine produced as a result of the antifungal immune response. Recently, elevated levels of CCL17 have been confirmed as a sensitive and specific marker of ABPA in patients with CF with diagnostic accuracy greater than total serum immunoglobulin E (IgE) [4]. If unrecognized or poorly treated, ABPA leads to airway destruction, bronchiectasis, and/or pulmonary fibrosis, resulting in significant morbidity and mortality [5]. Early diagnosis and treatment are essential in preventing end-stage progression. The development of ABPA is probably the combination of many genetic susceptibility factors, gene–gene interactions, and environmental exposure, which work together. There are different diagnostic criteria that have been used in the diagnosis of ABPA and dividing asthmatic patients into ABPA and non-ABPA.

Diagnostic criteria for allergic bronchopulmonary aspergillosis

Several diagnostic criteria for patients with asthma-ABPA have been suggested over the past

several decades (Table 1). In 1977, Rosenberg and colleagues formulated diagnostic criteria, which are still widely accepted and included seven relevant findings, defined as primary criteria [1]: asthma reactivity to *A. fumigatus* [2], serum eosinophilia [3], precipitating antibodies against *A. fumigatus*, and [4] central/proximal bronchiectasis. A few years later, eosinophilia and pulmonary infiltrates ceased to be considered essential for ABPA diagnosis, because these findings can be present only during exacerbations. In 1986, Patterson and colleagues highlighted that bronchiectasis is considered a major criterion for ABPA diagnosis and can be absent in early-stage ABPA. Therefore, in 1991, diagnostic criterion was revised by classifying patients with ABPA into two categories: ABPA with central bronchiectasis and ABPA-S (ABPA seropositive without bronchiectasis) [4]. Agarwal and colleagues proposed a new set of diagnostic criteria with the aim of overcoming the traditional classification and suggesting an optimum cutoff value for total serum IgE levels while not considering bronchiectasis, which can develop much later in ABPA natural history. Agarwal and colleagues established that ABPA diagnosis should first be formulated by the presence of two obligatory criteria, that is, positivity of skin prick test and/or elevated serum IgE to *A. fumigatus* and total serum IgE levels more than 1000 IU/ml, and at least two of three other criteria, including precipitins or IgG antibodies to *A. fumigatus*, radiological pulmonary findings consistent with ABPA, and total eosinophil count more than 500 cells/μl (in patients not treated with steroids) [1].

The aim of this study was to assess the value of TARC in diagnosis of ABPA.

Patients and methods

This study was approved by our institutional ethical committee. A total of 75 patients were subjected to laboratory investigations, including total IgE, serum specific IgE, and serum TARC marker between January 2018 and February 2020.

An informed consent was obtained from all individual participants included in the study.

Patients were referred to our Clinical Pathology Department from Chest Department, Faculty of Medicine, Assiut University Hospital, complaining of bronchial asthma and suspected to have ABPA.

All patients were subjected to the following: full medical history, full clinical examination, plain chest radiograph and/or computed tomography of chest, and laboratory investigations. These laboratory investigations included complete blood count, which was done on CELL-DYN Ruby, Abbott (Lake Bluff, Illinios, Unites States); total IgE, which was done on ADVIA centaur, Siemens (Munich, Germany, Europe); and serum TARC marker level and serum specific IgE by enzyme-linked immunosorbent assays (ELISA).

Table 1 Diagnostic criteria for asthma-allergic bronchopulmonary aspergillosis patients [4]

Rosenberg *et al.* [6]	Schwartz and Greenberger [7]	Agarwal *et al.* [1]
Primary criteria	ABPA-CB	Obligatory criteria
Asthma	Asthma	Positive skin test and/or serum IgE to *Aspergillus fumigatus*
Elevated total serum IgE levels	Positive skin test to *Aspergillus fumigatus*	Elevated total serum IgE levels
Immediate skin reactivity to *Aspergillus fumigatus*	Elevated total serum IgE levels	Other criteria (≥2)
Serum eosinophilia	Serum IgE and IgG to *Aspergillus fumigatus*	Precipitins or serum IgE to *Aspergillus fumigatus*
Precipitins	Central bronchiectasis	Serum eosinophilia
Central bronchiectasis	ABPA-S	Radiological pulmonary findings consistent with ABPA
History of pulmonary infiltrates	Asthma	
	Positive skin test to *Aspergillus fumigatus*	
	Elevated total serum IgE levels	
	Serum IgE and IgG to *Aspergillus fumigatus*	
	Additional findings	
Secondary criteria	Mucous plugs	
Aspergillus fumigatus in sputum	*Aspergillus. fumigatus* in sputum	
History of brown plugs or flecks expectoration	Precipitins	
Late skin reactivity to *Aspergillus fumigatus*	Parenchymal infiltrates	
	Delayed positive skin test	

ABPA, allergic bronchopulmonary aspergillosis; ABPA-S, ABPA seropositive without bronchiectasis; CB, central bronchiectasis; Ig, immunoglobulin.

Sample collection, storage, and handling

Random blood sampling was done, where 6 ml of venous blood was collected under complete aseptic conditions and divided into the following:

(1) 2 1 of venous blood was collected into EDTA-containing tube for complete blood count.
(2) 4 ml was collected into plain tube without anticoagulant. Blood was allowed to clot for 10–20 min at room temperature and centrifuged at the speed of 2000–3000 rpm for 20 min.
    (a) Serum was collected and divided into two aliquots: these two aliquots were stored at −80°C till time of assay of TARC marker, total IgE, and specific IgE levels.

Principle of the test of serum TARC marker level determination:

Serum TARC was measured by ELISA technique using human TARC ELISA Kit, Catalog No: SG-11462, purchased from SinoGeneClon Biotech Co. (China). The kit is used for the quantitative level of human TARC in the sample. Purified TARC antibody is coated on the microtiter plate, making solid-phase antibody, then TARC is added to the wells, then combine TARC antibody with labeled horseradish peroxidase to form antibody–antigen–enzyme–antibody complex, followed by washing completely, and then adding 3,3′,5,5′-tetramethylbenzidine substrate solution. 3,3′,5,5′-tetramethylbenzidine substrate becomes blue color at horseradish peroxidase enzyme catalyzed. The reaction is terminated by the addition of a stop solution, and the color change is measured at a wavelength of 450 nm. The concentration of TARC in the samples is then determined by comparing the optical density of the samples to the standard curve.

Statistical analysis

Date entry and data analysis were done using SPSS, version 24 (version 24; SPSS Inc., Chicago, Illinois, USA). Data were presented as number, percentage, mean, and SD. χ^2 test was used to compare between qualitative variables. Independent sample t test was used to compare quantitative variables between groups. P value was considered statistically significant when P value less than 0.05. Paired t test was used to compare between variables in the same group. Moreover, using Pearson correlation between variables in the same group.

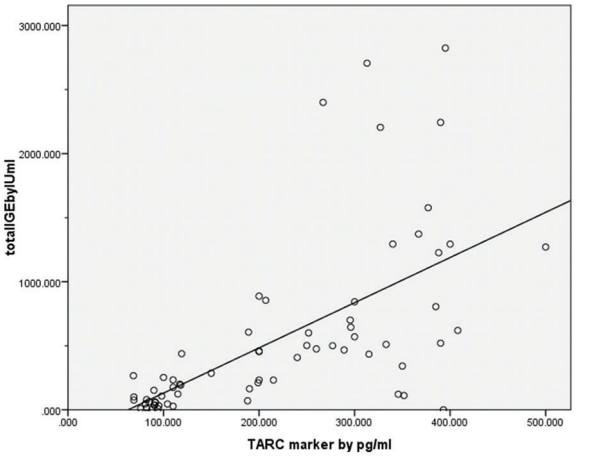
Results

A total of 75 patients were enrolled in the study who were divided into two groups: 67 patients in ABPA group and eight patients in non-ABPA group.

TARC marker achieved the best sensitivity, specificity, and diagnostic accuracy in comparison with other serological markers in diagnosis of ABPA (Tables 2–4). Moreover, it could diagnose the same number of patients who were diagnosed by other serological markers combined in definite criteria.

There was a positive correlation with highly significance difference ($P < 0.000$) between TARC, total IgE levels and specific IgE to *A. fumigatus* (Figs. 1 and 2).

Figure 1



Correlation between TARC and total IgE. IgE, immunoglobulin E; TARC, thymus and activation-regulated chemokine.

Table 2 Sensitivity and specificity of thymus and activation-regulated chemokine marker

Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
69.8	93.3	100	100	91.4	91.7

NPV, negative predictive value; PPV, positive predictive value.

Table 3 Sensitivity and specificity of total immunoglobulin E

Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
688.56	67.1	93.3	83.3	16.7	63

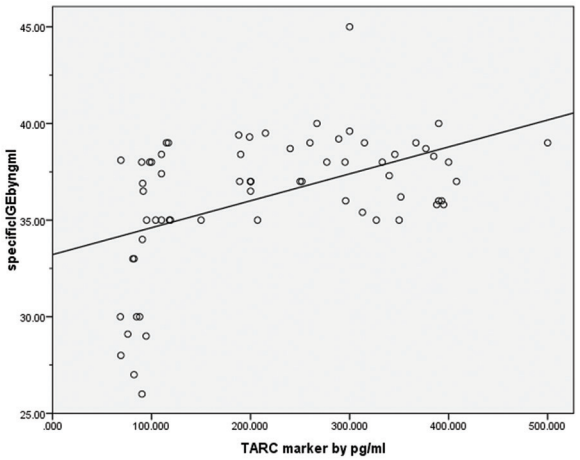
NPV, negative predictive value; PPV, positive predictive value.

Table 4 Sensitivity and specificity of specific immunoglobulin E

Cutoff	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Diagnostic accuracy (%)
36.39	60.0	100	100	33.3	62.4

NPV, negative predictive value; PPV, positive predictive value.

Figure 2



Correlation between TARC and specific IgE. IgE, immunoglobulin E; TARC, thymus and activation-regulated chemokine.

Discussion

Our study showed that the sensitivity of total IgE was 67.1%, the specificity was 93.3%, and diagnostic accuracy was 63%, when the cutoff value was 688.56 IU/ml. However, the study by Latzin *et al.* [8] showed the sensitivity was 64.7%, the specificity was 81%, and diagnostic accuracy was 74.3% when the cutoff value was 514 IU/ml. However, in the study of Singh *et al.* [9] using the cutoff of 1200 IU/ml, the sensitivity was 88.5% and the specificity was 70.5%. However, the study of Leonardi *et al.* [4] showed the sensitivity was 39% and the specificity was 100% when the cutoff value was more than 1000 IU/ml. The current study showed that specific IgE at cutoff value of 36.39 ng/ml had the sensitivity of 60%, the specificity of 100%, and the diagnostic accuracy of 62.4%, whereas the study of Agarwal *et al.* [10] showed that the sensitivity was 100% and the specificity was 69.3%, using cutoff value of more than 0.35 kUA/l. The present study showed that the sensitivity of TARC was 93.3%, the specificity was 100%, and diagnostic accuracy was 91.7%, using the cutoff value of 69.8 pg/ml. However, the study of Latzin *et al.* [8] showed that the sensitivity was 91.8%, the specificity was 94.7%, and diagnostic accuracy of 93.4% using the cutoff value of 386 pg/ml. Because TARC gave the best sensitivity, specificity, and diagnostic accuracy in comparison with other serological markers, so it could be used in the diagnosis of ABPA instead of other serological markers.

Conclusion

ABPA is an immunologic pulmonary disorder caused by hypersensitivity to *A. fumigatus*. ABPA in asthmatic patients is a devastating disease that requires early diagnosis and subsequent treatment. From these reports mentioned above, TARC marker achieved the best sensitivity, specificity, and diagnostic accuracy in diagnosis of ABPA in comparison with other serological markers. It would seem that TARC marker was superior to the other markers and can be used in diagnosis of ABPA instead of other markers.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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