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The usefulness of serum adenosine deaminase 2 (ADA_2) activity in adults for the diagnosis of pulmonary tuberculosis

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Abstract Rapid diagnosis of *Mycobacterium tuberculosis* remains an obstacle for therapy of tuberculosis (TB). Adenosine deaminase isoform 2 (ADA_2) is produced by activated macrophages and has been used for diagnosis of TB from extra-pulmonary sites. However, few studies adequately address whether serum ADA_2 activity is useful for diagnosis of active pulmonary tuberculosis (PTB). We prospectively measured serum ADA_2 activity in 110 patients with pulmonary disease (65 cases with active PTB and 45 cases with other respiratory diseases) and 78 healthy volunteers (eight with tuberculin skin test positive). The serum ADA_2 for the diagnosis of PTB had the sensitivity of 36·9%, the specificity of 84·5%, the positive predictive value of 10·9% and the negative predictive value of 96·2%. We concluded that serum ADA_2 activity is neither useful to diagnosis of active PTB nor to differentiate from other respiratory diseases.

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Keywords tuberculosis; adenosino deaminase; pulmonary infection; diagnosis.

INTRODUCTION

Pulmonary tuberculosis (PTB) remains one of the leading causes of morbidity and mortality worldwide accounting for approximately 8 million new cases and 2 million deaths each year (1). Although mycobacterial culture is sensitive and the standard for diagnosing tuberculosis (TB), the time to diagnosis requires a minimum of 2–3 weeks, and the acid-fast bacilli (AFB) smear, the rapid screening method for the diagnosis of PTB, is insensitivity for detecting mycobacteria among TB patients with non-cavitary disease (2). Compounding the need for better laboratory diagnosis of PTB is the difficulty to clinically discriminate TB from non-TB infectious respiratory diseases or lung cancer.

Adenosine deaminase (ADA, adenosine aminohydrolase, EC 3·5·4·4) is an enzyme released by monocyte/macrophages that transforms, respectively, adenosine and deoxyadenosine to inosine and deoxyinosine (3). The increased serum level of ADA has been reported for viral and bacterial pneumonia, for HIV-infection and for extra-pulmonary TB (3,4). In fact, diseases caused by intracellular micro-organisms are characterized by an elevated level of ADA in serum (3). Since *Mycobacterium tuberculosis* (Mtb) infects lung macrophages, ADA may be released and be detected in serum of patients with active PTB. There is limited data on the use of serum ADA levels to diagnose active PTB in adults (5). Two studies in children reported that serum ADA levels in PTB but they lacked appropriate comparison to other respiratory diseases (6,7). The purpose of this study was to evaluate whether total serum level of ADA or ADA_2 activity can discriminate active PTB from other lung diseases and from healthy volunteers.

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METHODS

Prospectively, 135 subjects (age 18 yrs and older) who presented for evaluation of pulmonary disease at the Pulmonary Diseases Unit, Hospital Universitario

Clementino Fraga Filho (HUCFF) of Universidade Federal do Rio de Janeiro (UFRJ), Brazil, and had respiratory symptoms and radiographic findings suggestive of PTB were enrolled after informed consent and blood was obtained. In addition, blood samples of 78 symptom-free healthy subjects without history of active PTB were also included as controls. The study was approved by the Ethics Committee of HUCFF/UFRJ. Serum separated from blood from each subject was stored at -20°C and thaw once for the ADA assay.

On study enrollment, the 135 patients with symptoms and signs of respiratory disease were administered a questionnaire, received a physical examination, chest radiograph, and HIV test (with Western Blot confirmation) and a sputum was collected for routine bacterial culture, mycobacterial culture and AFB smear. Patients unable to expectorate spontaneously underwent sputum induction (SI) or fibre optic bronchoscopy with bronchoalveolar lavage (BAL) performed by clinicians not involved with the research. All healthy controls received a tuberculin skin test (TST) using 2TU of Purified Protein Derivative (PPD Rt-23, 2 TU, Serum Institute, Denmark) and induration equal to or greater than 10 mm was considered positive.

All respiratory specimens were stained with haematoxylin and eosin, Papanicolaou, Ziehl-Neelsen stain, Grocot's methenamine silver stain, and were cultured in Löwenstein Jensen, Sabouraud medium, and routine bacterial culture following standard protocol. The specimens cultured positive for mycobacteria were speciated by standard biochemical methods. Active PTB was defined as a positive culture for Mtb in one or more pulmonary specimen and resolution of clinical and/or radiographic abnormalities after 3 months of standard anti-tuberculosis treatment. Inactive (pulmonary scarring) PTB was defined as negative mycobacterial culture with unchanged chest radiographic findings consistent with healed tuberculosis. A diagnosis of bacterial, fungal or viral infections was made on the basis of clinical, radiographic, and laboratory findings associated with clinical and/or radiographic resolution of abnormalities after treatment with antimicrobial therapy other than anti-TB drugs or only with supportive measures. Lung cancer was diagnosed by histopathology or cytopathology examination of specimen obtained by bronchoscopy. Patients were excluded if the HIV test was positive or if during the 3 months prior to enrollment have received any antibotic treatment for more than 1 week. All enrolled patients were followed for at least 6 months.

ADA activity was determined according to the Giusti's method (8). When high ADA activity was detected, isoenzymes were differentiated according to Gakis' methodology (3). The mean serum ADA levels for each group was assessed by one way ANOVA test. The diagnostic usefulness of ADA was assessed in terms of sensitivity and specificity. Positive and negative predictive values were calculated based on an estimated prevalence of 5% of active pulmonary TB among patients with respiratory symptoms in Brazil (9). The cutoff ADA_2 levels for diagnosis was obtained from the receiver operating characteristic (ROC) curve method.

RESULTS

One hundred and ten patients out of the 135 with pulmonary symptoms suggestive of PTB were evaluable. Twenty-five patients were excluded because of incomplete follow-up (18 cases), previous anti-TB treatment (three cases) and lost pulmonary specimens (four cases). The analysis of ADA levels used the 110 available patients. Sixty-five patients had active PTB and 45 patients were found to have other respiratory disease: inactive TB (12), acute infectious pneumonitis (21), lung cancer (11) and paracoccidioidomycosis (1). Of the 78 healthy controls, eight were tuberculin skin test positive. Patients with other respiratory diseases tended to be older than either TB patients or healthy controls (Table 1).

The ADA levels for each group are presented as means and as absolute value (Table 1, Fig. 1). The serum ADA activity cutoff value for the diagnosis of PTB established by the ROC curve method 14 U/l. Forty nine percent (32/

Table 1. Gender, age, total ADA value and number of cases of ADA and ADA_2 among 188 individuals studied

Groups (*n*)	Gender	Age	ADA		
	male	mean ± SD (yrs)	mean ± SD (U/l)	ADA > 14 U/l *n*	ADA_2 > 14 U/l *n*
Active PTB (65)	23	38·4 ± 14·9	16·5 ± 9·9 (a)	32	24
Other respiratory diseases (45)	19	44·5 ± 13·5	15·6 ± 9·1 (b)	20	14
Healthy volunteers (78)	39	23·9 ± 7·3	11·4 ± 6·3 (c)	23	5

PTB=pulmonary tuberculosis; SD=standard deviation; ADA=adenosine deaminase; ADA_2=adenosine deaminase isoform 2; *n*=number of cases; a *vs.* c (P=0·001); a *vs.* b (P=1·0); b *vs.* c (P=0·02)

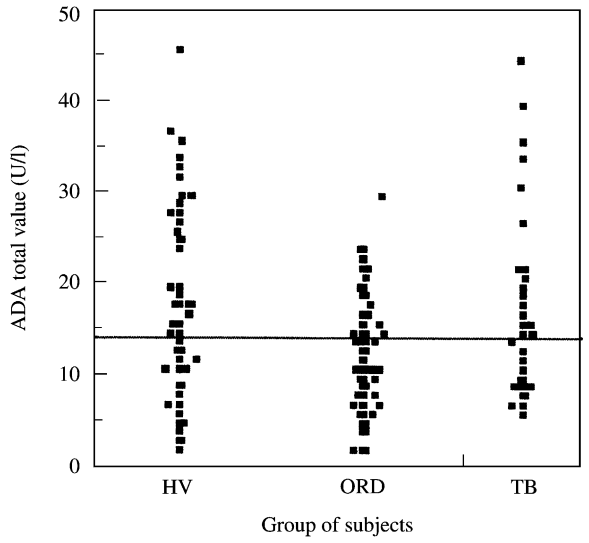

Fig. 1. ADA total value by group of subjects. HV: healthy volunteers; ORD: other respiratory diseases; TB: pulmonary tuberculosis.

65) of patients with active PTB had serum values of ADA levels above 14 U/l. In addition, 44·4% (20/45) of patients with other respiratory diseases had ADA levels above the cutoff point. Of the healthy controls, 29·5% (23/78) were above the cutoff value. Although ADA levels discriminated patients with active PTB from healthy controls, similar mean ADA levels were found between patients with active PTB and patients with other respiratory diseases. We also utilized the cutoff point of 14 U/l of total ADA and ADA isoform-2 (ADA_2) to evaluate the sensitivity and specificity of the assay. The sensitivity of ADA for diagnosing active PTB is 36·9% [95% confidence interval (CI); 33·3%–40·4%] and the specificity was 84·5% [95% CI; 81·8%–87·1%]. If the prevalence of PTB for patients with respiratory symptoms is 5%, the calculated positive predictive value (PPV) is 10·9% and the negative predictive value (NPV) is 96·2%.

DISCUSSION

Monocyte/macrophage activation by intracellular infection and inflammatory diseases leads to the release of ADA and elevated levels in serum. Although the ADA assay has been reported for TB using pleural, pericardial and meningeal fluids, few studies with appropriate comparison controls have critically evaluated serum ADA levels as a diagnostic tools for active PTB. Using an ADA cut-off obtained by ROC method of 14 U/l, we found that 49·2% (32/65) patients with active PTB had serum ADA value significantly elevated (P=0·001) compared to healthy controls. Similar findings were reported by Collazos *et al*. (5) in which 52% (13/25) patients with active PTB had serum ADA above the upper normal limit. However, in our study, the mean serum ADA value among patients with active TB was similar to patients with other respiratory diseases (P=1·0). This result is in disagreement with the report by Yasuhara *et al*. (10) in which serum ADA activity of children with active PTB was found to be significantly greater than those with bacterial or viral pneumonia. Possible explanations for the differences found between our study and that of Yasuhara *et al*. may include our older sample age, different ethnicity and the prevalence of different respiratory diseases.

Our findings of low sensitivity (36·9%) and low positive predictive value (10·9%) of serum ADA_2 activity for the diagnosis of active PTB suggest that ADA is not clinically relevant. Although two other studies have reported that serum ADA level in children had a sensitivity level of 100% in diagnosing active PTB, these series have not included a comparison group with other respiratory diseases (6,7). The finding of a negative predictive value of 96% means that most individuals with ADA_2 equal or below 14 U/l did not have active PTB. The negative predictive value of ADA is likely to be accurate because a large sample of controls was used in this study. However, additional studies are needed in order to evaluate the utility of serum ADA_2 as measured to exclude TB. We concluded that serum ADA_2 activity is increased in about half of all adults with active PTB, but was unable to differentiate active PTB from other respiratory diseases, and likely not to be clinically useful in TB prevalent areas.

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