NON-SPECIFIC BIOLOGICAL MARKERS AS A SCREENING TEST FOR DIAGNOSTIC OF EXTRAPULMONARY TUBERCULOSIS

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Abstract - Serum concentrations of adenosine deaminase were determined in 223 febrile patients. In 62, we discovered extrapulmonary tuberculosis. Serum levels of immunoglobulin G were monitored in 287 febrile patients, and 68 had extra-pulmonary tuberculosis. Serum concentrations of adenosine deaminase were significantly higher in patients with tuberculosis compared to other patients with fever of unknown origin. Serum concentrations declined during antituberculosis therapy. A correlation with the localization of infection was not found. Levels of immunoglobulin G were higher in patients with tuberculosis. Both tests had high sensitivity and specificity and could therefore be used for screening extrapulmonary tuberculosis; however, they can only be interpreted adequately following a full clinical investigation.

Key words: Tuberculosis, adenosine deaminase, immunoglobulin G, fever of unknown origin, laboratory tests

INTRODUCTION

Tuberculosis (TB) is an infection with human strains of *Mycobacterium tuberculosis* mycobacterium that is typified by a characteristic immune response. The most common form of the disease is pulmonary which is essential for the spread of TB. Tuberculosis can affect any organ in the body. The term extrapulmonary tuberculosis (EPTB) refers to isolated TB at any site in the body outside the lungs.

Until the appearance of human immunodeficiency virus (HIV) the incidence of tuberculosis in the world was declining, but in the 1980s, tuberculosis once again became a disease of increasing interest. (Dye et al.*,* 1999) In the period 1985-1992 there was an increased incidence of tuberculosis in both developing and developed countries, including the United States, and this trend continues (Golden and Vikram, 2005). It is estimated that around the world two billion people are infected with tuberculosis, of which about 8 million develop active TB annually, and around 2 million die (Dye et al. 1999). Of particular concern is the evidence that the incidence of tuberculosis and EPTB is increasing everywhere in the world including the HIV-negative population (Dye et al.*,* 1999, CDC 2005).

During the late 1990s, TB incidence leveled off after a long period of increasing trend during the preceding decades (Gledovic et al.*,* 2006, with an increasing incidence in elderly patients. (Pesut et al.*,* 2008)

The incidence of TB in Serbia, according to data of the Institute of Public Health in 2010 was 19.6 per 100,000. (www.batut.org.rs 2011). There are no data on the incidence of extrapulmonary tuberculosis.

Among patients with fever of unknown origin (FOU), the number of cases of extrapulmonary tuberculosis as an etiological factor increased. (Pelemis et al., 2009)

As TB can affect virtually any organ or organ system in the body, clinical EPTB varieties are numerous, which makes the diagnosis difficult. The determination of adenosine deaminase levels is used as one of the tests to prove serosal tuberculosis. There is also some elevation in serum concentrations that could be used as a screening test. (Gupta et al., 2010)

There are limited data about the elevation of serum IgG concentration during TB (Buckley and Dorsey, 1970). The majority of authors have studied populations of patients with pulmonary TB (Giasudin, 1990, Alarcon-Segovia and Fishbein, 1971). During the past years we have observed an elevation of immunoglobulin G (IgG) concentration in the serum of patients with EPTB. In the literature, there are also several tests for the detection of specific anti-TB antibodies, which are in use (Baig et al., 1986, Chandramuki et al., 1989)

The objective of this study was to compare the serum concentrations of adenosine deaminase and immunoglobulin G antibodies concentration in patients with EPTB and patients presenting fever of unknown origin, and to assess the existence of any predictive value of adenosine deaminase serum concentration to outcome.

PATIENTS AND METHODS

The subjects were patients of the Department of Clinical Pharmacotherapy, Institute for Infectious and Tropical Diseases, Clinical Center of Serbia, who were hospitalized for investigation of fever of unknown origin. During the period January 2007 to December 2011, the adenosine deaminase levels in 223 patients were measured during diagnostic FOU. In 62 of the patients tuberculosis infection was proved by some of the methods used (Group I), while 161 did not have tuberculosis (Group II). All patients were HIV negative.

During same period, the IgG serum concentration was measured in 287 patients. In 68 of them TB infection was diagnosed (Group III). Other causes of FUO were determined in 219 patients (Group IV).

All the patients underwent physical examinations and all the diagnostic procedures required for the diagnosis of FUO. In addition to standard examinations (X-ray of the lungs, abdomen echo), and standard biochemical tests, and depending on the presentation of illness, intravenous pielonephrography (IVP), abdomen, chest or pelvic CT scan, echocardiography, computed tomography (CT) and nuclear magnetic resonance (NMR) of the spine were carried out. The equipment and services of the Radiology Service of Clinical Center of Serbia were used. The histological processing of tissues of clinical interest (biopsy of the liver, LGL, peritoneum, small bowel and adnexa) were also conducted. Histological processing of tissue samples was performed at the Institute of Pathology, Medical Faculty, University of Belgrade.

The level of adenosine deaminase was measured by enzymatic method, the test "ADA tiazyme labs" on the device Olympus AU 680, from blood samples collected in a test tube without additives. The level of reference values specified by the laboratory was 0-24 U/L. We excluded from the study patients with proven infectious mononucleosis due to the specific pathogenesis of the disease where high levels of adenosine deaminase are expected. In all patients, the concentration of adenosine deaminase was measured in the initial diagnostic protocol. Control values were observed 1, 2, 6 and 12 months after the start of treatment in patients who proved to have specific infections. For immunoglobulin G serum concentration, a turbidimetric method on a Turbox-Orion device was used. The reference range of concentration was 7.0-15.0 g/L. Blood samples were collected in the same test tube as for adenosine deaminase measurement.

Patients with proven tuberculosis infection were treated for two months with four drugs (Rifampin, Isoniazid, Pyrazinamide, Ethambutol), and then an-

Graph 1. localisation of EPTB
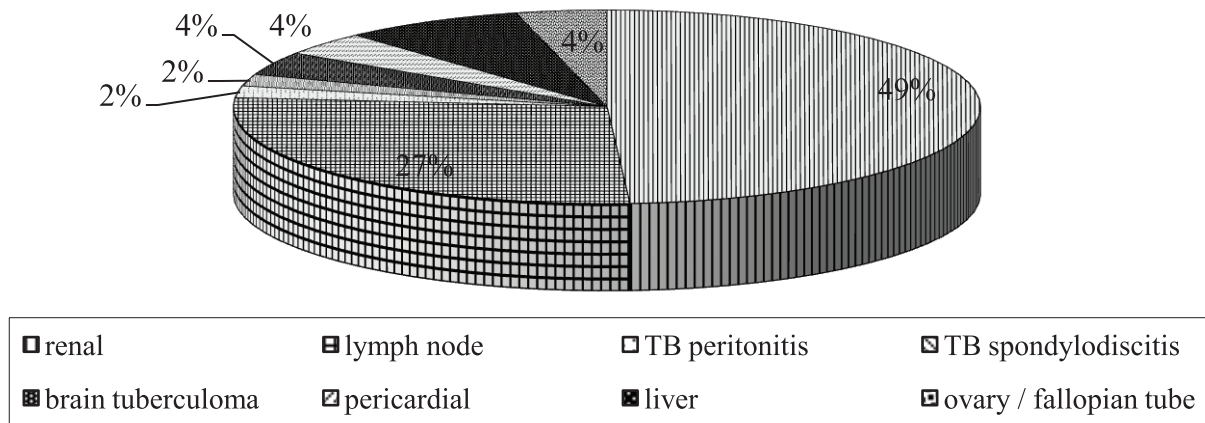
other four months with two drugs (Rifampin, Isoniazid).

Due to the heterogeneity of data, although the observed parameters were continuous, we used nonparametric tests (χ^2 test). The study was approved by the Clinical Center of Serbia Ethics Committee.

RESULTS

Adenosine deaminase was determined in 223 patients. Male and female distribution was approximately equal. In group I, there were 30 men and 32 women; in group II there were 79 men and 82 women. Most patients were between the ages of 40 and 60 years.

Serum IgG concentration was measured in 287 patients; the distribution by sex was also equal. Group III included 34 males and 34 females. In group IV, 105 males and 114 females were observed. The age pattern was the same as in the adenosine deaminase group.

Recognized risk factors for TB include concomitant chronic diseases and/or corticosteroid use, familial case of diagnosed TB family and/or personal history.

Risk factor distribution is shown in Table 1.

In our patients, EPTB was most frequently localized in the urogenital tract and lymph nodes: other localizations were less present (Graph 1).

Using a group of patients with fever of unknown origin (without proven specific infection or infectious mononucleosis) as a control for comparison with the group of EPTB patients, we obtained a highly statistical significant incidence difference (p <0.001) of elevated adenosine deaminase concentrations (Table 2).

From these data, the calculated sensitivity was 0.79, specificity 0.68, positive predictive value 0.48 and negative predictive value 0.89. In addition, the average concentrations of adenosine deaminase showed a statistically significant difference (a decline in value) as a function of time after treatment began (Table 3).

The initial concentration of adenosine deaminase did not show a statistically significant predictive value in the success of treatment.

The average serum IgG concentration was significantly higher in patients with EPTB (20.8±7,0 g/L)

Table 1. Risk factor distribution between patient groups.

	Chronic condition present	Positive TB history
Group I	9 (14.5%)	17 (27.4%)
Group II	39 (24.2%)	15 (9.3%)
Group III	11 (16.1%)	17 (25.0%)
Group IV	55 (25.1%)	23 (10.5%)

Table 2. The frequency of elevated values for adenosine deaminase in patients with fevers, for patients with proven EPTB, and patients without TB

		Adenosine deaminase concentration		Total
		elevated	normal	
Specific infection	was proven	49	13	62
	has not been proven	52	109	161
	Total	101	122	223

Table 3. Average concentration of adenosine deaminase in the beginning, one, two, six and twelve months after commencement of treatment in 62 patients with EPTB

Months of starting treatment	0	I	II	VI	XII
Mean concentration ± SD	30±11	19±9	11±3	8±3	5±2

Table 4. The frequency of elevated IgG concentration in patients with fevers, patients with proven EPTB, and patients without TB

		IgG serum concentration		Total
		elevated	normal	
Specific infection	was proven	53	15	68
	has not been proven	63	156	219
	Total	116	171	287

compared to other FUO patients (15.3±3.1g/L). The incidence of elevated IgG concentration had a highly statistical significance between the EPTB and other FUO patients (p<0,001) (Table 4).

The calculated sensitivity was 0.78, specificity 0.71, positive predictive value 0.45 and negative predictive value 0.91.

DISCUSSION

Definitive diagnosis of tuberculosis includes a demonstration of the presence of *M. tuberculosis* by microbiological, cytological or histopathological methods. Classical methods of TB diagnostics have significant limitations for the diagnosis of EPTB.

Cultivation is too long for these patients who often require rapid diagnosis. Material for PCR diagnosis is often not possible to be obtained (except cerebrospinal fluid and urine), and histopathological confirmation requires biopsies, which are invasive. The most reliable diagnostic criterion is still to confirm the presence of bacilli in the patient material by cultivation (Pelemis et al., 1999, Agadi, 2003, Radford, Rothel, 2003). In some forms, EPTB material can be taken for cultivation (renal, meningeal, pleural, and pericardial). In these cases, unfortunately, successful cultivation is very variable; the pleural fluid ranges from 12-70% (Valdes et al., 2003, Light, 2000, Ferrer, 1997), the pericardial fluid performance is 25-60% (Fowler, 1991, Trautner, Darouiche, 2001, Strang et al., 1987), and cerebrospinal fluid ranges from 40-

80% (Thwaites et al., 2002, Nikolic, 2007, Ibrarullah et al., 2002)

Because of these problems in exact diagnosis of tuberculosis, numerous additional tests are in use with the intention to facilitate the diagnosis.

In the recent use are, among others, tests that determine the biological (biochemical) markers of tuberculosis infection, such as levels of adenosine deaminase concentration (ADA) or the level of interferon gamma. Adenosine deaminase (ADA) is an enzyme in the purine salvage pathway that catalyzes the conversion of adenosine and deoxyadenosine to inosine and deoxyinosine with the release of ammonia. This is an important enzyme in T-lymphocytes, where it is in a 10 times higher concentration than in erythrocytes. Its activity increases during the reproduction and response to antigenic stimulation of lymphocytes. (Sharma et al., 2001) Therefore, its increased concentration may be found in all fluids occurring in the zones of tuberculosis serositis (Gupta et al., 2010, Mathur et al., 2006), used in diagnostics.

So far, in the literature and practice the measurement of the concentration of adenosine deaminase in effusions has proved to be a tuberculosis etiology marker of great importance.

Piras et al. were the first to report high ADA in tubercular pleural effusions (Piras et al., 1978). Meta-analysis of studies conducted between 1966 and 1999 concluded that the test performance was reasonably good (Goto et al., 2003) in diagnosing tuberculosis etiology in pleural effusion, with a sensitivity range of 47.1- 00%, and specificity 0-100%. In 2007, a systematic review of ADA by the NGS Health Technology Assessment Program concluded that there is no evidence to support the use of ADA tests for the diagnosis of pulmonary TB. However, there is considerable evidence to support their use in pleural fluid samples for the diagnosis of pleural TB, where sensitivity was very high, and to a slightly lesser extent for TB meningitis. In both pleural TB and TB meningitis, ADA tests had higher sensitivity than any other tests (Dinnes et al., 2007). Also, in Serbia, Zaric and

colleagues have shown the importance of ADA in the differential diagnosis of pleural effusions (Zaric et al., 2007). Other researchers have also observed the usefulness of ADA activity in the diagnosis of tuberculosis (Prasad et al., 1991, Mishra et al., 1995).

Other causes for the increase in ADA activity include bacterial infections, rheumatic disease and lymphoproliferative disorders. The article reviews of Gupta et al. present ADA estimation as an effective diagnostic criterion for tuberculous and non-tuberculous diseases in pleural, ascitic, synovial fluids and CSF (Gupta et al., 2010).

In extrapulmonary disease, this study the overall sensitivity to be 94.29%, specificity 92.16%, positive predictive value 89.00% and negative predictive value 95.92%; and in pulmonary disease, sensitivity was found to be 92.80%, specificity 90.00%, positive predictive value 92.86 % and negative predictive value 90.00%.

From the above it is evident that the determination of this enzyme was a reliable and useful test for the detection of tuberculosis infection in serous spaces (pleural, peritoneal, synovial space, cerebrospinal fluid) (Gupta et al., 2010, Zaric et al., 2007, Blake and Berman, 1982). Srinivasa et al. found elevated serum adenosine deaminase levels in patients with pulmonary tuberculosis (Srinivasa et al., 1982). There are no data in the literature that anyone from Serbia has measured the level of adenosine deaminase in serum in order to diagnose EPTB. In our sample, the average value of its concentration was elevated. Similar data were presented by Mishra and colleagues in child populations, where in addition to lung tuberculosis they had patients with miliary and extrapulmonary localization (Mishra et al., 2000).

In our sample, standard deviation was high, and consequently we had a relatively low but satisfactory resulting sensitivity and specificity. The negative predictive value of this test is high and this gives it a place as a widely usable screening test to exclude EPTB. In our results we could not compare with data from the literature since we did not find any data on

the sensitivity, specificity or the positive or negative predictive value of serum adenosine deaminase.

As the localization of TB infection was diverse, with a relatively small number of individual cases, we could not determine whether the higher value of this test correlated with particular localization.

Higher adenosine deaminase levels were found in patients with rheumatoid arthritis and other autoimmune diseases, which is in agreement with the findings of other authors (Hitoglou et al., 2001).

Serum immunoglobulin concentrations reflect the activity of humoral immunity that is active in a variety of infections and other pathological conditions (West et al., 1962). This concentration had some ethnic and age-related variations (Belldegrin et al., 1980; Al-Tawil, Thewaini, 1978). In other studies, correlations between IgG serum concentration and TB were found, but the majority of authors present only patients with pulmonary TB. In the Buckley study, the results included patients with extrapulmonary and pulmonary TB (Buckley and Dorsey, 1970). Elevated levels of IgG were founded in TB patients. We found similar results in our group of patients with EPTB. Relatively high values of specificity, sensitivity and negative predictive value give the serum IgG concentration a potential role in the differential diagnosis of patients with FUO, as biological marker of TB.

CONCLUSION

Increased concentrations of serum adenosine deaminase have shown potential as a usable screening test. To determine definitively the reference values for serum adenosine deaminase and to assess fully its diagnostic significance requires a significantly larger sample. Elevated serum concentrations of IgG in febrile patients can be an indicator of possible EPTB infections. For now, the serum concentration of adenosine deaminase and elevated serum IgG concentration can be used as an indicative EPTB parameter. These findings should be considered as part of a complete clinical description of the disease.

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