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THE VALUE OF ADENOSINE DEAMINASE (ADA) DETERMINATION IN THE DIAGNOSIS OF TUBERCULOUS ASCITES

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SUMMARY

In order to evaluate the role of the determination of adenosine deaminase activity (ADA) in ascitic fluid for the diagnosis of tuberculosis, 44 patients were studied.
Based on biochemical, cytological, histopathological and microbiological tests, the patients were divided into 5 groups: G1 - tuberculous ascites (n = 8); G2 - malignant ascites (n = 13); G3 - spontaneous bacterial peritonitis (n = 6); G4 - pancreatic ascites (n = 2); G5 - miscelaneous ascites (n = 15).
ADA concentration were significantly higher in G1 (133.50 ± 24.74 U/l) compared to the other groups (G2 = 41.85 ± 52.07 U/l; G3 = 10.63 ± 5.87 U/l; G4 = 18.00 ± 7.07 U/l; G5 = 11.23 ± 7.66 U/l).
At a cut-off value of >31 U/l, the sensitivity, specificity and positive and negative preditive values were 100%, 92%, 72% and 100%, respectively. ADA concentrations as high as in tuberculous ascites were only found in two malignant ascites caused by lymphoma.
We conclude that ADA determination in ascitic fluid is a useful and reliable screening test for diagnosing tuberculous ascites. Values of ADA higher than 31 U/l indicate more invasive methods to confirm the diagnosis of tuberculosis.

KEYWORDS: Tuberculous peritonitis; Adenosine deaminase; Ascites.

INTRODUCTION

Tuberculous peritonitis is a frequent cause of ascites in developing countries and may reach mortality rates of 50 to 60% when left untreated[1,4,14]. However, over the last few years, an increase in both the pulmonary and extrapulmonary forms of tuberculosis has been observed also in developed countries[5,13] due to the epidemics of Acquired Immunodeficiency Syndrome (AIDS). Recent data[13] have indicated that the incidence of tuberculosis among AIDS patients has been progressively increasing over the last ten years at a rate of 3 to 6% per year, and that the extrapulmonary forms have increased at a rate of 20% a year during the same period of time.

The insidious nature of tuberculous peritonitis often causes its diagnosis to be a challenge. Furthermore, it may occur in patients with other conditions presenting ascites, a fact that greatly hampers diagnosis. Noninvasive diagnostic methods have low sensitivity and specificity or are extremely time consuming. On the other hand, invasive methods such as laparoscopy and

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laparotomy increase mordibity among seriously ill patients.

Over the last few years it has been demonstrated that adenosine deaminase (ADA) determination has high sensitivity and specificity for the diagnosis of pleural, meningeal and pericardial tuberculosis when applied to pleural, cerebrospinal and pericardial fluid, respectively[2,4,6,11,14]. Recently, ADA determination has also been found to be an effective method for the diagnosis of tuberculous peritonitis[4,6,11,13,14]. ADA is an enzyme of purine catabolism which catalyzes the conversion of adenosine to inosine. It is secreted by lymphocytes and, to a lesser extent, by macrophages during activation of the cell immune response to mycobacterial antigens.

To evaluate the importance of ADA determination, we conducted a prospective study in which we determined its sensitivity and specificity for the diagnosis of tuberculous ascites.

CASES AND METHODS

The study was conducted on 53 patients with ascites who sought Hospital São Paulo, São Paulo, Brazil, from March to September 1994. The following tests were performed on ascitic fluid: biochemical determinations (glucose, DHL, total protein, albumin, amylase and triglycerides), cytology, culture for aerobes and mycobacteria, oncotic cytology, and search for *Mycobacterium tuberculosis* by the method of Ziehl-Nielsen.

ADA activity was determined by the colorimetric method of GIUSTI [7] which is based on the measurement of ammonia produced when adenosine deaminase acts on excess adenosine. The results are reported as U/l.

Of the 53 patients evaluated, 44 completed the study of ascitic fluid and were divided into 5 groups according to the following criteria:

Group 1

Tuberculous peritonitis: 8 patients with a positive *M. tuberculosis* search and/or culture or with histology of a peritoneal fragment (obtained by laparoscopy, laparotomy or at autopsy) compatible with a diagnosis of tuberculosis.

Group 2

Malignant peritoneal infiltration: 13 patients, 8 of whom presenting positive oncotic cytology and/or anatomopathological examination compatible with malignancy. In the remaining cases, laparoscopy or laparotomy revealed metastases to the peritoneum. Anatomophatological examination revealed a lymphoma in two cases.

Group 3

Spontaneous bacterial peritonitis (SBP): 6 patients with cirrhosis diagnosed by clinical, biochemical and histological criteria and with ascitic fluid cellularity showing more than 250 polymorphonuclears/mm^3 or more than 500 cells/mm^3 with a predominance of neutrophils and/or positive bacterial culture, according to RUNYON[10].

Group 4

Pancreatic ascites: 2 patients with amylase levels in ascitic fluid of more than 500 mg/dl and whose abdominal tomography confirmed the presence of acute or chronic pancreatitis.

Group 5

Miscellaneous: 15 patients with ascites of different causes: 12 descompensated chronic types of liver disease, 1 case of congestive heart failure, 1 case of chronic renal failure, and 1 case of pancreatitis with secondary bacte-

TABLE 1

Laboratory parameters concerning the ascitic fluid of the patients studied.

	Group 1	Group 2	Group 3	Group 4	Group 5
% lymphocytes	89 ± 8.9	78.1 ± 28.4	25.2 ± 24.2	450 ± 42.4	58.2 ± 38.6
N° of lymphocytes	663.8 ± 478.8	11072 ± 25453	543 ± 387.2	580.6 ± 287.2	227.2 ± 140
Total protein (mg/dl)	5.2 ± 2.5	3.7 ± 1.6	1.3 ± 0.6	-	2.7 ± 1.8
LDH (mg/dl)	935 ± 1228	945 ± 1464	86 ± 79.2	-	80.1 ± 47.7

Group 1, tuberculous peritonitis; Group 2: neoplasic ascitis; Group 3: SBP; Group 4 pancreatic ascitis; Group 5: miscelaneous.

rial peritonitis. When necessary, these diagnoses were based on clinical, biochemical, imaging and histological criteria.

Data were analyzed statistically by the Student t test, with the level of significance set at 5%. Sensibility, specificity, positive and negative predictive values (PPV and NPV) were calculated according to the formulas:

		disease	
		present	absent
test	(+)	A	C
	(-)	B	D

where: sensibility = A/A+B; specificity = D/C+D; PPV = A/A+C; NPV = D/D+B.

RESULTS

The mean values of the various parameters analyzed in the different groups are listed in Table 1. Mean ADA levels in the patients of the various groups studied are listed in Table 2 and were significantly higher in ascites of tuberculous etiology when compared to the remaining groups ($p< 0.0001$).

When the cut off value for ADA was established at 31 U/l, it was possible to differentiate ascites of tuberculous etiology from the remaining types, with 100% sensitivity, 92% specificity, 72% positive predictive value (PPV) and 100% negative predictive value (NPV) (Table 3). All patients with tuberculous peritonitis had ADA values about three times higher than the cut off value, and the two patients with lymphoma had ADA values superimposable to those detected in tuberculosis.

When we analyzed the remaining parameters utilized for the diagnosis of tuberculous peritonitis we observed that ADA determination was more sensitive than overall lymphocyte counts or total protein measurement and more specific than all other parameters, thus representing the best diagnostic test (Table 4).

TABLE 2

Mean ADA values U/l in the different groups studied.

Group	n	ADA
1	8	133.5 ± 24.7*
2	13	41.8 ± 52.1
3	6'	10.6 ± 5.9
4	2	18.0 ± 7.1
5	15	11.2 ± 7.7

Group 1: tuberculous peritonitis; Group 2: neoplastic ascitis; Group 3: SBP; Group 4: pancreatic ascitis; Group 5: miscelaneous.

TABLE 3

Sensitivity, specificity and predictive values of the various laboratory parameters determined in ascitid fluid.

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
% lymphocytes > 70%	100	47	37	100
Lymphocytes > 1000/mm³	16	73	16	73
Total protein > 3.5 mg/dl	71	53	29	27
LDH > 240 mg/dl	100	65	33	100
ADA > 31 U/l	100	92	72	100

DISCUSSION

The advances in diagnostic and chemotherapeutic techniques have not reduced the prevalence of tuberculosis in developing countries and the incidence of pulmonary and extrapulmonary forms has been progressively increasing in developed countries as a consequence of the AIDS epidemics.

The extrapulmonary forms, tuberculous peritonitis in particular, may present in an insidious manner with an atypical clinical picture, thus impairing early diagnosis. On the other hand, biochemical and direct bacteriologic tests are of low sensitivity and specificity and the techniques of mycobacterial culture are time consuming, with up to 4 to 6 weeks needed to obtain the final result.

In most cases the final diagnosis of tuberculosis is made on the basis of the presence of *M. tuberculosis* in ascitic fluid culture or its identification by histopathological study of the peritoneum. The search for the bacillus by the Ziehl-Nielsen method is of low sensitivity and culture is positive in 8 to 69% of cases[8], reaching rates of 83% when larger volumes of ascitic fluid are cultured[12]. Peritoneal biopsy by laparoscopy has 75 to 100% sensitivity[12], but is an invasive method not free from complications and not easily available at every hospital.

In our study, ADA determination in the ascitic fluid proved to be a good diagnostic method for tuberculous peritonitis, in agreement with recent data reported in the literature[4,6,11-14]. ADA levels higher than three times the cut off point established by us (31U/l) in ascitic fluid have 100% sensitivity and 92% specificity, which are values higher than those obtained for the other parameters normally used.

The ADA values obtained in non-tuberculous patiens were always below the cut off limit established, except for the two patients with peritoneal lymphoma (mean ADA = 145 U/l) and for one patient with a uterine neoplasia (ADA = 70 U/l), showing that false-positive results may occur in ascites with high levels of lymphocytes. Similarly, other investigators[13] also concluded that the absolute and relative numbers of lymphocytes lead to high rates of false-positive results, as is also the case for LDH determination and total protein measurement in ascitic fluid.

ADA is secreted by lymphocytes and, to a lesser extent, by macrophages. Thus, in ascites with a high neutrophil content, as observed in cirrhosis with SBP and in pancreatic ascites, we did not detect high ADA levels (15.5 ± 6.6 U/l). The data are similar to those reported by others[2,6,8]. On the other hand, ascites with a high lymphocyte content, as is the case for peritoneal lymphomas, course with considerably elevated ADA levels that are superimposable to those detected in peritoneal tuberculosis. In our study neoplastic ascites had more elevated levels of lymphocytes than tuberculous ascites (Table 1). However, levels of ADA were significantly higher in tuberculous ascites group, indicating that the lymphocyte activation iniciated by the infectious process is more important than the absolute number of lymphocytes.

We conclude that, due to the high sesitivity and specificity of the test, the determination of ADA in ascitic fluid should be used as a screening test, especially in countries where tuberculosis is endemic. ADA values higher than 31 U/l necessarily require invasive tests for confirmation of the diagnosis. If laparoscopy cannot be performed and a founded suspicion of tuberculosis persists, specific treatment should be started while waiting for the results of mycobacterial culture.

RESUMO

Valor da determinação da adenosina deaminase (ADA) no diagnóstico da ascite tuberculosa

Com o objetivo de avaliar o papel da determinação da atividade da enzima adenosina deaminase (ADA) no diagnóstico da peritonite tuberculosa, foram estudados 44 pacientes. De acordo com os resultados das determinações bioquímicas, citológicas, histopatológicas e microbiológicas, os pacientes foram divididos nos seguintes grupos: G1 - ascite tuberculosa (n = 8); G2 - neoplásica (n = 13), G3 - peritonite bacteriana espontânea (n = 6), G4 - ascite pancreática (n = 2), G5 - miscelânea (n = 15).

A concentração de ADA no grupo de pacientes com peritonite tuberculosa foi de 133.50 ± 24.74 U/l, significantemente mais elevada que nos outros grupos (G2 = 41.85 ± 52.07; G3 = 10.63 ± 5.87; G4 = 18.00 ± 7.07; G5 = 11.23 ± 7.66). Com um limite de corte de 31 U/l, a sensibilidade, especificidade, valor preditivo positivo e valor preditivo negativo para diagnóstico de tuberculose foram, respectivamente 100%, 92%, 72% e 100%.

Valores de ADA tão elevados quanto na tuberculose só foram encontrados nas ascites neoplásicas causadas por linfomas.

Com base nestes achados, consideramos que a determinação de ADA deve ser utilizada como um teste de triagem no diagnóstico diferencial das ascites. Valores de ADA acima de 31 U/l indicam a necessidade de testes invasivos (laparoscopia e/ou biópsia peritonial, para confirmação diagnóstica).

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