

Original articles

Value of adenosine deaminase in the diagnosis of tuberculous pleural effusions in young patients in a region of high prevalence of tuberculosis

Luis Valdés, David Alvarez, Esther San José, José R G Juanatey, Antonio Pose, José M Valle, Marcelino Salgueiro, José R R Suárez

Abstract

Background – Pleural biopsy is usually considered important for the diagnosis of pleural effusions, especially for distinguishing between tuberculosis and neoplasia, even though tuberculous pleural fluid contains sensitive biochemical markers. In regions with a high prevalence of tuberculosis, and in patient groups with a low risk of other causes of pleurisy, the positive predictive value of these markers is increased. The criteria for performing a pleural biopsy under these circumstances have been investigated, using adenosine deaminase (ADA) as a pleural fluid marker for tuberculosis.

Methods – One hundred and twenty nine patients with a pleural effusion aged ≤ 35 years (mean (SD) 25.2 (4.9) years) were studied. Seventy three were men. Eighty one effusions (62.8%) were tuberculous, 12 (9.3%) parapneumonic, and 10 (7.7%) neoplastic, five were caused by pulmonary thromboembolism, four by systemic lupus erythematosus, seven by empyema, three following surgery, one was the result of asbestosis, and one of nephrotic syndrome. In five cases no definitive diagnosis was reached. ADA levels were determined by the method of Galanti and Giusti.

Results – The diagnostic yield of procedures not involving biopsy was 94.5% (122/129). Pleural biopsy provided a diagnosis in a further two cases, but not in the remaining five. All tuberculous cases had pleural fluid levels of ADA of >47 U/l (mean (SD) 111.1 (36.6) U/l). The only other cases in which ADA exceeded this level were six of the seven patients with empyema. Cytological examination of the pleural fluid diagnosed eight of the 10 neoplastic cases, compared with six diagnosed by pleural biopsy.

Conclusions – In a region with a high prevalence of tuberculosis procedures not involving pleural biopsy have a very high diagnostic yield in patients with a pleural effusion aged ≤ 35 years, making biopsy

necessary only in cases in which pleural levels of ADA are below 47 U/l, pleural fluid cytology is negative and, in the absence of a positive basis for some other diagnosis, neoplasia is suspected.

(Thorax 1995;50:600-603)

Keywords: pleural effusion, tuberculosis, adenosine deaminase.

In the diagnosis of pleural effusions pleural biopsy is used to differentiate between tuberculosis and neoplasia. Although tuberculous pleural fluid contains sensitive biochemical markers of tuberculous pleurisy, including adenosine deaminase (ADA) and interferon γ ,¹⁻³ their specificity in the general population is not 100% and biopsy is accordingly still important.

In our region, however, the incidence of tuberculosis is high (95 cases per 100 000 inhabitants) with a mean (SD) patient age of 38 (20) years at presentation.⁴ The prevalence of tuberculosis among all patients with a pleural effusion is likewise high at 22.4%,³ and the pleura is affected in 23.3% of all tuberculous patients.⁵ These figures contrast with statistics from elsewhere such as the USA where the overall incidence of tuberculosis is only 9.3 cases per 100 000 inhabitants, the mean age at presentation is higher, and pleural effusions occur in less than 5%.^{6,7}

The high prevalence of tuberculous pleurisy among cases of pleural effusion in our region means that pleural fluid levels of ADA have a higher positive predictive value.⁸ This prompted us to investigate whether the concomitant increase in the diagnostic efficacy of non-biopsy based procedures would allow pleural biopsy to be used more selectively than hitherto among patients aged ≤ 35 years, for whom the positive predictive value of ADA levels for tuberculosis is further increased by a low incidence of neoplasia. Accordingly, in this study the cause of pleural effusions in patients in this age group was diagnosed using the usual criteria including pleural biopsy, but the diagnostic outcome was also evaluated if a more conservative approach

Sección de
Neumología
L Valdés
D Alvarez
J M Valle

Servicio de
Bioquímica
E San José

Servicio de Medicina
Interna
A Pose

Hospital de Conxo,
Santiago de
Compostela,
Spain

Faculty of Medicine,
University of Santiago,
Santiago de
Compostela, Spain
J R G Juanatey

Servicio de
Neumología, Hospital
General de Galicia,
Santiago de
Compostela, Spain
M Salgueiro
J R R Suárez

Presented in part at the
XVII World Congress on
Diseases of the Chest,
Amsterdam, The
Netherlands, June 1993.

Reprint requests to:
Dr L Valdés, Urbanización
Parque Montouto, C/Emilia
Pardo Bazán 26, 15894 Teo,
La Coruña, Spain.

Received 9 November 1993
Returned to authors
22 April 1994
Revised version received
8 July 1994
Accepted for publication
17 October 1994

had been used – namely, that pleural biopsy should be performed only if all other diagnostic tests gave negative results.

Methods

One hundred and twenty nine patients aged ≤ 35 years were studied in whom a pleural effusion was diagnosed before or after admission to our centre between January 1988 and June 1993. Their mean (SD) age was 25.2 (4.9) years (range 16–35); 73 were men (mean age 25.5 (4.1) years) and 56 women (mean age 24.5 (4.8) years).

Samples of pleural fluid and peripheral blood were taken from each patient and closed pleural biopsy was performed with a Cope⁹ or Abrams¹⁰ needle. The pleural fluid was examined macroscopically, cytologically, biochemically, and microbiologically (by Ziehl-Neelsen staining and culture in Lowenstein medium and in aerobic and anaerobic media). Biochemical evaluation of both pleural fluid and blood included determination of total protein concentration, lactate dehydrogenase, cholesterol, glucose, ADA, pH, erythrocyte count, and leucocyte subsets. In no case was thoracoscopy performed.

ADA activity (U/l at 37°C) was determined colorimetrically by the method of Galanti and Giusti.¹¹ The NH_4^+ released by deamination of adenosine added to the samples was quantified by incubation with phenol nitroprusside in an alkaline medium, followed by measurement of absorbance at 628 nm. The within-run precision of this method in our hands was evaluated using 30 replicate high ADA samples and 30 replicate low ADA samples. The corresponding coefficients of variation were 2.24% for low ADA samples (mean (SD) 22.93 (0.5) U/l) and 2.02% for high ADA samples (mean 102.48 (2.04) U/l). Between-run precision was evaluated using 17 pairs of duplicates and a coefficient of variation of 2.51% (mean 37.29 (0.94) U/l) was obtained.

The pleural effusions were classified as tuberculous if bacilli were identified in pleural fluid and/or biopsy cultures, or if the biopsy tissue exhibited caseating granulomas. Malignancy was diagnosed if neoplastic pleural tissue was identified or, in the case of AIDS patients already known to have cutaneous Kaposi's sarcoma, upon observation of serosanguineous pleural fluid.¹² Parapneumonic effusions were

diagnosed if the effusion was associated with bacterial pneumonia, pulmonary abscess or bronchiectasis, and empyema if pleural fluid cultures were positive.¹³ Postoperative effusions were diagnosed in patients who had normal chest radiographs before abdominal surgery and pleural effusions within the following 72 hours. Pulmonary thromboembolism was diagnosed from angiograms or perfusion scans¹⁴; systemic lupus erythematosus when the anti-nuclear antibody concentration was higher in pleural fluid than in serum in patients already known to have the condition¹⁵; asbestosis in the case of a patient exposed to asbestos and with signs of no other cause after three years' surveillance¹⁶; and nephrotic syndrome when the total protein concentration in 24 hour urine samples was $>3.5 \text{ g/l}$.¹⁷

For the evaluation of the non-biopsy based diagnosis of tuberculous pleurisy from levels of ADA in the pleural fluid we used a diagnostic threshold of 47 U/l, the lowest level in patients with tuberculous pleurisy aged ≤ 35 years. This choice was justified by comparison with the values found in our reference group of 407 patients with non-tuberculous, non-empyema pleural effusions of all ages seen in our centres between January 1988 and June 1993 (see Results section). Patients with empyema were excluded from this reference group to avoid meaningless bias; empyema is known to cause high levels of ADA in the pleural fluid¹⁸ but is easily distinguished from tuberculous pleurisy by means of other thoracocentesis based tests.

STATISTICAL ANALYSIS

For the purposes of this study "prevalence" refers to the number of cases of a given kind of pleural effusion divided by the total number of pleural effusions studied. Non-normal (non-Gaussian) distributions were identified from their skew and kurtosis. The statistical significance of differences between means was estimated by the Student's *t* test if the variables were normally distributed, and by Wilcoxon's T test otherwise.

The values of ADA levels for the diagnosis of tuberculous pleurisy were evaluated in terms of its sensitivity, specificity, and positive predictive value (PPV); $\text{PPV} = (\text{prevalence} \times \text{sensitivity}) / [(\text{prevalence} \times \text{sensitivity}) + (1 - \text{prevalence}) (1 - \text{specificity})]$, and negative predictive value (NPV) = $[(1 - \text{prevalence}) \times \text{specificity}] / [(1 - \text{prevalence}) \times \text{specificity} + \text{prevalence} \times (1 - \text{sensitivity})]$.

Table 1 Aetiology of pleural effusions among 129 patients aged ≤ 35 years and mean (SD) concentrations of adenosine deaminase (ADA) in the pleural fluid

Aetiology	n	%	ADA (U/l)
Tuberculosis	81	62.8	111.1 (36.6)
Parapneumonic	12	9.3	28.9 (8.1)*
Neoplastic	10	7.7	17.2 (4.9)*
Metastatic	6	4.6	
Kaposi's sarcoma	2	1.5	
Lymphoma	2	1.5	
Empyema	7	5.4	139.7 (100.7)
Pulmonary thromboembolism	5	3.8	15.4 (10.7)*
Systemic lupus erythematosus	4	3.1	15.7 (3.2)*
Postoperative	3	2.3	24.6 (4.6)*
Benign asbestosis	1	0.8	31
Nephrotic syndrome	1	0.8	4
Idiopathic	5	3.8	19.6 (5.2)*

* $p < 0.001$ with respect to the tuberculous pleurisy group.

Results

The diagnoses of the 129 patients are listed in table 1. As expected, among our patients with pleural effusions aged ≤ 35 years the prevalence of tuberculous effusions was very high (63% (81/129) compared with 22.4% for patients of all ages with effusions in this region).³ The prevalence of neoplastic effusions was low at 7.7% (10/129) compared with 27.1% for patients of all ages with effusions.³

Table 2 summarises the microbiological and histological results among the patients for

Table 2 Sensitivities of microbiological and histological procedures for diagnosis of tuberculous pleurisy among 81 patients aged ≤ 35 years with tuberculous pleurisy

Procedure	n	%
Ziehl-Neelsen staining of pleural fluid	6	7.4
Culture of pleural fluid in Lowenstein medium	31	38.3
Ziehl-Neelsen staining of biopsy tissue	17	20.9
Culture of biopsy tissue in Lowenstein medium	46	56.8
Observation of caseating granulomas	62	76.5

whom at least one of these tests was positive – that is, the 81 patients considered to have tuberculosis. The joint sensitivity of the analyses of biopsy tissue was 83.9% (68/81), and that of pleural fluid analyses 38.2% (31/81). The most frequent finding (76.5%, 62/81) was the observation of caseating granulomas in biopsy tissue.

In the patients with tuberculous effusions the mean ADA concentration was 111.1 (36.6) U/l (median 112 U/l, range 47–247 U/l). These values were significantly greater ($p < 0.001$) than in any other group except those with empyema (table 1). The pleural ADA concentrations of the whole reference group (407 non-tuberculous, non-empyema patients of all ages) ranged from 1 to 77 U/l (mean 19.8 (12.4) U/l, median 19 U/l), and those of the 41 patients in the subgroup aged ≤ 35 years ranged from 4 to 40 U/l (mean 21.1 (8.9) U/l, median

20 U/l). Thus, all the patients aged ≤ 35 years (and 96.7% of the whole reference group) had pleural ADA concentrations below 47 U/l, the lowest level seen in the patients with tuberculous pleurisy aged below 35 years. Since in this context false negatives are preferable to false positives (because patients for whom all non-biopsy based analyses are negative will be subjected to biopsy), the diagnostic threshold chosen was 47 U/l. With this threshold the sensitivity of pleural ADA concentrations for tuberculosis among patients aged ≤ 35 years with an effusion was 100% and its specificity 87.5%. All the false positives were empyemas. The positive predictive value was 93.1% and the negative predictive value 100% (figure).

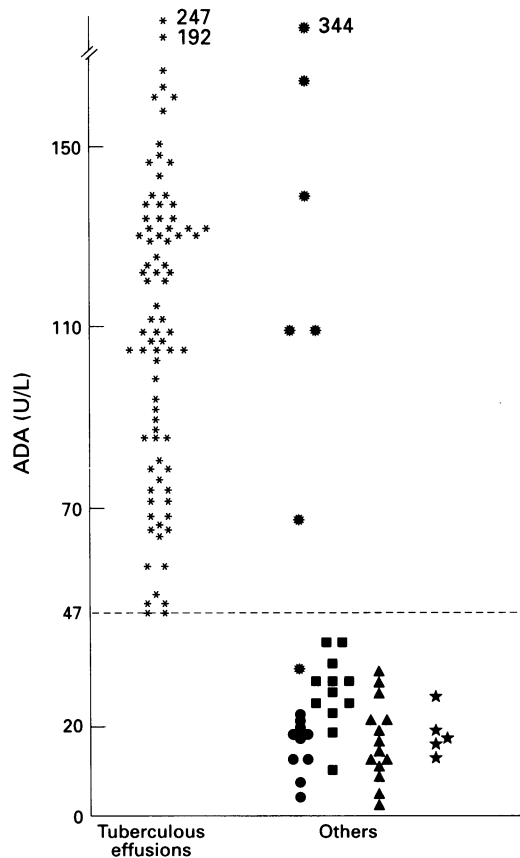
For eight of the 10 patients with neoplastic effusions diagnosis was reached by pleural fluid analysis, and included two with Kaposi's sarcoma, two with non-Hodgkin's lymphoma, and four with metastatic adenocarcinomas. Analysis of biopsy tissue diagnosed six metastatic effusions (the above four plus one metastatic seminoma and one metastatic bronchogenic epidermoid), but not the other neoplastic cases.

Discussion

Thoracentesis and pleural biopsy are both performed routinely for the diagnosis of pleural effusions. Although the determination of pleural fluid levels of ADA and interferon γ for the diagnosis of tuberculous pleurisy is increasing, analysis of biopsy tissue is often considered obligatory for definitive diagnosis of tuberculous and neoplastic effusions,^{19,20} despite the diagnostic sensitivity of biopsy for these two conditions being only 75% and 57% respectively.²¹

As a result of the epidemiological characteristics of tuberculosis in our region, its prevalence among cases of pleural effusion is high at 22.4%.³ We therefore reasoned that the positive predictive value of pleural fluid levels of ADA for tuberculous pleurisy should be better than in regions of lower prevalence, especially among patients with a low probability of neoplasia (who may also have high ADA levels³).

With a concentration of ADA in pleural fluid of >47 U/l as a diagnostic criterion, the sensitivity and negative predictive value of ADA levels for the diagnosis of tuberculous pleurisy were both 100%. The only other effusions with pleural fluid concentrations of ADA above this level occurred with empyemas, making the specificity of pleural ADA concentrations for tuberculous pleurisy in this age group 87.5%. Since empyemas are easily distinguishable by appearance and culture of pleural fluid, the specificity of pleural fluid analysis for tuberculosis was 100%. The positive predictive value of pleural fluid concentrations of ADA for tuberculosis was 93.1%, rising to 100% when empyemas were excluded. Other authors have also reported high levels of ADA in patients with other causes of pleural effusion (mainly lymphomas, adenocarcinomas, systemic lupus erythematosus, or pneumonia)²²;



Concentrations (U/l) of adenosine deaminase (ADA) in 129 patients aged less than 35 years with pleural effusions. * = tuberculous effusions; ● = empyema; ● = neoplastic effusions; ■ = parapneumonic effusions; ▲ = other known diagnoses; ★ = idiopathic effusions.

all our young patients with these conditions had pleural ADA concentrations below our diagnostic threshold.

It is traditionally considered that for diagnosis of neoplastic effusions the sensitivity of pleural fluid cytology is greater than that of analysis of biopsy tissue (66% versus 46%),²³ and that their joint sensitivity can reach 73%. Our findings for the 10 patients aged ≤ 35 years with neoplasia are in keeping with this.

The only effusions that were diagnosed at biopsy but not by other procedures were two of the six patients with metastatic disease. Both these cases fulfilled the conservative criteria for biopsy that we wished to test (ADA concentration below 47 U/l, negative cytology, and the absence of other positive indications for the cause of the effusion). In this sample of patients the conservative criteria would therefore have left no cases undiagnosed that were not also left undiagnosed by immediate biopsy.

In general, the only cases in which our conservative criteria would have led to misdiagnosis, but which might conceivably be diagnosed correctly using immediate biopsy, are cases of neoplastic effusion with negative cytological results and high pleural concentrations of ADA. It is necessary to stress "might" because it is estimated that the 66% sensitivity of cytology for neoplastic effusions is only increased to 73% if both biopsy and cytology are used,²³ and it is questionable whether any of the patients making up the 7% difference will have high pleural concentrations of ADA. Since this is an important issue we feel that more data from other areas with high rates of tuberculosis are needed to establish an accurate estimate of the probability that a patient with a pleural effusion aged ≤ 35 years with negative cytological results, a high concentration of ADA in the pleural fluid, and no other diagnosis has a malignancy rather than tuberculosis.

Nevertheless, our results show that, in a region of high tuberculosis prevalence, routine determination of pleural fluid concentrations of ADA in young patients with pleural effusions can provide a very reliable basis for deciding whether to start antituberculous therapy before

the results of biopsy or culture become available.

- Ocaña I, Martínez Vázquez JM, Segura RM, Fernández de Sevilla T, Capdevila JA. Adenosine deaminase in pleural fluid. Test for diagnosis of tuberculous pleural effusion. *Chest* 1983;84:51-3.
- Ribera E, Ocaña I, Martínez Vázquez JM, Rossell M, Español T, Ruibal A. High level of interferon gamma in tuberculous pleural effusion. *Chest* 1988;93:308-11.
- Valdés L, San José E, Alvarez D, Sarandeses A, Pose A, Chomón B, et al. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme and gamma-interferon. *Chest* 1993;103:458-65.
- Salgueiro M, Zamarrón C, Alvarez-Calderón P, Tumbeiro M, Penela P, Valle JM, et al. Estudio epidemiológico de la tuberculosis en el Area Sanitaria de Santiago de Compostela durante los años 89, 90 y 91. *An Med Intern (Madrid)* 1993;9:427-32.
- Vidal R, de Gracia J, Ruiz J, Fite E, Monsó E, Martín N. Estudio controlado de 637 pacientes con tuberculosis. Diagnóstico y resultados terapéuticos con esquemas de 6 y 9 meses. *Med Clin (Barc)* 1986;87:368-70.
- Bloch AB, Rieder HL, Kelly GD, Cauthen GM, Hayden CH, Snider DE. The epidemiology of tuberculosis in the United States. Implications for diagnosis and treatment. *Clin Chest Med* 1989;10:297-313.
- Seibert AF, Haynes J Jr, Middleton R, Bass JB Jr. Tuberculous pleural effusion. Twenty-year experience. *Chest* 1991;99: 883-6.
- Pettersson T, Ojala K, Weber TH. Adenosine deaminase in the diagnosis of pleural effusions. *Acta Med Scand* 1984; 215:299-304.
- Cope C. New pleural biopsy needle. *JAMA* 1958;167: 1107-8.
- Abrams LD. New inventions: a pleural biopsy punch. *Lancet* 1958;i:30-1.
- Giusti G. Adenosine deaminase. In: Bergmeyer HU, ed. *Methods of enzyme analysis*. New York: Academic Press, 1974:1092-9.
- O'Brien RF, Cohn DL. Serosanguineous pleural effusions in AIDS-associated Kaposi's sarcoma. *Chest* 1989;96:460-6.
- Light RW. Parapneumonic effusions and infections of the pleural space. In: Light RW, ed. *Pleural diseases*. Philadelphia: Lea & Febiger, 1983:101-18.
- McNeill BJ. Ventilation-perfusion studies and the diagnosis of pulmonary embolism: concise communication. *J Nucl Med* 1980;21:319-23.
- Good JT Jr, King TE, Antony VB, Sahn SA. Lupus pleuritis: clinical features and pleural fluid characteristics with special reference to pleural fluid antinuclear antibodies. *Chest* 1983;84:714-8.
- Hillerdal G. Non-malignant asbestos pleural disease. *Thorax* 1981;36:669-75.
- Glasscock RJ. The nephrotic syndrome. In: Brenner B, Rector FL, eds. *The kidney*, 3rd edn. Philadelphia: WB Saunders, 1986:955-63.
- Sahn SA. State of the art: the pleura. *Am Rev Respir Dis* 1988;138:184-234.
- Health and Public Policy Committee. American College of Physicians. Diagnostic thoracentesis and pleural biopsy in pleural effusions. *Ann Intern Med* 1985;103:799-802.
- Hall WJ, Mayewsky RJ. Diagnostic thoracentesis and pleural biopsy (letter). *Ann Intern Med* 1986;105:298.
- Tomlinson JR, Sahn SA. Invasive procedures in the diagnosis of pleural disease. *Semin Respir Med* 1987;9:30-6.
- Ena J, Valls V, Pérez de Oteyza C, Enriquez de Salamanca R. Utilidad y limitaciones de la adenosina desaminasa en el diagnóstico de la pleuresia tuberculosa. Estudio metaanalítico. *Med Clin (Barc)* 1990;95:333-5.
- Sahn SA. Malignant pleural effusions. In: Fishman A, ed. *Pulmonary diseases and disorders*, 2nd edn. New York: McGraw Hill, 1988:2159-70.