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RESEARCH ARTICLE

PROTEINS, LACTATE DEHYDROGENASE AND ADENOSINE DEAMINASE LEVELS IN PLEURAL FLUID - USEFUL MARKERS FOR DIFFERENTIATING TUBERCULOSIS FROM LUNG CANCER

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ABSTRACT

To evaluate the clinical and laboratory characteristics of pleural effusions in to tuberculosis (TB) or cancer (CA). A total of 385 patients with pleural effusion due to TB (n=175) or CA (n=112) were studied. The following parameters were analyzed: patient gender, age and pleural effusion characteristics (size, location, microscopic fluid aspect protein concentration, lactate dehydrogenase (DHL) and adenosine deaminase activity (ADA) and nucleated cell counts).

The objective of this study was to evaluate the utility of the determination of protein concentration, lactate dehydrogenase (DHL) and adenosine deaminase (ADA) level in pleural fluid for the differential diagnosis between tuberculous pleural effusion (TPE) and malignant pleural effusion (MPE). We retrospect tively reviewed the clinical records of 385 patients with pleural effusion and investigated their pleural protein concentration, DHL and ADA levels as determined by an auto analyzer (Micro Lab 300). The study included patients with TPE (n=175), MPE (n=112), benign non-tuberculous pleural effusion (n=81), and pleural effusion of unknown etiology (n=17). Although the protien concentration, DHL and ADA activity in pleural fluid can help in the diagnosis of TPE and MPE patients.

Fluid with higher protein ($p < 0.001$) levels predominated in effusions from the tuberculosis group (5.3 ± 0.8 g/dL) when compared to the CA group (4.2 ± 1.0 g/dL), whereas DHL levels were more elevated in CA (1,177 ± 675 x 1,030 ± 788 IU; $p = 0.003$) than in TB. As expected, ADA activity was higher in the TB group (107.6 ± 44.2 X 30.6 ± 57.5 U/L; $p < 0.001$).

Our results demonstrate that in lymphocytic pleural exudate obtained from patients with clinical and radiological evidence of tuberculosis, protein concentration, DHL and ADA were the parameters that better characterize these effusions. In the same way, when the clinical suspicion is malignancy, serous- hemorrhagic lymphocytic fluid should be submitted to oncotic cytology once this easy and inexpensive exam reaches a high diagnostic performance (80%). In this context, we suggest thoracocentesis with fluid biochemical and cytological examination as the first diagnostic approach for these patients.

INTRODUCTION

The first step in the etiological investigation of a pleural effusion is to determine whether the effusion is a transudate or an exudate. Transudates reflect the presence of systemic disease with repercussions on the mechanisms of pleural fluid production and resorption[1]. In contrast, exudates reflect the presence of primary pleural disease and require etiological investigation[1]. However, considering worldwide epidemiological aspects, most cases of pleural effusion result as a consequence of tuberculosis or cancer[2,3]. In this respect, although differential diagnosis must be a priority, it is often difficult due to the similar biochemical profiles and the predominance of lymphocytes in both conditions.

The gold standard for diagnosis of pleural tuberculosis is the identification of *Mycobacterium tuberculosis* in pleural fluid or tissue[4]. However, in clinical practice this identification is problematic because of the low identification rate of the bacillus (less than 30% in pleural fluid and approximately 50% in the pleura) and the slow growth of mycobacterium in culture (about 60 days)[4]. Therefore, in regions with a high prevalence of tuberculosis, pleural biopsy demonstrating a granulomatous inflammatory process is used for diagnosis since other granulomatous diseases such as sarcoidosis,

mycosis and rheumatoid arthritis account for less than 10% of granulomatous findings in the pleura[5].

On the other hand, the diagnosis of neoplastic pleural effusion is made based on the presence of malignant cells in the pleural fluid or tissue. The positivity rate of the cytological exam ranges from 40 to 87%, higher than that obtained with a needle biopsy which ranges from 35 to 65%[1,6].

In this context, the objective of the present study was to describe the characteristics and laboratory performance of pleural fluid biochemical and cytological parameters from patients with tuberculosis or cancer.

METHODS

A total of 385 consecutive patients from the Pleural Outpatient Clinic with a diagnosis of pleural effusion due to tuberculosis (n=175) or cancer (n=112) were included. The criteria for establishment of the diagnosis of tuberculosis were: a) pleural biopsy demonstrating a granulomatous process, b) detection of *M. tuberculosis* in pleural fluid or tissue, and c) a compatible clinical history and radiologic exams, in patients with a lymphocytic exudate and fluid with higher protein ($p < 0.001$) levels predominated in effusions from the tuberculosis group (5.3 ± 0.8 g/dL) when compared to the CA group (4.2 ± 1.0 g/dL), whereas DHL levels were more elevated in CA (1,177 ± 675 x 1,030 ± 788 IU; $p = 0.003$) ADA activity was higher in the TB group (107.6 ± 44.2 x 30.6 ± 57.5 U/L; $p < 0.001$). Pleural fluid (yellow-citrine, serohemorrhagic or chylous) were also evaluated. Total protein concentration (colorimetric biuret reaction, Microlab 300) and lactate dehydrogenase (LDH) levels (kinetic ultraviolet method, Microlab 300) were assayed in pleural fluid samples collected without the addition of anticoagulant, used for oncotic cytology and cell count and for the quantification of ADA (colorimetric method of ADA-MTB [7]).

RESULTS

The demographic data of the patients and the characteristics of the pleural effusions are shown in table 1. Patients with tuberculosis were significantly ($p < 0.001$) younger (35.7 ± 12.6 years) than those with cancer (57.5 ± 13.5 years).

	Tuberculosis	Cancer	p
N	**175**	**112**	**NS**
Age (years)	**35.7 ± 12.6**	**57.5 ± 13.5**	**< 0.001**
Gender (M/F)	127/48 (72.57/27.43 %)	39/74 (34/66 %)	< 0.001
Size(% of the hemithorax)			
<25	32 (18.29 %)	1 (0.9 %)	< 0.001
25 – 75	138 (78.86 %)	80 (71.4 %)	0.187
>75	5 (2.85 %)	31 (27.7 %)	< 0.001
Location			
Unilateral	167 (95.4 %)	104 (92.8 %)	0.381
Bilateral	8 (4.6 %)	8 (7.2 %)	0.342
Aspect			
Yellow – citrine	154 (88 %)	53 (47.32 %)	<0.0001
Serohemorrhagic	20 (11.4 %)	57 (50.9 %)	<0.0001
Chylous	1 (0.6 %)	2 (1.78 %)	

Among patients with tuberculosis, there was a predominance ($p < 0.001$) of males (127/175; 72.57%) over females (48/175; 27.43%), while the opposite was observed in the cancer group (females: 74/112, 66% versus males: 39/112, 34%; $p < 0.001$). This finding reflects the characteristics of our outpatient clinic, where mainly breast cancer patients are seen.

With respect to fluid volume, we observed a predominance of moderate effusion in both groups (tuberculosis: 137/175; 78.2%, and cancer: 80/112; 71.4%), with no significant difference between them ($p = 0.187$). Although most effusions were moderate, the lowest volumes (<25%) were observed in the tuberculosis group (30/175; 17.14%), whereas more voluminous effusions (>75%) were most frequent in the cancer group (31/112; 27.67%). Unilateral effusions predominated in both tuberculosis (167/175; 95.42%) and cancer (104/112; 92.85%) groups.

Macroscopically, most pleural effusions presented a yellow-citrine aspect (203/287; 70.71%), demonstrating a significant difference ($p < 0.001$) between the tuberculosis (154/175; 88.0%) and cancer (53/112; 47.32%) groups. Although half of these effusions were yellow-citrine, serohemorrhagic effusions predominated ($p< 0.001$) in the cancer group (56/112; 50.0%) when compared to the tuberculosis group (20/175; 11.4%).

Table 2 - Biochemical and cytological charecteristics of the pleural fluid.

Investigation	Unit	Tuberculosis	Cancer	p
Protein	(g/dl) (U/L)	5.3 ± 0.8	4.2 ± 1.0	< 0.001
DHL		1090 ± 788	1177 ± 575	0.003
ADA	(U/L)	107.6 ± 44.2	30.6 ± 57.5	< 0.001
Nucleated cell count	(mm) (%)	2860 ± 2194	3068 ± 1807	< 0.001
Mesothelial cells		1.3 ± 2.2	6.1 ± 1.6	0.005
Microphages	(%)	10.5 ± 10.2	33.2 ± 23.4	< 0.001
Leukocytes	(%)	87.8 ± 11.7	57.5 ± 26.8	< 0.001
Granulocytes	(%)	7.6 ± 166	13.3 ± 19.2	< 0.001
Lymphocytes	(%)	88.9 ± 19.7	31.7 ± 22.4	< 0.001

Table 2 shows the biochemical and cytological characteristics of the pleural fluids. Protein concentrations were significantly higher ($p < 0.001$) in the tuberculosis group (5.3 ± 0.8 g/dL) compared to the cancer group (2 ± 1.0 g/dL). In contrast, LDH levels were higher ($p = 0.003$) in the cancer group (1,177 ± 675 IU) than in the tuberculosis (1,030 ± 788 IU) group. Finally, as expected, ADA levels were significantly higher in the tuberculosis group (107.6 ± 44.2 U/L versus 30.6 ± 57.5 U/L; $p < 0.001$).

Although the pleural effusions were characterized by high cellularity, neoplastic effusions were more richly cellular than tuberculous effusions (3,058 ± 1,807 versus 2,860 ± 2,194 cells/mm^3; $p < 0.001$). Analysis of the proportion of nucleated cells revealed a significantly lower percentage of mesothelial cells in the tuberculosis group (1.3 ± 2.2% versus 6.1 ± 1.6%; $p = 0.005$), whereas the percentage of macrophages was significantly higher ($p < 0.001$) in the cancer group (33.2 ± 23.4% versus10.5 ± 10.2%). A higher proportion of leukocytes ($p < 0.001$) was observed in the tuberculosis group (87.8 ± 11.7%) when compared to the cancer group (57.5 ± 26.8%). Although both effusions were lymphocytic, the number of lymphocytes was significantly higher ($p < 0.001$) in the tuberculosis (88.9 ± 19.7%) compared to the cancer group (81.7 ± 22.4%).

In cancer effusions, oncotic cytology was positive in 70.5% (79/112) of cases and highly suggestive in 19.6% (22/112), permitting suspicion or confirmation of diagnosis in approximately 90% of the patients. A second pleural approach (thoracocentesis) confirmed the diagnosis of cancer in four suspected cases and in additional two whose cytology was negative. In the remaining 24 patients, cytology was inconclusive. The diagnosis of cancer was confirmed by

immunophenotyping of pleural fluid in four cases (lymphoma), by closed pleural biopsy in 13 (carcinoma), and by thoracoscopy-guided pleural biopsy in seven (carcinoma). Closed pleural biopsy was conclusive in 68/78 (87.1%) and 21/37 (56%) of tuberculosis and cancer patients, respectively. A second pleural biopsy increased the cancer rate to 23/37 (62.1%).

DISCUSSION

A systematic approach to the classification of pleural effusion permits the diagnosis of a large number of pleural diseases, especially when considering the high incidence of tuberculosis and cancer. Diagnostic exploration is based on the analysis of clinical variables (gender, age and symptoms), imaging characteristics (volume and location of fluid), and laboratory (biochemical and cytological) data.

A detailed clinical history of previous neoplasms or complaints related to consumptive states, as well as the presence of fever, night sweats, cough and contact with tuberculous patients are fundamental. Since thoracocentesis is the first approach proposed in cases of pleural effusion, analysis of the removed fluid is the easiest and fastest way of assessment[8]. Although the cause of pleural effusion remains indeterminate in about 20% of cases, cytological, biochemical and microbiological analysis of pleural fluid is fundamental for adequate screening, avoiding more expensive exams that would increase the morbidity and/or mortality of the patient[9].

Tuberculosis is a ubiquitous disease with a high prevalence[3]. This disease usually predominates among individuals younger than those affected by cancer, a fact confirmed in the present study in which only 4.2% of the cases of tuberculosis occurred in patients older than 35 (only one patient older than 60) years.Pleural effusion due to tuberculosis indicate that 40% of cases occurred in male individuals older than 40 years.[2] Thus, although important, age should only be considered as a complementary variable in the diagnosis of tuberculosis.

With respect to gender, it is known that men are more predisposed to both tuberculosis[10] and lung cancer,[11,12] although the incidence of cancer has been increasing among women over the last few decades. The predominance of females among patients with cancer observed in the present study is in contrast to international epidemiological data.

The pleural effusions were moderate in most patients with tuberculosis or cancer, with unilateral effusions predominating in more than 90% of cases. This finding agrees with literature showing that pleural effusions are preferentially unilateral and moderate.[1,12] Despite this similarity, a higher incidence of voluminous collections is found in malignant pleural effusions, a fact also observed in the present study[1].

After evaluation of demographic variables and chest images, the pleural space should be submitted to invasive investigation. The first step is thoracocentesis, i.e., thoracic puncture for pleural fluid collection, with the objective, in addition to the determination of macroscopic aspects, of material collection for adequate laboratory examination.
Yellow-citrine fluid (sometimes slightly turbid) predominates in tuberculosis while serohemorrhagic fluid predominates in cancer. Our results confirmed this observation[8,13] but also showed overlaps between the groups concerning the classification of pleural effusion based only on this variable.

As a rule, thoracocentesis should always be considered for collection of pleural fluid for diagnosis. Only in exceptional cases, when diagnosis has already been established or during an emergency (respiratory insufficiency), will it be performed when no laboratory infrastructure is available. Otherwise, thoracocentesis should include fluid collection and subsequent biochemical and cytological exams.

The first parameters analyzed (LDH and protein) should permit the differentiation between a transudate and an exudate (Light's criteria).[14] Clinically, this discrimination is extremely valuable, since transudative effusions generally reflect systemic diseases and therefore do not require a complementary diagnostic approach. On the other hand, exudates indicate pleural involvement resulting from systemic disorders or primary pleural diseases. In these cases, diagnostic exploration should be complemented by other pleural fluid exams or histological evaluation of the pleura.[1,8]

Since our objective was to differentiate between tuberculosis and cancer, the first step was to recognize the presence of an exudate. All pleural effusions included in the study were exudates and, although not specific, protein levels were significantly higher in the tuberculosis group, in agreement with the findings of Liam *et al.*[15]Since the protein concentration in pleural tuberculosis is frequently higher than 4.5 g/dL, Melo *et al.*[16] proposed this value as a cutoff for diagnostic presumption. As confirmation of this proposal, this was the minimum protein level detected in the present study.

Lactic dehydrogenase, a nonspecific inflammatory marker,[8] is in general discretely elevated in pleural tuberculosis. Neoplasms present higher levels, suggesting a greater extent of pleural disease or the presence of blood in the pleural cavity.[17] Despite this difference and, as is the case for the macroscopic evaluation of effusion, these biochemical parameters do not permit differentiation between the two diseases because of overlapping values.

The next step is the quantification of ADA, an enzyme produced by macrophages and activated T lymphocytes, [18]which is usually elevated in tuberculosis. In this situation, the activity of the enzyme is generally higher than 40 U/L, although similar levels are observed in lymphocytic pleural effusions secondary to rheumatoid arthritis and certain lymphoproliferative diseases. Other lymphocytic exudates, such as those secondary to pulmonary edema or metastatic tumors, generally show levels below 40 U/L.[19- 22] In the present study, using a cutoff value of 40 U/L as a presumptive diagnosis, all cases of tuberculosis were correctly classified. This cutoff value shows high efficiency in the classification of lymphocytic effusions (sensitivity: 87 to 100% and specificity: 81 to 97%).[19-25]Despite the high diagnostic sensitivity of ADA, its specificity is influenced by other clinical conditions and by the regional prevalence of tuberculosis.[26,27]

In the present study, 13 (9%) patients with cancer presented an ADA activity higher than 40 U/L. Five of these patients had lymphomas (diagnosed by immunophenotypying) and eight had solid tumors. Despite increased ADA levels, etiological

diagnosis was established in seven of these cases by pleural fluid cytology. In one case, both cytology and pleural biopsy were negative. However, chest X-ray demonstrated a pulmonary mass and the diagnosis of undifferentiated lung carcinoma was established by transbronchial biopsy. In the remaining five cases, a closed pleural biopsy (n=3) or thoracoscopy-guided biopsy (n=2) led to a definitive diagnosis.

Considering the cytological profile of the pleural fluids in these two diseases, no cellular finding is characteristic, with exception to the presence of neoplastic cells in cancer effusions.

Oncotic cytology of the pleural fluid is considered to be the most sensitive method for the diagnosis of malignant pleural effusion.[1,8] However, its performance varies according to the histological type and exfoliative capacity of the tumor, the techniques used to process the pleural samples, the number of slides analyzed per case and, finally, the cytologist expertise. These factors explain the variation in the efficacy of the exam (40 to 87%) reported in the literature.[28] In this study, the first fluid examination led to the diagnosis of cancer in 70.1% (79) of cases. In 19.4% (22), oncotic cytology was highly suspicious of malignancy and in 10.5% (12) it was negative. 4; 13 7)

This study demonstrates that although the pleural effusion exudates secondary to tuberculosis or cancer were predominantly lymphocitic, some clinical and laboratory aspects could aid in their differentiation. In general, tuberculosis occurred in younger persons, and effusions were unilateral and of a median size. The pleural fluid was frequently yellow (citrine or turbid) with high protein and ADA levels and with a low number of mesothelial cells. In contrast, patients with malignant effusions were generally older and, although the effusions were predominantly unilateral, the presence of a bilateral effusion was more associated with malignancy. In addition, the fluid was predominantly turbid or serohemorrhagic. The level of protein was lower than in tuberculosis, while ADA was normal and LDH was frequently higher. Cytological examination revealed a predominance of lymphocytes and macrophages. In patients with clinical and radiological evidence of tuberculosis presenting an exudative effusion rich in lymphocytes, protein and ADA levels are important to the etiological diagnosis.

References

1. Maskell NA,Butland RJA Guidelines for the Investigation of aUnilateral Pleual Effusion in Adults. Throax 2003:58:8 - 17
2. Centro de Vigilancia Epidemiológica Alexandre Vranjac. Secretaria da Saúde de São aulo. (20/08/2006)
3. Instituto Nacional de Câncer - Ministério da Saúde. (20/08/2006)
4. Gopi A, Madhavan SM, Sharma SK and Sahn SA. Diagnosis and Treatment of Tuberculous Pleural Effusion in 2006. Chest 2007; 131:880-89.
5. Valdés L, Pose A, San José E and Martínez Vázquez JM.Tuberculous Pleural Effusions.EurJ Intern Med 2003;14:77-88.
6. Di Bonito I, Falconieri G, Colautti I, Bonifacio D and Dudine S. The Positive Pleural Effusions. A Retrospective Study of Cytopathologic Diagnosis with Autopsy Confirmation. Acta Cytol 1992; 36:329-32.
7. Giust G. Adenosine Deaminase In: Bergmeyer HU. Methods of Enzymatic Analysis. New York Academic Press 1974; 1092-99.
8. Light RW. Clinical Manifestations and Useful Tests. In: Pleural Diseases. 4thed, Philadelphia, Lippincott-Williams & Wilkins, 2001. p. 42-86.
9. Villena V, López Encuentraz A, Echave – Sustaeta J, Alvarez Martinez C, Martin Escribano P. Prospective study of 1,000 consecutive patients with pleural effusion. Etiology the effusion and characteristics of the patients. Arch. Bronconeumol. 2002;38:21-6.
10. World Health Organization (20/08/2006)
11. Parkin DM. Bray F, Ferlay J and Pisani P. Global Cancer Statistics, 2002. CA Cancer J Clin 2005; 55:74-108.
12. Antunes G, Neville E, Duffy J, Ali N. BTS Guidelines for the Management of Malignant Pleural Effusions. Thorax 2003; 58:29-38.
13. Porcel-Perez JM, Vives Soto M, Esquerda Serrano A, Jover Saenz A. Cutoff Values of Biochemical Tests on Pleural Fluid: Their Usefulness in Differential Diagnosis of 1040 Patients with Pleural Effusion. Ann Med Intern 2004; 21:113-17.
14. Light RW, MacGregor MI, Luchsinger PC *et al*. Pleural Effusions: The Diagnostic Separation of Transudates and Exudates. Ann Intern Med 1972; 77:507-13.
15. Liam CK, Lim KH, Wong CM. Differences in Pleural Fluid Characteristics, white Cell Count a Biochemistry of Tuberculous and Malignant Pleural Effusions. Med J Malaysia 2000; 55:21-28.
16. Melo FAF, AJB Santos ML *et al*. Diagnóstico da Tuberculose Pleural pela ADA, Isolada ou Combinada a Outras Variáveis, Inclusive em HIV-Positivos. Folha Med 2000; 119:19-21.
17. Vergnon JM, Guidollet J, Gateau O, Ripoll JP, Collet P, Louisot P *et al*. Lactic dehydrogenase isoenzyme electrophoretic patterns in the diagnosis of pleural effusion. Cancer 1984; 54:507-11.
18. Gaga M, Papamichalis G, Bakakos P, Latsi P, Samara I, Koulouris NG, *et al*. Tuberculous Effusion: ADA Activity Correlates with CD4+ Cell Numbers in the Fluid the Pleura. Respiration 2005; 72:160-65.
19. Pettersson T, Ojala K and Weber TH. Adenosine Deaminase in the Diagnosis of Pleural Effusions. Acta Med Scand 1984; 215:299-304.
20. Ocanã I, Martinez-Vasquez JM, Ribera E, Segura RM and Pascual C. Adenosine Deaminase Activity in the Diagnosis of Lymphocytic Pleural Effusions of Tuberculous, Neoplastic and Lymphomatous Origin. Tubercle 1986; 67:141-45.
21. Lee YC, Rogers JT, Rodriguez RM, Miller KD and Light RW. Adenosine Deaminase Levels in Nontuberculous Lymphocytic Pleural Effusions. Chest 2001; 120:356-61.
22. Jimenez Castro D, Diaz Nuevo G, Perez Rodríguez E and Light RW. Diagnostic Value of Adenosine Deaminase in Nontuberculous Lymphocytic Pleural Effusions. Eur Respir J 2003; 21:220-24.
23. Goto M, Noguchi Y, Koyama H, Hira K, Shimbo T and Fukui T. Diagnostic Value of Adenosine

Deaminase in Tuberculous Pleural Effusion: A meta-analysis. Ann Clin Biochem 2003; 40:374-81.

24. Chen ML, Yu WC, Lam CW, Au KM, Kong FY and Chan AY. Diagnostic Value of Pleural Fluid Adenosine Deaminase in Tuberculous Pleurisy. Clin Chim Acta 2004; 341:101-107.
25. Smach MA, Garouch A, Charfeddine B, Ben Abdelaziz A, Dridi H, Krayem B, *et al*. Diagnostic Value of Serum and Pleural Fluid Adenosine Deaminase Activity in Tuberculous Pleurisy. Ann Biol Clin (Paris) 2006; 64:265-70.
26. Valdés L, Alvarez D, San Jose E, Juanatey JR, Pose A, Valle JM *et al*. Value of Adenosine Deaminase in the Diagnosis of Tuberculous Pleural Effusion in Young Patients in a Region of High Prevalence of Tuberculosis. Thorax 1995; 50:600-603.
27. Diacon AH, Van de Wal BW, Wyser C, Smedema JP, Bezuidenhout J and Bolliger CT *et al*. Diagnostic Tools in Tuberculous pleurisy: A Direct Comparative Study. Eur Respir 2003; 22:589-91.
28. Antonangelo L. Desempenho Diagnóstico das Biopsias versus Citologia das Punções Aspirativas dos Derrames. In Aspectos Clínicos e Laboratoriais dos Derames Cavitários. 1st ed., Rio de Janeiro, Revinter 2001. p 135-44.