Meaning and Diagnostic Value of Determining the Lysozyme Level of Pleural Fluid*  

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We determined the levels of lysozyme in pleural fluid and serum in 141 patients with the following different causes for their pleural effusions: tuberculosis; neoplasias; transudates; parapneumonic, not complicated; empyemas; and miscellaneous. The lysozyme level of the pleural fluid and the ratio of that level over the serum level of lysozyme (PL/SL ratio) was meaningfully increased in patients with empyema (p<0.01). The groups with tuberculous and neoplastic effusions showed significant differences in the PL/SL ratio (p<0.01). The existence of a raised PL/SL ratio suggested important local synthesis of lysozyme, and it came up in empyemas and tuberculosis, unlike the other groups. Excluding the patients with empyemas, a PL/SL ratio of 1.2 showed a sensitivity of 100 percent, specificity of 94.9 percent, positive predictive value of 94.7 percent, negative predictive value of 100 percent, and accuracy of 97.3 percent for the diagnosis of tuberculous pleural effusion. All of this suggests that the determination of the lysozyme level can be an easy method of great usefulness in the initial diagnosis of pleural effusions.

Lysozyme (muramidase) is a bacteriolytic protein with low molecular weight distributed extensively in organic fluids.1 In 1976, Klockars et al8 found high levels in tuberculous pleural effusions, but later, the lysozyme level was stated to be on limited diagnostic usefulness.9 For these reasons, we intended to evaluate the diagnostic value of the determination of the lysozyme level in our environment, in which there is a high incidence of tuberculosis.

MATERIALS AND METHODS  

One hundred forty-one patients with pleural effusions who were admitted consecutively to our hospital were studied. They were subdivided into the following groups:

Tuberculous effusions were found in 54 patients (27 men and 27 women) with a mean age of 32±14 years (±SD). The diagnosis was determined by culture of fluid or pleural biopsy or both (15 patients) or by the presence in the pleural biopsy of granulomas with necrosis and compatible clinical and therapeutic evolution.

Malignant effusions occurred in 35 patients (16 men and 19 women) with a mean age of 62±16 years. The diagnosis was obtained by pleural biopsy with typical histopathologic or cytologic findings (or both) with compatible clinical findings and evolution. The origin of neoplasia was bronchogenic carcinoma in 13 patients (ten epidermoid, two adenocarcinomas; one unclassified), metastatic carcinoma in six patients (three breast, two ovary; one melanoma), carcinoma with the primary tumor of unknown origin in nine patients (eight adenocarcinomas; one poorly differentiated unclassified), malignant mesothelioma in one patient, malignant neurinoma in one patient, lymphoma in four patients, and chronic myeloid leukemia in one patient.

Transudates were found in 12 patients (six men and six women) with a mean age of 71±14 years. Nine of them had cardiac failure, two had renal failure, and one had nephrotic syndrome.

Parapneumonic effusions occurred in six men with a mean age of 53±23 years. The effusion was associated with a bacterial pneumonia and presented polymorphonuclear predominance, pH of 7.20 or more, glucose level of 40 mg/dl, and lactic dehydrogenase level of less than 1,000 milliunits/ml, with negative Gram's staining and culture of pleural fluid.

Empyemas were found in eight men (with a mean age of 48±10 years) with presence of purulent pleural fluid. The cultures were positive for anaerobes in six cases, for Streptococcus pneumoniae in one case, and for Staphylococcus aureus in another case.

A miscellaneous group consisted of four men and two women with a mean age of 50±20 years whose diagnoses were effusions associated with pulmonary embolism in two cases, pancreatitis in one case, posttraumatic effusion in one case, viral pleuropneumonitis in one case, and systemic lupus erythematosus in the last case.

We did not achieve diagnostic confirmation in 11 patients; seven of them were diagnosed with probable tuberculosis by clinical criteria, two with probable carcinoma, one with possible lymphoma, and one with possible embolism. In another nine cases the diagnoses were not clear; two of them had eosinophilic effusion. All of these 20 cases were excluded from the statistical evaluations.

The serum level of lysozyme and the level of lysozyme in the pleural fluid were determined by the turbidimetric kinetic method using as substrate a suspension of 2.2 g of Micrococcus lyodekkticus per liter of phosphate buffer (66 mmol/L) with a pH of 6.3 and standardizing with pure lysozyme from hens' egg white.4 The extractions of blood and pleural fluid were performed simultaneously; the samples were withdrawn before the treatment was started and were collected in EDTA. The floating was frozen at −20°C.

Statistics  

Analyses of variance followed by the Neuman-Keul procedure3 were used to determine significant (p<0.05) differences in the serum level of lysozyme, the lysozyme level of the pleural fluid, and the ratio of the pleural fluid level over the serum level (PL/SL ratio).

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among all groups. The degree of association between the pleural fluid level and the serum level of lysozyme, as well as between the pleural fluid level and other biochemical and cytologic parameters, was calculated by the coefficient of correlation. The relation between the pleural fluid level and the serum level of lysozyme in the groups with tuberculous and malignant effusions was analyzed by linear regression analysis by the least-square method. The value of the PL/SL ratio for the diagnosis of pleural tuberculosis was established according to the following indices: sensitivity (true-positive/true-positive + false-negative), specificity (true-negative/true-negative + false-positive), positive predictive value (true-positive/true-positive + false-positive), negative predictive value (true-negative/false-negative + true-negative), and diagnostic accuracy (true-positive + true-negative/true-positive + false-positive + false-negative).

**RESULTS**

The mean concentration of lysozyme in the serum was as follows in each of the groups: tuberculous effusions, 9.42 ± 3.92 mg/dl (± SD) (range, 1 to 25 mg/dl); malignant effusions, 10.58 ± 5.62 mg/dl (range, 4 to 27 mg/dl); transudates, 11.55 ± 6.97 mg/dl (range, 6 to 30 mg/dl); parapneumonic effusions, 10.70 ± 5.21 mg/dl (range, 5 to 13 mg/dl); empyemas, 10.33 ± 3.38 mg/dl (range, 8 to 33.5 mg/dl); and miscellaneous, 8.91 ± 2.48 mg/dl (range, 7.5 to 12 mg/dl). The groups did not show significant differences. Figure 1 shows the lysozyme levels of the pleural fluid in different groups. Only one case in the empyemas had lysozyme in low levels in the pleural fluid. This patient presented with a nephrotic syndrome, too. Significant differences were only found between the empyemas and the rest of the groups (p<0.01).

Figure 2 shows the levels of the PL/SL ratio. It was possible to demonstrate statistically significant differences between the mean of the group with empyemas and the other groups (p<0.01), as well as between the tuberculous effusions and the malignant effusions (p<0.01).

The correlation between the lysozyme level of the pleural fluid and other biochemical parameters, as well as the lymphocyte count and polymorphonuclear neutrophils in the pleural fluid, was not significant. The relation between the pleural fluid level and serum level of lysozyme in all groups can be seen in Table 1. The group with malignant effusions has a better correlation index (r = 0.95) than the tuberculous group (r = 0.57).

<table>
<thead>
<tr>
<th>Type of Effusion</th>
<th>r</th>
<th>p Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous</td>
<td>0.57</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Malignant</td>
<td>0.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Transudate</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Parapneumonic</td>
<td>0.79</td>
<td>NS</td>
</tr>
<tr>
<td>Empyema</td>
<td>-0.0016</td>
<td>NS</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0.95</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*NS, Not significant.
and the regression line follows a way near that of identity in the group with malignant effusions; however, the line in the group with tuberculous effusions moves remarkably to the left (Fig 3 and 4).

In order to assess the PL/SL ratio for the diagnosis of pleural tuberculosis, we have chosen a cutoff point of 1.2. So, excluding the patients having fluid with pus (empyemas), all of the patients in the tuberculous group had a PL/SL ratio of 1.2 or more. By contrast, only one patient from the group with malignant effusion and another one with a parapneumonic effusion exceeded this limit. Then the value of a PL/SL ratio of 1.2 or more as a diagnostic test of pleural tuberculosis shows a sensitivity of 100 percent, specificity of 94.9 percent, positive predictive value of 94.7 percent, negative predictive value of 100 percent, and accuracy of 97.3 percent.

**DISCUSSION**

Our results agree with those obtained by Klockars et al in a more limited series of cases; and we use, along with this, more accurate statistical criteria. According to our data, a PL/SL ratio higher than 1.2 strongly suggests the diagnosis of tuberculosis when empyema has been excluded. On the other hand, the existence of a neoplasia would be improbable with that ratio. On the contrary, a PL/SL ratio less than 1.2 would be infrequent in tuberculosis and empyema, but it would not point to a specific cause.

The presence of a raised PL/SL ratio suggests local production of lysozyme in the pleural cavity, such as would occur in tuberculous pleurisies and empyemas. This synthesis has been demonstrated in epithelioid cells and mononuclear phagocytes of tuberculous granulomas, as well as in granulocytes of empyemas. A contrary situation to these two processes must occur in neoplastic effusions. In these effusions, just as in the group with transudates, the existence of a PL/SL ratio lower than or equal to one with a raised coefficient of correlation between the pleural fluid level and the serum level, suggests that the pleural fluid level belongs to the filtration or diffusion of the systemic circulation. According to this, we could think that the ratio of the pleural fluid and serum levels of protein in the group with malignant effusions (0.64 ±10) could be correlated with the PL/SL ratio. This could not be proved (r = 0.21, not significant), perhaps because of the low molecular weight of lysozyme (15,000 daltons). Nevertheless, in spite of the previously mentioned findings and the findings of Klockars et al, who did not find lysozyme in tumoral cells of their patients, we found this enzyme in the specimen from biopsy in one case of the group with malignant effusions. This was

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21556/...)

**FIGURE 3.** Relation between lysozyme levels of pleural fluid (PL) and of serum (SL) in patients with neoplasia.

![Figure 4](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21556/...)

**FIGURE 4.** Relation between lysozyme levels of pleural fluid (PL) and of serum (SL) in patients with tuberculosis.

Lysozyme Level of Pleural Fluid (Veree Hernandez et al)
one of the patients with adenocarcinoma whose primary tumor was not located, and he is also the only patient with neoplasia with a PL/SL ratio higher than the cutoff point (1.75) (Fig 2). Although in this patient the pleural fluid level of lysozyme was 7 mg/dl (a low value in our tuberculous group), there is no doubt that the determination of the lysozyme level in pleural effusions should not be employed as a diagnostic test of security. In spite of this, the validity of this test cannot be underestimated, as has been done,^ according to work by Wu et al. In this series, only the lysozyme levels of pleural fluid, that are juxtaposed to ours, are reported, but the PL/SL ratio is not stated. Moreover, only two cases of tuberculous pleurisy are included. Our findings confirm that the value of this test does not lie in the distinction between malignant and nonmalignant effusions,^ but in the distinction between tuberculous and nontuberculous effusions, of which malignant are the great majority, as shown by other studies.19

This determination, therefore, seems to be useful as an initial clinical orientation in the study of pleural effusion, mainly in populations with a high incidence of tuberculosis. The lysozyme would behave in a similar way to adenosine deaminase.17,18 Both enzymes would originate in different cells, although they would be produced more or less simultaneously during the process. In a preliminary study19 with 134 patients with pleural effusions, from whom empyemas were excluded, we have found a high correlation between pleural lysozyme and adenosine deaminase (r = 0.80; p<0.001). Similarly, we13 found that if we add to the diagnostic approach of a PL/SL ratio higher than 1.2, that of a pleural level of adenosine deaminase higher than 33 international units/L, then the sensitivity and specificity for the diagnosis of tuberculosis was 100 percent.

In the effusion due to collagen disease, the results of these determinations were little homogeneous. The only case of systemic lupus erythematosus in our series had a PL/SL level of 1.04; and in another case, which was not reported herein, the PL/SL level, was 0.94. Asseo et al20 found a high level only in one of their patients. So, at this moment, we can not draw a conclusion about this type of pathologic abnormality. These authors do not obtain conclusions either about the infectious nonmycobacterial pathologic abnormalities. We have found only one patient in the group with empyemas who had a decreased pleural fluid level of lysozyme and PL/SL ratio; it was a nephrotic syndrome, with levels of protein in both the pleural fluid and serum being very decreased. The rest of the empyemas had very high pleural fluid levels and PL/SL ratios, contrasting with the group with parapneumonic effusions. All of the patients of the group with empyemas had fluid with pus. We could not prove if the nonpurulent pleural effusions with bacterial activity reach intermediate pleural fluid levels of lysozyme and PL/SL ratios between the groups with empyemas and with parapneumonic effusions. Therefore, these levels could superpose the tuberculous group.

In summary, we prove that determination of the lysozyme level in the pleural fluid and serum provides an easy method with high sensitivity and specificity for the diagnosis of pleural tuberculosis, but emphasize the fact that the validity as a method of diagnostic accuracy is limited by the presence of isolated false-positive values.

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REFERENCES