Childhood Parapneumonic Effusions*  
Biochemical and Inflammatory Markers  
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Study objectives: Biochemical and inflammatory markers in pleural inflammation were evaluated in pediatric cases of parapneumonic effusions, and interleukin (IL)-8 and tumor necrosis factor (TNF)-α concentrations were tested for possible differentiation of the complicated nature of effusions.

Patients: Twenty-eight patients (12 female) who were admitted to Hacettepe University Children’s Hospital over a 2-year period were included in the study.

Measurements: Patients were grouped according to the stage of effusion. Pleural fluid leukocyte count, neutrophil ratio, pH, protein, glucose levels, lactate dehydrogenase (LDH) levels, TNF-α levels, IL-8 levels, and nitrite levels were obtained.

Results: Of these patients, 13 had empyema, 10 had complicated parapneumonic effusions (CPEs), and 5 had uncomplicated parapneumonic effusions (UPEs). Protein and glucose levels decreased, leukocyte count, neutrophil ratio, TNF-α levels, nitrite levels, and IL-8 levels increased progressively as the stage of the disease progressed. IL-8, TNF-α, and nitrite levels all correlated positively with each other (all p < 0.001), and pH correlated negatively with these markers (all p < 0.001). At a cutoff value of 76.6 pg/mL, TNF-α discriminated between CPEs and UPEs with a sensitivity of 50%, a specificity of 100%, and an accuracy of 78%. At a cutoff value of 701.6 pg/mL, IL-8 differentiated CPE and UPE with a sensitivity of 80%, a specificity of 80%, and an accuracy of 86%.

Conclusions: Progressive changes in common biochemical markers (ie, pH, and protein, glucose, and LDH levels) are interrelated during stages of pleural inflammation. IL-8 may be used as an alternative marker for discriminating between CPEs and UPEs in pediatric parapneumonic effusions.

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Key words: interleukin-8; inflammation; nitric oxide; pediatric parapneumonic effusions; tumor necrosis factor-α

Abbreviations: CPE = complicated parapneumonic effusion; IL = interleukin; LDH = lactate dehydrogenase; NO = nitric oxide; TNF = tumor necrosis factor; UPE = uncomplicated parapneumonic effusion

Parapneumonic effusions constitute a major portion of childhood pleural effusions.¹ In clinical practice, a biochemical analysis of the pleural fluid including pH, and protein, glucose, and lactate dehydrogenase (LDH) levels helps to classify and diagnose parapneumonic effusions correctly and to manage them optimally.² Traditionally, pleural pH, and glucose and LDH levels are used to differentiate complicated parapneumonic effusions (CPEs) and uncomplicated parapneumonic effusions (UPEs).³ Although these criteria are widely used, there may be significant overlap among groups leading to an absence of reliable cutoff values.

In many branches of life sciences, various studies are being performed to elucidate the inflammatory process. The pleural response to inflammation has been inadequately investigated until now, and most of the studies were done on animal models or mesothelial cell cultures.³⁻⁵ These investigations mainly concern cytokines like tumor necrosis factor (TNF)-α and interleukin (IL)-8,⁶⁻¹¹ and intermediate metabolites like nitric oxide (NO).¹²⁻¹³ However, the clinical significance of these variables is unclear, and determining the diagnostic accuracy of these markers may provide useful information for patient management in clinical practice.
Serum

The previous properties were present. According to the criteria of pleural effusions, and to determine whether TNF-α, IL-8, and NO are useful markers in discriminating between subgroups of parapneumonic effusions.

**Materials and Methods**

**Patients**

Twenty-eight consecutive pediatric patients with parapneumonic pleural effusions whose pleural collections were sampled for diagnostic and/or therapeutic purposes were included in the study between June 2001 and June 2003 on admission to Hacettepe University Children’s Hospital. The patients were classified according to the biochemical and inflammatory properties of pleural fluid in pediatric patients with parapneumonic effusions, and to determine whether TNF-α, IL-8, and NO are useful markers in discriminating between subgroups of parapneumonic effusions.

**Laboratory Measurements**

The samples obtained by thoracentesis or thoracostomy were immediately analyzed for pH (AVL Omni blood gas analyzer; Diamond Diagnostics; Holliston, MA), total cell counts (Beckman Coulter; Fullerton, CA), differential cell count (manually under microscope using Wright-stained smears), protein, glucose, and LDH (Modular P800; Roche Diagnostics; Basel Switzerland). Total protein, glucose, and LDH levels were measured in serum also. pH measurement was done in pleural fluid samples, which arrived in the laboratory on ice within 10 min following the procedure, that were obtained anaerobically using heparinized syringes. The supernatant from each sample was stored at −70°C after centrifugation of the fluid to measure TNF-α, IL-8, and nitrite levels later. TNF-α and IL-8 levels in pleural fluid were measured by an immunoenzymometric assay (BioSource Inc; Worcester, MA). As NO is not a stable molecule and has a short half-life, nitrite was measured as its metabolite in pleural fluids, using the Griess reaction.

**Statistical Analysis**

The data are presented as the mean ± SD, median, and range. Nonparametric tests were used for data without normal distribution. The Mann-Whitney U and Kruskal-Wallis tests were used to compare groups according to the group number. For the comparison of nominal data the χ² test was used. As the number of samples is low, analysis of bivariate correlations was done using the Spearman correlation test. A two-tailed p value of ≤ 0.05 was considered to be significant. A statistical software package (SPSS, version 11.5 for Windows 11.5; SPSS; Chicago, IL) was used for the statistical analysis of the results.

**Results**

The patient group consisted of 12 female patients (43%) and 16 male patients (57%) with a mean age of 6.28 ± 4.61 years (age range, 15 months to 18 years). Of these patients, 13 patients had empyema, 10 patients had CPEs, and 5 patients had UPEs, according to the above criteria.

### Table 1—Comparison of Serum and Pleural Fluid Characteristics, and Clinical Features of the Patients According to the Stage of Pleural Effusion

<table>
<thead>
<tr>
<th>Variables</th>
<th>Empyema Group (n = 13)</th>
<th>CPE Group (n = 10)</th>
<th>UPE Group (n = 5)</th>
<th>p Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, g/dL</td>
<td>6.5 (5.0–8.3)</td>
<td>6.55 (5.4–7.8)</td>
<td>7.71 (7.3–8.4)</td>
<td>0.031</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>89 (63–128)</td>
<td>89.5 (61–113)</td>
<td>88 (80–157)</td>
<td>0.692</td>
</tr>
<tr>
<td>LDH, IU/L</td>
<td>656 (310–1,280)</td>
<td>622 (421–1,680)</td>
<td>612 (445–668)</td>
<td>0.621</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocyte count, cells/μL</td>
<td>10,400 (1,000–100,000)</td>
<td>2,200 (700–94,100)</td>
<td>1,400 (1,400–3,000)</td>
<td>0.026</td>
</tr>
<tr>
<td>Neutrophil ratio, %</td>
<td>80 (60–95)</td>
<td>73 (42–97)</td>
<td>36 (27–83)</td>
<td>0.034</td>
</tr>
<tr>
<td>Protein, g/dL</td>
<td>4.00 (3.0–5.7)</td>
<td>4.45 (3.3–5.8)</td>
<td>5.71 (5.0–7.2)</td>
<td>0.014</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>73 (2–121)</td>
<td>55 (2–125)</td>
<td>86 (61–103)</td>
<td>0.324</td>
</tr>
<tr>
<td>pH</td>
<td>7.26 (6.33–7.43)</td>
<td>7.26 (6.74–7.98)</td>
<td>7.40 (7.38–7.53)</td>
<td>0.037</td>
</tr>
<tr>
<td>HCO₃⁻, mmol/L</td>
<td>20.55 (15–30.6)</td>
<td>19.10 (10–51.1)</td>
<td>22.40 (21.2–25.3)</td>
<td>0.221</td>
</tr>
<tr>
<td>PCO₂, mm Hg</td>
<td>40.70 (22–79.90)</td>
<td>41.20 (20.8–76.0)</td>
<td>38.10 (25.9–42.8)</td>
<td>0.647</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>111.2 (5.4–1,363.6)</td>
<td>72.1 (32.4–1,000)</td>
<td>34.7 (2.0–56.7)</td>
<td>0.245</td>
</tr>
<tr>
<td>IL-8, pg/mL</td>
<td>990.70 (95.4–1,781.6)</td>
<td>1,015.50 (44.4–1,700.8)</td>
<td>260.30 (5.0–810.8)</td>
<td>0.051</td>
</tr>
<tr>
<td>Nitrite, μmol/L</td>
<td>10.5 (3.1–146.5)</td>
<td>14.2 (6.1–79.3)</td>
<td>11.8 (4.0–16.8)</td>
<td>0.779</td>
</tr>
</tbody>
</table>

*Values given as median (range), unless otherwise indicated. FF = pleural fluid. p Values denote the statistical significance of differences in median values of the three groups (Kruskal-Wallis test). A p value of ≤ 0.05 was considered to be significant.

†Significantly different from the respective values in empyema and CPE groups.

‡Significantly different from the respective values in CPE group.
Twenty-three patients (82%) received antibiotic therapy before admission to our hospital. The mean duration of time between the onset of symptoms and hospital admission was 10.5 ± 11.5 days (range, 1 to 60 days).

The pleural fluid characteristics of the three groups are shown in Table 1. Leukocyte count and neutrophil ratio increased as the inflammation progressed, and these markers reached their highest values in fluid samples from empyemas. All 28 samples were classified as exudates according to the criteria of Light and Rodriguez.2 Protein and glucose concentrations decreased, and LDH levels increased as inflammation proceeded. The protein concentration in the UPE group was significantly higher than that in the empyema group (p = 0.006) and in the CPE group (p = 0.005). A strong positive correlation was present between protein levels in serum and pleural fluids (r = 0.831; p < 0.001) [Fig 1, top, A]. No such correlation was found between LDH levels (r = 0.265; p = 0.086) and glucose levels (r = 0.326; p = 0.090) in serum and pleural fluid (Fig 1, bottom, B). Pleural pH gradually decreased as the stage of inflammation progressed, relevant to the changes in HCO₃ and PCO₂. The pH value was positively correlated to glucose levels (r = 0.692; p < 0.001), and negatively correlated to leukocyte count (r = -0.473; p = 0.007), neutrophil ratio (r = -0.416; p = 0.014), PCO₂ (r = -0.525; p = 0.003), TNF-α (r = -0.705; p < 0.001), IL-8 (r = -0.685; p < 0.001), and nitrite (r = -0.549; p = 0.001) [Fig 2].

The median IL-8 level was significantly lower in the UPE group than in the empyema group (p = 0.026) and the CPE group (p = 0.028). TNF-α and nitrite levels were not statistically different among the groups. When the three inflammatory mediators were compared, all were positively and significantly correlated with each other as follows: IL-8 and TNF-α (r = 0.585; p = 0.001); IL-8 and nitrite (r = 0.671; p < 0.001); and TNF-α and nitrite (r = 0.683; p < 0.001). Biochemical markers were correlated with inflammatory markers as follows: pleural fluid glucose was negatively correlated with IL-8 (r = -0.732; p < 0.001), to TNF-α (r = -0.612; p < 0.001), and to nitrite (r = -0.671; p < 0.001); the pleural fluid leukocyte count was correlated positively with IL-8 (r = 0.462; p = 0.009), to TNF-α (r = 0.454; p = 0.010), and to nitrite (r = 0.421; p = 0.016); and the neutrophil ratio was positively correlated to IL-8 (r = 0.434; p = 0.010), but there were no correlations with TNF-α and nitrite.

The pleural IL-8 and TNF-α concentrations for the individual patients in the CPE and UPE groups are shown in Table 2. Although there was some overlap in the individual values of these two groups, concentrations of IL-8 and TNF-α were higher in the CPE group in general. The diagnostic accuracies of these two markers in the differentiation of CPE and UPE were tested by receiver operating curves. IL-8 level differentiated CPEs and UPEs with 80% sensitivity, 80% specificity, and 86% accuracy when a cutoff value of 701.6 pg/mL was applied (p = 0.027; 95% confidence interval, 67 to 100%). These values changed to 70%, 100%, and 86%, respectively, when...
the cutoff point was raised to 885.65 pg/mL. For TNF-α, a cutoff value of 76.6 pg/mL resulted in a sensitivity, specificity, and accuracy of 50%, 100%, and 78%, respectively (p = 0.086; 95% confidence interval, 52 to 100%). These values changed to 90%, 60%, and 78%, respectively, when the cutoff value was 37.5 pg/mL. Nitrite values had much lower sensitivity, specificity, and accuracy in differentiating CPEs and UPEs.

**Discussion**

In clinical practice, pleural fluid analysis provides the most accurate estimation of the stage of pleural inflammation, because the inflammatory process follows a characteristic cascade of events. It is already known that a decrease in pleural pH and glucose levels and an increase in pleural LDH level show ongoing bacterial and cellular metabolic activity and are used as major differentiation criteria of parapneumonic effusion subtypes. In the present study, we have observed that IL-8 may also be used as an alternative marker for the complication of parapneumonic effusions.

After classifying our patients according to the above-mentioned criteria, some additional aspects of pleural inflammation were observed in our data. These include the initiation of pleural inflammation with filtration of proteinaceous fluid and chemotaxis of inflammatory cells into the pleural space following the progressive increase in IL-8 concentration, which is known as the major neutrophil chemoattractant molecule. This was evident in the strong positive correlations between IL-8 and neutrophil ratio, and IL-8 and leukocyte count. One more aspect of pleural inflammation is that the predominant cell type found in the pleural fluid of patients who are symptomatic during the very early stages are polymorphonuclear leukocytes. High leukocyte counts with neutrophil predominance in our group of parapneumonic effusions reflect this acute stage of inflammation.

Protein, glucose, and LDH concentrations in pleural fluid are determined by the nature of the inflammatory process. The initially high pleural protein level reflects the filtration of serum proteins into the pleural space due to increased vascular permeability.
positive correlations among IL-8, TNF-α levels to show that they work interactively. When stimulated,7,8 and TNF-α types including mesothelial cells produce TNF-α/H9251 of other cytokines including IL-8,6,7 and to induce acute-phase reactions by inducing the release of collagen synthesis9,19 during inflammation. Many cell types including mesothelial cells produce TNF-α when stimulated,7,8 and TNF-α is found in high concentrations in many pleural conditions including pleural infections and malignancy,8 limiting its role in differential diagnosis. Likewise, NO functions through all stages of inflammation,13 affects vascular permeability and local blood flow,3 induces the onset of regeneration during wound healing,13 induces fibroblast proliferation,13 and has both inflammatory and anti-inflammatory functions depending on the type and stage of inflammation and on its local concentration.4,12 NO functions through the whole process probably by regulating the cytokine production, including IL-8 and TNF-α,13 and it also has a chemotactic effect on monocytes and neutrophils, which in turn synthesize other cytokines.13 Our data did not show statistically significant differences in TNF-α and NO levels among groups, but there were positive correlations among IL-8, TNF-α, and nitrite levels to show that they work interactively.

To our knowledge, whether TNF-α and NO could differentiate subgroups of parapneumonic effusions has been investigated in only two studies in the past few years.20,21 In both studies, it was shown that TNF-α may contribute to the identification of patients with CPE, although the latter study21 indicated a much lower cutoff point when compared to the former and to the present study. Our results show that IL-8 may be used as a marker with relatively high sensitivity and specificity in differentiating CPE and UPE in pediatric patients with parapneumonic pleural effusions. According to our data, TNF-α is a marker that is less informative than IL-8 in differentiating CPE and UPE, since TNF-α values did not differ significantly between the two groups, and its sensitivity, specificity, and accuracy are markedly lower than those for IL-8.

To our knowledge, studies regarding the role of pleural IL-8, TNF-α, and nitrite concentrations in the differentiation of CPEs and UPEs in pediatric patients have not been reported previously. Our results are important as initial findings demonstrating the sensitivity and specificity of IL-8 in discriminating the complicated nature of pleural effusions. The therapeutic approach may possibly be guided by measurements of IL-8 concentrations in future clinical practices, depending on the results of further studies, which will improve our findings.

As our hospital is a referral center for pediatric patients, we usually receive patients in whom previous antibiotic therapy for pneumonia and UPEs has failed. This resulted in a patient group including more empyemic fluids than CPEs and UPEs, and there was a total of five patients with UPEs seen in this 2-year period. Therefore, the inclusion of a larger number of patients in our study was impossible. Previous antibiotic therapy was also a limitation of our study in that the therapy most probably interfered with the inflammatory process, although no clinical improvement was observed in the patients.

Both biochemical and inflammatory markers of pleural effusions should be continuously used for clinical purposes in further studies in pediatric patients. Considering the combination of pH, glucose,
and LDH as the “gold standard” test in differentiating CPEs and UPEs, we concluded that a high concentration of IL-8 is a feature of CPEs and may be used as an alternative marker. Also, the predominant function of IL-8 as a neutrophil chemoattractant has already clearly been established but understanding the diverse functions of TNF-α and NO during inflammation needs further analysis.

REFERENCES