Epithelial Neutrophil-Activating Peptide-78 Recruits Neutrophils into Pleural Effusion

Short running head: Pleural ENA-78 induces neutrophil influx

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This study was supported in part by research grants 30660064 and 30872343 from National Natural Science Foundation of China; in part by research grant 0728137 from Natural Science Foundation of Guangxi Zhuang Autonomous Zone; and in part by research grant 200621 from the Bureau of Health, Guangxi Zhuang Autonomous Zone, China.
ABSTRACT

The aim of this study was to investigate the presence of epithelial neutrophil-activating peptide (ENA)-78 in pleural effusions, as well as the chemoattractant activity of pleural ENA-78 on neutrophils.

Pleural effusion and serum samples were collected from 75 patients who presented to the respiratory institute (19 with malignant pleural effusion, 21 with tuberculous pleural effusion, 18 with infectious pleural effusion, and 17 with transudative pleural effusion). The concentrations of ENA-78, myeloperoxidase and neutrophil elastase were determined, and the chemoattractant activity of ENA-78 for neutrophils both in vitro and in vivo was also observed.

The concentrations of ENA-78, myeloperoxidase and neutrophil elastase in infectious pleural effusion were significantly higher than those in malignant, tuberculous, and transudative groups, respectively (all p < 0.01). Infectious pleural fluid was chemotactic for neutrophils in vitro, and anti-ENA-78 antibody could inhibited partly this chemotactic effects. Intrapleural administration of ENA-78 produced a marked progressive influx of neutrophils into pleural space.

Compared to non-infectious pleural effusion, ENA-78 appeared to be increased in infectious pleural effusion. Our data suggested that ENA-78 was able to induce neutrophil infiltration into pleural space, and might be responsible for pleural neutrophil degranulation.

Key words: Infections; Inflammatory cell; Neutrophil migration; Pleural effusion.
INTRODUCTION

The inflammatory process results in increased pleural vascular permeability, leading to the accumulation of fluid enriched in proteins and to the recruitment of cells with the development of exudative pleural effusion (PE) (1). PE is characterized by the presence of specific subsets of leukocytes (1, 2), which, together with pleural mesothelial cells, contribute to the local production of cytokines and chemokines (3, 4). Analysis of malignant and tuberculous PEs usually shows a lymphocytic preponderance; whereas infectious PEs, including empyema and parapneumonic effusion, are typically associated with an influx of neutrophils (5, 6).

Neutrophils are recruited to sites of inflammation by a variety of chemoattractants, including complement factors (C5a), interleukin (IL)-8, arachidonic acid metabolites (leukotriene B4), and infectious peptides (N-formyl-methionyl-leucyl-phenylalanine) that are generated and released locally at sites of injured tissue. Neutrophils release reactive oxygen and nitrogen species into the extracellular millieu, in addition to releasing their granular content, which contains several serine and neutral proteases that can produce tissue injury (7). Chemokines are potent proinflammatory molecules, and their regulation must therefore be tightly controlled (8). One of these CXC chemokines is epithelial neutrophil-activating peptide (ENA)-78 (also named CXCL5) that contains a Glu-Leu-Arg motif essential for neutrophil-stimulating activity (9, 10). ENA-78 has been implicated in the pathogenesis of a number of pulmonary diseases (11-13). The present study was performed to determine total and differential leukocyte counts, concentrations of ENA-78 and two indicators of neutrophil
degranulation (myeloperoxidase [MPO] and neutrophil elastase [NE]) in pleural fluid and
serum of patients with PEs of various etiologies, in order to: (1) determine whether PE
ENA-78 is produced in the pleural space; (2) establish the relation of this chemokine to the
neutrophil number and activation state in PE; and (3) explore the chemoattractant activity of
ENA-78 on neutrophils.
MATERIALS AND METHODS

Subjects

A prospective design study was performed in our institute of respiratory diseases from October, 2006 through March, 2008. The study protocol was approved by our institutional review board for human studies, and informed consent was obtained from all subjects.

Seventy-five consecutive patients with PE of various causes were recruited in the present study.

Malignant PE was collected from 19 patients (age range: 34 to 74 yr) with newly diagnosed lung cancer with PE. Histologically, 14 cases were adenocarcinoma and 5 were squamous cell carcinoma. A diagnosis of malignant PE was established by demonstration of malignant cells in PE and/or on closed pleural biopsy specimen.

Twenty-one patients (age range: 17 to 68 yr) were proven to have tuberculous PE, as evidenced by 1) a compatible clinical history associated with presence of acid fast bacilli in PE specimen or by demonstration of granulomatous pleurisy on closed pleural biopsy specimen in the absence of any evidence of other granulomatous diseases; 2) an exudative lymphocytic effusion with an adenosine deaminase level of > 40 U/L, along with a positive purified protein derivative skin test result and the exclusion of any other potential causes of pleurisy; 3) after anti-tuberculosis chemotherapy, the resolution of PE and clinical symptoms was observed.

Eighteen PE patients (age range: 16 to 57 yr) were classified as infectious PE (including 16 empyema and 2 parapneumonic effusion). Empyema was defined as an effusion that met one
or more of the following criteria: purulent fluid on macroscopic examination, positive Gram
stain and/or growth of organisms in culture, and PF pH < 7.2 or glucose < 3.3 mmol/L in
association with pneumonia. Parapneumonic effusion was those with a glucose concentration
> 3.3 mmol/L and pH > 7.2, and no organisms seen on Gram stain or found on PE culture in a
patient with pneumonia.

Seventeen patients (age range: 17 to 70 yr) with PE were classified as transudates on the
basis of Light’s criteria (14).

The patients were excluded if they had received any invasive procedures directed into the
pleural cavity or if they had suffered chest trauma within 3 months prior to hospitalization or
had a PE of undiagnosed cause. At the time of sample collection, none of the patients had
received any anti-tuberculosis therapy, anti-cancer treatment, corticosteroids, or other
nonsteroid anti-inflammatory drugs.

**Sample collection and processing**

The PE samples were collected in heparin-treated tubes from each subject, using a standard
thoracocentesis technique within 24 h after hospitalization. Ten milliliters of venous blood
were drawn simultaneously for obtaining sera. The PE specimens were immersed in ice
immediately and were then centrifuged at 1,200 g for 5 min. The cell-free supernatants of PE
and serum were frozen at –80 °C immediately after centrifuge for later determining
concentrations of ENA-78, MPO, and NE. Total and differential cell counts, detections of
concentrations of pleural protein, glucose, lactate dehydrogenase, and adenosine deaminase
were performed in addition to cytologic and microbiological examination of pleural fluid. A pleural biopsy was performed when the results of pleural fluid analysis were suggestive of tuberculosis or malignancy.

**Measurement of ENA-78, MPO and NE**

The concentrations of ENA-78, MPO and NE in both PEs and sera were measured by sandwich enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer’s protocols. The ELISA kits for ENA-78 and MPO were purchased from R & D Systems Inc. (Minneapolis, MN, USA), and the ELISA kits of NE were purchased from Cell Sciences® (Canton, MA, USA). All samples were assayed in duplicate. The lower detection limits were: ENA-78 (15 ng/L); MPO (0.1 µg/L); NE (0.4 µg/L).

**Neutrophil chemotaxis assay**

Human neutrophils were prepared from peripheral blood by Ficoll-Hypaque density gradient in 0.9% saline (Sigma Chemical Co., St. Louis, MO, USA). Neutrophils were separated from erythrocytes by hypotonic lysis and then suspended in Hanks' balanced salt solution (HBSS) with calcium/magnesium (Gibco, Grand Island, NY, USA) at $2.0 \times 10^6$ cells/ml with greater than 95% viability by tryblue exclusion. 200 µl of the neutrophil suspension ($1.0 \times 10^6$ cells/ml in RPMI 1640) were loaded into the upper well of the chamber, while 30 µl pleural fluid from 5 patients with malignant PE, 5 with tuberculous PE, 5 with empyema, or 5 with transudates was placed in the bottom chamber. The two wells were separated by a polycarbonate filter
paper with a pore size of 5 µm, and the chamber was incubated at 37 °C for 45 min. At the end of incubation, the filter was fixed, stained, and mounted on a glass microscope slide. Migration was assessed by counting the number of cells that had migrated beyond a certain depth into the filter (50 µm). To correct for donor to donor variation, migration data of test samples were compared with their corresponding control values (HBSS alone) and expressed as the percentages above the control value. To demonstrate that ENA-78 was responsible for neutrophil migration, blocking experiments were performed by mixing the PE with 10 µg/ml of anti-ENA-78 monoclonal antibody (mAb) or IgG2a irrelevant isotype control (R & D System Inc.).

**Effects of intrapleural injected ENA-78 on neutrophil recruitment**

After additional study protocol was approved by our institutional review board and informed consent was obtained from the subjects studied, a total of 10 patients with tuberculous PE were included in this section of study. Right after collection of PE samples, ten µg of recombinant human ENA-78 (R & D Systems Inc.) in vehicle (0.1% human serum albumin in 0.9% saline) was injected into the pleural space of 5 patients, and vehicle only was injected into the pleural space of the other 5 patients. Intrapleural injection of ENA-78 or vehicle was randomized. The dose of ENA-78 was based upon a preliminary study involving two PE patients. PE collection for determining neutrophil numbers was repeated 6, 12, and 24 h after the injection of ENA-78 or vehicle.
**Statistical analysis**

Data were expressed as mean ± standard error of mean (SEM) or median (25th to 75th percentiles). Comparisons of the data between different groups were performed using Kruskal-Wallis one-way ANOVA on ranks. For the levels of ENA-78, MPO and NE in PE and in corresponding serum, paired data comparisons were made using a Wilcoxon signed-rank test. The correlations between variables were determined by Spearman rank correlation coefficients. The effects of intrapleural injected ENA-78 or vehicle on neutrophil recruitment were compared through one-way repeated-measures ANOVA. Analysis was completed with SPSS version 14.0 Statistical Software (Chicago, IL, USA), and p values of less than 0.05 were considered to indicate statistical significance.
RESULTS

Characteristics in PEs

Cytological characteristics in PEs are illustrated in Table 1. Subjects with lung cancer showed a large proportion of lymphocytes and macrophages in PE. Importantly, on cytologic examination, malignant cells were found in 13 subjects. Subjects with tuberculosis showed a marked elevation of total cell counts, and a large proportion of these cells were lymphocytes, with some neutrophils and macrophages. Absolute lymphocyte counts evidenced the highest values in tuberculous PE, showing a significant increase in comparison with each of PEs induced by the other causes (all p < 0.05). Also as expected, total cell counts in infectious PE were the greatest among the four groups (all p < 0.05), and neutrophil was the most predominant cell type, the numbers of this kind of white cells were significantly greater than those in the PEs with any other causes (all p < 0.01).

Concentrations of ENA-78, MPO and NE in PEs

As shown in Table 2 and Figure 1, the concentration of ENA-78 in infectious PE (median, 2,639.2 ng/L; 25th to 75th percentile, 448.8 to 5,014.6 ng/L) was significantly higher than those in malignant PE (88.0 ng/L; 52.2 to 337.8 ng/L), tuberculous PE (73.0 ng/L; 33.9 to 131.5 ng/L), and transudative PE (36.1 ng/L; 22.3 to 74.2 ng/L), with all p < 0.01. It should be mentioned that although ENA-78 concentration in infectious PE were much higher than those
in the other PEs, we were unable to identify a cutoff value for ENA-78 that could be used to diagnose infectious PE.

The similar results were observed with MPO and NE studies, and the concentrations of both these enzymes changed in parallel among the groups. Also as shown Table 2, the concentrations of both MPO and NE in infectious PE were significantly higher than those in the any other groups (all \( p < 0.01 \)).

The comparisons of levels of ENA-78, MPO, and NE in PEs with their corresponding compartments in sera are also shown in Table 2. The concentrations of ENA-78, MPO and NE in sera were not different with one another among 4 groups, respectively (all \( p > 0.05 \)). ENA-78 concentration was much lower in PEs than in sera in from patients with malignant, tuberculous, and transudative PEs (all \( p < 0.05 \)); in contrast, ENA-78 in infectious PE tended to be increased when compared the corresponding serum, but the difference did not reach statistical significance (\( p = 0.102 \)). MPO concentration was much lower in malignant and transudative PEs, and higher in tuberculous and infectious PEs than in their corresponding sera (all \( p < 0.05 \)). NE concentration in malignant, tuberculous, and infectious, but not transudative PE, was much higher than that in their corresponding serum.

**Correlation between ENA-78 and inflammatory cells, MPO, as well as NE**

We noted that pleural ENA-78 levels were positive correlated with the number of neutrophils (Figure 2A, \( n = 75, r = 0.526, p < 0.001 \)), but not with lymphocytes (\( n = 75, r = 0.167, p = 0.153 \)), macrophages (\( n = 75, r = 0.022, p = 0.854 \)), mesothelial cells (\( n = 75, r = -0.250, p = \))
and malignant cells (n = 13, r = 0.017, p = 0.957). We further noted that the pleural ENA-78 levels were positive correlated with PE MPO levels (Figure 2B, n = 75, r = 0.714, p < 0.001) and PE NE levels (Figure 2C, n = 75, r = 0.739, p < 0.001). The correlations between pleural ENA-78 and neutrophil numbers, MPO levels, as well as NE levels within the different diagnostic groups are shown in Table 3. It was still noted that PE ENA-78 levels are positive correlated with MPO levels and NE levels even when PEs with various etiologies were analyzed separately.

**Infectious PE was chemotactic for neutrophils**

The finding that the concentration of pleural ENA-78 correlated best with the number of neutrophils prompted us to test the chemoattractant activity of PEs on human neutrophils. Our results showed that exudates, especially empyema, but not transudates exerted a potent chemoattractant activity for neutrophils (Figure 3). To determine whether ENA-78 was responsible for the migration of neutrophils, the ability of an anti-ENA-78 mAb to neutralize the chemoattraction of neutrophils was tested. The anti-ENA-78 mAb significantly suppressed neutrophil chemotaxis in all exudative PE groups. These data provided indirect evidences that ENA-78 was capable of inducing neutrophil recruitment into the pleural space.

**Recruitment of neutrophils into PE caused by ENA-78**

To investigate the direct chemoattractant capacity of ENA-78 to recruit neutrophils *in vivo*, we injected 10 µg human recombinant ENA-78 into pleural space of patients with tuberculous
pleurisy, and then observed the changes in neutrophil numbers. Compared with baseline value (0.38 ± 0.06 × 10^9/L), a significant increase in the number of neutrophils started to be observed at 6 h (0.72 ± 0.10 × 10^9/L, p < 0.01) after intrapleural ENA-78 injection; The number of neutrophils increased with time, reaching a maximum at 12 h (0.82 ± 0.10 × 10^9/L, p < 0.01), and lasting for at least 24 h (0.79 ± 0.10 × 10^9/L, p < 0.01) (Figure 4). After vehicle only was injected into the pleural cavity, we did not observe increases of neutrophil counts in PE obtained at 3 time points when compared with baseline measurement before injection (all p > 0.05).
DISCUSSION

The development of PE is often associated with an increase of inflammatory cells in the pleural space. PEs caused by diverse disease entities are usually present with the predominance of a certain type of leukocytes. Infectious PEs, including empyema and parapneumonic effusion, are typically associated with an influx of neutrophils, whereas tuberculous and malignant PEs are rich in lymphocytes (5, 6).

Inflammation within the pleural space could be mediated by a number of proinflammatory molecules. ENA-78 is a 78-amino acid protein displaying the four highly conserved cysteine residues that are common feature of all CXC chemokines, including IL-8. The overall amino acid sequence identity of ENA-78 with IL-8 is only 22% (15). ENA-78 is a potent activator of neutrophil function, inducing chemotaxis, enzyme release, and a rise of intracellular calcium (9) by acting via the type-B IL-8 receptors (16), and thus may play an important role in the recruitment of leukocytes to inflammatory lesions in a manner similar to that demonstrated for IL-8 and other neutrophil-activating proteins (17, 18).

We did not identify the cell origins of pleural ENA-78 in the present study. The primary aim of this study was to explore the presence of ENA-78 in PE, the chemoattractant activity of PE ENA-78 on neutrophils, as well as the relation of ENA-78 to the activation state of neutrophils in PE. Originally, ENA-78 was characterized from the IL-1β- and tumor necrosis factor-α-stimulated alveolar type II epithelial cell line A549 (9). Now, it is well known that ENA-78 expression is inducible by a variety of inflammation mediators, including
lipopolysaccharide, IL-1, and tumor necrosis factor-α, in epithelial cells (9, 19), monocytes (20, 21), platelets (22), endometrial stromal cells (23), endothelial cells (24), and macrophage (25). In the present study, our data have shown for the first time that ENA-78 could be detected by ELISA in PEs, and that the concentration of ENA-78 in infectious PE was significantly higher than those in malignant, tuberculous, and transudative PEs. These findings suggested that more pleural sources of ENA-78 exist in infectious patients. Local production has been reported for some chemokines such as IL-8 (26, 27), monocyte chemotactic peptide-1 (27, 28), and macrophage-derived chemokine (29), etc., in inflammatory or neoplastic pleural disorders. Likewise, PE ENA-78 can also be produced by inflammatory cells recruited into the pleural space. Interestingly, although ENA-78 concentration was much lower in PEs than in sera in from patients with malignant, tuberculous, and transudative PEs, ENA-78 in infectious PE tended to be increased when compared the corresponding serum. These results taken together favor the concept of a local production of ENA-78 in infectious PE, rather than a passive diffusion of this chemokine from plasma to the pleural compartment. On the other hand, it was not possible for ENA-78 to exude into peripheral circulation from the pleural space in patients with malignant, tuberculous, or transudative PE, since ENA-78 concentrations in these PEs were much lower than those in the their corresponding sera.

The mechanism by which neutrophils infiltrate into pleural cavity is not elucidated completely so far. The finding of higher concentration of ENA-78 in infectious PE than in any of the other groups was consistent with the massive neutrophilic infiltration of PE in this condition. If ENA-78 was the major factors contributing to neutrophil influx into the pleural
space, strong correlations between PE neutrophil counts and ENA-78 would be expected. As a matter of fact, our results have demonstrated a positive correlation between neutrophil numbers and ENA-78 level, when all PEs studied were analyzed together. Indeed, an *in vitro* migration assay in the present study further confirmed that exudative PEs could induce the migration of neutrophils, and that an anti-ENA-78 mAb inhibited the ability of the PEs to stimulate neutrophil chemotaxis. The important findings in this study also included that intrapleural administration of 10 µg human recombinant ENA-78, not vehicle, of patients with tuberculous PE produced a marked progressive influx of neutrophils into pleural space. Therefore, we have provided the direct evidence in the present study for the first time that intrapleural injection of ENA-78 was able to chemoattract neutrophil recruitment into pleural space.

We noted that the effect of inhibiting ENA-78 in infectious pleural fluid only reduced neutrophil chemotaxis by around 50%, suggesting that ENA-78 is one of several chemokines that aid neutrophil recruitment into the pleural space. Actually, it has been reported in the earlier studies that IL-8 concentrations in infectious PE were much higher than those in the PEs with any other causes, and that there was a significant positive correlation between pleural IL-8 concentrations and neutrophil counts (27, 30). Moreover, it has also been demonstrated that IL-8 was a major neutrophil chemotactic factor in pleural liquid of patients with empyema (27, 30). Since ENA-78 and IL-8 exhibit different patterns of expression in cells, such as human monocytes and endothelial cells (20, 24), the comparison of chemotactic activity between ENA-78 and IL-8 need to be established in the future study. In addition, it is likely
that backup redundancy exists among the many chemokines. It is also necessary to design *in vivo* animal experiments using gene knockout mice or chemokine antagonists to explore such a back redundancy in pleural infection.

Neutrophils release reactive oxygen and nitrogen species into the extracellular milieu, in addition to releasing their granular content, which contains several serine and neutral proteases that can produce tissue injury. Among the neutrophil proteases, NE has been implicated in both chronic and acute inflammatory damage, and oxidant species, partly produced by the action of another granular enzyme, MPO, potentiate its effects (31, 32). Therefore, it is likely that the products derived from neutrophil activation are related to the evolution of infectious PE from the noncomplicated to the complicated state. The effect of ENA-78 on neutrophil degranulation has been demonstrated *in vitro* (9, 33), and there are also data to support its occurrence *in vivo* (25). In agreement with earlier studies reported by the other authors, we found the highest PE concentrations of both MPO and NE in infectious PEs (34) with a profile similar to that for ENA-78. In the present study, we have shown that both MPO and NE correlated positively with ENA-78, thus suggesting a role for ENA-78 in pleural neutrophil degranulation.

It should be noted that although ENA-78 level in infectious PE was higher than that in PE caused by the other causes, it is not possible to differentiate infectious PE from other kinds of PEs due to the wide distribution and obvious overlap of these chemokine concentrations among each group.
In conclusion, compared to non-infectious PE, ENA-78 appeared to be increased in infectious PE. ENA-78 was able to induce neutrophil infiltration into pleural space, and might be responsible for pleural neutrophil degranulation.
Conflict of interest statement

None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.
References


Figure legends

**Figure 1** Comparison of concentrations of epithelial neutrophil-activating peptide-78 (ENA-78) in pleural effusions with different etiologies. Statistical analysis was done by Kruskal-Wallis one-way ANOVA on ranks.
The graph shows the distribution of ENA-78 (ng/L) levels in different types of pleural effusions: Malignant, Tuberculous, Infectious, and Transudative.

- Malignant: The data points are spread across the range, with no significant difference indicated.
- Tuberculous: The data points are also spread, with no significant difference.
- Infectious: The data points are clustered together, indicating a significant difference at a p value of 0.001.
- Transudative: The data points again show a significant difference at a p value of 0.001.

The y-axis represents ENA-78 levels ranging from 1 to 100,000 ng/L.
**Figure 2** The concentrations of epithelial neutrophil-activating peptide-78 (ENA-78) correlated with neutrophil numbers (A), the concentrations of myeloperoxidase (MPO) (B) and neutrophil elastase (NE) (C) in pleural effusions. Correlations were determined by Spearman rank correlation coefficients.
Figure 3  Exudative pleural effusions are chemotactic for neutrophils *in vitro*. Pleural effusions from patients with lung cancer (n = 5), tuberculous pleurisy (n = 5), empyema (n = 5) and with transudates (n = 5) were used to stimulate chemotaxis of peripheral blood neutrophils isolated from healthy adults. ENA-78 = epithelial neutrophil-activating peptide; PE = pleural effusion. Data are expressed as percent of control. Open bars represent chemotaxis in the absence of anti-ENA-78 monoclonal antibody; hatched bars represent an irrelevant isotype control; closed bars represent chemotaxis in the presence of anti-ENA-78 monoclonal antibody. *p < 0.01 compared with the corresponding group without anti-ENA-78 monoclonal antibody; †p < 0.01 compared with each of the other pleural effusion groups. The comparisons were determined by Kruskal-Wallis one-way ANOVA on ranks.
Figure 4  Changes of neutrophil numbers in pleural effusion from patients with tuberculous pleurisy, who were intrapleurally injected with recombinant human epithelial neutrophil-activating peptide-78 (ENA-78) (■) or vehicle (□). n = 5 for each group. Points represent mean values, with error bars depicting SEM at each time point. *p < 0.01 compared within-group change from baseline measurements determined by one-way repeated-measures ANOVA.
<table>
<thead>
<tr>
<th></th>
<th>Malignant PE</th>
<th>Tuberculous PE</th>
<th>Infectious PE</th>
<th>Transudative PE</th>
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<td>19</td>
<td>21</td>
<td>18</td>
<td>17</td>
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<td>Total cell counts, $\times 10^9$/L</td>
<td>1.48 ± 0.10</td>
<td>2.52 ± 0.16†</td>
<td>6.46 ± 0.89‡</td>
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<td>3.8 ± 0.8</td>
<td>13.1 ± 1.0‡</td>
<td>77.5 ± 3.5‡</td>
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<td>Macrophage§</td>
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<td>Malignant cell</td>
<td>6.5 ± 1.2</td>
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</table>

* Values are presented as mean ± SEM; PE = pleural effusion.

† $p < 0.05$ compared with transudative group; ‡ $p < 0.01$ compared with each of the three groups; § $p < 0.05$ compared with one another among four groups; ¶ $p < 0.05$ compared with malignant group. The comparisons were determined by Kruskal-Wallis one-way ANOVA on ranks.
Table 2  Concentrations of ENA-78, MPO and NE in pleural effusions and sera*

<table>
<thead>
<tr>
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<td>Pleural effusion</td>
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<td>52.2 – 337.8</td>
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<td>448.8 – 5,014.6</td>
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NE (µg/L)

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</table>

* Values are presented as median (25th – 75th percentiles); ENA-78 = epithelial neutrophil-activating peptide; MPO = myeloperoxidase; NE = neutrophil elastase.

† p < 0.05 compared with the corresponding compartments in sera, determined by Wilcoxon signed-rank test.
‡ p < 0.01 compared with each of the other pleural effusion groups determined by Kruskal-Wallis one-way ANOVA on ranks.
Table 3  Correlation of ENA-78 and neutrophil numbers or neutrophil activation markers in pleural effusion

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Neutrophil count</th>
<th>MPO</th>
<th>NE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ENA-78</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>r = 0.526, p &lt; 0.001</td>
<td>r = 0.714, p &lt; 0.001</td>
<td>r = 0.739, p &lt; 0.001</td>
</tr>
<tr>
<td>Malignant PE</td>
<td>19</td>
<td>r = –0.227, p = 0.349</td>
<td>r = 0.772, p &lt; 0.001</td>
<td>r = 0.786, p &lt; 0.001</td>
</tr>
<tr>
<td>Tuberculous PE</td>
<td>21</td>
<td>r = 0.324, p = 0.152</td>
<td>r = 0.645, p = 0.002</td>
<td>r = 0.463, p = 0.035</td>
</tr>
<tr>
<td>Infectious PE</td>
<td>18</td>
<td>r = 0.521, p = 0.027</td>
<td>r = 0.922, p &lt; 0.001</td>
<td>r = 0.723, p = 0.001</td>
</tr>
<tr>
<td>Transudative PE</td>
<td>17</td>
<td>r = 0.136, p = 0.603</td>
<td>r = 0.574, p = 0.016</td>
<td>r = 0.914, p &lt; 0.001</td>
</tr>
</tbody>
</table>

ENA-78 = epithelial neutrophil-activating peptide; MPO = myeloperoxidase; NE = neutrophil elastase.