

## ROLE OF INTERLEUKIN-8 IN DIFFERENTIATION OF UNCOMPLICATED FROM COMPLICATED PARAPNEUMONIC EFFUSION

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**Abstract:** The aim of this study was to investigate the value and significance of interleukin-8 in differentiation of uncomplicated parapneumonic effusion (UCPPE) from complicated parapneumonic effusion (CPPE).

Using an IMMULITE 1000 Analyzer, with chemiluminescent immunometric assay, levels of interleukin-8 (IL-8) were measured in the pleural fluid of patients with UCPPE (n = 30), and CPPE (n = 30), and three classical parameters (pH, glucose, and LDH) in these two groups. Receiver-operating curves were to assess the sensitivity and specificity of interleukin-8 for differentiating between the two patient groups.

IL-8 levels were statistically higher in the CPPE group. A positive significant correlation, was found between levels of IL-8 and lactate dehydrogenase (LDH) ( $r = 0.68$ ,  $p < 0.05$ ). There was also a positive significant correlation between IL-8 and protein level in pleural effusion ( $r = 0.306$ ,  $r < 0.01$ ). There was a significant negative correlation between levels of IL-8 and pH ( $r = -0.83$ ,  $p < 0.05$ ), and of IL-8 and glucose in pleural fluid ( $r = -0.61$ ,  $p < 0.05$ ). A cut-off value of 1805.81 pg/ml, differentiated CPPE from UPPE with a sensitivity of 100% and a specificity of 98%.

IL-8 may be used as an alternative marker for the complication of parapneumonic effusion.

**Key words:** uncomplicated parapneumonic effusion (UCPPE), complicated parapneumonic effusion (CPPE), cytokin, interleukin-8.

### *Introduction*

Parapneumonic effusions usually occur as a result of community-acquired pneumonia, but they may occur as a complication of nosocomial pneumonia, lung abscesses or inflamed bronchiectasiones, lung cancer or lung infarction with development of infection. They can frequently appear if dental or sinus focus is present.

In the United States of America (USA) there are one million adult patients who are being hospitalised because of community-acquired pneumonia yearly, 20–40% with parapneumonic effusion, uncomplicated parapneumonic effusion (UCPPE) or complicated parapneumonic effusion (CPPE) according to evolution [1, 2, 3]. Uncomplicated parapneumonic effusions occur in 36–57% of all hospitalised patients with pneumonia [1, 3, 4].

The importance of early detection and diagnosis of parapneumonic effusions is quite considerable, particularly in the initial stage of sterile exudate (parapneumonic uncomplicated effusion) the resolution of which occurs by antibiotic treatment without the need for drainage or other surgical interventions to prevent loculation and their progression to empyema [5].

If pneumonia is not treated correctly and has not come to the inhibition of the antimicrobial agent, bacteria multiply uncontrollably and invade through the lung interstitium in the pleural space and exceed the capacity of intrapleural phagocytes (neutrophils and macrophages), resulting in a positive culture and Gram stain. This bacterial invasion or fibropurulent stage is characterized with increased migration of neutrophils and increased metabolic activity during phagocytosis, resulting in increased utilization of glucose and production of carbon dioxide and lactic acid that leads to cell death. These pathophysiological changes result in pleural fluid acidosis, low glucose and increased concentrations of LDH (6.2). Therefore, in determining the basic stages of the parapneumonic effusions and direction of treatment, values of LDH, concentration of glucose in pleural fluid and pH values have a very important role. Metabolic activities of bacteria and inflammatory cells influence these parameters.

According to evolution, parapneumonic effusions are separated into two groups [12, 4, 12]:

- Uncomplicated parapneumonic effusions (UCPPE)
- Complicated parapneumonic effusions (CPPE)

The time needed for the effusion to pass over from the first to the second stage is about 2–3 weeks when the effusion is inadequately treated [13, 14]. Certainly, the patient's comorbidity and age have a great importance in the evolutionary process of parapneumonic effusions.

Uncomplicated parapneumonic effusions are marked as simple parapneumonic effusion and are generally sterile exudates with negative bacterial culture, a glucose level above 60 mg/dl, pH above 7.20 and level of LDH under

1000 UI/ml. In this case resolution is accomplished by treatment with antibiotics without drainage [4, 15].

In the case of inadequate diagnosis and treatment, the uncomplicated parapneumonic effusions can progress to complicated, characterized by deposits of fibrin, the presence of microorganisms in the pleural fluid, which may not always be isolated, with values of glucose below 60 mg/dl, pH below 7.2 and values of LDH that are three times greater than the normal limit or above 1000 UI/ml. CPPE may result with an accumulation of pus in the pleural space and the development of empyema [5, 16, 19].

Pathophysiological changes in the process of inflammation into the pleural space result in pleural fluid acidosis, a low level of glucose and an increased concentration of LDH [6]. Because of this LDH, glucose and pH in pleural fluid are significant parameters in the determination of the stage of parapneumonic effusion [1, 2, 3, 4, 6].

In some studies the old parameters have low specificity and sensitivity [9, 10, 20]. In the study of Porcel *et al.*, LDH has a sensitivity of 74%, specificity 78%, glucose 58% sensitivity and specificity 93%, and a pH sensitivity of 58% and specificity of 93% [10]. Our study examines the possibility of IL-8 being used as an alternative marker for determining the complexity of parapneumonic effusion because of possible dismissal in the separation of parapneumonic effusions by classical parameters [10, 17, 19]. Interleukin-8 has an important influence on the pathophysiology of parapneumonic effusion [7, 8, 9, 11, 20].

Pleural mesothelial cells are partly responsible for the accumulation of neutrophils and mononuclear phagocytes in the pleural space. They release interleukin-8 (IL-8) which is a member of the C-X-C (chemotactic-chemokine) family. It is the mediator and the regulator of the chemotaxis of the neutrophils and leucocytes during the inflammatory process and is important in the pathology of many infectious diseases [7, 18]. It is also established by its critical role in the pathogenesis of other lower respiratory infections [21]. Apart from mesothelial cells IL-8 is released from alveolar macrophages and lung fibroblasts, as well as lymphocytes and neutrophils that are stimulated by microorganisms and their toxins [8, 9, 22]. The accumulation of fluid in the pleural space is the result of an increase in vascular permeability. In these processes IL-8 has a central role [7, 22]. The concentration of IL-8 in the inflammatory process was significantly higher in the pleural space than in the blood.

### *Objective*

The aim of this study was to investigate the value and significance of interleukin-8 in differentiation between uncomplicated parapneumonic effusion (UCPPE) and complicated parapneumonic effusion (CPPE).

### Materials and Methods

The study included 60 patients treated at the Clinic for Infectious Diseases and Febrile Conditions in Skopje, with pneumonia and parapneumonic effusion, during the period from January 2010 until February 2011.

The diagnoses of parapneumonic effusion are based on: the standard clinical, bio-chemical laboratory and microbiological findings, lung x-ray, diagnostic thoracentesis and analysis of the pleural fluid. Radiographic findings were realized at the Institute of Radiology. All the patients underwent an ultrasound examination of the lungs and pleura with a two-dimensional echo at the Infectious Diseases Clinic for diagnosis of pleural effusion and implementation of diagnostic or therapeutic thoracentesis if the size of the effusion was more than 10 mm. After verification of pneumonia and pleural effusion, the distinction between the transudates and exudates was done according to Light's criteria. Exudative pleural effusion is one that meets at least one of the criteria of Light. The transudate is the effusion that meets all three criteria at the same time: 1. to have intercourse protein p/s below 0.5, intercourse LDH p/s below 0.6, and LDH in pleural fluid under 282 U/L which is the lowest limit in our laboratory.

The examined patients were divided into two groups:

1. The first examined group consisted of 30 patients with uncomplicated parapneumonic effusion.
2. The second examined group consisted of 30 patients with complicated parapneumonic effusion.

Both groups were formed on the basis of pH, glucose and LDH values in pleural fluid obtained by thoracentesis.

Parameters for division of parapneumonic effusions according to their evolution:

- Uncomplicated parapneumonic effusions: pH > 7.2 Glucose > 60 mg/dl LDH < 1000 UI/ml;
- Complicated parapneumonic effusions: pH ≤ 7.2 Glucose ≤ 60 mg/dl LDH ≥ 1000 UI/ml

*Interleukin 8* was determined using an IMMULITE 1000 Analyzer with chemiluminescent immunometric assay at the Public Health Institute of the Republic of Macedonia. To perform the test 50 micro litres of pleural fluid were needed. The range of calibration with the highest values of interleukin-8 was 7500 pg/ml. Analytical sensitivity was 2 pg/ml. Positive values were those above 70 pg/ml. The samples taken were frozen at the Public Health Institute at -20°C.

Determination of biochemical parameters (LDH, glucose) was performed on a multi-channel Roche Hitachi analyzer in the biochemical laboratory of the Infectious Diseases Clinic. Ph values were determined in the same laboratory by a blood gas machine.

*Statistical analysis* – Qualitative variables between groups were analysed using the  $\chi^2$  test. The significance of differences between the two arithmetic proportions was done using the Student's t test. The relationship between the variables of pleural fluid was estimated using the correlation graphs. An ROC curve was created in order to determine the specificity and sensitivity of interleukin-8. Statistical analysis was conducted using SPSS 19.0 For Windows.

### Results

In the first group of patients with uncomplicated parapneumonic effusion 17 (56.67%) patients were male, 13 (43.33%) were female. In the group of patients with complicated parapneumonic effusion 24 (80%) were male and only 6 (20%) patients were female. There were more male patients in both groups, but there was no significant statistical difference between groups ( $\chi^2 = 0.04493$ ,  $p = 0.8321$ , NS).

In the group with uncomplicated effusion, the patients were aged between 23 and 86 with an average age of  $58.06 \pm 18.44$ , and the group with complicated effusions were aged between 18 and 78 with an average age of  $49.07 \pm 17.76$  years. There was no statistically significant difference regarding the average values of the age of patients with uncomplicated and complicated parapneumonic effusions ( $t = 1.925$ ,  $p = 0.8428$ ).

The median values of IL-8 in the group of UCPPE were  $248.21 \pm 137.13$  pg/ml, in CPPE group  $4047.71 \pm 2608.64$  pg/ml. This clearly indicates the statistically significant difference in favour of larger values in the group of CPPE ( $t = -7.967$ ,  $P = 0.000000$ ) (Figure 1)

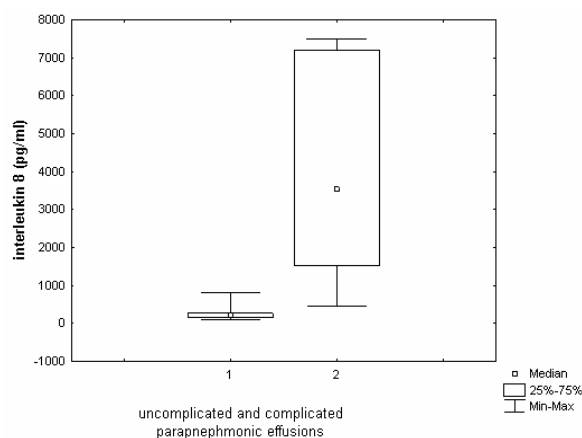


Figure 1 – Concentration of interleukin 8 (IL-8 pg/ml) in both patient groups

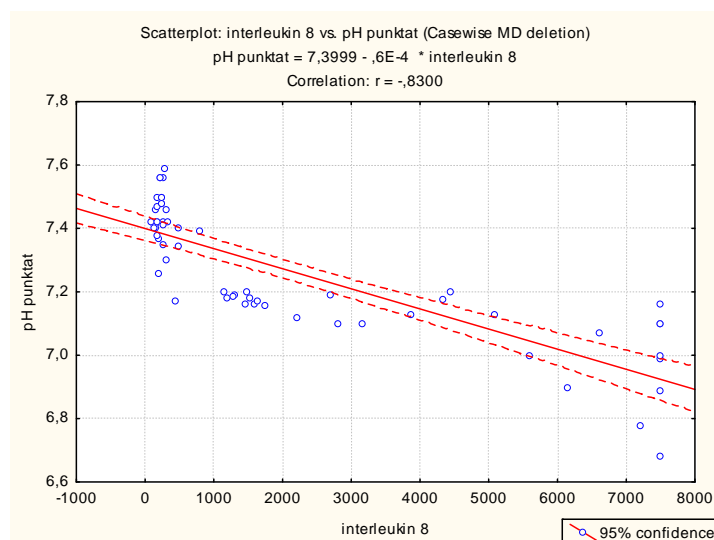
In the three classical parameters used to distinguish both the groups of parapneumonic effusions statistically significantly higher values of LDH ( $t = -7.17118$ ,  $p = 0.00000$ ) were seen among the group with CPPE. There were statistically significant lower values of glucose ( $t = 12.418$ ,  $P = 000.000$ ) and pH ( $t = 12.418$ ,  $P = 000.000$ ) in the pleural fluid in CPPE (Table 1).

Table 1

*Values of LDH, glucose and pH in both groups' parapneumonic effusion*

Mean values and SD	UCPPE	CPPE
LDH U/L	439,53 (193,78)	1900,23 (1098,69)
Glucose mg/dl	111,06 (43,32)	45,18 (12,27)
pH	7,343 (0,076)	7,092 (0,13)

Interleukin-8 correlated significantly positively with LDH ( $r = 0.68$ ,  $p < 0.05$ ) and protein ( $r = 0.306$ ,  $p < 0.01$ ) in pleural fluid, but a significant strong inverse correlation existed in pH ( $r = -0.83$ ,  $P, 0.05$ ) and values of glucose in pleural effusion ( $r = -0.63$ ,  $P < 0.05$ ) (Figure 2).



*Figure 2 – Correlation between interleukin 8 (IL-8 pg/ml) and Glucose (mg/dl) in both patients groups*

These pathophysiological changes appear as a result of the inflammatory process that occurs in the pleural space.

The ROC curve was created in order to determine the diagnostic value of IL-8. From this it can be clearly seen that the IL-8 with a sensitivity of 100% and specificity of 98% with an IL-8 value of 1805.81 pg/ml, with 95% confidence interval can distinguish uncomplicated from complicated parapneumonic effusion. (Figure 3)

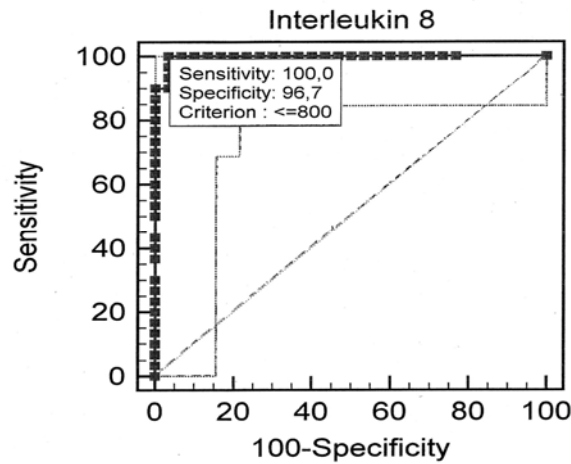


Figure 3 – Roc curve for interleukin-8 with 95% confidence

Figure No 4 demonstrates the positive and negative predictive value of IL-8 (Figure 4).

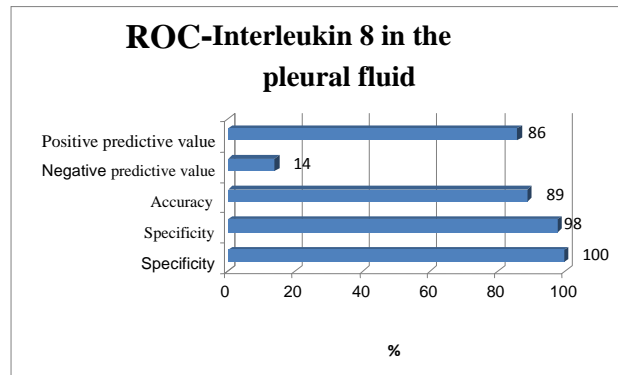


Figure 4 – Predictive value of IL-8

### Discussion

In our study significantly higher values of LDH were found in the group with CPPE. This value of LDH correlates with the degree of inflammation that occurs in the pleural space. Concentration of pH and glucose in this study are lower during the inflammatory process in complicated effusions, which corresponds to acidosis in the pleural space and the spread of infection.

According to Akarsu *et al.*, IL-8 concentrations are statistically higher in complicated parapneumonic effusions than in uncomplicated parapneumonic effusions, and were highest in cases of empyema [9]. Also the concentration of IL-8 in pleural fluid significantly correlates positively not only with the number of neutrophils, but also with the concentration of LDH in the pleural fluid, i.e. the degree of inflammation [9, 22]. Also the concentration of IL-8 correlates significantly, but inverted, with the level of glucose and pH [7, 10, 20]. This significant inverted correlation with pH and glucose and the significant positive correlation with LDH are also confirmed in the examination conducted in patients with parapneumonic effusions in our study.

Anthony *et al.*, also detected statistically higher levels of IL-8 concentrations in parapneumonic complicated effusions than in uncomplicated effusions [7, 10]. Our study certifies that the concentration of IL-8 is significantly higher in complicated parapneumonic effusions compared to uncomplicated.

Receiver-operating characteristic curves were used to assess the sensitivity and specificity of IL-8 for differentiating between the two patient groups. Unlike the study by Porcel *et al.*, conducted in Spain and published in 2008, where the sensitivity was 84% and specificity 82%, in our examination of the sensitivity values of 100% and specificity of 97% are higher. The difference is the IL-8-determination method. Porcel uses the ELISA method, we tested our method of enzyme-amplified chemiluminescence by use of a Siemens Immulite 1000 analyzer.

Yet, compared with the old markers of differentiation of the parapneumonic effusions, the sensitivity and specificity of which were 100%, the IL-8 has no increased value, but is similar.

### Conclusion

This study explains, in part, the pathophysiology of complications in parapneumonic effusion and the role of interleukin-8. Pleural fluid IL-8 is a marker of differentiation with a diagnostic accuracy at least similar to that of classical parameters such as pH, glucose or LDH levels.

Although the classical parameters have a better specificity and sensitivity than IL-8, its value is important because it is only one parameter for deter-



mination versus three biochemical parameters (LDH, glucose and pH) and because it has a specific method for taking pH and its determination is only by the blood gas machine. IL-8 may be used as an alternative marker for the complication of parapneumonic effusion but its diagnostic value needs further analysis.

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#### Резиме

### УЛОГАТА НА ИНТЕРЛЕУКИН-8 ВО РАЗГРАНИЧУВАЊЕ НА НЕКОМПЛИЦИРАНИ ОД КОМПЛИЦИРАНИ ПАРАПНЕВМОНИЧНИ ИЗЛИВИ

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Оваа студија е спроведена да ја утврди вредноста и значењето на интерлеукин-8 во разграничување на некомплицирани од комплицирани парапневмонични изливи.

Со користење на анализаторот Immulite 1000 со методот на имунометриска хемилуминисценција беше одредувано нивото на интерлеукин-8 (ИЛ-8)

во плевралната течност кај група од 30 пациенти со некомплицирани и 30 пациенти во групата на комплицирани парапневмонични изливи, како и класичните параметри за разграничување (пХ, гликоза и ЛДХ) во двете групи. ROC (Receiver-operating) кривата беше конструирана за одредување на специфичноста и сензитивноста на интерлеукин-8 за разграничување на овие две групи.

Нивото на ИЛ-8 беше сигнификантно повисоко во групата на комплицирани парапневмонични изливи. Позитивна сигнификантна корелација е најдена помеѓу нивото на ИЛ-8 и лактат дехидрогеназата (ЛДХ) ( $p = 0,68$ ,  $p < 0,5$ ). Исто така позитивна, сигнификантна корелација е верифицирана помеѓу ИЛ-8 и нивото на протеините во плевралниот излив ( $p = 0,306$ ,  $p < 0,01$ ). Значителна негативна корелација постои помеѓу нивото на ИЛ-8 и вредностите на пХ ( $r = -0,83$ ,  $p < 0,05$ ), како и помеѓу ИЛ-8 и гликозата во плевралната течност ( $r = -0,61$ ,  $p < 0,05$ ). Cuttoff вредностите од 1805,81 пг/мл, ги одвојуваат некомплицираниите од комплицираните парапневмонични изливи со сензитивност од 100% и специфичност од 98%.

Интерлеукин-8 може да се користи како алтернативен маркер за одредување на компликациите кај парапневмоничните изливи.

**Клучни зборови:** некомплицирани парапневмонични изливи, комплицирани парапневмонични изливи, цитокин, интерлеукин-8.

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