ORIGINAL ARTICLE

# The IL-6 value in the differential diagnosis of the exudative pleural effusion

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Interleukin 6 (IL-6) is locally produced at the sites of inflammation of the pleural space. It is also known that some malignant cells produce IL-6. Pleural IL-6 leaks to systemic circulation and causes systemic effects. In this study we measured IL-6 in serum/pleural fluid paired samples in various groups of patients with exudative pleural effusions. In the serum were detected significantly higher IL-6 values in patients with parapneumonic effusions (n=32, mv 12.3±6.1U/ml) in comparison to tbc effusions n=23 mv 4±3 U/ml, (p=0.00464) as well as to malignant effusions n=20, mv 5.4±3.9U/ml (p=0.02). In the pleural fluid the tbc effusions presented with significantly higher IL-6 values (mv 498±276 U/ml), in comparison to malignant effusions (mv 42±56.2 U/ml), {p=0.0046} as well as to parapneumonic effusions (mv 244±192 U/ml), {p=0.01}. The serum / pleural IL-6 ratio, was: 1) tbc effusions ≤0.01, 2) parapneumonic effusions ≥0.02, 3) malignant effusions ≥0.1. Ninety six per cent of the malignant effusions were presented with a pleural fluid' s IL-6 value < 100 U/ml, whereas all the empyemas and the tbc effusions were presented with a value > 100 U/ml. These results suggest that the serum /pleural fluid's IL-6 ratio ( IL-6 criterium) together with pleural fluid's IL-6 values can lead to quick differential diagnosis of the exudative pleural effusion, which will be confirmed later with the traditional practice methods.
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Interleukin 6 (IL-6) is a multifunctional protein, with a molecular weight of 26KDa , produced by lymphoid and non-lymphoid cells and by normal and transformed cells, including T- cells, monocytes/macrophages, fibroblasts, hepatocytes, vascular endothelial cells, cardiac myxomas, bladder cell carcinomas, myelomas, astrogliomas, glioblastomas, adenocarcinomas and mesotheliomas cells[1]. The production of IL-6 in these various cells is regulated, either positively or negatively, by a variety of signals including mitogens, antigenic stimulation, lipopolysaccharides, IL-1, TNF, PDGF and viruses[2].

The effects of IL-6 on different cells are numerous and variable. Clinically it is important that IL-6 has a pivotal role in inflammatory responses such as the induction of acute phase proteins and the synthesis of immunoglobulins[3]. Elevated IL-6 levels have been reported to be associated with a variety of diseases, including autoimmune diseases, mesangial proliferative glomerulonephritis, psoriasis, inflammatory bowel disease and malignancies[4,5].

Most authors agree, that in case of inflammation of the pleura, IL-6 is locally produced in the pleural space and high concentrations are detected in exudative pleural effusions[6,7]. High concentrations of IL-6 were also detected, in carcinomatous pleural effusions[8-10]. From the pleural space, IL-6 could leak to circulation and cause systematic effects as the induction of C-reactive protein[11]. This is supported by the fact that serum concentrations of IL-6 in the same patients were detected at much

lower levels, not significantly increased in comparison to normal controls[5-11].

From the above mentioned, it is obvious that the final IL-6 values in the pleural effusions depend each time upon the specific disease, since different signals activate various groups of cells in different degree. In contrast, serum IL-6 values depend upon the pleural values, as well as the lymphatic flow in the pleural lymphatic vessels. In case of a malignant or a tuberculous effusion, the lymphatic flow is reduced because of the infiltration of the pleural lymphatic vesels and the mediastinal nodes[12]. In this study we measured IL-6 concentration in the serum and the pleural fluid of various patients with exudative pleural effusions to determine whether IL-6 values have any diagnostic usefulness in the difficult differential diagnosis of the exudative pleural effusions.

## MATERIAL - METHOD

### Patients

One hundred patients with pleural effusion were included in this study. Pleural fluid, after diagnostic thoracocentesis and serum samples were examined on the same day. The effusions were classified as transudates or exudates according to the Light R.W criteria (pleural fluid's protein / serum protein > 0.5, pleural fluid's LDH / serum LDH > 0.6, pleural fluid's LDH > 200 IU/l)[13]. The diagnosis of malignant effusion was made by cytopathologic detection of malignant cells in the effusion or in other samples resulted from bronchoscopy, fine needle aspiration (FNA), pleural biopsy or sputum specimens. For the diagnosis of a tuberculous effusion the detection of Mycobacterium Tuberculosis in the pleural fluid was not considered as necessary and it was based on the Zeihl-Nielsen coloring or the positivity of the cultures in Lowenstein-Jensen medium of other respiratory tract samples or pleural biopsy specimens. Parapneumonic effusions were divided into uncomplicated and complicated following the next criteria: 1) macroscopic view of pus, 2) pleural fluid's glucose < 50mg/ml, 3) pleural fluid's pH 0.30 or more below blood's pH, 4) positive Gram coloring or cultures. To examine the correlation between serum and pleural IL-6 concentrations, we obtained serum/pleural fluid paired samples from all the patients.

### Determination of IL-6 Concentration

The serum and pleural levels of IL-6 were measured using the sandwich Enzyme Amplified Sensitivity ImmunoAssay (EASIA) method with two monoclonal antibodies against distinct epitopes of IL-6. We used the commercially available kit by Biosource Europe S.A. (ISO 9001). The protocol was as following: 100 μL standard plus 100 μL control plus 100 μL sample were incubated in shaker for 1 hour at room temperature (18-25 °C). Then the excess of antigen was removed by washing. Next, we added 100 μL anti-IL-6 conjugate and after an incubation period of 1 hour in a dark place at room temperature, we removed the unbound enzyme labeled antibodies by washing. The bound antibodies were measured through a chromogenic reaction (200 μL chromogenic solution for 30 min) that was terminated by the addition of 50 μL stop solution. Finally we read absorbance at 450 nm of spectrophotometer. The sensitivity of the method was 2 pg/ml (1 pg/ml corresponding to 100 μIU/ml) and the expected range 0-1000 pg/ml. As it is a research product, no reference values have been established.

### Statistical Analysis

Data was expressed as mean value  SD. The difference between groups was tested by the students' T test. A p value of < 0.05 was used to denote statistical significance.

## RESULTS

Twenty-five effusions were classified as transudates and seventy-five as exudates. The IL-6 values in the transudates were measured very low (mean value 2 1.7 U/ml), whereas in the serum could not be detected. Exudates had a significantly higher IL-6 level than transudates ($p<0.01$). As tuberculous were classified 23 exudates, as parapneumonic 32 and as malignant 20 effusions.The levels of IL-6 in the serum and the pleural fluid, as well as their ratio, in every group of patients are shown in table 1.

In the serum significantly higher IL-6 values were observed in patients with parapneumonic

**Table 1. Patient serum and pleural exudate IL-6 values and their ratio (pts 75).**

| Patiens Group | No of pts | Serum IL-6 (Mean+/-SD, U/ml) | P.fluid IL-6 | S/P ratio IL-6 criterium | No of > 100U/ml |
|---|---|---|---|---|---|
| Tuberculous | 23 | 4±3 | 498±276 | ≤ 0.01 | 23 |
| Parapneumonic | 32 | 12.3±6.1 | 244±192 | ≥ 0.02 | 32 |
| Complicated | 9 | 15.1±3.2 | 301±285.5 | | 9 |
| Uncomplicated | 23 | 9.9±6.1 | 187±107 | | 23 |
| Malignant | 20 | 5.4±3.9 | 42±56.2 | ≥ 0.1 | 1 |
| Adenocacinoma | 14 | 5.7±2.6 | 45±39.2 | | |
| Small cell cancer | 4 | 1.6±1.3 | 7.9±10.4 | | |
| Mesothelioma | 2 | 6.7±5.9 | 388±254 | | |

effusions in comparison to patients with tuberculus (p=0.00464) or malignant effusions (p=0.02). In the pleural fluid the tuberculous effusions were presented with significantly higher values in comparison to malignant (p=0.0046) or to parapneumonic effusions (p=0.01). It is noticable, that all the patients with malignant effusion were presented with IL-6 values bellow the level of the 100 U/ml, with the exception of one patient suffering from malignant pleural mesothelioma. In all the other groups of patients, values above the level of the 100 U/ml were detected.

## DISCUSSION

Our results are in agreement with previous studies, which indicate that IL-6 is produced locally at the site of active inflammation, in our case being the pleural space[4-11]. Its concentration in pleural effusions is a useful complementary marker in differentiating exudates from transudates. Tuberculous effusions, parapneumonic effusions and some malignant effusions had quite high IL-6 levels. It is known that T lymphocytes are activated during immunologic reactions in the pleural space and these cells are responsible for the IL-6 production in tuberculus patients[14,15]. We speculate that the continuing activation of the macrophages by the bacterial lipopolysaccharide leads to the release of cytokines (IL-1, TNF-a), which in turn promote the production of IL-6 by the stroma cells in the case of the parapneumonic effusion[16]. In the case of malignant effusion it is known that some malignant cells produce IL-6 by themselves[7,8,10,17]. It has been observed that IL-6 level was higher in inflammatory than in malignant exudates, although high levels of IL-6 in malignant pleural fluid have been reported after pleurodesis or in cases of mesothelioma patients[8,17-19].

Even though in many of the above mentioned studies IL-6 values similar to ours have been reported, to our knowledge these values have not been introduced into clinical practice as complementary index in the differential diagnosis of pleural effusions. To our oppinion the reason is the overlapping of the values detected in some cases of complicated parapneumonic effusions or mesotheliomas with the values detected in tuberculous effusions. Having in mind the differences in the kinetics of the IL-6 from the pleural space to the serum, because of the reduced lymphatic flow in the case of tuberculous or malignant effusions, we suggest the use of the serum/pleural (S/P) IL-6 ratio as a sensitive marker in the differential diagnosis of the pleural effusions. Preliminary data suggest that S/P IL-6 ratio ≤0.01 is compatible with tuberculous effusion. S/P IL-6 ratio ≥0.02 is compatible with parapneumonic or malignant effusion and the differentiation between them could be put forward by the pleural fluid's IL-6 levels. IL-6 values were >100 U/ml in all the parapneumonic effusions in contrast to the malignant effusions where detected values < 100 U/ml was the rule.

In summary the serum/pleural IL-6 ratio (IL-6 criterium) in combination with pleural fluid's IL-6 values can lead to a quick differential diagnosis of the exudative effusions, which will be confirmed later using the traditional practice methods.

## ΠΕΡΙΛΗΨΗ

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**Η σημασία της IL-6 στη διαφορική διάγνωση του εξιδρωματικού πλευριτικού υγρού.** Ιπποκράτεια 2001, 5 (4): 172-175

Η ιντερλευκίνη - 6 παράγεται τοπικά σε θέσεις ενεργού φλεγμονής της υπεζωκοτικής κοιλότητας και εισέρχεται στη συστημική κυκλοφορία, προκαλώντας συστηματικά αποτελέσματα. Είναι επίσης γνωστό ότι ορισμένα νεοπλασματικά κύτταρα παράγουν ιντερλευκίνη - 6. Σε αυτή τη μελέτη, μετρήσαμε τα επίπεδα ιντερλευκίνης – 6 σε ανά ζεύγη δείγματα ορού και πλευριτικού υγρού σε διάφορες ομάδες ασθενών με εξιδρωματικές πλευριτικές συλλογές. Στον ορό ανιχνεύθηκαν υψηλότερες τιμές ιντερλευκίνης – 6 σε ασθενείς με παραπνευμονικές συλλογές (32 ασθενείς, μ.ό. 12.3 ±6.1 U/ml) σε σύγκριση με φυματιώδεις (23 ασθενείς, μ.ό. 4±3 U/ml, p=0.00464) ή κακοήθεις (20 ασθενείς, μ.ό. 5.4±3.9 U/ml, p=0.02) πλευριτικές συλλογές. Στο πλευριτικό υγρό βρέθηκαν στατιστικά σημαντικά υψηλότερα επίπεδα ιντερλευκίνης – 6 σε ασθενείς με φυματιώδη πλευριτική συλλογή (μ.ό. 498±276 U/ml) συγκριτικά με εκείνους που είχαν κακοήθη (μ.ό. 42±56.2 U/ml, p=0.0046) ή παραπνευμονική (μ.ό. 244±192 U/ml, p=0.01) πλευριτική συλλογή. Ο λόγος της ιντερλευκίνης – 6 στον ορό προς το πλευριτικό υγρό ήταν: α) ≤0.01 σε φυματιώδεις, β) ≥0.02 σε παραπνευμονικές και γ) ≥0.01 σε νεοπλασματικές πλευριτικές συλλογές. Στο 96% των νεοπλασματικών συλλογών ανιχνεύθηκαν τιμές ιντερλευκίνης – 6 στο πλευριτικό υγρό < 100 U/ml, ενώ σε όλα τα εμπυήματα και τις φυματιώδεις συλλογές βρέθηκαν τιμές > 100 U/ml. Τα αποτελέσματα δείχνουν ότι ο λόγος της ιντερλευκίνης – 6 στον ορό προς το πλευριτικό υγρό σε συνδυασμό με τα επίπεδα ιντερλευκίνης – 6 στο πλευριτικό υγρό μπορούν να χρησιμεύσουν ως συμπληρωματικοί δείκτες στη διαφορική διάγνωση μιας εξιδρωματικής πλευριτικής συλλογής.

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