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Role of C-C chemokines in the determination of pleural effusion etiology

Abstract

Introduction: Pleural effusion secondary to various diseases is associated with the presence of different inflammatory cells. The C-C chemokines (MCP-1 and MIP-1α), produced by pleural mesothelial cells, plays an important role in the recruitment of inflammatory cells to the pleural space. The purpose of the study was to evaluate predictive value of MCP-1 and MIP-1α in the differential diagnosis of pleural effusion.

Material and methods: Based on Light's criteria in 29 cases exudates and in 10 transudates were recognized. We investigated 39 patients with pleural effusion (congestive heart failure — 10, parapneumonic — 11, tuberculous — 6, malignant — 12). The C-C chemokines MCP-1 and MIP-1α levels in pleural effusion and serum were measured by ELISA.

Results: The MCP-1 was significantly higher ($p = 0.009$) in the patient with exudates than in patients with transudates (2436 pg/ml and 794 pg/ml respectively). ROC curve analysis revealed however that this parameter has limited value in the differentiation of exudates an transudates (MCP-1 cut off value 1060 pg/ml, sensitivity 48%, specificity 90%, PPV 93%, NPV 37%).

The chemokine MIP-1α were significantly higher ($p = 0.001$) in tuberculous than in the malignant effusion (405 pg/ml and 30 pg/ml respectively). Based on the ROC curve analysis, as a cut off value in the differentiation of tuberculous and malignant pleural effusion a value 120 pg/ml was accepted. The sensitivity of this test was 66% and specificity 99%, PPV 80%, NPV 84%.

Conclusions: The chemokine MCP-1 has a limited value in the differentiation between transudate and exudates; MIP-1α could be helpful in the differentiation between tuberculous and malignant pleural effusion.

Key words: malignant effusion, tuberculous effusion, C-C chemokines, pleural effusion

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Introduction

Pleural effusion is a frequent abnormality requiring diagnostic evaluation, the first step involves establishing whether the effusion is an exudate or a transudate. Light's criteria, based on the measurement of lactate dehydrogenase (LDH) and protein in the pleural fluid and the serum, have been used most commonly to achieve this goal for nearly 30 years [1]. In the recent years other useful biochemical markers have been proposed (cholesterol, bilirubin, albumin), but their diagnostic value was not superior to Light's criteria, which are characterised by high accuracy and simplicity [1–4]. Confirmation of the inflammatory nature of the effusion determines further diagnostic steps [1]. In certain situations this routine procedure fails, as evidenced by the presence of transudate in about 10% of patients with pleural metastases [5]. Examination of the cellular composition of pleural effusions is similarly characterised by low specificity. While high lymphocyte counts usually support the suspicion of tuberculosis or malignancy, they are merely found in half of such cases [6]. Cytological examination of the pleural effusion confirms the diagnosis in only 43–72% of cases of pleural metastases

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[7, 8]. Despite the expanding battery of tests, in many cases the aetiology of pleural effusion cannot be established, hence the ongoing search for new markers that would prove useful in the differential diagnosis of pleural effusion.

One of the mechanisms responsible for the accumulation of pleural fluid involves increased permeability of capillaries induced by proinflammatory cytokines [9]. Pleural effusion is characterised by the presence of various inflammatory cells. Neutrophils predominate in pleural empyema and parapneumonic effusions, while tuberculous and malignant effusions abound in mononuclear cells (lymphocytes, monocytes) [8]. The migration of inflammatory cells partly results from the formation and release of various cytokines by mesothelial cells. These cytokines include cytokines from the C-C chemokine family, such as MCP-1 (monocyte chemotactic protein 1) and MIP-1α (macrophage inflammatory protein 1α) [10-15]. Chemokines are recognised inflammatory mediators [15]. They have been implicated in the pathogenesis of chronic bronchitis [16, 17], sarcoidosis [18] and interstitial lung disease [19]. Concentrations of C-C chemokines are elevated in bronchoalveolar lavage fluid (BALF) from pulmonary tuberculosis patients [20]. The few reports so far have demonstrated involvement of C-C chemokines in the pathogenesis of pleural effusion [10, 11, 14, 15]. The chemokines MCP-1 and MIP-1α express chemotactic effects on monocytes, lymphocytes, basophils and eosinophils [21, 22]. Mesothelial cells, which produce these cytokines, are actively involved in the migration of mononuclear inflammatory cells to the pleura [10, 11, 14, 15]. Although the concentrations of C-C chemokines have been measured in the pleural fluid [10, 14, 15], no attempts have been made so far to establish their role in the diagnostic evaluation of pleural effusion.

Based on the above presumptions we proposed a hypothesis that measuring concentrations of certain chemokines might play a role in establishing the aetiology of pleural effusion and verified this hypothesis by measuring MCP-1 and MIP-1α concentrations in various clinical cases of pleural fluid accumulation.

Material and methods

We tested pleural fluids and sera from 39 patients who were hospitalised at our Pneumology Department and underwent, with previous consent, standard diagnostic procedures to establish the cause of their pleural effusion.

The study group comprised 30 men (76.9%) and 9 women (23.1%) aged 47–74 years (mean 61.97 ± 8.5 years) with no significant differences between men and women ($p = 0.2$). The causes of pleural fluid accumulation included: lung cancer (12 patients, mean age 59.2 ± 8.3 years), pneumonia (11 patients, mean age 61.3 ± 9.0 years) and tuberculosis (6 patients, mean age 61.3 ± 9.4 years). Transudate was present in 10 patients and was associated with congestive heart failure (mean age 66.3 ± 7.1 years). No significant differences were observed with respect to age ($p = 0.2$).

Pleural fluid was collected during diagnostic thoracocentesis and routine biochemical, bacteriological and cytological tests were performed [23]. Parapneumonic effusion was defined as an exudate accompanied by pulmonary inflammatory infiltrates on radiographs consistent with the clinical picture. Malignant effusion was diagnosed on the basis of positive cytology in patients with histopathologically confirmed lung cancer. The tuberculous aetiology was confirmed by histological examination of pleural tissue samples obtained during pleuroscopy and/or by positive bacteriology of the pleural fluid. Transudate was diagnosed in accordance with Light's criteria, taking into account clinical data and the results of diagnostic investigation suggestive of congestive heart failure [1–3].

Pleural fluid and serum samples, 5 ml each, were centrifuged at 1500 g for 10 minutes and stored at –70°C until the measurement of MCP-1 and MIP-1α.

The concentrations of the chemokines MCP-1 and MIP-1α were determined with a ready-to-use ELISA kit (R&D). The range of measurable concentrations was 31.2–2000 pg/ml for MCP-1 and 2.8––500 pg/ml for MIP-1α.

Statistical analysis

The statistical analysis was conducted using procedures available in the software Statistica 7.1 (licence number ABDP610A903827AR). The analysis was based on simple procedures of descriptive and analytical statistics. Descriptive statistics were used to present the distributions of quantitative variables (arithmetic mean, standard deviation, median, mode) and qualitative variables (frequencies). The Shapiro-Wilk test was used to verify normal distribution. The differences in the distributions of the quantitative variables were evaluated by means of the t-Student test or non-parametric tests (the Mann-Whitney test for independent samples). The analysis of variance (ANOVA) or its counterpart for

Table 1. The mean value of MCP-1 in serum and pleural effusion

Patient group	Mean ± SD [pg/ml] (serum)	Mean ± SD [pg/ml] (effusion)	p-value#
P (n = 10)	291,13 ± 113,17	794,25 ± 508,19	0,005
W (n = 29)	272,90 ± 100,61	2436,10 ± 2560,83	0,00003
Wrp (n = 12)	313,76 ± 103,53	3147,25 ± 2948,21	0,002
Wtbc (n = 6)	244,70 ± 38,60	2197,82 ± 2712,49	0,02
Wpp (n = 11)	243,72 ± 111,32	1790,27 ± 2005,56	0,004

P — transudate; W — exudate all cases; Wrp — exudate in lung cancer; Wtbc — tuberculous exudate; Wpp — parapneumonic exudate; # — the result of Mann-Whitney U test

non-parametric tests (the Kruskal-Wallis test) were used for comparisons of more than two groups for quantitative variables. The differences in the frequency of distribution for qualitative variables were assessed by the χ^2 test. Due to the small size of the groups and the nature of the distribution of variables the analysis of the simple relationships was conducted on the basis of Spearman's rang correlation. *P* values below 0.05 were adopted to reflect statistical significance. The diagnostic value of the tests was assessed taking into consideration the conventional parameters (sensitivity, specificity, positive predictive value and negative predictive value) and the ROC (receiver operator curve) analysis.

Results

The concentrations of MCP-1 and MIP-1α in the serum and in the pleural fluid were measured in all the patients. The mean serum MCP-1 concentrations in all the study groups did not differ significantly and the mean pleural fluid MCP-1 concentrations were significantly higher than those in the serum in all the study groups (Table 1).

We found no significant differences in the concentration of MCP-1 between individual exudates (p = 0.4). The overall MCP-1 concentration in the exudate was significantly higher than that in the transudate (p = 0.009).

Based on the ROC analysis, the cutoff value that best differentiated exudate from transudate was the MCP-1 concentration of 1060 pg/ml at the sensitivity of 48%, specificity of 90%, positive predictive value of 93% and the negative predictive value of 37% (Figure 1).

We also analysed the ratio of pleural fluid MCP-1 to serum MCP-1 (MCP-1 P/S) and its value was significantly higher in the exudate than in the transudate (9.89 ± 9.95 vs 3.29 ± 2.55, p = 0.009).

Based on the ROC analysis, we adopted the cutoff value of 7.2 for MCP-1 P/S as best differentiating exudates from transudates. The sensitivity of the test was 55% and the specificity 80% with the positive predictive value of 91% and the negative predictive value of 33%.

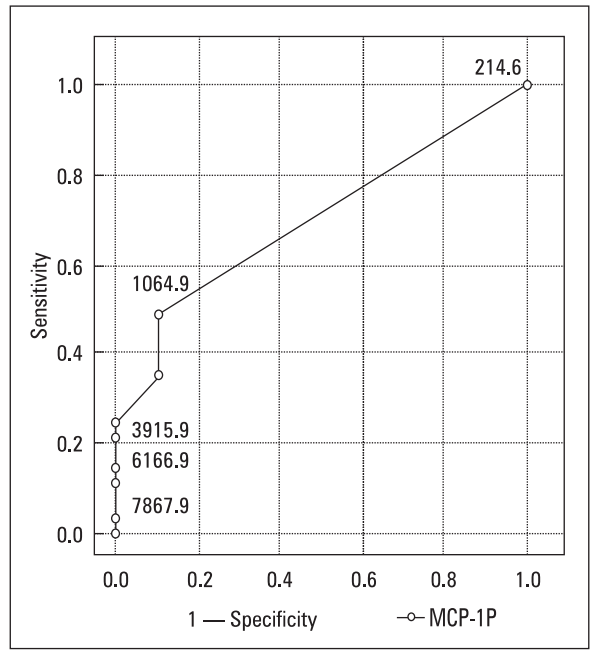

Figure 1. ROC curve — differential diagnosis of exudate vs. transudate based on MCP-1 value [pg/ml]

We also showed a positive correlation between the concentration of MCP-1 and the activity of LDH in pleural fluid in the entire study group (r = 0.54, p = 0.01) (Figure 2).

The pleural fluid MIP-1α concentration in patients with lung cancer was significantly higher than that in the serum (p = 0.003). Similar differences were found in the case of pleuropneumonia (p = 0.004), tuberculosis (p = 0.02) and overall in exudates (p = 0.00003) (Table 2).

MIP-1α concentrations in exudates and transudates did not differ significantly (p = 0.6). No significant difference in MIP-1α concentration was found between parapneumonic exudates and malignant exudates (p = 0.2).

Table 2. The mean value of MIP-1α in serum and pleural effusion

Patient group	Mean ± SD [pg/ml] (serum)	Mean ± SD [pg/ml] (effusion)	p-value#
P (n = 10)	40,28 ± 72,02	53,24 ± 78,57	0,5
W (n = 29)	17,44 ± 47,98	126,78 ± 250,07	0,00003
Wrp (n = 12)	3,10 ± 5,35	30,16 ± 38,64	0,003
Wtbc (n = 6)	67,14 ± 92,15	405,78 ± 457,92	0,02
Wpp (n = 11)	5,98 ± 17,23	80,00 ± 91,13	0,003

P — transudate; W — exudate all cases; Wrp — exudate in lung cancer; Wtbc — tuberculous exudate; Wpp — parapneumonic exudate; # — the result of Mann-Whitney U test

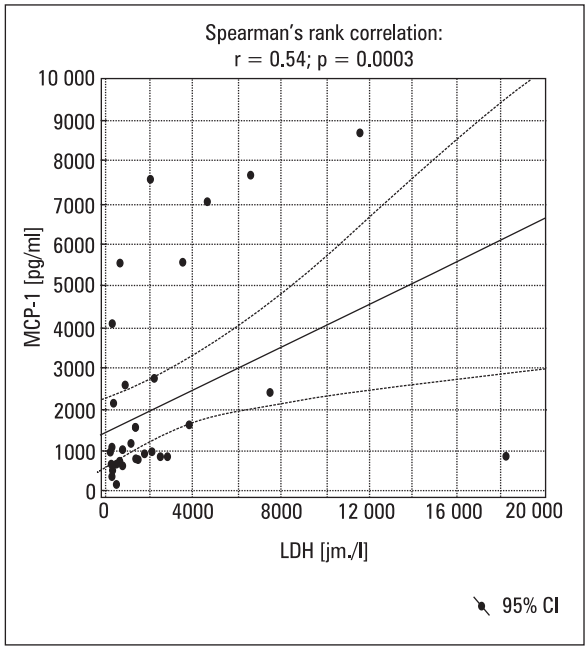

Figure 2. Relationship between pleural fluid LDH and MCP-1 levels

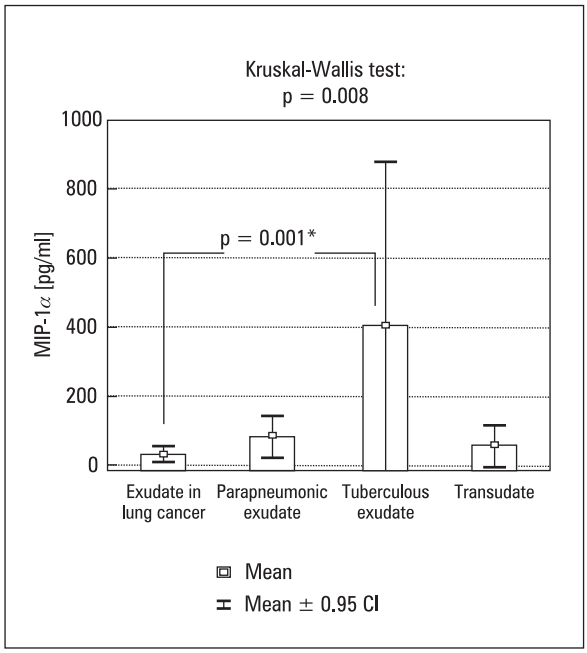

Figure 3. The chemokine MIP-1α concentration in pleural effusion

Pleural fluid MIP-1α concentrations were significantly higher in tuberculous exudates than those in exudates from lung cancer patients (p = 0.001).

MIP-1α concentration in the tuberculous exudate was also significantly higher than that in the parapneumonic exudate (p = 0.03) or in the transudate (p = 0.009) (Figure 3).

Based on the ROC analysis, we adopted an MIP-1α concentration cutoff value differentiating tuberculous from malignant exudates at 120 pg/ml (sensitivity 66%, specificity 99%, positive predictive value 80% and negative predictive value 84%) (Figure 4).

Determination of serum MIP-1α and the pleural fluid to serum MIP-1α ratio did not improve the diagnostic efficacy.

Discussion

The mean serum MCP-1 concentrations did not differ between the aetiologies of pleural effusion in our study (p = 0.35). The concentration of this chemokine in the pleural fluid was significantly higher than that in the serum, which suggests a local release of MCP-1 into the pleural cavity. At the same time, the pleural fluid MCP-1 concentration in the exudate was significantly higher than that in the transudate. However, the low sensitivity of the proposed cutoff value of 1060 pg/ml renders the usefulness of this marker limited in differentiating exudate from transudate. Currently, the distinction between the exudate and transudate is made on the basis of Light's criteria, whose diagnostic value is higher (sensitivity 98%, specificity 93%) than that of the pleural fluid MCP-1 concentration proposed by us (sensitivity 48%, specificity 90%) [1].

Although the pleural fluid to serum MCP-1 concentration ratio in exudates was significantly higher than that in transudates, this did not improve the sensitivity or specificity of this marker

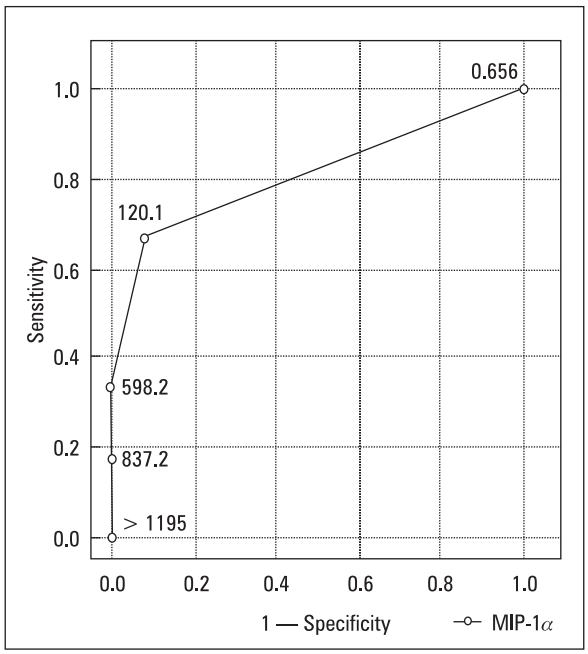

Figure 4. ROC curve — differential diagnosis of exudate vs. transudate based on MIP-1α value [pg/ml]

in the aspect of differential diagnosis. Antony et al. [10] found the highest MCP-1 concentrations in malignant effusions and showed that sources of MCP-1 may also include neoplastic cells present in the pleural fluid. The authors did not provide information about the sites of primary tumours causing the pleural metastases they reported. In our study lung cancer was the cause of all malignant effusions. The activity of tumour cells as regards the formation of MCP-1 may vary and this may be the reason for discrepant results. The positive correlation between the MCP-1 concentration and the LDH activity we demonstrated in the pleural fluid ($r = 0.54$) allows the conclusion that high MCP-1 levels are suggestive of increased pleural inflammation.

Our study demonstrated significant differences in pleural fluid MIP-1α concentrations between studied groups of patients. The MIP-1α concentration in the tuberculous effusion was significantly higher than that in the transudate ($p = 0.0009$), which is consistent with the findings obtained by Mohammed et al. [14]. In another study by these authors the MIP-1α concentration was significantly higher in parapneumonic exudates than in malignant exudates [15], and higher concentrations of MIP-1α were found in complicated parapneumonic exudates. The study group we described included patients with uncomplicated parapneumonic effusion only, which may be the reason for the lower values of MIP-1α. The low pleural fluid MIP-1α concentration in patients with lung cancer suggests a more limited involvement of this cytokine in the pathogenesis of this effusion type, as emphasised by other researchers as well [15]. Patients with transudate did not show any significant differences in MIP-1α concentrations between the serum and the pleural fluid, which indicates the absence of local release of this cytokine into the pleural cavity and proves that it is not involved in the accumulation of exudate in the pleural cavity.

The mean MIP-1α concentration was significantly higher in tuberculous effusions compared to malignant effusions ($p = 0.001$) and parapneumonic effusions ($p = 0.03$). We adopted 120 pg/ml as the cutoff MIP-1α value differentiating tuberculous from malignant exudates at the sensitivity of 66% and the specificity approaching 100%.

Differentiating malignant from tuberculous effusions is an important and frequent issue. Determination of adenosine deaminase (ADA) [3, 24] or interferon-γ [25] may be useful in this process. Unequivocal cases MIP-1α may be determined, and high values of this marker suggest tuberculous aetiology.

Conclusions

1. The usefulness of MCP-1 for establishing the aetiology of pleural effusion is limited.
2. When differentiating between tuberculous and malignant effusions, an MIP-1α concentration equal to or greater than 120 pg/ml may suggest tuberculous pleurisy.

References

1. Light R.W., Macgregor M.I., Luchsinger P.C., Ball W.C. Jr. Pleural effusions: the diagnostic separation of transudates and exudates. Ann. Intern. Med. 1972; 77: 507–513.
2. Burgess L.J., Maritz F.J., Taljaard F.F.J. Comparative analysis of the biochemical parameters used to distinguish between pleural transudate and exudates. Chest 1995; 107: 1604––1609.
3. Burgess L.J., Maritz F.J., Le Roux I., Taljaard J.J. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 1995; 50: 672–674.
4. Meisel S., Shamiss A., Thaler M., Nussinovitch N., Rosenthal T. Pleural fluid to serum bilirubin concentration ratio for the separation of transudates from exudates. Chest 1990; 98: 141––144.
5. Porcel J.M., Alvarez M., Salud A., Vives M. Should a cytologic study be ordered in transudative pleural effusions? Chest 1999; 116: 1836–1837.
6. Sahn S.A. Malignant pleural effusions. Semin. Respir. Med. 1987; 9: 43–53.
7. Martenson G., Pettersson K., Thiringer G. Differentiation between malignant and non-malignant pleural effusion. Eur. J. Resp. Dis. 1985; 67: 326–334.
8. Salyer W.R., Eggelston J.C., Erozan Y.S. Efficacy of pleural needle biopsy and pleural fluid cytopathology in the diagnosis of malignant neoplasm involving the pleura. Chest 1975; 67: 158–164.
9. Kroegel C., Anthony V.B. Immunobiology of pleural inflammation: potential implications for pathogenesis, diagnosis and therapy. Eur. Respir. J. 1997; 10: 2411–2418.

10. Antony V.B., Alvarez M., Salud A., Vives M. Recruitment of inflammatory cells to the pleural space. Chemotactic cytokines, IL-8 and monocyte chemotactic peptide-1 in human pleural fluids. J. Immunol. 1993; 151: 7216–7223.
11. Antony V.B., Godbey S.W., Kunkel S.L. et al. Pleural mesothelial cell expression of C-C (monocyte chemotactic peptide) and C-X-C (interleukin 8) chemokines. Am. J. Respir. Cell. Mol. Biol. 1995; 12: 581–588.
12. Anthony V.B. Immunological mechanisms in pleural disease. Eur. Respir. J. 2003; 21: 539–544.
13. Marchi E., Broaddus C.V. Mechanisms of pleural liquid formation in pleural inflammation. Curr. Opin. Pulm. Med. 1997; 3: 305–309.
14. Mohammed K.A. Mycobacterium-mediated chemokine expression in pleural mesothelial cells: role of C-C chemokines in tuberculous pleurisy. J. Infect. Dis. 1998; 178: 1450–1456.
15. Mohammed K.A., Nasreen N., Ward M.J., Antony V.B. Macrophage inflammatory protein-1α C-C chemokine in parapneumonic pleural effusions. J. Lab. Clin. Med. 1998; 132: 202–209.
16. Barczyk A., Pierzchala W., Sozanska E. Stężenia C-C chemokin (MCP-1, MIP-1α, MIP-1β) w indukowanej plwocinie u chorych na przewlekłą obturacyjną chorobę płuc i u chorych na przewlekłe zapalenie oskrzeli. Pneumonol. Alergol. Pol. 2001; 69: 40–49.
17. Capelli A., Di Stefano A., Gnemmi I. et al. Increased MCP-1 and MIP-1 beta in bronchoalveolar lavage fluid of chronic bronchitis. Eur. Respir. J. 1999; 14: 160–165.
18. Petrek M., Kolek V., Szotkowská J., du Bois R.M. CC and C chemokine expression in pulmonary sarkoidosis. Eur. Respir. J. 2002; 20: 1206–1212.
19. Yoshioka S. High-BAL fluid concentration of Rantes in nonspecific interstitial pneumonia compared with usual interstitial pneumonia. Respir. Med. 2004; 98: 945–951.
20. Kurashima K., Mukaida N., Fujimura M. et al. Elevated chemokine levels in bronchoalveolar lavage fluid in tuberculosis patients. Am. J. Respir. Crit. Care Med. 1997; 155: 1474–1477.
21. Oppenheim J.J., Zachariae C.O., Mukaida N., Matsushima K. Properties of the novel proinflammatory supergene intercrine cytokine family. Annu. Rev. Immunol. 1991; 9: 617–48.
22. Schall T.J., Bacon K.B. Chemokines, leukocyte trafficking and inflammation. Curr. Op. Pneum. 1994; 6: 865–873.
23. Pierzchala W., Trzaska M. Interpretacja wyników rutynowego badania płynu opłucnowego. Wiadomości Lekarskie 2000; 53: 191–199.
24. Safianowska A., Krenke R., Dmowska-Sobstyl B., Bogacka-Zatorska E., Domagala-Kulawik J., Chazan R. Aktywność deaminazy adenozyny w gruźliczym i nowotworowym wysięku w opłucnej. Pneumonol. Alergol. Pol. 2006; 74: 5–9.
25. Okamoto M., Kawabe T., Iwasaki Y. et al. Evaluation of interferon-gamma, interferon-gamma inducing cytokines, and interferon-gamma-inducible chemokines in tuberculous pleural effusions. J. Lab. Clin. Med. 2005; 145: 88–93.