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Stephan Fricke*, Gerhard Hoheisel, Christian Gessner, Katrin Bauer, Hans-Jürgen Seyfarth, Stefan Hammerschmidt, Franka Kahlenberg, Kin Chan, Andreas Boldt, Hubert Wirtz, Thomas Keller and Ulrich Sack

Mediators in pleural effusions of different origin: a two-step diagnostic study

Mediatoren in Pleuraergüssen unterschiedlicher Genese – eine Studie in zwei Schritten

Abstract

Background: Many mediators in pleural effusions give diagnostic information. This study therefore aimed to find out whether the repeated determination of interleukin (IL)-6, IL-15, vascular endothelial growth factor (VEGF), and tissue inhibitors of metalloproteinase (TIMP)-2 would confirm previous results and, furthermore, whether a combination of these parameters could achieve a higher diagnostic yield.
Methods: Two consecutive groups of patients with pleural effusions were included. The underlying disease entities in series I vs. series II were: 12 vs. 0 tuberculosis (TB), 19 vs. 30 primary lung cancer, 7 vs. 14 secondaries to the lung, 5 vs. 13 congestive heart failure, and 2 vs. 7 parapneumonic effusion.
Results: VEGF could not differentiate values of series I to the same degree as in series II. For IL-15, totally deviating levels were obtained in series II. IL-6, however, showed comparable results in both series. Values of TIMP-2 were generally higher in series II. The diagnostic accuracies of VEGF and IL-6 were comparable. With logistic regression, the data of VEGF and IL-6 could be used as a classifying tool when TB cases were excluded. The classifying tool based on the data of series I showed a high degree of diagnostic accuracy (area under the curve, AUC=0.94) in contrast to the calculation based on the data of series II (AUC=0.56). Combining both series, VEGF was superior.
Conclusions: VEGF was confirmed as a valid marker for malignant diseases. VEGF in combination with IL-6 could serve as a classifying tool. TB cases, however, should be excluded. The previously proposed relevance of TIMP-2 as a diagnostic parameter could, similarly to IL-15, not be confirmed.

Keywords: biomarkers; cytokines; pleural effusion.

Zusammenfassung

Hintergrund: Zahlreiche Mediatoren im Pleuraerguss sind diagnostisch nutzbar. Die vorliegende Arbeit wurde durchgeführt, um vorliegende Beschreibungen zur Messung von IL-6, IL-15, VEGF und TIMP-2 zu bestätigen und zu überprüfen, ob durch Kombination bessere diagnostische Aussagen möglich sind.
Methoden: Es wurde zwei Gruppen von Patienten nacheinander untersucht. In den beiden Kohorten lagen folgende Diagnosen vor (I vs. II): Tuberkulose (12/0), Bronchialkarzinom (19/30), Lungenmetastasen (7/14), Rechtsherzinsuffizienz (5/13) und parapneumonische Ergüsse (2/7).
Ergebnisse: VEGF war in der ersten Serie weniger gut als in der zweiten. Bei IL-15 fanden sich in der II. Serie völlig

***Korrespondenz: Dr. Stephan Fricke,** Translationszentrum für Regenerative Medizin (TRM) Leipzig, Universität Leipzig, Philipp-Rosenthal-Straße 55, 04103 Leipzig, Deutschland,
Tel.: + 49 341 97 25500, Fax: + 49 341 97 25509,
E-Mail: stephan.fricke@trm.uni-leipzig.de
Gerhard Hoheisel: Praxis für Innere Medizin, Pneumologie und Allergologie, Leipzig, Deutschland
Christian Gessner: Praxis für Innere Medizin und Pneumologie, Leipzig, Deutschland
Katrin Bauer: Institut für Klinische Immunologie, Medizinische Fakultät der Universität Leipzig, Leipzig, Deutschland
Hans-Jürgen Seyfarth, Stefan Hammerschmidt and Hubert Wirtz: Department für Innere Medizin, Neurologie und Dermatologie, Abteilung Pneumologie, Universitätsklinikum Leipzig, Leipzig, Deutschland
Franka Kahlenberg, Andreas Boldt and Ulrich Sack: Translationszentrum für Regenerative Medizin (TRM), Universität Leipzig, Leipzig, Deutschland; und Institut für Klinische Immunologie, Medizinische Fakultät der Universität Leipzig, Leipzig, Deutschland
Kin Chan: Abteilung für Pneumologie Pallliativmedizin, Haven of Hope Hospital, Hong Kong
Thomas Keller: ACOMED statistik, Leipzig, Deutschland

abweichende Ergebnisse. Die Ergebnisse für IL-6 waren in beiden Serien gleich. In Serie II lagen grundsätzlich höhere TIMP-2-Werte vor. Die diagnostische Genauigkeit von VEGF und IL-6 war vergleichbar. Mittels logistischer Regression war es möglich, IL-6 und VEGF als Klassifikator zu nutzen, wenn die Tuberkulosefälle ausgeschlossen wurden. Dieser zeigte mit den Werten der ersten Kohorte eine gute Genauigkeit (AUC=0.94), während er in der 2. Serie nicht befriedigte (AUC=0.56). In Kombination beider Serien überzeugte VEGF.

Schlussfolgerung: VEGF konnte als valider Marker für maligne Erkrankungen bestätigt werden. In Kombination mit IL-6 könnte es als Klassifikator dienen, allerdings sollten Tuberkulosefälle ausgeschlossen werden. Die in Vorarbeiten vorgeschlagene Relevanz von TIMP-2 und IL-15 konnte nicht bestätigt werden.

Schlüsselwörter: Biomarker; Pleuraerguss; Zytokine.

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Background

In recent publications we investigated the value of the determination of cytokines and proteases in the differential diagnosis of lung disorders by assessing pleural effusions of different origins. Increased levels of interleukin (IL)-6 and IL-8 but decreased levels of the soluble form of the interleukin 6 receptor (sIL-6R) were found in pleural effusion fluid of patients with lung cancer and malignant pleural effusions (CA) and could be used for differential diagnosis against effusions of tuberculous origins (TB) [1, 2]. In another study matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) were examined. MMP-2, TIMP-1, and TIMP-2 concentrations were increased in CA pleural fluid vs. plasma, in contrast to MMP-9 being higher in plasma, but only pleural fluid MMP-1 and MMP-8 were significantly increased in CA vs. congestive heart failure (CHF) effusions [3]. In patients with TB pleural effusions compartmentalized MMP-1, MMP-2, TIMP-1, and TIMP-2 and, compared to CHF, a surplus of MMP-1, MMP-2, MMP-8, and MMP-9 in the pleural space was found [3]. The determination of vascular endothelial growth factor (VEGF) in pleural effusions of patients with CA, tumours with secondaries to the lung (TM), congestive heart failure (CHF), TB, and acute infections (INF) showed VEGF to be higher in effusions of patients with CA as well as TM in comparison to INF, TB, or CHF [4]. In another study we investigated immunoreactive concentrations of IL-4, IL-6, IL-11, IL-15, IL-17, IL-18, and tumor necrosis factor-α (TNF-α) in pleural effusions from patients with TB, CA, TM, CHF, and INF. Statistical analysis based on singular cytokines did not provide evidence for diagnostic relevance. However, fuzzy-logic analysis was able to assign patients to the correct diseases with an 80% accuracy using IL-6 and IL-15 measurements [4].

Various parameters have been studied by other groups for better differentiation of pleural effusions, including high molecular weight proteins, acute phase reactants, and cytokines [5]. TIMP-2 is considered as an interesting marker in malignant pleural effusions due to a primary tumour elsewhere in the body [6]. VEGF is another important mediator in exudative pleural effusions [7]. VEGF has been studied in human cancer cell lines and is considered to play a critical role in the production of malignant pleural effusions [8, 9]. VEGF was shown to be useful as an adjunct of the conventional algorithm in the diagnosis of malignant pleural effusions [10]. The comparison of VEGF with adenosine deaminase (ADA) and interferon-γ (IFN-γ) suggested that high amounts of pleural fluid IFN-γ may indicate an increased rate of residual pleural thickening in tuberculous pleurisy [11]. IFN-γ, IL-12p40, and IL-6 proved useful in differentiating tuberculous and malignant effusions [12]. C-reactive protein (CRP), IL-6, and TNF-α provided useful information for the differentiation of parapneumonic, tuberculous and malignant effusions in clinical practice [2]. Applying a decision tree analysis that contained age, temperature, pleural fluid ADA, and LDH was helpful in the differential diagnosis of tuberculous and malignant pleural effusions [13]. Finally, the determination of ADA, INF-γ, CRP, carcinoembryonic antigen (CEA), IL-6, TNF-α and VEGF in pleural effusions of different origin and in addition, the application of receiver operating characteristic curve (ROC) analysis and further statistical methods showed that the combination of ADA and CRP levels might be sufficient for discriminating between malignant, tuberculous and parapneumonic pleural effusions [14].

The combination of various parameters and the application of advanced statistical tools seemed therefore promising in achieving a higher diagnostic yield, improved diagnostic accuracy, and differential diagnostic capability. This observation encouraged us to expand and combine previous investigations. We therefore, in a first step, intended to find out whether the determination of the so far most promising parameters IL-6, IL-15, VEGF, and TIMP-2 would confirm previous results in a new set of patients. In a second step we intended to find out whether

the use of a combination of these parameters would lead to a higher diagnostic yield.

Materials and methods

Patients were consecutively enrolled into the study (series I) after informed consent was obtained following the ethical standards of our institutional guidelines. In order to test the validity of parameters measured and the diagnostic accuracy obtained a second series of patients (series II) was examined. In series I altogether 38 patients (19 women, 19 men, age 67±19 years) and in series II altogether 50 patients (18 women, 32 men, age 64±13 years) were enrolled. Twelve patients with primary lung cancer and malignant pleural effusion (CA) in series I (six women, six men, age 75±8 years) and 16 patients in series II (five women, eleven men, age 66±8 years) were included; the diagnosis was based on radiological, clinical, histological, and/or cytological evidence, and on malignant cells in pleural fluid or pleural biopsy specimens. Seven patients with secondaries to the lung from a primary tumour (two breast, two gastric, two colon and one kidney cancer; TM) in series I (six women, one man, age 69±19 years) and 14 patients in series II (nine women, five men, age 60±10 years) were included; the diagnosis was based on radiological, clinical, histological, and/or cytological evidence, and on malignant cells in pleural fluid or pleural biopsy specimens. Twelve patients with tuberculous pleurisy (TB) in series I (eight women, four men, age 52±24 years) were included, in series II no TB cases were available; the diagnosis was based on the clinical and radiological appearance, microbiological proof of acid fast bacilli and/or growth of *M. tuberculosis* in sputum or pleural effusion fluid, or on histological proof of granuloma formation in pleural biopsy specimens. Two patients with pneumonia and parapneumonic effusions (PNEU) in series I (two men, age 76±29 years) and seven patients in series II (two women, five men, age 62±25 years) were included; the diagnosis was based on radiological, clinical, and/or microbiological evidence. Finally, five patients with congestive heart failure and pleural effusion (CHF) in series I (three women, two men, age 76±9 years) and 13 patients in series II (two women, eleven men, age 69±12 years) were included; the diagnosis was based on radiological and clinical findings, the transudate nature of the effusion, and exclusion of other etiologies.

Pleural effusion samples were collected during thoracocentesis performed for diagnostic or therapeutic purposes. Plain tubes were used to collect samples for determination of lactate dehydrogenase (LDH) and total protein by standardised automated methods; Light's criteria were calculated as described before [15]. EDTA tubes (final concentration: 1 mg/mL) were used to collect and store samples at –70°C for later determination of VEGF, IL-15, IL-6, and TIMP-2 by Enzyme Linked Immunosorbent Assays (ELISA) using commercially available kits (human VEGF, IL-15: Quantikine™ Immunoassays, R&D Systems, Wiesbaden, Germany; human IL-6: Biosource International, Camarillo, CA, USA; TIMP-2: Biotrak™, Amersham, Freiburg, Germany) as described before [1, 4, 15]. The lower detection limits were: 5 pg/mL (IL-6), 1 pg/mL (IL-15), 3.13 ng/mL (TIMP-1) and 2.5 pg/mL (VEGF).

The results of series I and II were used for determination of differential diagnosis accuracy. Box-plot-format was chosen to present data (boxes show median and 25th/75th percentiles, horizontal bars represent 5th/95th percentiles). Diagnostic characteristics were calculated as ROC curves by an Excel application (ROC-tools, details see http://www.acomed-statistik.de). Per study, the occurrence of tumour disease (yes/no) in dependence on all markers was investigated in logistic regression models (complete models without selection processes).

Results

The results of series I in comparison to series II show differences to an unexpected degree (Figure 1). The values obtained in series II can no longer differentiate the entities examined as sharply as in series I. For VEGF lower values for CA, TM, and PNEU were obtained in contrast to higher values for CHF effusions in series I. As for IL-15, a totally deviating level of results was obtained with barely detectable values in series II. The values of TIMP-2, however, were at a higher level in series II than in series I for all entities examined. Only for IL-6 the distribution pattern of results in series I was comparable to that obtained in series II. Based on these observations, the diagnostic accuracies of IL-6 and to a lesser degree of VEGF seem both high when comparing the two series. The diagnostic accuracy of TIMP-2, however, seems low, while that of IL-15 was only minimal.

Table 1 shows the results for the ROC curves. Especially, the results of series II demonstrate a diagnostic potential for IL-6 and VEGF. The data of VEGF and IL-6 were therefore used to create a classifying tool by applying the method of logistic regression. TB cases were excluded, as samples were only available in series I. The classifying tool calculated in this way based on the data of series I shows a high degree of diagnostic accuracy (AUC=0.94); whereas the calculation based on the data of series II does

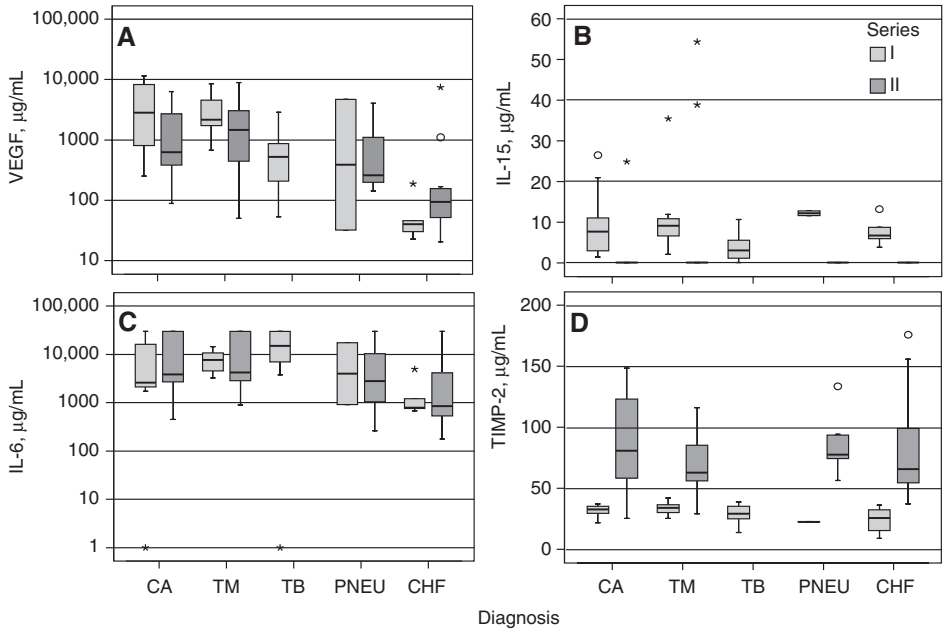

Figure 1 Levels of VEGF (A), IL-15 (B), IL-6 (C), and TIMP-2 (D) in pleural effusion fluid separated by disease entities.
For each parameter two values are given: series I and II. Circles and stars indicate outliers. CA, primary lung cancer and malignant pleural effusion; TM, secondaries to the lung from a primary tumour; TB, tuberculous pleurisy; PNEU, pneumonia and parapneumonic effusions; CHF, congestive heart failure and pleural effusion.

not (AUC=0.56). This could further be demonstrated by presenting the classification values (values >0 and <1) in a box-plot format (Figure 2A). It should be noted, however, that the number of values for development and for testing was rather low (Table 2). Therefore, it seemed appropriate to use series II as the development data base and series I as the testing data base, with the result of an AUC=0.866 (development) and 0.78 (testing), respectively. The results shown in box-plot format are depicted in Figure 2B.

Combining data, VEGF is the best parameter discriminating between underlying pathologies (Figure 3).

Discussion

Results of numerous publications in pulmonology and immunology describing cytokines and other markers in pleural effusions suggest a diagnostic benefit of such

Table 1 Results of ROC analyses separated by disease entities and series I and II.

	Area under curve (AUC)	Standard error	Significance level (p)	95% Confidence interval	
				Lower limit	Upper limit
VEGF					
Series I	0.859	0.062	0.000	0.736	0.981
Series II	0.770	0.076	0.002	0.621	0.920
IL-15					
Series I	0.644	0.090	0.129	0.467	0.821
Series II	0.550	0.082	0.552	0.389	0.711
IL-6					
Series I	0.461	0.098	0.683	0.269	0.654
Series I (TB excluded)	0.774	0.121	0.035	0.537	1.012 (??)
Series II	0.726	0.076	0.007	0.576	0.876
TIMP-2					
Series I	0.693	0.092	0.045	0.513	0.873
Series II	0.484	0.084	0.851	0.319	0.649

Series I: first group; series II: second group.

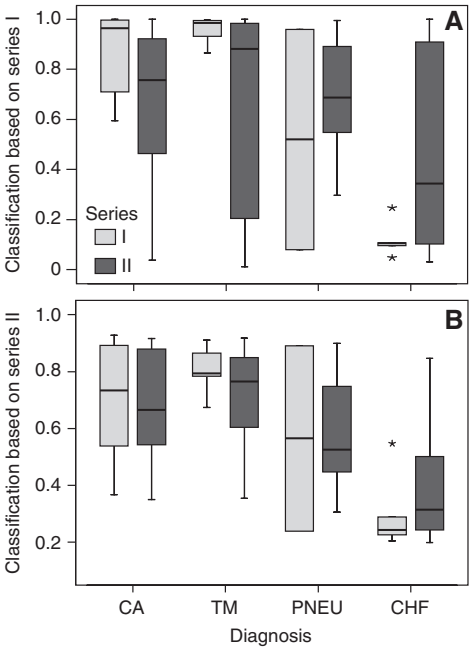

Figure 2 Classification values presented in box-plot format. Calculation Figure 2A based on the data of series I, and classification values in Figure 2B were developed on the basis of series II. The appearance is more homogenous in comparison to the presentation in Figure 2A. CA, primary lung cancer and malignant pleural effusion; TM, secondaries to the lung from a primary tumour; PNEU, pneumonia and parapneumonic effusions; CHF, congestive heart failure and pleural effusion.

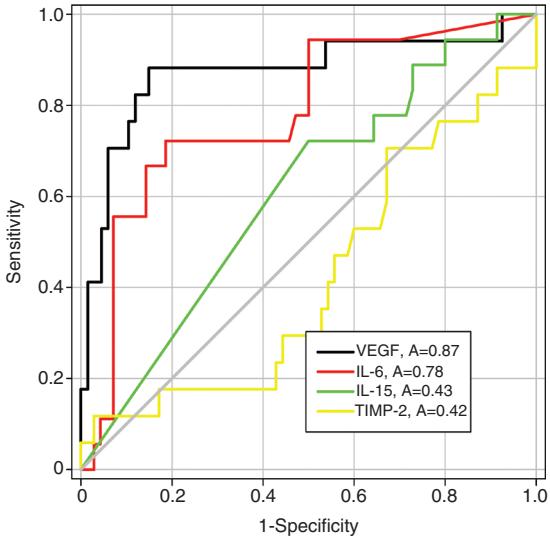

Figure 3 ROC analysis of cytokines for detection of malignant pleural effusions in comparison to non-malignant ones. VEGF is shown to be superior to other parameters IL-15, IL-6 or TIMP-2.

parameters in patients' care. A multitude of observations is available. However, it seems important not only to confirm previous findings but, in addition, to search for tools improving the diagnostic accuracy based on results already obtained. We therefore decided to investigate IL-6, IL-15, TIMP-2, and VEGF in pleural effusions repeatedly and evaluated results by application of expanded calculatory methods. We aimed 1) to confirm pre-existing data and 2) to check whether a combination of these data could improve the process of differential diagnosis.

This study shows that IL-6 has a high diagnostic accuracy when determined repeatedly in different series of patients. IL-6 has a wide spectrum of biological activities and was found to be increased in malignant pleural effusions of patients with pulmonary carcinoma and also tuberculosis [16–19]. IL-6 also seems to play an important anti-inflammatory role in pleural effusions, breaking with the traditional idea of IL-6 being a pro-inflammatory acute phase reaction cytokine only [20]. IL-6, IL-8, and VEGF also play a role in the pathogenesis of prenatal chylothoraces in human fetuses [21]. The diagnosis of tuberculous pleural effusions by determination of CRP, IL-1α, IL-6, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ has been the focus of numerous studies [2, 22, 23].

Table 2 Data base for classification tool development.

n	Diagnostic group		Total
	Non-malignant (without TB)	Malignant	
Series I	7	19	26
Series II	20	30	50
Total	27	49	76

This study further shows that IL-15 has a low diagnostic accuracy, with deviating results when tested in different series of patients. This deviation was considered as not being related to the methods or tests used but rather to the unstable nature of IL-15 itself. Recent studies report the occurrence of IL-15 in autoimmune as well as in malignant diseases, including pleural effusions [24–26]. IL-15 is an interesting parameter and should be studied further, however, seems not suitable as a candidate to improve diagnostic accuracy in the differential diagnosis of pleural effusions.

This study demonstrated that the determination of TIMP-2 is of low diagnostic accuracy. Although MMP and their endogenous inhibitors TIMP play an important role in the homeostasis of the pleural space in various disease states, their contribution to differential diagnosis of pleural effusions of different origins seems limited [27, 28].

VEGF demonstrated a high diagnostic accuracy in this study similar to that of IL-6. VEGF has potent angiogenic,

mitogenic, and vascular permeability enhancing properties specific for endothelial cells, was found at high levels in exudative inflammatory and neoplastic pleural effusions [28].

A limitation of this study was that TB cases were not available in both series. The results for TB could therefore only demonstrate that the respective parameters could be detected in the entities studied. The classifying tool could only be used when TB cases were excluded. With regards to the calculation of the classifying tool the number of values for development and for testing was low. In general, 10 to 20 values are necessary per group and per parameter. The study demonstrates that pilot studies for investigation of diagnostic potential of biomarkers as currently widely undertaken in terms of "individualized medicine" might be of limited validity and need confirmation in independent trials.

It should be noted and has been shown strikingly by our results that discriminative power of biomarkers highly depends on clinical question, compared groups, and patients' entities. Inhomogeneous sample distribution in different studies makes comparisons very hard.

In summary, VEGF was not only confirmed as a valid marker for malignant diseases. Furthermore it was shown that the results of VEGF and IL-6 could be used to create a classifying tool. The diagnostic relevance of IL-15 could not be confirmed in the second dataset. In studies based on a small case numbers, however, such effects can be expected. The previously proposed relevance of TIMP-2 as a diagnostic parameter could, similarly to IL-15, not be confirmed when the two series were compared. Although its functions stay controversely discussed, VEGF stays the best suitable diagnostic parameter [29].

Conclusions

VEGF as well as IL-6 are both parameters demonstrating a diagnostic potential for differentiation between non-malignant effusions (e.g., CHF, parapneumonic) vs. malignant effusions (e.g., lung cancer and secondary cancer type effusions). Both parameters could be used in combination. Whether an accuracy of AUC=0.8 with a sensitivity of 75% and a specificity of 75% is clinically relevant or not depends on the clinical situation of the individual patient. However, this degree of diagnostic accuracy is comparable to that of other known tumour markers. IL-15 and TIMP-2 demonstrated detrimental results in the two consecutive series and were therefore not considered to be useful for differential diagnosis.

Conflict of interest statement

Authors' conflict of interest disclosure: T.K. owns the ACOMED statistik. All other authors stated that there are no conflicts of interest regarding the publication of this article.
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References

1. Hoheisel G, Izbicki G, Roth M, Chan CH, Leung JC, Reichenberger F, et al. Compartmentalization of pro-inflammatory cytokines in tuberculous pleurisy. Respir Med 1998;92:14–7.
2. Kiropoulos TS, Kostikas K, Oikonomidi S, Tsilioni I, Nikoulis D, Germenis A, et al. Acute phase markers for the differentiation of infectious and malignant pleural effusions. Respir Med 2007;101:910–8.
3. Hoheisel G, Sack U, Hui DS, Huse K, Chan KS, Chan KK, et al. Occurrence of matrix metalloproteinases and tissue inhibitors of metalloproteinases in tuberculous pleuritis. Tuberculosis (Edinb) 2001;81:203–9.
4. Chomej P, Bauer K, Bitterlich N, Hui DS, Chan KS, Gosse H, et al. Differential diagnosis of pleural effusions by fuzzy-logic-based analysis of cytokines. Respir Med 2004;98:308–17.
5. Ruiz GR, Marquez de Prado Urquia MM, Borque de LL. Distinguishing pleural transudates and exudates through the quantification of biochemical parameters. Rev Clin Esp 2004;204:511–20.
6. Giarnieri E, Alderisio M, Mancini R, Falasca C, Ricci A, Mariotta S, et al. Tissue inhibitor of metalloproteinase 2 (TIMP-2) expression in adenocarcinoma pleural effusions. Oncol Rep 2008;19:483–7.
7. Economidou F, Antoniou KM, Tzanakis N, Sfiridaki K, Siafakas NM, Schiza SE. Angiogenic molecule Tie-2 and VEGF in the pathogenesis of pleural effusions. Respir Med 2008;102:774–9.
8. Stathopoulos GT, Kollintza A, Moschos C, Psallidas I, Sherrill TP, Pitsinos EN, et al. Tumor necrosis factor-alpha promotes malignant pleural effusion. Cancer Res 2007;67:9825–34.
9. Tomimoto H, Yano S, Muguruma H, Kakiuchi S, Sone S. Levels of soluble vascular endothelial growth factor receptor 1 are elevated in the exudative pleural effusions. J Med Invest 2007;54:146–53.
10. Shu J, Sun G, Liu H, Liu J. Clinical utility of vascular endothelial growth factor in diagnosing malignant pleural effusions. Acta Oncol 2007;46:1004–11.
11. Gerogianni I, Papala M, Tsopa P, Zigoulis P, Dimoulis A, Kostikas K, et al. Could IFN-gamma predict the development of residual pleural thickening in tuberculous pleurisy? Monaldi Arch Chest Dis 2008;69:18–23.
12. Yang CS, Lee JS, Lee HM, Shim TS, Son JW, Jung SS, et al. Differential cytokine levels and immunoreactivities against

Mycobacterium tuberculosis antigens between tuberculous and malignant effusions. Respir Med 2008;102:280–6.
13. Porcel JM, Aleman C, Bielsa S, Sarrapio J, Fernandez de ST, Esquerda A. A decision tree for differentiating tuberculous from malignant pleural effusions. Respir Med 2008;102:1159–64.
14. Daniil ZD, Zintzaras E, Kiropoulos T, Papaioannou AI, Koutsokera A, Kastanis A, et al. Discrimination of exudative pleural effusions based on multiple biological parameters. Eur Respir J 2007;30:957–64.
15. Moche M, Hui DS, Huse K, Chan KS, Choy DK, Scholz GH, et al. Matrix metalloproteinases and their inhibitors in lung cancer with malignant pleural effusion. Pneumologie 2005;59:523–8.
16. Hirano T, Taga T, Nakano N, Yasukawa K, Kashiwamura S, Shimizu K, et al. Purification to homogeneity and characterization of human B-cell differentiation factor (BCDF or BSFp-2). Proc Natl Acad Sci USA 1985;82:5490–4.
17. Hoheisel G, Izbicki G, Roth M, Chan CH, Reichenberger F, Schauer J, et al. Proinflammatory cytokine levels in patients with lung cancer and carcinomatous pleurisy. Respiration 1998;65:183–6.
18. Sack U, Scheibe R, Wotzel M, Hammerschmidt S, Kuhn H, Emmrich F, et al. Multiplex analysis of cytokines in exhaled breath condensate. Cytometry A 2006;69:169–72.
19. Sack U, Biereder B, Elouahidi T, Bauer K, Keller T, Trobs RB. Diagnostic value of blood inflammatory markers for detection of acute appendicitis in children. BMC Surg 2006;6:15.
20. Chibante AM, Vaz MC, Vargas FS. IL-6 anti-inflammatory activity in pleural effusion post-coronary artery bypass graft surgery. Rev Port Pneumol 2007;13:319–34.
21. Chen M, Hsieh CY, Shih JC, Chou CH, Ma GC, Chen TH, et al. Pro-inflammatory macrophage migratory inhibition factor and interleukin-6 are concentrated in pleural effusion of human fetuses with prenatal chylothorax. Prenat Diagn 2007;27:435–41.
22. Wong CF, Yew WW, Leung SK, Chan CY, Hui M, Au-Yeang C, et al. Assay of pleural fluid interleukin-6, tumour necrosis factor-alpha and interferon-gamma in the diagnosis and outcome correlation of tuberculous effusion. Respir Med 2003;97:1289–95.
23. Xirouchaki N, Tzanakis N, Bouros D, Kyriakou D, Karkavitsas N, Alexandrakis M, et al. Diagnostic value of interleukin-1alpha, interleukin-6, and tumor necrosis factor in pleural effusions. Chest 2002;121:815–20.
24. Yanagawa H, Takeuchi E, Miyata J, Maniwa K, Suzuki Y, Ogushi F, et al. Rheumatoid pleural effusion with detectable level of interleukin-15. J Intern Med 1998;243:331–2.
25. Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. Blood 2001;97:14–32.
26. Chen YM, Ting CC, Peng JW, Yang WK, Yang KY, Tsai CM, et al. Restoration of cytotoxic T lymphocyte function in malignant pleural effusion: interleukin-15 vs. interleukin-2. J Interferon Cytokine Res 2000;20:31–9.
27. Park KJ, Hwang SC, Sheen SS, Oh YJ, Han JH, Lee KB. Expression of matrix metalloproteinase-9 in pleural effusions of tuberculosis and lung cancer. Respiration 2005;72:166–75.
28. Cheng D, Rodriguez RM, Perkett EA, Rogers J, Bienvenu G, Lappalainen U, et al. Vascular endothelial growth factor in pleural fluid. Chest 1999;116:760–5.
29. Bradshaw M, Mansfield A, Peikert T. The role of vascular endothelial growth factor in the pathogenesis, diagnosis and treatment of malignant pleural effusion. Curr Oncol Rep 2013;15:207–16.