Cytokine/Chemokine/Growth Factor Levels in Malignant Pleural Effusion of Non-small Cell Lung Cancer

Naoki HAYAMA, Shigeaki HATTORI, Genki TAKAHASHI, Fuminari TAKAHASHI, Tomoe TAKEUCHI, Jun TANAKA, Yukihiro HORIO, Hiroto TAKIGUCHI, Katsuyoshi TOMOMATSU, Asako KITAHARA, Takahisa TAKIHARA, Kyoko NIIMI, Tsuyoshi OGUMA and Koichiro ASANO

Division of Pulmonary Medicine, Department of Medicine, Tokai University School of Medicine

(Received June 30, 2020; Accepted September 7, 2020)

Objective: Malignant pleural effusions (MPEs) deteriorate the quality of life in patients with advanced stages of cancer. Although vascular endothelial growth factor (VEGF) is known to be a key factor for MPE formation, it is not fully clarified whether there are other components related to its appearance.
Methods: Pleural effusion and serum samples were collected from patients with MPEs of non-small cell lung cancer. Cellular analysis of pleural effusion was performed using fluorescence flow cytometry. The concentrations of 12 cytokines, chemokines, and growth factors in MPEs and serum samples were analyzed using the cytometric bead array method.
Results: Fifteen patients (median age: 70 years, 11 males) with non-small cell lung cancer (13 adenocarcinoma, 2 squamous cell carcinoma) were enrolled in this study. Concentrations of VEGF, interleukin (IL)-5, IL-6, IL-8, IL-12/IL-23p40, and C-C motif chemokine ligand (CCL) 2 were significantly higher in MPE than in serum. Pleural IL-5 levels correlated with malignant cell numbers in MPE. There was no factor related to the total amount of drained effusion or period of chest tube insertion.
Conclusions: Production of six molecules were increased in the pleural cavity with MPE of non-small cell lung cancer. Complex interactions among these molecules may regulate MPE formation.

Key words: interleukin-5, IL-12/IL-23p40, pleural effusion, non-small cell lung cancer, vascular endothelial growth factor

INTRODUCTION

A number of malignant tumors induce malignant pleural effusion (MPE), which is associated with high morbidity and mortality. Lung and breast cancers are the leading causes of MPEs, accounting for approximately 50% of cases, and MPE due to lung cancer has the poorest prognosis [1]. In addition, MPE causes many symptoms such as cough, dyspnea, fatigue, chest pain, and deterioration of the quality of life of patients. Therefore, almost all patients with MPE require therapeutic interventions, such as thoracentesis, pleural catheter indwelling, and pleurodesis [1–3].

There are two major mechanisms by which MPEs accumulate in the pleural cavity. One is the blockade of the pleural effusion drainage system. Draining lymph vessels are filled with tumor cells, and pleural effusion cannot be absorbed from the chest cavity. Another mechanism, which is more important, is enhanced production of pleural effusion. Tumor-host interactions play a key role in regulating the production of pleural fluid. Tumor cells secrete inflammatory and vasoactive mediators, stimulating inflammation, angiogenesis, and vascular hyperpermeability. These mediators activate host cells such as lymphocytes, macrophages, and mesothelial cells, which then produce more cytokines and chemokines [4]. Several cytokines or growth factors, such as vascular endothelial growth factor (VEGF) [5–8], C-C motif chemokine ligand (CCL) 2 [9, 10], and interleukin (IL)-8 [11, 12], have been reported as key molecules involved in MPE formation.

Here, we analyzed the correlation between the concentrations of cytokines, chemokines, and growth factors and the amount of pleural effusion or cell fraction in MPE in non-small cell lung cancer (NSCLC) patients. The purpose of this study was to identify the key molecules that regulate the production of MPEs.

MATERIALS AND METHODS

Patients

This study was performed between September 2013 and March 2017. Patients with cytologically proven MPEs due to non-small cell lung cancer at the Tokai University Hospital in Japan were enrolled in this study. MPEs were drained though chest tubes inserted in all patients. After complete drainage of the pleural effusion, they received chemical pleurodeses. The number of days and total amount of drained pleural effusion between the chest-tube insertion and the pleurodesis were evaluated retrospectively from medical records.

Naoki HAYAMA, Division of Pulmonary Medicine, Department of Medicine, Tokai University School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, Japan Tel: +81-463-93-1121 Fax: +81-463-93-0381 E-mail: hayama@is.icc.u-tokai.ac.jp

Table 1 Patient characteristics and cellular analysis of pleural effusion

Age, years[1]	70 (60 - 93)
Male/Female	11 / 4
Histology	
Adenocarcinoma	13
Squamous cell carcinoma	2
Days from the day of chest-tube insertion till pleurodesis[1]	8 (4 - 19)
Total amount of drained effusion, mL[2]	3799.3 ± 1673.5
Cell counts in pleural effusion, /μL[2]	
Total cell count	1872.1 ± 1247.0
Neutrophil	122.2±114.0
Lymphocyte	804.7 ± 916.7
Macrophage	472.1 ± 699.2
Eosinophil	118.6 ± 134.9
Malignant cell	349.4 ± 565.3

[1] median (range), [2] mean ± SD

This study was approved by the Institutional Review Board of Tokai University Hospital (13R-137) and was implemented in compliance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

Sample acquisition

Samples of pleural effusion for the measurement of cytokines, chemokines, and growth factors were obtained by thoracentesis when MPE was diagnosed. Cell analysis of the pleural effusion was performed immediately after sample acquisition with Sysmex XN-3000 (Sysmex, Kobe, Japan). Peripheral blood samples were collected just before pleurodesis. Pleural effusion and blood samples were centrifuged at 1710 × g for 5 min at 4˚C, and the supernatants were stored at -80˚C until analysis.

Cytokine/chemokine/growth factor analysis

MPE and serum concentrations of 12 cytokines, chemokines, and growth factors, including interleukin (IL)-2, IL-5, IL-6, IL-8, IL-9, IL-12/IL-23p40, interferon-α, interferon-γ, CCL2, CCL11, basic fibroblast growth factor, and VEGF, were measured using the cytometric bead array method (Human CBA flex Kit, Beckton Dickinson, San Jose, CA, USA) with BD LSR Fortessa (Beckton Dickinson, San Jose, CA, USA) according to the manufacturer's instructions. The levels of cytokines, chemokines, and growth factors were analyzed with FCAP Array software (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analyses

Data are expressed as the mean ± standard deviation (SD) or median and range. Group comparisons were made using the Wilcoxon signed-rank sum test. Spearman's rank correlation coefficient was used to examine the association between the cytokines and the cellular population in pleural fluids. We used the Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM Corp., Armonk, N.Y., USA) for the analyses.

RESULTS

Patient characteristics

Fifteen patients (11 males and 4 females) with non-small cell lung cancer were enrolled in this study. The median age was 70 years (range: 60–93 years). A histological diagnosis of adenocarcinoma was made in 13 patients and squamous cell carcinoma in 2 patients. Patient characteristics are summarized in Table 1. The median duration from chest-tube insertion to pleurodesis was 8 days (range: 4–19 days). The total amount of drained effusion from each patient was 3.8 ± 1.6 L.

Cellular analysis of pleural effusion

The mean total cell count (TCC) in the pleural effusion was 1872 ± 1247/μL, and the malignant cell count was 349 ± 565/μL. The cell count analysis of the pleural effusion is summarized in Table 1.

Cytokine/chemokine/growth factor levels in pleural effusion and serum

Cytokine/chemokine/growth factor levels in pleural effusion and serum from patients are shown in Figure. The levels of six molecules, that is, VEGF, IL-5, IL-6, IL-8, IL-12/IL-23p40, and CCL2, were significantly higher in the pleural effusion than in serum ($p < 0.01$). Respective pleural levels (mean ± SD) of these six molecules were 510.2 ± 349 pg/mL for VEGF, 36.5 ± 43.2 pg/mL for IL-5, 16.0 ± 6.9 ng/mL for IL-6, 9.33 ± 10.93 ng/mL for IL-8, 587 ± 488 pg/mL for IL-12/IL-23p40, and 6.18 ± 6.95 ng/mL for CCL2. Among these six molecules, pleural CCL2 levels correlated with the levels of IL-8 in MPE ($\rho = 0.62$, $p < 0.05$; Table 2).

Correlation between cell count and cytokine/chemokine/growth factor in the pleural effusion

The correlation between cell counts in the MPE and the six molecules whose levels in MPE were higher than in serum is shown in Table 3. TCC in the pleural effusion showed a significant correlation with IL-8 levels in the pleural effusion ($\rho = 0.52$, $p < 0.05$). Eosinophil counts in the pleural effusion were correlated with the IL-6 levels in MPE ($\rho = 0.64$, $p < 0.01$). There was no correlation between neutrophil, lymphocyte, or macrophage counts in the pleural effusion and the concentrations of any molecule. Malignant cell counts in MPE significantly correlated with IL-5 levels in the pleural effusion ($\rho = 0.61$, $p < 0.05$).

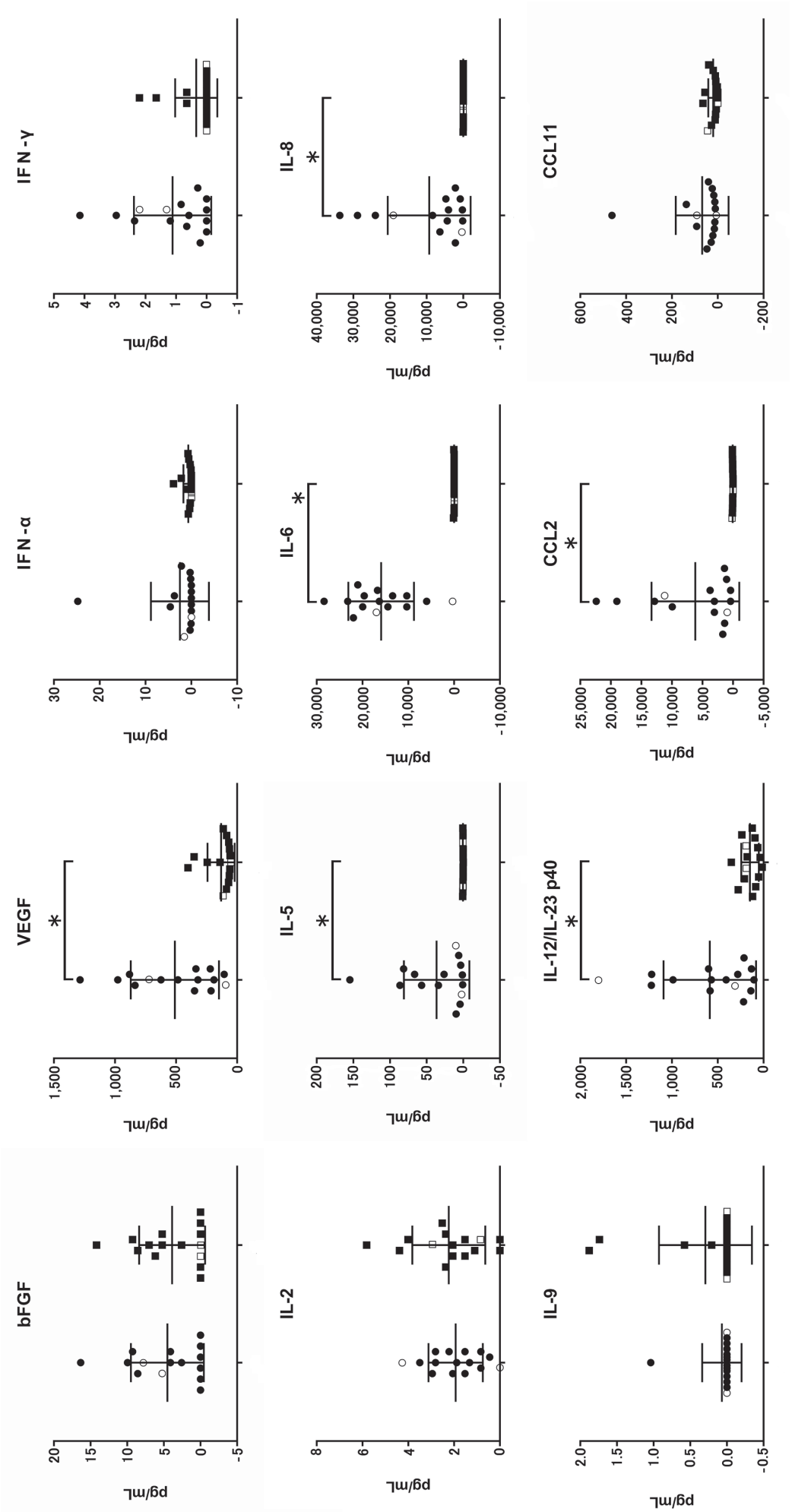

Figure Comparison between the concentrations of cytokines/chemokines/growth factors in the pleural effusion and serum
The concentrations of 12 molecules in the pleural effusion (closed circle: adenocarcinoma, open circle: squamous cell carcinoma) and serum (closed square: adenocarcinoma, open square: squamous cell carcinoma) samples were analyzed using the cytometric bead array method. Dots = raw data points; lines = mean; bars = SD. * $p < 0.01$
bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; IFN, interferon; IL, interleukin; CCL, C-C motif chemokine ligand

Table 2 Correlation among the six cytokine/chemokine/growth factor levels in pleural effusion

	VEGF	IL-5	IL-6	IL-8	IL-12p40	CCL2
VEGF	1	0.45	0.33	0.17	0.03	0.40
IL-5		1	0.43	0.38	0.01	0.31
IL-6			1	0.15	-0.45	0.35
IL-8				1	-0.06	0.75*
IL-12p40					1	0.11
CCL2						1

Numbers are ρ values. *$p < 0.01$.
VEGF, vascular endothelial growth factor; IL, interleukin; CCL, C-C motif chemokine ligand

Table 3 Correlation between cell counts and cytokine/chemokine/growth factor levels in pleural effusion

	TCC	*Neutrophils*	*Lymphocytes*	*Macrophages*	*Eosinophils*	*Malignant cells*
VEGF	-0.01	-0.20	0.25	-0.07	0.34	0.022
IL-5	0.41	-0.12	0.26	-0.08	0.49	0.61*
IL-6	0.24	0.17	-0.13	0.05	0.64**	0.25
IL-8	0.52*	-0.04	0.13	0.28	0.23	0.23
IL-12 p40	-0.14	0.13	0.13	-0.24	-0.51	0.01
CCL2	0.19	-0.20	-0.12	0.03	0.25	0.31

Numbers are ρ values. *$p < 0.05$. **$p < 0.01$.
TCC, total cell count; VEGF, vascular endothelial growth factor; IL, interleukin; CCL, C-C motif chemokine ligand

Correlation between clinical parameters and cytokine/chemokine/growth factor levels in the pleural effusion

The total amount of drained effusion or the period of chest tube insertion showed no correlation with any of the 12 molecule levels in MPE. VEGF level in the pleural effusion also exhibited no correlation with the total amount of drained effusion ($\rho = 0.07$, $p = 0.80$) or the period of chest tube insertion ($\rho = -0.01$, $p = 0.95$).

DISCUSSION

MPE accumulates in response to an imbalance between the production and absorption of pleural effusion. Excessive production of pleural effusion caused by pleural vascular hyperpermeability is thought to play a major role in MPE formation. Tumor-derived VEGF was first experimentally reported to regulate MPE formation [13]. Recently, many molecules such as IL-6, tumor necrosis factor (TNF)-α, and CCL2 have been proposed as possible promoters of MPE formation [4, 14]. These molecules are released from various cells under tumor-host interactions in the pleural microenvironment. In this study, the levels of six molecules — VEGF, IL-5, IL-6, IL-8, IL-12/IL-23p40, and CCL2 — were significantly higher in the malignant pleural effusion than in serum, suggesting that these molecules were produced locally in the pleural cavity. Previous studies have also reported that VEGF [12], IL-5 [15], IL-6 [16, 17], IL-8 [18], and CCL2 [19] in MPEs were higher than those in serum.

To the best of our knowledge, this is the first report demonstrating the possible production of IL-12/IL-23p40 in MPEs. IL-12/IL-23p40 is a common 40kDa sub-unit shared by IL-12 and IL-23. In our study, the level of IL-12/IL-23p40 in MPE was higher than that in serum. Previous reports demonstrated that IL-12 was barely detectable in MPE [20, 21], suggesting that the levels of IL-12/IL-23p40 in the MPE in our study reflected the levels of IL-23. IL-23 is a pro-inflammatory cytokine that enhances the differentiation of naive T cells to Th17 cells, especially in the presence of IL-1β and IL-6 [17, 22]. Previous reports demonstrated the high levels of IL-1β and increased numbers of Th17 cells in MPE [17, 23, 24]. Th17 cells can be induced from pleural naive $CD4^+$ T cells in the presence of 10 ng/ml of IL-23 [17]. CCL2 also enhances recruitment of Th17 cells, and IL-6 with IL-23 facilitate differentiation of Th17 cells, which secrete IL-17 that promotes angiogenesis and augments IL-6, IL-8, and VEGF release in various types of non-tumor and tumor cells [25, 26]. Thus, CCL2, IL-6, IL-8, IL-12/IL-23p40, and VEGF, high in MPE in our study, interact together to promote Th17 immunity in pleural cavity and MPE formation by enhancing hyperpermeability.

The IL-6 level in MPE correlated with the eosinophil numbers in our study. It has been demonstrated that IL-6 is released by several cell populations in MPE, including cancer cells, macrophages, pleural mesothelial cells [27], and eosinophils [28]. Moreover, eosinophils activated by IL-17 and IL-23 synthesized and secreted IL-6 *in vitro* [29]. In our cases, IL-6 might be secreted from eosinophils in MPE which was Th17 dominant microenvironment. Because there are no reports on the correlation between eosinophils and IL-6 in MPE, further studies on this relationship are needed.

IL-8 levels in MPE were correlated with CCL2 levels in MPE in our study, as previously reported by Yokoyama *et al.* [30]. IL-8 and CCL2 can be co-produced *in vitro* by pleural mesothelial cells stimulated with lipopolysaccharide, TNF-α, and IL-1β [31]. Although a previous study demonstrated that the concentration of IL-8 in the pleural effusion correlated with the number of lymphocytes in the pleural effusion of patients with cancer, tuberculosis, and chronic heart failure [11], our study revealed that pleural IL-8 levels had no correlation with the number of either neutrophils or lymphocytes. Regarding CCL2, it was reported that CCL2 levels correlated with monocytes numbers in MPE [32]. However, we found no correlation between CCL2 levels and the number of

inflammatory or malignant cells. Therefore, we speculated that IL-8 and CCL2 were dominantly produced by mesothelial cells lining the pleural cavity under tumor-host interactions.

We found that the pleural levels of IL-5, but not VEGF, correlated with the number of malignant cells in the pleural effusion. In an eosinophilic pleural effusion with more than 10% eosinophils, there was a correlation between IL-5 levels and the number of eosinophils in the pleural fluid [33]. However, the correlation between IL-5 levels and malignant cell counts in MPEs has not been examined in previous studies. Although there is few report about IL-5 in MPE, it has been reported that IL-5 levels are significantly elevated in MPEs than in transudative effusions caused by chronic heart failure [15]. When human adenocarcinoma cells that did not secrete IL-5 were administered into the pleural cavity of mice, host cell-derived IL-5 enhanced the recruitment of myeloid suppressor cells, enhanced vascular permeability, and promoted MPE [15].

VEGF is known as a vascular permeability factor and is an important mediator of MPE formation. Although Yano *et al*. [13] clarified that VEGF produced by the tumor cells is important for the formation of MPE, we could not find any correlation between VEGF levels and the number of cancer cells in MPEs. VEGF can be secreted from lymphocytes, macrophages, and mesothelial cells, however, the mRNA expression of VEGF detected in T cells, monocytes/macrophages, and tumor cells isolated from MPE was relatively low [34]. It has been reported that pleural mesothelial cells produce VEGF by TGF-β simulation *in vivo* and *in vitro* [35], and our results demonstrate no relationship between pleural VEGF levels and inflammatory or malignant cell counts in MPEs, which indicated that VEGF was mainly produced by pleural mesothelial cells lining the chest cavity under tumor-host interactions.

We could not find any factor that was associated with the total amount of drained effusion or the period of chest tube insertion. From our results, it is considered that not a single molecule, such as VEGF, controls the amount of MPE, but complex interactions among cytokines, chemokines, and growth factors in the thoracic cavity might regulate it.

In conclusion, we have shown the cytokine, chemokine, and growth factor levels in the pleural effusion in patients with MPE due to non-small cell lung cancer. VEGF, IL-5, IL-6, IL-8, IL-12/IL-23p40, and CCL2 were likely to be synthesized in the pleural cavity, but none of these molecules could explain the total amount of effusion. Further studies are required to clarify the regulation of MPE possibly based on the complex interaction of multiple molecules.

ACKNOWLEDGMENTS

The authors thank Yoshinori Okada for his valuable help with cytokine analysis, and we are grateful to the Support Center for Medical Research and Education, Tokai University.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

1) Roberts ME, Neville E, Berrisford RG, Antunes G, Ali NJ, Group BTSPDG. Management of a malignant pleural effusion: British Thoracic Society Pleural Disease Guideline 2010. Thorax 2010; 65 Suppl 2: ii32–40.
2) Thomas R, Francis R, Davies HE, Lee YC. Interventional therapies for malignant pleural effusions: the present and the future. Respirology 2014; 19(6): 809–22.
3) Feller-Kopman DJ, Reddy CB, DeCamp MM, Diekemper RL, Gould MK, Henry T, *et al*. Management of Malignant Pleural Effusions. An Official ATS/STS/STR Clinical Practice Guideline. Am J Respir Crit Care Med 2018; 198(7): 839–49.
4) Spella M, Giannou AD, Stathopoulos GT. Switching off malignant pleural effusion formation-fantasy or future? J Thorac Dis 2015; 7(6): 1009–20.
5) 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(3): 207–16.
6) Fafliora E, Hatzoglou C, Gourgoulianis KI, Zarogiannis SG. Systematic review and meta-analysis of vascular endothelial growth factor as a biomarker for malignant pleural effusions. Physiol Rep 2016; 4(24): e12978.
7) Yanagawa H, Takeuchi E, Suzuki Y, Ohmoto Y, Bando H, Sone S. Vascular endothelial growth factor in malignant pleural effusion associated with lung cancer. Cancer Immunol Immunother 1999; 48: 396–400.
8) Thickett DR, Armstrong L, Millar AB. Vascular endothelial growth factor (VEGF) in inflammatory and malignant pleural effusions. Thorax 1999; 54: 707–10.
9) Stathopoulos GT, Psallidas I, Moustaki A, Moschos C, Kollintza A, Karabela S, *et al*. A central role for tumor-derived monocyte chemoattractant protein-1 in malignant pleural effusion. J Natl Cancer Inst 2008; 100(20): 1464–76.
10) Qian Q, Sun WK, Zhan P, Zhang Y, Song Y, Yu LK. Role of monocyte chemoattractant protein-1, tumor necrosis factor-alpha and interleukin-6 in the control of malignant pleural effusion and survival in patients with primary lung adenocarcinoma. Int J Biol Markers 2012; 27(2): e118–24.
11) Pac E, Gjomarkaj E, Melis M, Profita M, Spatafora M, Vignola AM, *et al*. Interleukin-8 Induces Lymphocyte Chemotaxis into the Pleural Space Role of Pleural Macrophages. Am J Respir Crit Care Med 1999; 159: 1592–99.
12) Hsu IL, Su WC, Yan JJ, Chang JM, Lai WW. Angiogenetic biomarkers in non-small cell lung cancer with malignant pleural effusion: correlations with patient survival and pleural effusion control. Lung Cancer 2009; 65(3): 371–76.
13) Yano S, Shinohara H, Herbst RS, Kuniyasu H, Bucana CD, Ellis LM, *et al*. Production of experimental malignant pleural effusions is dependent on invasion of the pleura and expression of vascular endothelial growth factor/vascular permeability factor by human lung cancer cells. Am J Patho 2000; 157(6): 1893–03.
14) Stathopoulos GT, Kalomenidis I. Malignant pleural effusion: tumor-host interactions unleashed. Am J Respir Crit Care Med 2012; 186(6): 487–92.
15) Stathopoulos GT, Sherrill TP, Karabela SP, Goleniewska K, Kalomenidis I, Roussos C, *et al*. Host-derived interleukin-5 promotes adenocarcinoma-induced malignant pleural effusion. Am J Respir Crit Care Med 2010; 182(10): 1273–81.
16) Alexandrakis MG, Coulocheri SA, Bouros D, Mandalaki K, Karkavitsas N, Eliopoulos GD. Evaluation of inflammatory cytokines in malignant and benign pleural effusions. Oncol Rep 2000; 7: 1327–59.
17) Ye ZJ, Zhou Q, Gu YY, Qin SM, Ma WL, Xin JB, *et al*. Generation and differentiation of IL-17-producing CD4+ T cells in malignant pleural effusion. J Immunol 2010; 185(10): 6348–54.
18) Kotyza J: Interleukin-8 (CXCL8) in tumor associated non-vascular extracellular fluids: its diagnostic and prognostic values. A review. Int J Biol Markers 2012; 27(3): 169–78.
19) Trzaska-Sobczak M, Pierzchała W, Brożek G, Farnik M. Role of C-C chemokines in the determination of pleural effusion etiology. Pol Pneumonol Allergol 2008; 76: 415–20.
20) Chen Y-M, Yang W-K, Whang-Peng J, Kuo BI-T, Pemg R-P.

Elevation of interleukin-10 levels in malignant pleural effusion. Chest 1996; 110: 433–36.
21) Wu DW, Chang WA, Liu KT, Yen MC, Kuo PL. Vascular endothelial growth factor and protein level in pleural effusion for differentiating malignant from benign pleural effusion. Oncol Lett 2017; 14(3): 3657–62.
22) Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, *et al*. Development, cytokine profile and function of human interleukin 17-producing helper T cells. Nat Immunol 2007; 8(9): 950–57.
23) Shimokata K, Saka H, Murate T, Hasegawa Y, Hasegawa T. Cytokine content in pleural effusion. Chest 1991; 99(5): 1103–07.
24) Ye ZJ, Zhou Q, Yin W, Yuan ML, Yang WB, Xiong XZ, *et al*. Differentiation and immune regulation of IL-9-producing CD4 + T cells in malignant pleural effusion. Am J Respir Crit Care Med 2012; 186(11): 1168–79.
25) Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. Nat Rev Immunol 2008; 8(5): 337–48.
26) Huang Q, Duan L, Qian X, Fan J, Lv Z, Zhang X, *et al*. IL-17 promotes angiogenic factors IL-6, IL-8, and vegf production via Stat1 in lung adenocarcinoma. Sci Rep 2016; 6: 36551.
27) Yamaguchi T, Kimura H, Yokota S, Yamamoto Y, Hashimoto T, Nakagawa M, *et al*. Effect of IL-6 elevation in malignant pleural effusion on hyperfibrinogenemia in lung cancer patients. Jpn J Clin Oncol 2000; 30(2): 53–8.
28) Hamid BQ, Barkans J, Meng Q, Ying S, Abrams JS, Kay AB, *et al*. Human eosinophils synthesize and secrete interleukin-6, in vitro. Blood 1992; 80(6): 1496–1501.
29) Cheung PF, Wong CK, Lam CW. Molecular mechanisms of cytokine and chemokine release from eosinophils activated by IL-17A, IL-17F, and IL-23 : implication for Th17 lymphocytes-mediated allergic inflammation. J Immunol 2008 ; 180(8): 5625–35.
30) Yokoyama A, Kohno N, Ito M, Abe M, Hiwada K, Yamada H, *et al*. Eotaxin levels in pleural effusions: comparison with monocyte chemoattaractant protein-1 and IL-8. Intern Med 2000; 39(7): 547–52.
31) Antony VB, Hott JW, Kunkel SL, Godbey SW, Burdick MD, Strieter RM. 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(6): 581–8.
32) Antony VB, Godbey SW, Kunkel SL, Hott JW, Hartman DL, Burdick MD, *et al*. 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(12): 7216–23.
33) Mohamed KH, Abdelhamid AI, Lee YCG, Lane KB, Conner B, Hawthorne M, *et al*. Pleural fluid levels of interleukin-5 and eosinophils are closely correlated. Chest 2002; 122(2): 576–80.
34) Atanackovic D, Cao Y, Kim JW, Brandl S, Thom I, Faltz C, *et al*. The local cytokine and chemokine milieu within malignant effusions. Tumour Biol 2008; 29(2): 93–104.
35) Lee YCG, Melkerneker D, Thompson PJ, Light RW, Lane KB. Transforming growth factor beta induces vascular endothelial growth factor elaboration from pleural mesothelial cells in vivo and in vitro. Am J Respir Crit Care Med 2002; 165(1): 88–94.