

The concentration of Interleukin-27 in the pleural fluid of patients with exudative pleural effusion and its diagnostic value in differentiating between benign and malignant pleural effusion

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ABSTRACT

Introduction: The excessive accumulation of pleural fluid in the pleural cavity results in pleural effusion, which is either exudative or transudative. Effective treatment of pleural effusion calls for the differential diagnosis of benign pleural effusion (BPE) and malignant pleural effusion (MPE). This study objective was to measure the concentration of IL-27 and its diagnostic value in differentiating BPE from MPE in pleural fluid of patients with exudative pleural effusion.

Materials and Methods: Samples were obtained from 130 patients with exudative pleural effusion. The concentration of IL-27 in the pleural fluid was measured using ELISA. Statistical analysis was carried out using descriptive statistics tests and independent t-test in SPSS 19. The significance level in all calculations was set to 0.05. The ROC

curve analysis was carried out to determine the sensitivity of IL-27 to diagnose benign pleural effusion.

Findings: Of the 130 patients included in this research, 88 were MPE and 42 were BPE. The average age of the MPE and BPE groups was 57 and 59 years, respectively. The average IL-27 concentration in BPE group (344.15 ± 236.42) was significantly higher than MPE group (203.05 ± 76.03) ($P=0.000$). The area under the ROC curve was 0.803, which reflected the ability of IL-27 measurement to differentiate between BPE and MPE.

Conclusion: Given the significant difference between the level of IL-27 in the two study groups, the measurement of this biomarker in exudative pleural effusion cases can differentiate between BPE and MPE with good sensitivity and specificity.

Keywords: IL-27, Malignant Pleural Effusion, Pleural Fluid, Benign Pleural Effusion, Pleural Effusion

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INTRODUCTION

Pleural effusion refers to the excessive accumulation of fluid following a lack of balance in production and excretion of the pleural fluid in the pleural cavity. Although this condition is not considered a disease, it can reflect a pathologic disorder. Since various factors such as pulmonary problems and systemic disorders are involved in the outbreak of this disorder, the effective treatment of pleural effusion calls for the diagnosis of its causes. Each year over 1.5 million people develop pleural effusion in the United States.^{1,2} Pleural effusion has become a medical challenge because it is caused by various diseases and factors. The first step in treating a patient with pleural effusion is to differ between benign pleural effusion (BPE) and malignant pleural effusion (MPE). However, before taking this step it is necessary to determine whether the pleural effusion is exudative or transudative. Some of the causes of transudative pleural effusion include heart failure, hypoproteinemia, and atelectasis. On the other hand, exudative pleural effusion which needs

more case and examination than the transudative type, may be caused by inflammatory processes or malignancies that increase capillary permeability or impair lymph drainage. Some of the non-invasive methods to determine the benignancy or malignancy of pleural effusion are a computed tomography (CT) and positron emission tomography (PET).^{3,4} There is no need to perform invasive diagnostic methods to the pleural tissue in patients with transudative pleural effusion. However, if the patient is diagnosed with exudative pleural effusion, additional and more invasive examinations such as closed pleural biopsy and thoracoscopy are required for a more accurate diagnosis of the background causes. Most MPE cases (90-97%) are exudative, which are caused by damages to the pleural membrane.⁵ The progression of MPE is accompanied with extremely poor prognosis, and thus life expectancy varies from 4 months to less than one year in these patients.⁶ The most common malignancies in men and women with malignant

pleural effusion are lung and breast cancer.⁷ From the medical perspective, it is substantially important to diagnose malignancies as soon as possible using the most reliable methods.^{8,9} Differentiating between BPE and MPE is still debatable. Moreover, the cytological analysis of the pleural fluid is the most common method to prove malignancy and the use of invasive methods are necessary to obtain the sample required for the analysis.^{10,11} Although the specificity of the cytological findings is 100%, only about 60% of malignant pleural effusions are diagnosed with this method.¹² It is more necessary to use invasive methods to diagnose exudative pleural effusions with negative cytology that are suspected with malignancy.¹¹ Closed pleural biopsy and thoracoscopy are among the preferable invasive methods for this purpose, but they are not available in all centers.^{11,13-15} Given the consequences of the invasive methods, noninvasive diagnostic tests capable of accurately differentiating BPE from MPE are highly necessary. Therefore, numerous studies have examined the potential of biomarkers to improve the diagnosis of MPE. However, none of the studied biomarkers offers the adequate sensitivity and specificity required for the diagnosis of MPE.^{10,16,17} Research results have revealed that interleukin-27 (IL-27), as a member of the Interleukin-12 family, is involved in the malignancy and different types of infections such as tuberculosis, which is a common cause of exudative pleural effusion along with cancer.¹⁸ Apparently, IL-27 plays a role in the pathogenesis of pleural effusion, and this molecule could also be used as a potential marker for the diagnosis of malignant pleural effusion. One study revealed that IL-27 differentiates tuberculous pleural effusion (TPE) from MPE with a sensitivity and specificity of 92.7% and 98.4%, respectively.¹⁹ Given the importance of differentiating between BPE and MPE in the early diagnosis and treatment of patients and the necessity of the existence of a satisfactory diagnostic marker to attain this goal, this research was carried out with the aim of measuring the concentration of IL-27 in the pleural fluid of patients with exudative pleural effusion and assessing its diagnostic value in differentiating between BPE and MPE.

MATERIALS AND METHODS

A total of 130 patients with exudative pleural effusion who visited Shahid Sayad Shiraz and 5 Azar hospitals of Gorgan City from 2015 to 2016 were selected using the simple convenience sampling method after their informed consent was obtained. Prior to the research, common diagnostic procedures such as radiography, CT scan, and

ultrasonography were taken for all of the patients. The research inclusion criteria were the existence of exudative pleural effusion, and the exclusion criteria were transudative pleural effusion, diabetes mellitus, self-immune diseases, and rheumatologic diseases. To analyze the pleural fluid, thoracocentesis was performed after obtaining the patient's information and conducting precise examinations. Therefore, 5 mL of the pleural fluid of each patient was collected. The samples were stored at -20°C for further examinations following 15 minutes centrifugation. To differentiate the exudative pleural effusion from the transudative type, the biochemical analysis of the samples includes measurement of pH, glucose level, and protein level. Patients with transudative pleural effusion were excluded from the research. Using the VATS (video-assisted thoracoscopic surgery) technique and the results of the microbiological, pathological, and cytological examinations, the patients with exudative pleural effusion were divided into the groups of malignant pleural effusion (MPE) and benign pleural effusion (BPE) by etiology. The IL-27 levels were measured using ELISA method and the Padtan Zist Pajooh commercial kit in accordance with the manufacturer's guidelines. An analysis was conducted to determine the sensitivity of IL-27 in diagnosing benignity. Statistical data analysis was carried out using descriptive statistics tests, Kolmogorov-Smirnov test, Mann-Whitney test, and the independent t-test in SPSS 19. The significance level in all calculations was set to 0.05.

RESULTS

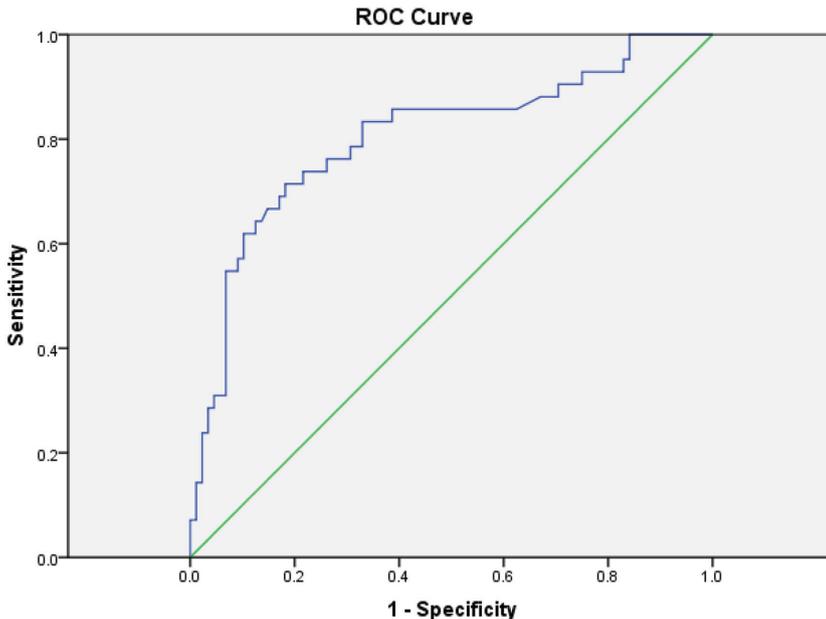
Of the 130 patients included in this research, 88 (67.7%) patients were put in the MPE group as the experimental group, of whom 44 were male, and 44 were female (50%). A total of 42 patients (32.3%) including 26 men (61.9%) and 16 women (38.1%) were put in the BPE group as the control group. The average age of the patients of the MPE and BPE groups was 57 and 59 years, respectively. A total of 62 patients (47.7%) were smokers, and 68 patients (52.3%) were non-smokers. Concerning the distribution of the smokers in the two groups, 41 patients (46.6%) in the MPE group and 21 (53.4%) in the BPE group had a history of smoking. [Table \(1\)](#) shows the frequency of the causes of exudative pleural effusion. Moreover, 66 patients (50.8%) had a history of malignancy as presented in [table \(2\)](#). The average IL-27 level in the pleural fluid of patients in the MPE and BPE groups was 203.05 ± 76.03 and 344.15 ± 236.42 , respectively. The difference between the IL-27 levels was significant ($P=0.000$). The average IL-27 level in patients with and without

Table 1 Extreme comparison of causes of exudative pleural effusion

Rank	Disease	Frequency	%
	Esophageal cancer	5	3.8
	Breast cancer	31	23.8
	Metastatic carcinoma	5	3.8
	Gastric cancer	13	10.0
	Lung cancer	22	16.9
	TB	42	32.3
	Ovarian cancer	7	5.4
	Colon cancer	5	3.8

Table 2 Comparison of the frequency of secondary cancers in patients with previous history of malignancy

Rank	Disease	Frequency	%
	Esophageal cancer	5	7.6
	Breast cancer	31	47.0
	Metastatic carcinoma	5	7.6
	Gastric cancer	13	19.7
	Ovarian cancer	7	10.6
	Colon cancer	5	7.6

**Figure 1** Row chart for Interleukin 27

a history of malignancy was 202.9 ± 36.6 and 321 ± 75.5 , respectively. This difference was also statistically significant ($P=0.001$). The comparison between mean IL-27 levels in the smokers (280.76 ± 80.15) and nonsmokers (250.78 ± 19.59) revealed no significant difference ($P=0.35$). As for gender, the average IL-27 level in the male and female patients was 256.719 ± 36.43 and 274.838 ± 76.42 ,

respectively. This difference was not statistically significant ($P=0.33$). In this research, pleural fluid protein level was also measured in the patients. The results indicated that the average concentration of protein in the pleural fluids of patients of the MPE and BPE groups was 4373.8 ± 419.3 and 4411.7 ± 493.0 mg/dl, respectively. The correlation between IL-27 concentration and pleural fluid protein concentration was also analyzed, but it was not statistically significant ($r=0.15$). There was also no statistically significant relationship between patient age and IL-277 level ($r=1.00$). Figure (1) depicts the ROC analysis performed to determine the sensitivity of IL-27 in the diagnosis of benignancy. The area under the curve is equal to 0.803 (with 95% CI=0.716-0.890), which reflects the differentiation power of this test. In this curve, sensitivity shows the ability to diagnose a benign disorder accurately and specificity shows the potential for accurately diagnosing a malignant disorder.

DISCUSSION AND CONCLUSION

The presence of neoplastic cells in the pleural fluid indicated malignant pleural effusion (MPE). It is estimated that approximately 40,000 people in the UK develop MPE annually.²⁰ However, the incidence rate for the United States is over 1.5 million people a year.² It is estimated that about 50% of patients with metastatic malignancies contract MPE and lack proper prognosis.²⁰ Diagnosing the cause of pleural effusion is necessary for its effective treatment. Hence, experimental investigations help determine whether the condition is exudative or transudative. The treatment of exudative pleural effusion completely depends on its causes.² If the patient is suffering exudative pleural effusion, invasive experiments may be needed for more accurate diagnosis findings. This diagnosis is important because most MPE cases of exudative pleural effusion are lack of satisfactory prognosis, and require accurate early diagnosis.^{5,21} On the other hand, although cytologic analysis of the pleural fluid is the method commonly used to prove malignancy, this method fails to accurately diagnose MPE in 40% of the cases.^{10,11} Since differentiating MPE from BPE has remained a debatable diagnostic challenge, the identification and discovery of biomarkers that are accessible and offer satisfactory sensitivity and specificity are necessary. IL-27, which is produced in response to microbial contamination, is a heterodimeric cytokine. The results of several studies have unveiled the substantial role of IL-27 in regulating the performance of human macrophages in infections.²² This cytokine is involved in various

immunity diseases because of its dual pro-inflammatory and anti-inflammatory effects on immunity responses.¹⁹ In this research, the diagnostic value of the concentration of IL-27 in the pleural fluid was assessed in 130 patients with exudative pleural effusion to differentiate between MPE and BPE. The patients were examined by age, gender, family history of cancer, smoking history, and the level of the pleural fluid protein. The main findings from this research showed a statistically significant difference between the concentrations of IL-27 in the MPE and BPE groups ($P=0.02$), with higher IL-27 concentration in the BPE patients. To our knowledge, no study has examined the difference between the concentrations of IL-27 in these two groups and the diagnostic value of IL-27 in differentiating MPE from BPE. Yang et al. (2012) examined the concentration of IL-27 in the pleural fluid of patients with tuberculous pleural effusion, MPE, infectious pleural effusion, and transudative pleural effusion. Their findings revealed the higher concentration of IL-27 in the TPE group as compared to other groups. Hence, IL-27 was introduced as a suitable diagnostic biomarker that could differentiate TPE from non-tuberculous pleural effusion with a cut-off point, sensitivity, and specificity of 1.007ng/L, 92.7%, and 99.1%, respectively.¹⁸ Nevertheless, in the study by Valdes et al. (2014) IL-27 was less effective than ADA (adenosine deaminase) in diagnosing TPE. However, the use of ADA with IL-27 increased sensitivity.²² A study reported by Liu et al. (2016) indicated the higher diagnostic effectiveness of IL-27 in differentiating TPE from non-TPE pleural effusion as compared to interferony (IFN- γ) or ADA. Furthermore, if IL-27 is used with ADA or IFN- γ , the accuracy of the differential diagnosis escalates.¹⁹ Xu et al. (2014) assessed the prognostic and diagnostic values of IL-17 in MPE. Their findings suggested that the IL-17 levels in MPE were significantly higher than nonmalignant effusion and TPE. Therefore, the researchers stated that IL-17 can be a suitable tumor marker for diagnosing MPE.²³ Concerning IL-17, the findings from a study by Klimatsidas et al. (2012) showed high levels of serum and pleural fluid IL-17 in MPE patients.²⁴ Results of the present research indicated that variables such as age, gender, smoking, cancer history, and pleural fluid protein levels do not significantly affect the IL-27 level. However, the significant difference between the levels of IL-27 in the two study groups reflects the potential of IL-27 for differentiating between BPE and MPE, but further research is required to achieve this goal.

AUTHORS CONTRIBUTION

Mohammad Reza Hashempour, Ali Aryannia, Mahshid Mehrjerdian and Seyyed Sadegh Baniaghil collected the data and analyzed it. Arash Rezaie wrote a primary draft, and Reza Alipoor revised it. After that Mohammad Reza Hashempour submitted the manuscript.

CONFLICTS OF INTEREST

This study had no conflict of interest for the authors.

REFERENCES

1. Karkhanis VS, Joshi JM. Pleural effusion: diagnosis, treatment, and management. *Open access emergency medicine: OAEM.* 2012;4:31.
2. Saguil A, Wyrick K, Hallgren J. Diagnostic approach to pleural effusion. *Am Fam Physician.* 2014;90(2):99-104.
3. Sun Y, Yu H, Ma J, Lu P. The role of 18F-FDG PET/CT integrated imaging in distinguishing malignant from benign pleural effusion. *PloS one.* 2016;11(8):e0161764.
4. Na MJ. Diagnostic tools of pleural effusion. *Tuberculosis and respiratory diseases.* 2014;76(5):199-210.
5. Heffner JE. Diagnosis and management of malignant pleural effusions. *Respirology.* 2008;13(1):5-20.
6. Burrows CM, Mathews WC, Colt HG. Predicting survival in patients with recurrent symptomatic malignant pleural effusions: an assessment of the prognostic values of physiologic, morphologic, and quality of life measures of extent of disease. *CHEST Journal.* 2000;117(1):73-8.
7. Khaleeq G, Musani AI. Emerging paradigms in the management of malignant pleural effusions. *Respiratory medicine.* 2008;102(7):939-48.
8. Fishman AP, Elias JA. *Fishman's pulmonary diseases and disorders:* McGraw-Hill Medical;1988;2:1411-38.
9. Frey D. *Harrison's principles of internal medicine.* LWW; 2002; 2:1513-15.
10. Hooper C, Lee YG, Maskell N. Investigation of a unilateral pleural effusion in adults: British Thoracic Society pleural disease guideline 2010. *Thorax.* 2010;65(Suppl 2):ii4-ii17.
11. Light RW. The undiagnosed pleural effusion. *Clinics in chest medicine.* 2006;27(2):309-19.
12. Alemán C, Sanchez L, Alegre J, Ruiz E, Vázquez A, Soriano T, et al. Differentiating between malignant and idiopathic pleural effusions: the value of diagnostic procedures. *Journal of the Association of Physicians.* 2007;100(6):351-9.
13. Roberts ME, Neville E, Berrisford RG, Antunes G, Ali NJ. Management of a malignant pleural effusion: British Thoracic Society pleural disease guideline 2010. *Thorax.* 2010;65(Suppl 2):ii32-ii40.
14. Neragi-Miandoab S. Malignant pleural effusion, current and evolving approaches for its diagnosis and management. *Lung cancer.* 2006;54(1):1-9.
15. Wang X-F, Wu Y-H, Jiao J, Guan C-P, Yang X-G, Wang M-S. Diagnostic value of superoxide dismutase in tuberculous and malignant pleural effusions. *Asian Pacific Journal of Cancer Prevention.* 2013;14(2):821-4.
16. Botana-Rial M, Casado-Rey P, Leiro-Fernandez V, Andrade-Olivie M, Represas-Represas C, Fernandez-Villar A. Validity of procalcitonin and C-reactive protein measurement when differentiating between benign and malignant pleural effusion. *Clinical laboratory.* 2010;57(5-6):373-8.
17. Jin D, Chen Y, Wang Z, Wang S, Bunjhoo H, Zhu J, et al. Diagnostic value of interleukin 22 and carcinoembryonic

- antigen in tuberculous and malignant pleural effusions. *Experimental and therapeutic medicine*. 2011;2(6):1205-9.
18. Yang W-B, Liang Q-L, Ye Z-J, Niu C-M, Ma W-L, Xiong X-Z, et al. Cell origins and diagnostic accuracy of interleukin 27 in pleural effusions. *PLoS One*. 2012;7(7):e40450.
 19. Liu Y-L, Wu Y-B, Zhai K, Wang X-J, Shi H-Z. Determination of Interleukin 27-Producing CD4+ and CD8+ T Cells for The Differentiation Between Tuberculous and Malignant Pleural Effusions. *Scientific reports*. 2016;6:19424.
 20. Egan A, McPhillips D, Sarkar S, Breen D. Malignant pleural effusion. *QJM: An International Journal of Medicine*. 2014;107(3):179-84.
 21. Colt H. Drainage and biopsy techniques. *Textbook of Pleural Diseases* Arnold, London. 2003:481-98.
 22. Valdés L, San José E, Ferreiro L, Golpe A, Gude F, Álvarez-Dobaño JM, et al. Interleukin 27 could be useful in the diagnosis of tuberculous pleural effusions. *Respiratory care*. 2014;59(3):399-405.
 23. Xu C, Yu L, Zhan P, Zhang Y. Elevated pleural effusion IL-17 is a diagnostic marker and outcome predictor in lung cancer patients. *European journal of medical research*. 2014;19(1):23.
 24. Klimatsidas M, Anastasiadis K, Foroulis C, Tossios P, Bisiklis A, Papakonstantinou C, et al. Elevated levels of anti-inflammatory IL-10 and pro-inflammatory IL-17 in malignant pleural effusions. *Journal of cardiothoracic surgery*. 2012;7(1):104.



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