

Diagnostic Accuracy of Pleural Fluid Soluble Interleukin 2 Receptor in Patients with Tuberculous Pleural Effusion

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Article information	Abstract
<p>Article history: Received: 8 Dec 2012 Accepted: 26 Dec 2013 Available online: 12 Mar 2013 ZJRMS 2014; 16 (4): 19-23</p> <p>Keywords: Pleural effusion IL 2 Receptor Tuberculosis Cytokines</p> <p>*Corresponding author at: Department of Ophthalmology, Ophthalmology Research Center, Rasoul Akram hospital, Tehran University of Medical Sciences, Tehran, Iran. E-mail: kourosh.shahyar@gmail.com</p>	<p>Background: Pleural tuberculosis occurs in 4% of newly diagnosed cases of tuberculosis. T-cells have an important role on the immunity against mycobacterial infections and as a result, the level of soluble interleukin 2 receptors (SIL-2R) as a marker of T-cell activation is elevated in patients with tuberculous pleural effusion.</p> <p>Materials and Methods: In this cross sectional study, the diagnostic accuracy of SIL-2R level was assessed in separating tuberculous from non-tuberculous effusions in Zahedan, Iran. From 112 patients fulfilled entrance criteria for exudative pleural effusion, 88 patients were included and underwent diagnostic procedures to identify the origin of pleural effusion. The SIL-2R was evaluated at various cut-off levels by nonparametric receiver operating characteristic (ROC) curve, and values affording greatest diagnostic accuracy were selected.</p> <p>Results: SIL-2R level in TB group was 9147 ± 3573 while this level in non-TB group was 2724 ± 1326 and the difference was statistically significant ($p=0.001$). The cut-off point in our study was 4200 U/ml and the area under curve was 0.930 with 95% CI: 0.881–0.979 ($p=0.001$). The sensitivity and specificity for this level is 86 and 89%.</p> <p>Conclusion: Several factors lead to the variation in the level and cut-off point of SIL-2R in different regions. Our cut-off point was lower than other studies. The level of SIL-2R in patients with tuberculosis is significantly higher than parapneumonic effusions. We suggest that measuring the SIL-2R level in pleural fluid of tuberculous patients is a useful diagnostic tool in diagnosing tuberculous pleural effusion.</p> <p>Copyright © 2014 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It typically affects the lungs (pulmonary TB) but can affect other sites as well (extrapulmonary TB) [1]. TB is the second leading cause of death from an infectious disease worldwide (after HIV, which caused an estimated 1.8 million deaths in 2008) [1]. Despite the availability of highly efficacious treatment for decades, TB remains a major global health problem [2].

Pleural effusion in patients with tuberculosis is caused by infection of pleura or is associated with pulmonary tuberculosis [3]. Pleural tuberculosis occurs in 4% of newly diagnosed cases of tuberculosis and its frequency differs among countries [3, 4]. The HIV (human immunodeficiency virus) infection is associated with doubling of pleural tuberculosis incidence [5]. The diagnosis of pleural tuberculosis infection is based on demonstration of *Mycobacteria* in sputum, pleural biopsy specimen or pleural fluid [3, 5]. The most sensitive diagnostic test is biopsy of pleura with thoracoscope. Granulomatous inflammation, caseous necrosis and/or acid-fast bacilli may be present in the histological findings of pleural biopsy [5].

Tuberculous pleural effusion (TPE) is resulted from a cell mediated delayed-type hypersensitivity reaction and T-cells have an important role on the immunity against mycobacterial infections [6]. As a result of T-cell activation, soluble interleukin 2 receptors (SIL-2R) are released in the blood and subsequently an elevated level of SIL-2R provides a good marker of ongoing cell activation. Elevated serum levels of SIL-2R increasingly have been found in patients with adult T-cell leukemia, Hodgkins disease, Sezary syndrome, acute and chronic lymphocytic leukemia and acquired immunodeficiency syndrome [7]. Several studies show elevation of other biomarkers of T-cell activation in pleural fluid of patients with pleural tuberculosis. The elevated level of interferon gamma (IFN- γ) and adenosine deaminase (ADA) is reported in different studies [8, 9]. Hiraki et al. showed that IFN- γ has the most sensitivity among these markers [9]. Some studies have demonstrated high level of SIL-2R in TPE but the cut of points vary due to the profile of the center where the study was conducted, the local epidemiological situation and the difference in diagnostic procedures [8]. According to high prevalence of tuberculous infection in our country, it is important to

identify the diagnostic level of SIL-2 in Iran. In the present study, therefore, we aimed to determine if pleural fluid levels of soluble IL-2R were elevated in patients with tuberculous pleurisy, compared with patients with pleural effusions of nontuberculous etiologies.

Materials and Methods

This cross sectional study was designed to assess diagnostic accuracy of SIL-2R level in separating TB from non TB effusions. Over a 16 months period, 224 patients with pleural effusion were selected from internal medicine, infectious diseases, cardiology, surgery, and pediatrics wards of -AliEbn-e-Abitaleb, Boo Ali and Khatam-Al-Anbia hospitals in Zahedan, Iran.

Patients underwent history taking and physical examination. Then posteroanterior and lateral decubitus chest X-ray were performed. Patients who had pleural effusion in posteroanterior chest X-ray or more than 1 centimeter pleural effusion in lateral decubitus X-ray and who had not congestive heart failure underwent diagnostic thoracentesis (pleural fluid tap). Diagnostic thoracentesis was performed in patients who had congestive heart failure and fever, chest pain or one-sided pleural effusion simultaneously. With a written informed consent, thoracentesis was performed in posterior axillary line, two intercostals space beneath scapula with a 50 cm³ heparinized syringe. Pleural fluid was assayed for cell count and cell differentiation, protein level, LDH, gram staining, smear, BK culture and cytology.

Laboratory examination: Pleural fluid samples were collected in dried tubes and transferred to the laboratory immediately. Samples were centrifuged with 500 rpm for 15 minutes and froze at -80°C. Biochemical tests of pleural fluid were assessed. Protein and LDH levels of serum were measured simultaneously. Patients divided to transudative or exudative pleural effusion according to Light's criteria. Exudative pleural effusion defines by three criteria: 1) pleural fluid protein level to serum protein level more than 0.5, 2) pleural fluid lactate dehydrogenase level to serum lactate dehydrogenase level more than 0.6, 3) pleural fluid LDH level to serum LDH level more than 2.3 of normal laboratory upper limit.

Second clinical assessment: Patients with exudative pleural effusion underwent more diagnostic procedures to identify the origin of their pleural effusion. Some of these procedures are listed below: pleural biopsy, PCR assessment of pleural fluid, measuring rheumatologic factors like RF, Anti dsDNA and ANA with suspicion to collagen vascular diseases, evaluation of anti-TB treatment in patients with clinical suspicion to tuberculosis, spiral CT or ventilation scan in suspicion to pulmonary emboli, bronchoscopy and bronchoalveolar lavage (BAL).

Diagnosis of tuberculous, malignant, and miscellaneous pleural effusions: Tuberculous effusions were diagnosed when any of the following criteria were met: 1) finding tuberculous bacilli in the pleural fluid or pleural biopsy, 2) finding tuberculous bacilli in the culture of pleural fluid or pleural biopsy, 3) finding tuberculous granulomas in the pleural biopsy, 4) positive response of

pleural fluid PCR for tuberculosis, 5) positive response to anti-TB treatment in patients with clinical and radiologic findings of tuberculous pleural effusion which is not approved with diagnostic tests, 6) positive sputum culture for tuberculosis. Malignant pleural effusion was diagnosed either with pleural fluid cytology or biopsy specimen histology. Parapneumonic pleural effusion was defined by presence of pleural effusion in an acute febrile disease with pneumonia or lung abscess in the absence of tuberculosis or malignancy which responded to antibiotic therapy. Empyema was diagnosed with purulent pleural fluid or isolation of bacterial specimen in parapneumonic effusion. A spiral CT scan was assessed for diagnosis of pulmonary emboli.

Measurement of SIL-2R level: Samples were collected in tubes containing ethylene-diamine-tetra-acetic acid disodium (EDTA) with maximum concentration of 3mmol/L and were centrifuged at 3 degree centigrade at 500 rpm for 15 minutes. The level of SIL-2R was measured on the basis of kit instructions.

Classification: Patients were excluded from this study by following criteria: 1) non-Iranians, 2) with transudative pleural effusion, 3) with hemothorax, 4) diagnosis was not revealed despite performing diagnostic procedures. Patients were classified in two groups, TB group and non-TB group. Non-TB groups include patients with parapneumonic effusion or empyema.

Analytical methods: For descriptive variables mean, standard deviation and percent were calculated. Statistical techniques were used to calculate sensitivity, specificity, negative and positive predicted value. We used independent samples *t*-test to compare differences between TB and non-TB group parameters. The utility of SIL-2R as a diagnostic tool for tuberculosis was evaluated at various cut-off levels by nonparametric receiver operating characteristic (ROC) curve, and values affording greatest diagnostic accuracy were selected. $p < 0.05$ was considered significant. All parameters calculated with 95% confidence interval using SPSS-18 software.

Results

Two hundred and twenty four patients with pleural effusion in a 16-month period were studied. One hundred and two patients were excluded from our study because they were not Iranian or had transudative effusion or final diagnosis was not ascertained. From 112 patients fulfilled entrance criteria for exudative pleural effusion, 24 patients were excluded; 14 patients with malignancy (12.5%), 3 patients with pulmonary emboli (2.6%), 2 patients with subdiaphragmatic abscess (1.7%) and 5 patients with collagen vascular disease (4.4%). There were 30 patients in TB group (34%) and 58 patients in non-TB group (66%). Non-TB or bacterial group included 48 patients with parapneumonic effusion (42.8%) and 10 patients with empyema (9%). There were 21 men and 9 women with the mean age of 44±2.7 in TB group and 41 men and 14 women with the mean age of 49±3.5 in non-TB group. As it is shown in table1, protein levels, LDH

levels, cell count and cell differentiation has been compared in TB and non-TB groups. There was no significant difference in protein levels, LDH levels and white blood cell counts but there was significant difference in lymphocyte count and percentile between two groups ($87\pm 16\%$ in TB group and $54\pm 27\%$ in non-TB group, $p<0.001$). SIL-2R level in TB group was 9147 ± 3573 while this level in non-TB group was 2724 ± 1326 and the difference was statistically significant ($p<0.001$).

Decision thresholds derived from ROC curves, in figure1, shows that tuberculous effusions are associated with pleural fluid SIL-2R values greater than 4200 U/ml. The area under curve (AUC) calculated with the ROC curve on the basis of ROC analysis (AUC=0.930; CI 95%: 0.881-0.979, $p=0.001$). The discriminatory properties of this cut-off level in separating tuberculous from non-tuberculous effusions were as follows: sensitivity 86%, specificity 89%, positive predicted value 81%, negative predicted value 92%, positive likelihood ratio 7.81 and negative likelihood ratio 0.15%.

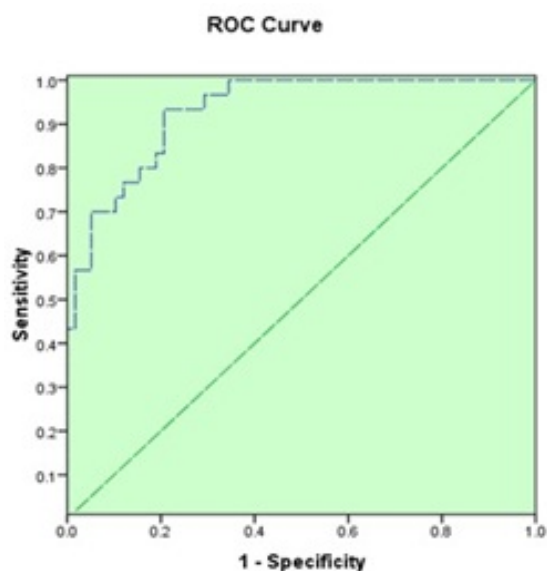


Figure1. ROC curve showing sensitivity and 1-specificity values at various cut-off points for SIL-2R levels in pleural fluid. (SIL-2R: AUC=0.930; 95% CI: 0.881-0.979; $p<0.001$)

Discussion

It is clear that T-lymphocytes are essential in the immunity against tuberculosis [6, 10, 11]. Activation of

T-lymphocytes is caused by interaction of Mycobacteria with alveolar macrophages. Interleukin-2 is a lymphokine which is essential to the proliferation of antigen-stimulated T-cells. As a result IL-2R molecules are expressed on their cell surface [12] and SIL-2R molecules are released into the circulation [13]. As this soluble receptor retains some of the biological activities of the cell-associated IL-2R molecule, including its capacity to bind IL-2 efficiently [14], it is possible that SIL-2R could play a regulatory role in the immune response. Elevated level of SIL-2R is not specific for TPE, and it has been seen in other types of pleural effusion like malignancy and parapneumonic effusion but this level in TPE is higher than other disorders [15]. Pleural effusion is present in 4% of patients with non-pulmonary tuberculosis and the mean age is 34 [16, 17]. This form of infection is more frequent in areas of high prevalent HIV infection [18].

The most common clinical features of TPE are fever, cough and pleuritic chest pain [19]. Other clinical features like night sweating, weight loss and weakness are correlated with extent of effusion. Generally, TPE is one sided in little amounts but in 30% of patients is massive. In a study, pleural effusion was third reason of massive pleural effusion (12%) after malignancy (55%) and pneumonia (22%) [20]. Acid-fast bacilli are present in 5% of TB pleuritis cases in microscopy of pleural fluid because of the paucibacillary nature of the disease [21, 22]. The culture of pleural fluid for Mycobacteria has low sensitivity (24-58%) [22, 23] and it is not useful because it makes an 8 week delay in diagnosis.

The most sensitive available diagnostic method is biopsy of pleural tissue for histological examinations (80%) and culture of pleural fluid and tissue for Mycobacteria (85%) [21, 24] but it is not always useful because pleural biopsy is invasive. In addition, complications of biopsy are dependent on the skill of the operator because it is technically difficult, particularly in children.

Routine collection of induced sputum has been proposed for the diagnosis of pleural TB. Since patients with pleural TB rarely produce sputum spontaneously. In a prospective study of 113 patients with confirmed pleural TB, induced sputum had a sensitivity of 52%, compared with 12% sensitivity of pleural fluid culture [5, 25]. Because of these ineffective diagnostic tools, the use of biomarkers like ADA, INF- γ and SIL-2R are widely spread.

Table1. Comparison of protein level, LDH level and cell count between tuberculous and non-tuberculous pleural fluid

Groups	TB group Mean \pm SD	Non-TB group Mean \pm SD	p-Value
PF* protein level (g/dl)	5.1 \pm 0.58	4 \pm 1.2	NS
PF LDH level (IU/L)	3831 \pm 2460	4378 \pm 3215	NS
PF WBC count (cells/mm ³)	2973 \pm 1886	6454 \pm 4011	NS
PF lymphocyte count (cells/mm ³)	2157 \pm 1499	1584 \pm 1287	$p<0.001$
Lymphocyte percentile (%)	87 \pm 16	54 \pm 27	$p<0.001$

*PF: pleural fluid

In present study, we measured the level of SIL-2 receptor in the pleural fluid of 112 patients with pleural effusion. The level of SIL-2R in patients with tuberculosis is significantly higher than parapneumonic effusions and malignancies. The sensitivity of SIL-2R in our study is 86% and specificity is 89% and the cutoff point for this biomarker assumed 4200 U/ml. Other studies have shown this relationship but the cut of points in different studies are not same.

Ito et al. reported elevated levels of SIL-2 receptor in 10 patients with TPE in comparison with 23 patients with non-TPE. In addition; this study indicated a positive correlation between SIL-2R and ADA levels in tuberculous pleural effusion [15]. In the study of Hiraki et al. comparison of six biological markers in TPE showed that SIL-2 receptor has the most sensitivity (AUC, 0.990) after interferon- γ in comparison with ADA, IL-18, IL-12 p40 and IAP [9]. In another study in Spain which was done on 23 patients with TPE and 119 patients with non-TPE, Porcel et al. showed that there is elevated serum level of SIL-2 receptors in 91.3% of patients with TPE while 5% of non-TPE patients had this level. The cut of point in this study was 4700 u/ml with sensitivity of 91% and specificity of 94% [26]. Another study shows that SIL-2R levels are significantly elevated in pleural fluid of patients with active pulmonary TB. Although this level declines after anti-TB chemotherapy, but it is markedly higher in cases than control group after six months of treatment indicating a delay in resolution of inflammatory response despite anti-TB chemotherapy [27]. According to result of different studies, the variation in the level and cut-off point of SIL-2R is due to several factors. Prevalence of tuberculous infection in different regions, difference in clinical and laboratory procedures, prevalence of concomitant diseases or infections like HIV

infection, the progression of underlying infection, the rate of drug resistance to tuberculous treatment and patient's socioeconomic status are among these factors. As a result, we cannot use a single cut-off point in different countries and regions. Due to difference in cut-off points and high prevalence of tuberculous infection in our country, Iran, measuring the level of SIL-2R and its cut-off point is necessary and this level in our study is lower than other studies and countries.

Overall, we suggest measuring the level of SIL-2 in pleural fluid of patients with pleural effusion who are suspicious with tuberculous infection. This measurement can be used as a diagnostic tool and repeating the measurement after 6 months evaluates the effectiveness of therapy. In addition, we can use SIL-2R level in diagnosing extrapulmonary tuberculosis and in immunodeficient patients like HIV positive patients rather than conventional diagnostic tools. Concurrent measurement of SIL-2 in serum, pleural fluid and bronchoalveolar lavage is also suggested.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

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References

1. Global tuberculosis control: WHO report 2011 WHO/HTM/TB/2011.16.
2. Global tuberculosis control, 10th revision (ICD-10). 2nd ed. Geneva, World Health Organization; 2007.
3. Perez-Rodriguez E, Light RW. Effusions from infections: tuberculosis. In: Textbook of pleural diseases. Light RW, Lee G. 2nd ed. London; Hodder Arnold; 2008: 367-378.
4. Baumann MH, Nolan R, Petrini M, et al. Pleural tuberculosis in the United States. *Chest* 2007; 131(4): 1125-1132.
5. Gopi A, Madhavan SM, Sharma SK and Sahn SA. Diagnosis and treatment of tuberculous pleural effusion in 2006. *Chest* 2007; 131(3): 880-889.
6. Maurice JL. Delayed hypersensitivity and immunity in tuberculosis. *Am Rev Respir Dis* 1975; 111(4): 243-246.
7. Kloster BE, John PA, Miller LE, et al. Soluble interleukin-2 receptors are elevated in patients with AIDS or at risk of developing AIDS. *Clin Immunol Immunopathol* 1987; 45(3): 440-46.
8. Krenke R, Safianowska A, Paplinska M, et al. Pleural fluid adenosine deaminase and interferon gamma as diagnostic tools in tuberculous pleurisy. *J physiol Pharmacol* 2008; 59(suppl 6): 349-360.
9. Hiraki A, Keisuke A, Ryosuke E, et al. Comparison of six biological markers for the diagnosis of tuberculous pleuritis. *Chest* 2004; 125(3): 987-989.
10. Edwards D, Kirkpatrick CH. The immunology of mycobacterial diseases. *Am Rev Respir Dis* 1986; 134(5): 1062-1071.
11. Ellner JJ, Wallis RS. Immunologic aspects of mycobacterial infections. *Rev Infect Dis* 1989; 11(suppl 3): S455-S459.
12. Cantrell DA, Smith KA. Transient expression of interleukin-2 receptors: Consequences for T-cell growth. *J Exp Med* 1983; 158(6): 1895-1911.
13. Rubin LA, Kurman CC, Fritz ME, et al. Soluble interleukin-2 receptors are released from activated human lymphoid cells in vitro. *J Immunol* 1985; 135(5): 3172-3177.
14. Rubin LA, Jay G, Nelson DL. The released interleukin-2 receptor binds interleukin-2 efficiently. *J Immunol* 1986; 137: 3841-3844.
15. Ito M, Kojiro N, Shirasaka T, et al. Elevated levels of soluble interleukin-2 receptors in tuberculous pleural effusions. *Chest* 1990; 97(5): 1141-1143.
16. Hass DW. Mycobacterial Disease. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of

- infectious diseases. 6th ed. New York: Churchill Livingstone; 2005; 2852-86.
17. Sharma SK, Mohan A. Extrapulmonary tuberculosis. *Indian J Med Res* 2004; 120(4): 316-353.
 18. Harries AD. Tuberculosis and human immunodeficiency virus infection in developing countries. *Lancet* 1990; 335(8686): 387-390.
 19. Chakravorty S, Sen MK, Tyagi JS. Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technology. *J Clin Microbiol* 2005; 43(9): 4357-4362.
 20. Valdes L, San Jose E, Alvarez D and Valle JM. Adenosine deaminase (ADA) isoenzyme analysis in pleural effusions: Diagnostic role and relevance to the origin of increased ADA in tuberculous pleuritis. *Eur Respir J* 1996; 9(4): 747-751.
 21. Light RW. Useful tests on the pleural fluid in the management of patients with pleural effusions. *Curr Opin Pulm Med* 1999; 5(4): 245-249.
 22. Bueno EC, Clemente GM, Castro CB, et al. Cytologic and bacteriologic analysis of fluid and pleural biopsy specimens with Cope's needle. Study of 414 patients. *Arch Intern Med* 1990; 150(6): 1190-1194.
 23. Seibert AF, Haynes J Jr, Middleton R, Bass JB Jr. Tuberculous pleural effusion. Twenty-year experience. *Chest* 1991; 99(4): 883-886.
 24. Palomino JC, Leao SC, Ritacco V, eds. Tuberculosis 2007. From Basic Science to Patient Care. 1st ed. www.tuberculosis textbook.com/tuberculosis;2007.
 25. Conde MB, Loivos AC, Rezende VM, et al. Yield of sputum induction in the diagnosis of pleural tuberculosis. *Am J Respir Crit Care Med* 2003; 167(5): 723-725.
 26. Porcel JM, Gázquez I, Vives M et al. Diagnosis of tuberculous pleuritis by the measurement of soluble interleukin 2 receptor in pleural fluid. *Int J Tuberc Lung Dis* 2000; 4(10): 975-979.
 27. Chan CH, Lai CK, Leung JC, et al. Elevated interleukin-2 receptor level in patients with active pulmonary tuberculosis and the changes following anti-tuberculosis chemotherapy. *Eur Respir J* 1995; 8(1): 70-73.