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Are soluble IL-2 receptor and IL-12p40 levels useful markers for diagnosis of tuberculous pleurisy?

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Abstract

Background: The differential diagnostic utilities of the levels of soluble interleukin (IL)-12p40 and the IL-2 receptor in sera and pleural effusions were evaluated in patients with exudative pleural effusions. *Methods:* We enrolled a total of 120 patients with exudative pleural effusions. The clinical, radiological, and histopathological diagnoses were tuberculous pleurisy in 52, malignant pleurisy in 39, and parapneumonic effusions in 29 patients. *Results:* We measured serum IL-12p40 and adenosine deaminase (ADA) levels in patients with tuberculous pleurisy and in a control group treated for pleural effusion to determine if such levels were useful in the diagnosis of pleural effusion ($p < 0.005$). Definite microbiological or histopathological diagnoses of tuberculous pleurisy or pleural effusion were recorded, and we found that ADA and serum soluble IL-2 receptor levels aided in diagnosis ($p < 0.001$). The levels of ADA and soluble IL-2 in pleural effusions afforded sensitivities and specificities of 84.62% and 82.69% and of 70.59% and 80.88%, respectively. The soluble IL-2 receptor level afforded a sensitivity and specificity of 82.69% and 52.9%. IL-12p40 levels in pleural effusions and sera afforded sensitivities and specificities of 80.77% and 80.77% and of 60.29% and 39.71%, respectively. *Conclusion:* Soluble IL-2 receptor levels in patients with tuberculous pleurisy serve as markers of disease in non-endemic countries, similarly to ADA levels.

Keywords: *Tuberculosis, pleural effusion, soluble IL-2 receptor, IL-12p40, adenosine deaminase*

Introduction

Tuberculous pleurisy is the second most common (23%) form of tuberculosis featuring extrapulmonary involvement (lymph nodes being the most affected tissues) [1,2]. To achieve a definite diagnosis of tuberculous pleurisy, *Mycobacterium tuberculosis* bacilli must be noted in sputum or pleural fluid, or typical caseous granulomas must be evident in pleural membranes [1]. The incidence of tuberculous pleurisy is not similar to that of tuberculosis in the community, but different countries have reported varying rates of pleural involvement [2]. Even when all appropriate biochemical, bacteriological, and histopathological diagnostic methods are applied, disease etiology cannot be determined in 15–25% of cases [3]. Therefore, a diagnosis of tuberculosis and definition of the relevant etiological factors should ideally be possible via measurement of parameters in pleural fluid or serum. Such an approach is less invasive. The success of

biopsy used to diagnose tuberculous pleurisy was 51–84% in various studies [4,5]. Microbiological evidence of *M. tuberculosis* in pleural fluid was used to diagnose 25% of cases, and 40–71% of biopsies yielded positive bacillus cultures [6,7]. Because diagnosis can be difficult, many possible diagnostic methods, including assessment of the levels of interleukin (IL)-2, IL-6, IL-12, adenosine deaminase (ADA), interferon (IFN)-γ, and tuberculostearic acid, have been explored [8–10]. Today, the preferred diagnostic marker is ADA, an enzyme that can be assayed at low cost and rapidly. Although false-positive results are common and additional parameters must be measured to confirm diagnosis, modern diagnostic methods have become less invasive. To contribute to such efforts, we measured the levels of soluble IL-2 receptor and IL-12p40 in pleural fluids and sera.

IL-12 is a heterodimeric protein composed of two subunits (p35 and p40), produced by phagocytic

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cells, such as dendritic cells and macrophages, that have phagocytosed *M. tuberculosis* [11]. Differentiation of naive T cells into Th1 cells, increased secretion of IFN-γ, and enhanced cytotoxicity of antigen-specific T cells and natural killer (NK) cells represent protective immune responses against *M. tuberculosis*. Such protective changes are triggered by IL-12 [11–15].

IL-2 plays a central role in the physiology of the immune system. CD4 + T-helper cells produce IL-2, which increases clonal proliferation of T lymphocytes and accelerates the development of B lymphocytes. IL-2 stimulates NK cell function and activates macrophages [16] via binding to a surface receptor on target cells. Soluble IL-2α receptor levels particularly increase in patients with inflammatory, immune, and neoplastic diseases. Such increases in serum levels reflect disease activity [17]. In the present study, soluble IL-2 receptor and IL-12p40 levels in serum and pleural fluid were investigated for their utility in the diagnosis of tuberculous pleurisy. Also, we explored the diagnostic utilities of IL-12p40 and soluble IL-2 receptor levels (in both serum and pleural fluid) in terms of the differential diagnosis of exudative pleural effusions, and we compared our results with diagnoses made via ADA levels, which afford high sensitivity and specificity in the differential diagnosis of tuberculous pleurisy.

Materials and methods

Patients

Between October 2010 and February 2011, a total of 120 patients with exudative pleural effusions admitted to the hospital were included in the study. All of our patients had pleural effusion during the study. The mean patient age was 46.0 ± 18.8 years (range 18–70 years), and 48 were female. The patients were divided into those with nontuberculous versus tuberculosis pleurisy: 52 patients exhibiting clinical and histopathological features of tuberculous pleurisy and 68 with non-tuberculous pleurisy. Diagnosis of tuberculous pleurisy was made by showing acid-fast bacilli in pleural fluid and/or demonstration of necrotizing granulomatous inflammation of pleural tissue. A complete resolution of pleural fluid was observed after antituberculosis treatment for 6 months in all cases. Medical histories were recorded and physical examinations performed. Blood samples were taken for complete blood counts, determination of erythrocyte sedimentation rate, biochemical tests, and assessment of bleeding/clotting parameters. Sputum specimens for acid-resistant bacilli (ARB) and culture, and pleural fluid samples (obtained via thoracentesis) were obtained from all patients. Pleural

fluids were subjected to bacteriological and biochemical testing. The institutional ethics committee approved the study. All patients provided oral and written informed consent.

Laboratory investigations

Patients whose exudates fulfilled Light's criteria were included in the study. Parietal pleura biopsies were performed using video-assisted thoracoscopic surgery (VATS) or a Ramel needle. Samples destined for histopathological examination were stored in 10% (v/v) formaldehyde. Samples of pleural fluid (at least 10 ml volumes) were transported to the laboratory under sterile conditions for measurement of ADA activities. Serum and pleural fluid samples (5 ml) were centrifuged for 10 min at 2000 rpm and the supernatants were stored at –80°C before measurements (which were all performed on the same day). ADA activities in pleural fluid were measured using the Guisti method, which measures the conversion of adenosine to inosine and ammonia by ADA. Pleural fluid and serum levels of IL-12p40 and soluble IL-2 receptor antibody were measured using semi-quantitative ELISA, and the results were collected spectrophotometrically.

Statistical evaluation

Results are presented as means ± standard deviations (SDs) or as medians. The Mann–Whitney U test was used to compare median values and the Student's t test to compare averages and SDs. For nonparametric values, the chi-squared test was used to compare data between groups. Soluble IL-2 receptor and IL-12p40 concentrations in pleural fluids and sera and their correlations with ADA levels were evaluated using the Spearman's rank correlation test. The sensitivity, specificity, positive predictive value, negative predictive value, and positive and negative likelihood probabilities were determined using standard formulae. Receiver operating characteristic (ROC) curve analysis was employed to determine the areas under the curves of serum and pleural fluid IL-12p40 and soluble IL-2 receptor levels and to derive descriptive characteristics and biochemical parameters of various boundary levels. MedCalc 9.2.0.1 (Mariakerk, Belgium) was used to compare ROC data (areas under curves).

Results

In the tuberculous pleurisy group, 21 patients were female (40.4%) and 31 male (59.6%), with a mean age of 30.7 ± 13.8 years. In the non-tuberculosis

pleurisy group, 27 patients were female (39.7%) and 41 male (60.3%), of mean age 59.5 years (22–70 years). Thus, a significant age difference was apparent: tuberculous pleurisy occurred in young-to-middle-aged patients, but most malignant pleural effusions (in the control group) occurred in elderly patients. The non-tuberculosis pleurisy group showed malignant pleural effusions (39 patients, 57.4%) or parapneumonic pleural effusions (29 patients, 42.6%). The average pleural fluid ADA level in the tuberculous pleurisy group was 82.20 ± 25.90 U/ml, and that in the control group was 49.40 ± 16.50 U/ml. The pleural fluid ADA levels differed significantly between the two groups ($p < 0.001$).

In tuberculous pleural fluid, using a cut-off value of 62.44 U/ml, ADA afforded a diagnostic sensitivity of 84.62% and a specificity of 80.88%. The positive and negative predictive values of ADA status were 79.19% and 87.30%, respectively. Results and comparison of parameters in the tuberculosis and control groups are shown in Table I. Upon ROC analysis, a soluble IL-2 receptor cut-off level of 4.8 ng/ml in tuberculous pleural fluid afforded 82.69% sensitivity and 70.59% specificity. At a serum soluble IL-2 receptor cut-off level of 0.6 ng/ml in the tuberculous group, the sensitivity was 82.69% and the specificity 52.94%. The lactate dehydrogenase (LDH), total protein, and albumin levels in the pleural fluids of patients with exudative pleural effusion are shown in Table III, with reference to the Light criteria.

At a cut-off value of 210 pg/ml, the IL-12p40 level in tuberculous pleural fluid afforded a sensitivity of 80.77% and a specificity of 60.29%. In patients with tuberculosis, the serum IL-12p40 level, at a cut-off value of 42pg/ml, afforded 80.77% sensitivity and

Table II. Correlation values of the markers analyzed.

	Pleural fluid ADA	
Marker	r value	p value
Serum sIL-2 receptor	0.208	0.023
Serum IL-12p40	0.198	0.030
Pleural fluid sIL-2 receptor	0.377	0.001
Pleural fluid IL-12p40	0.362	0.001

ADA, adenosine deaminase.

39.71% specificity (Table II). ROC curves for the markers evaluated are shown in Figures 1 and 2. Correlations between pleural fluid marker levels and those of ADA are shown in Table III. Soluble IL-2 receptor and IL-12p40 statuses of serum or pleural fluid did not yield diagnostic data additional to those afforded by the ADA levels, upon Kruskal–Wallis testing. These parameters, alone or in addition to ADA levels, were shown to contribute to diagnosis.

Discussion

In the present study, differential diagnosis using the examined parameters (pleural fluid soluble IL-2 receptor, pleural fluid IL-12p40, serum soluble IL-2 receptor) was shown to be as meaningful as ADA. Serum ADA levels were correlated with IL-12p40 levels in pleural fluid. In the present study, the difference created by studying the parameters in pleural fluid and serum allowed us to reach a diagnosis using less invasive methods. Pleural soluble IL-2 receptor in particular was found to be close to ADA activity in the differential diagnosis of malignant pleural effusion and can be recommended for use as an indicator.

Table I. Results and comparison of parameters in the tuberculosis and control groups.

Parameter	Tuberculosis group	Control group*	p value
Number (*n*)	52 (43.3%)	68 (56.7%)	
Age (years)†	25 (18–68)	59.5 (22–70)	< 0.001
Sex			
Male	31 (59.6%)	41 (60.3%)	NS
Female	21 (40.4%)	27 (39.7%)	NS
LDH	491.5 (341–710)	318.5 (217–559)	< 0.001
Total protein	5.20 ± 0.50	4.70 ± 1.0	< 0.001
Albumin	2.87 ± 0.29	2.73 ± 0.64	NS
Pleural fluid ADA (U/ml)‡	82.2 ± 25.9	49.4 ± 16.5	< 0.001
Serum sIL-2 receptor†	0.85 (0.69–3.8)	0.6 (0.4–1.1)	< 0.001
Serum IL-12p40†	67 (48–88.5)	52 (36.2–70.5)	0.041
Pleural fluid sIL-2 receptor (ng/ml)†	11 (0.5–20)	3.45 (0.1–13)	< 0.001
Pleural fluid IL-12p40 (pg/ml)†	500 (246–500)	166 (97–350)	< 0.001

ADA, adenosine deaminase; LDH, lactate dehydrogenase; NS, not significant.
*Control: malignant pleural effusions + parapneumonic effusions.
†Data in range of median (25–75%).
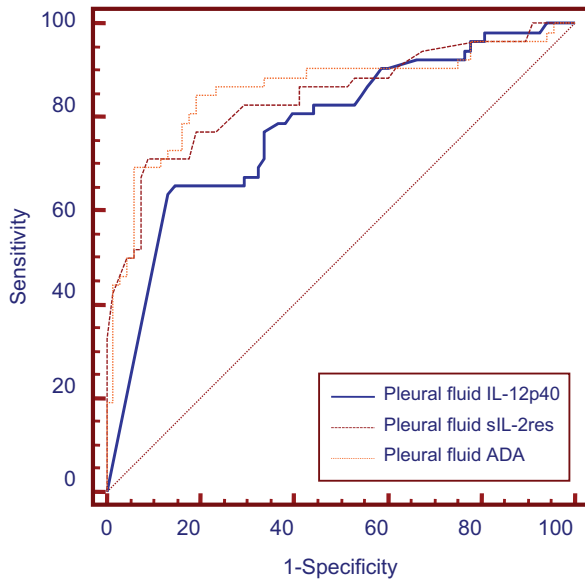‡The average of data ± SD.

Figure 1. Pleural fluid IL-12p40, sIL-2 receptor, and adenosine deaminase (ADA) levels in patients with tuberculous pleurisy visualized using receiver operating characteristic (ROC) curves.

Pleural effusion caused by tuberculosis is one of the most common causes of extrapulmonary tuberculosis and pleurisy in Turkey [18]; 3% of all pleurisies are tuberculous in nature, and 3.5% of tuberculosis cases also show pleurisy [4,6]. In Turkey, 25–50% of pleurisies are tuberculous in nature [19]. Pleural biopsy is the most common method used initially to diagnose exudative pleural effusion, with a diagnostic yield of 50–80%. Performance of a second biopsy affords a diagnostic contribution of 10–40% [19,20]. Bacilli evident in pleural fluid or granulomas of a pleural biopsy are valuable in terms of definitive diagnosis. However, fluid from patients with tuberculous pleurisy is of low diagnostic value when smears are made or cultures attempted. PCR

affords more reliable and rapid diagnosis, but only 70–86% of cases can be diagnosed in this manner [21]. Pleural biopsy is invasive and associated with a risk of complications. Thus, many clinicians and researchers measure markers in blood and pleural fluid for rapid diagnosis [22,23].

The effects of IL-2 on other cells are of secondary importance [16]. IL-2 acts by binding to IL-2 receptors on the surface of target cells. The soluble IL-2α receptor is expressed constitutively in CD16-NK cells. In addition, the receptor can be expressed after stimulation of peripheral blood T cells, intrathymic T-cell precursors, B lymphocytes, dendritic and oligodendritic cells, CD16 + NK cells, mast cells, monocytes/macrophages, liver Kupffer cells, and skin Langerhans cells [16]. The serum levels of the soluble IL-2α receptor are especially increased in patients with inflammatory, immune, and neoplastic diseases, and these levels reflect the extent of underlying disease activity [17].

Thus, the availability of a test to diagnose tuberculous pleurisy that is both simple and accurate would provide a great advantage. In the present study, we measured the levels of the soluble IL-2 receptor and IL-12p40 in pleural effusions and sera to determine their utility in the diagnosis of tuberculosis, either in combination with ADA levels or alone.

Valdes et al. [23] investigated whether the IL-12p40 level could be used to diagnose tuberculous pleurisy in 92 patients with pleural effusions. Levels of the ADA isoform ADA-2, IFN-γ, and IL-12p40 and numbers of CD3/DR T lymphocytes were compared among 39 patients with tuberculous pleurisy, 42 with malignant pleural effusions, and 15 with parapneumonic effusions. The IL-12p40 level was more useful for diagnosis of tuberculous pleurisy than the other biomarkers, as revealed by comparison of the areas under the ROC curves. An IL-12p40 level
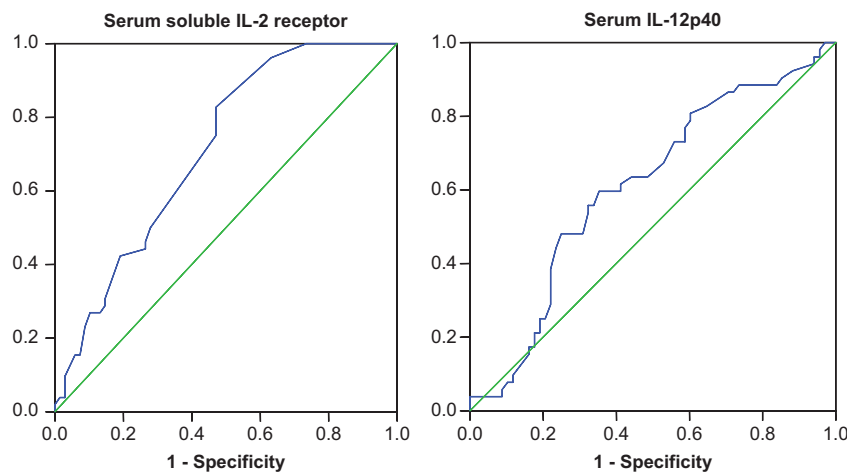

Figure 2. Receiver operating characteristic (ROC) curves for serum levels of the soluble IL-2 receptor and IL-12p40.

Table III. Specificity, sensitivity, and positive predictive and negative predictive values of various parameters, with cut-off levels.

Parameter	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
ADA >62.44 U/ml	84.62	80.88		
Pleural fluid soluble IL-2 receptor >4.8 ng/ml	82.69	70.59	68.25	84.21
Serum soluble IL-2 receptor >0.6 ng/ml	82.69	52.94	57.33	80
Pleural fluid IL-12p40 >210 pg/ml	80.77	60.29	60.87	80.39
Serum IL-12p40 >42 pg/ml	80.77	39.71	50.60	72.97

ADA, adenosine deaminase.

of 550 pg/ml afforded a sensitivity of 92.3% and a specificity of 70.2% [23]. We determined a sensitivity of 80.77% and specificity of 60.29% using an IL-12p40 cut-off level of 210 pg/ml. In our study numbers of tuberculous pleurisy patient were lower. Hiraki et al. [24] compared the levels of six biochemical markers among 55 patients with pleural effusions, 20 tuberculous pleurisy patients, and 35 patients with non-tuberculosis effusions. INF-γ was the most sensitive and specific biochemical marker upon ROC analysis. The sensitivities and specificities afforded by the protein levels were as follows, in descending order: soluble IL-2 receptor, ADA, IL-18, IL-12p40, and the immunosuppressive acidic protein [24]. We found that the soluble IL-2 receptor levels (in both serum and pleural fluid) were more sensitive than those of IL-12p40. Pleural fluid parameters were more specific than the same parameters in serum.

In 2008, Yang et al. measured the levels of nine cytokines and chemokines synthesized during the mounting of an antibody response against five antigens of *M. tuberculosis* in 41 patients with malignant pleural effusions and 81 with tuberculous pleurisy [25]. IFN-γ, IL-12p40, and IL-6 contributed (with statistical significance) to differentiation of tuberculous pleurisy from malignant pleural effusions, as shown by logistic regression [25]. A point that differs from the present study is that the differential diagnosis of the parameters was only analyzed using serum . In areas such as Turkey, where tuberculosis is endemic, the pleural levels of the soluble IL-2 receptor are not very useful in diagnosis, but in countries where the disease is not endemic, such levels can be used to differentially diagnose malignant pleural effusion and tuberculous pleurisy [9]. A cut-off level of 4.8 ng/ml afforded a specificity of 70.59%, similar to that when the ADA level was used as an indicator. On the other hand, the serum levels of the soluble IL-2 receptor lack specificity, restricting their utility. The limitations of our study include the small number of cases evaluated and the facts that the work was performed in a country where tuberculosis is endemic, that ADA levels were measured only in pleural fluids, and that the tuberculosis group was significantly younger than the control group.

Conclusion

In the present study, we examined the levels of soluble IL-2 receptor and IL-12p40 in pleural fluids and sera. The markers were as useful as the ADA level in terms of differential diagnosis. ADA levels were correlated with IL-12p40 levels in pleural fluid. These new markers can be used for differential diagnosis, replacing ADA, with a reduced risk of complications. In particular, the soluble IL-2 receptor level in serum is recommended as an indicator of pleural tuberculosis in non-endemic countries, just as the ADA level is used for the differential diagnosis of malignant pleural effusion.

Acknowledgment

References

[1] WHO. Global tuberculosis control. Surveillance, planning, financing. Communicable diseases. World Health Organization, Geneva; 2002. WHO/CDS/TB/2002; 295.

[2] Frank W. Tuberculous pleural effusions. Eur Respir Mon 2002;22:219–33.

[3] Collins TR, Sahn SA. Thoracocentesis: clinical value, complications, technical problems and patient experience. Chest 1987;91:817.

[4] Kumar S, Seshadri MS, Koshi G, John JT. Diagnosing tuberculous pleural effusion: comparative sensitivity of mycobacterial culture and histopathology. Br Med J (Clin Res Ed) 1981;283:20.

[5] Diacon AH, Van de Wal BW, Wyser C, Smedema JP, Bezuidenhout J, Bolliger CT, et al. Diagnostic tools in tuberculous pleurisy: a direct comparative study. Eur Respir J 2003;22:589–91.

[6] Perez-Rodriguez E, Castro DJ, Light RW. Effusions from tuberculosis. In: Light RW, Lee YCG, editors. Textbook of pleural diseases. Arnold Medical Press; 2003 pp 329–44.

[7] Roth BJ. Searching for tuberculosis in the pleural space. Chest 1999;116:3–5.

[8] Valdés L, San José E, Alvarez D, Sarandeses A, Pose A, Chomón B, et al. Diagnosis of tuberculous pleurisy using the biologic parameters adenosine deaminase, lysozyme, and interferon gamma. Chest 1993;103:458–65.

[9] Chang SC, Hsu YT, Chen YC, Lin CY. Usefulness of soluble interleukin 2 receptor in differentiating tuberculous and carcinomatous pleural effusions. Arch Intern Med 1994; 154:1097–101.

[10] Yew WW, Chan CY, Kwan SY, Cheung SW, French GL. Diagnosis of tuberculosis pleural effusion by detection of tuberculostearic acid in pleural aspirates. Chest 1991;100:1261–3.

[11] Magram, J, Sfarra J, Connaughton S, Faherty D, Warrier R, Carvajal D, et al. Ann N Y Acad Sci 1996;795:60–70.

[12] Altare F, Durandy A, Lammas D, Emile JF, Lamhamedi S, Le Deist F, et al. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. Science 1998; 280:1432–5.

[13] Mcdyer JF, Hackley MN, Walsh TE, Cook JL, Seder RA. Patients with multidrug-resistant tuberculosis with low CD4 + T cell counts have impaired Th1 responses. J Immunol 1997;158:492–500.

[14] Holscher C, Atkinson RA, Arendse B, Brown N, Myburgh E, Alber G, et al. A protective and agonistic function of IL-12p40 in mycobacterial infection. J Immunol 2001;167:6957–66.

[15] Kroemer G, Andreu JL, Gonzala JA, Ramos JCG, Martinez AC. Interleukin-2, autotolerance, and autoimmunity. Adv Immunol 1991;50:147–222.

[16] Rubin LA, Nelson DL. The soluble interleukin-2 receptor: biology, function, and clinical application. Ann Intern Med 1990;113:619–27.

[17] Rubin LA, Jay G, Nelson DL. The released interleukin-2 receptor binds interleukin-2 efficiently. J İmmunol 1986; 137:3841–4.

[18] Bahar B, Demir R, Ozesmi M, Kayseri Nuh Naci Yazgan Chest Diesases Hospital analysis of cases hospitalized in the last 8 years. Tuberkuloz ve Toraks 1989;37:59–64.

[19] Poppius H, Kokkola K. Diagnosis and differential diagnosis in tuberculous pleurisy. Scand J Respir Dis 1968;63(Suppl): 105–10.

[20] Fontan Bueso J, Verea Hernando H, Garcia-Buela JP, Dominguez Juncal L, Martin Egana MT, Montero Martinez MC. Diagnostic value of simultaneous determination of pleural adenosine deaminase and pleural lysozyme/ serum lysozyme ratio in pleural effusion. Chest 1988; 93:303–7.

[21] Kim CH, Woo H, Hyun IG, Kim C, Choi JH, Jang SH, et al. A comparison between the efficiency of the Xpert MTB/RIF assay and nested PCR in identifying Mycobacterium tuberculosis during routine clinical practice. J Thorac Dis 201;6:625–31.

[22] Wongtim S, Silachamroon U, Ruxrungtham K, Udompanich V, Limthongkul S, Charoenlap P, et al. Interferon gamma for diagnosing tuberculous pleural effusions. Thorax 1999; 54:921–4.

[23] Valdés L, San José E, Alvarez Dobaño JM, Golpe A, Valle JM, Penela P, et al. Diagnostic value of interleukin-12 p40 in tuberculous pleural effusions. Eur Respir J 2009; 33:816–20.

[24] Hiraki A, Aoe K, Eda R, Maeda T, Murakami T, Sugi K, et al. Comparison of six biological markers for the diagnosis of tuberculous pleuritis. Chest 2004;125:987–9.

[25] 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.