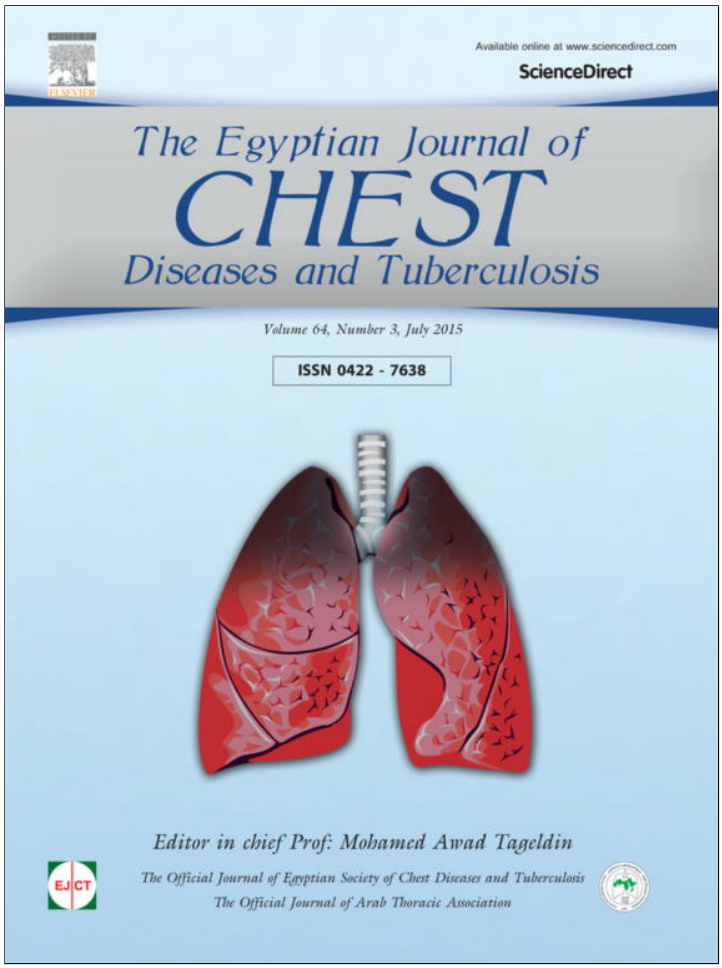

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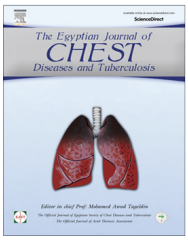

ORIGINAL ARTICLE

Differential diagnostic efficiency of T cells subsets versus interferon-gamma, tumor necrosis factor-alpha and adenosine deaminase in distinguishing tuberculous from malignant pleural effusions

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Abstract *Background:* The differential diagnosis of tuberculous and malignant pleural effusion (PE) is extremely difficult and continues to pose clinical challenges.

Aim of the study: To evaluate the utility of pleural fluid interferon gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), adenosine deaminase (ADA) levels with T cells subsets in differential diagnosis of malignant (MPE) and tuberculous pleural effusions (TPE).

Methods: Forty patients with pleural effusion (20 tuberculous and 20 malignant) were included in the study. The percentages of CD3+ lymphocytes, CD4+ lymphocytes and Treg (CD4+ CD25+) cells in pleural effusion from patients with tuberculous and malignant PE were determined by flow cytometry. The concentrations of IFN-γ, TNF-α, and ADA were simultaneously determined in pleural fluids by enzyme linked immunosorbent assay and colorimetric methods.

Results: IFN-γ, TNF-α and ADA concentrations were significantly higher in TPE than MPE (2.26 ± 1.62 vs. 0.3 ± 0.20 IU/ml: $P < 0.0001$, 122.45 ± 47.69 vs. 35.03 ± 31.88 pg/ml: $P < 0.0001$ and 84.22 ± 41.47 vs. 23.19 ± 17.93 U/l: $P < 0.0001$ respectively). T-cells markers (CD3+ T-cells, CD4+ T-cells and T reg cells) were significantly higher in TPE than MPE (76.46% vs. 65.29%; P 0.004, 51.21% vs. 43.50%; P 0.044 and 14.60% vs. 12.43%; P 0.032

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respectively). CD3+ plus CD4+ as well as CD3+ plus CD4+ plus T reg combinations were all 100% specific for discriminating TPE from MPE. TNF-α plus IFN-γ, TNF-α plus ADA, as well as IFN-γ plus TNF-α plus ADA, were 100% specific for discriminating TPE from MPE. Furthermore, the specificity of combined-diagnostic value of IFN-γ, TNF-α and ADA with T cells subsets was >95%.

Conclusions: The combinations of pleural fluid IFN-γ, TNF-α and ADA levels and T cells subsets could effectively address the challenge of distinguishing tuberculous pleural effusion from malignant pleural effusion.

Introduction

Tuberculosis is a common disease in Egypt, with an incidence rate of 19/100,000 and 5530 new cases diagnosed in 2011 [1]. Tuberculosis typically attacks the lungs, but can also affect other parts of the body. Extra pulmonary infection sites include the pleura, the central nervous system, the lymphatic system, the genitourinary system, and the bones and joints [2].

TPE is caused by the rupture of a pulmonary sub pleural caseous focus which releases mycobacterium into the pleural cavity, thereby triggering an immune response involving mainly macrophages, CD4+ T lymphocytes, and the cytokines released by these cells (especially interleukin 1, interleukin 2, and interferon γ) [3].

It has been well documented that CD4 (+) T lymphocytes are the dominant leukocytes present in TPE. Traditionally, CD4 (+) T cells have been classified into two functionally distinct subsets, helper T-cell type 1 (Th1) and Th2 cells, based on their cytokine secretion profiles. Recently, regulatory T cells, Th17 cells, Th9 cells, and Th22 cells have been added to the 'portfolio' of this ever enlarging subset [4]. CD4+CD25+ T cell numbers in TPE were much higher than those in peripheral blood from patients with TPE and from healthy subjects, also CD4+CD25+ T cells infiltrating pleural space were regulatory T cells as they expressed high levels of Foxp3 transcription factor. Moreover, CD4+CD25+ T cells could potently suppress the proliferation of responding T cells [5], also it was found that CD4+CD25+ T-cell numbers in MPE were much higher than those in PE from patients with lung cancer without malignant effusion and higher than numbers in peripheral blood [6].

Various cytokines have been demonstrated in pleural fluid from patients with TB pleural effusions, interferon γ, which is released by activated CD4+ T cells is capable of activating macrophages, increasing their bactericidal capacity against *Mycobacterium tuberculosis* and is involved in granuloma formation. Several studies have found elevated concentrations of INF-γ in TB pleural effusions, which are related to increased production at the disease site by effector T cells. Another highly efficient marker is ADA, has been reported to accumulate in the pleural fluid of TB patients and can predict TPE with high sensitivity and specificity at 95% and 90% respectively [7], Also high concentrations of TNF-α in pleural fluid have been observed in several diseases, including tuberculosis [4].

This prompted us to conduct this study to assess the role of T Cells subsets, IFN-γ, TNF α and ADA in the differential diagnosis of TPE and MPE, by determining the best cutoff levels of pleural fluid T Cells subsets, IFN-γ, TNF α and ADA for differentiating between TPE and MPE.

Patients and methods

Patients' selection

Our study was carried out in the Departments of Chest, Medical Microbiology and Immunology and Clinical pathology, Faculty of Medicine, Sohag University during the period from November 2012 to December 2013.The study was approved by the research ethics committee and consents were obtained from patients in the study.

Collection of pleural fluid was carried out to patients who were indicated for thoracocentesis. Patients were subsequently included if the examinations of pleural effusion and/or biopsy specimens established a diagnosis of TPE or MPE. Twenty patients were shown to have tuberculous PE, as evidenced by the growth of *M. tuberculosis* from PE or by demonstration of granulomatous pleuritis on a closed pleural biopsy specimen in the absence of any evidence of other granulomatous diseases. Malignant PE was collected from 20 patients with newly diagnosed lung cancer. Histologically, among the patients with malignancies, 14 had adenocarcinoma, 3 had squamous cell carcinoma, 1 had Hodgkin Lymphoma and 2 had distant metastasis.

At the time of sample collection, none of the patients had received any antituberculosis therapy, anticancer treatment, corticosteroids, or other nonsteroid anti-inflammatory drugs.

Methods

All patients were subjected to:

Complete history taking; personal history and family history.
Thorough clinical evaluation.
Radiological evaluation (Chest X rays).
Complete blood count, serum biochemical tests.

Pleural fluid samples were obtained by intercostal needle aspiration. All pleural fluids were stained and cultured for the presence of bacteria and analyzed cytologically for the presence of tumor cells. Total and differential white cell counts, proteins, glucose and lactate dehydrogenase were determined in all pleural fluids. The pleural fluid samples were centrifuged at 2000 rpm for 10 min. ADA activity was

measured promptly according to Giusti's colorimetric method, since the activity of ADA tends to decrease during storage; the remaining supernatant was frozen at −20 °C until assayed for IFN-γ and TNF α.

Analysis of lymphocyte markers

The cell pellets were resuspended in phosphate buffered saline (PBS) and analyzed by flow cytometry. 10 µl monoclonal antibodies (MoAb) were added to 100 µl pleural fluid then vortexed and incubated for 15 min at room temperature at 22 °C in the dark, and then 1 ml PBS was added, immediately vortexed then incubated for 10 min in room temperature at 22 °C in the dark.

Fluorescein-conjugated monoclonal antibodies (MoAbs) were used for staining lymphocytes namely anti-CD4 labeled with fluorescein isothiocyanate (FITC), anti-CD25, labeled with phycoerythrin (PE), anti-CD3 labeled with electron coupled dye (ECD) and anti CD127 labeled with phycoerythrin-cyanin5 (PE–Cy™5) (Beckman Coulter Immunotech, Marseille, France).

The Lymphocyte markers analyzed were CD3+(Pan T marker) CD3+ CD4+ (total T helper cells), CD4+ CD25hi (T regulatory cells). The pleural fluid samples were analyzed using Epics-XL flow cytometry (Coulter, USA). Lymphocytes were analyzed using a gate set on forward scatter versus side scatter, with a standard 4-color filter configuration using System II software version 3.0 (Coulter, USA).

Determination of cytokines' concentrations and enzyme activity of ADA

Enzyme-linked immunosorbent assay (ELISA) was performed according to manufacturer's instructions to determine the pleural concentrations of IFN-γ, TNF-α. Pleural enzyme activity of ADA was determined by spectrophotometric method.

IFN-γ ELISA Kit, manufactured by Cellestis Ltd., Laboratories (Victoria, Australia) was used for IFN-γ assay. This assay employs the quantitative sandwich enzyme immunoassay technique according to manufacturer's instructions using the Stat fax 2600 microplate reader (Awareness Technologies, Palm City, USA).

AviBion Human TNF-α ELISA Kit, manufactured by orgenium Laboratories (Helsinki, Finland) was used for TNF α. This assay employs the quantitative sandwich enzyme immunoassay technique according to manufacturer's instructions using the Stat fax 2600 microplate reader (Awareness Technologies, Palm City, USA).

ADA Enzymatic assay kit, manufactured by BQKITS Diagnostics (San Diego, USA) was used to measure ADA activity using Cintra 2020 spectrophotometer (GBC Scientific, Victoria, Australia).

Statistical analysis

Data analyses were performed using SPSS statistical package version 10 (SPSS, Inc., Chicago, IL, USA). Values are presented as mean ± standard deviation (SD). Sensitivity and specificity values providing the best test performance and the area under the curve (AUC) were calculated using a receiver operating characteristic (ROC) curve analysis. The *P*-value of <0.05 was considered as statistically significant.

Results

Patient demographics

The demographic data of the patients and the characteristics of the pleural effusions are shown in Table 1. Forty patients were included in the study: 20 TPE patients (8 men and 12 women; median age: 35 (14–59) years and 20 malignant PE patients (15 men and 5 women; median age: 57 (28–70) years. Patients with TPE were significantly younger (36.95 ± 12.77 years) than those with MPE (54.5 ± 10.59 years) with *P* value <0.001. Protein concentrations were significantly higher ($P < 0.001$) in the TPE group (5.38 ± 1.63 g/dL) compared to the MPE group (4.06 ± .760 g/dL). In contrast, LDH levels were higher ($P = 0.003$) in the MPE group (983.5 ± 264 IU) than in the TPE (726.6 ± 256 IU) group. A higher proportion of leukocytes ($P < 0.001$) were observed in the TPE group (1908 ± 2063) when compared to the MPE group (1143.4 ± 973.64). Although both effusions were lymphocytic, the number of lymphocytes was higher in the TPE compared to the MPE group. In cancer effusions, oncotic cytology was positive in 10% of cases.

Lymphocytes subsets in pleural fluid

Evaluation of the lymphocyte subsets showed that T-cells, especially CD3+ T-cells, were significantly higher in TPE (76.46%) than that in the MPE (65.29%; *P* 0.004). Similarly, significant rise of CD4+ T-cells cells were observed in tuberculous PE (51.21%) in comparison with malignant PE (43.50%;

Table 1 Patients' demographic data and pleural effusions characteristics.

	Pleural effusion		
	Tuberculous	Malignant	*P* value
Patients	20	20	
Age	36.95 ± 12.77	54.50 ± 10.59	0.0001
Sex(M/F)	(8/12)(40/60%)	(15/5)(75/25%)	0.025
Size (% of the hemithorax)			
Small	4 (20%)	5 (25%)	0.70
Moderate	13 (65%)	8 (40%)	0.11
Massive	3 (15)	7 (35%)	0.14
Location			
Unilateral	16 (80%)	18 (90%)	0.37
Bilateral	4 (20%)	2 (10%)	0.37
Protein g/L	5.38 ± 1.36	4.06 ± 0.766	0.001
LDH IU/L	726.6 ± 256.41	983.5 ± 264.54	0.003
Glucose	75.65 ± 36.59	65.60 ± 25.74	0.32
Total cell counts (×109/L)	1908 ± 1063	1143.4 ± 973.64	0.02
Differential cell counts (%)			
Lymphocytes	80.45 ± 18.43	72.2 ± 22.06	0.23
Neutrophils	19.55 ± 9.43	18.6 ± 22.06	0.23
Malignant cells	–	9.2 ± 1.5	0.001

P 0.044). Significant increase of T regs in TPE compared to MPE (*P* value 0.032) (Table 2).

Cytokine concentrations and ADA in pleural fluid

Data presented in (Table 2) shows that the mean IFN-γ concentration for the tuberculous group was 2.26 ± 1.61 IU/mL (range: 0.54–3.49 IU/mL), compared with a mean level of 0.29 ± .19 IU/mL (range: 0.02–0.47 IU/mL) in the cancer group ($P < 0.0001$). The mean ADA activity for the tuberculous group was 84.22 ± 41.47 U/L, compared with a mean level of 23.18 ± 17.93 U/L in the cancer group ($P < 0.0001$). Serum TNF α levels in TB patients were significantly higher than those in patients with cancer (122.45 ± 41.47 vs. 35.03 ± 31.88 pg/ml: $P < 0.0001$).

Differential diagnostic values of pleural fluid cytokines and T cells

ROC curve analysis was introduced to evaluate the cut-off values of pleural TNF-α, IFN-γ, ADA (Fig. 1). The thresholds were found to be 50 pg/mL, 0.5 IU/mL, and 35.5 U/L respectively (Table 3). An analysis of the ROC curve for ADA activity showed that at the most accurate cut-off level of 35.5 U/L. The sensitivity of the test for tuberculous PE was 85% and the specificity 85% (Fig. 1). Assuming 0.5 IU/ml as the threshold value for IFN-γ concentration, the diagnostic sensitivity for tuberculous PE was 90% with the corresponding specificity of 95% (Fig. 1), With a cut-off value of 50 pg/mL, the sensitivity of TNF-α for differential diagnosis of TPE from MPE was 85%, with the specificity of 100% and accuracy of 92% (Table 3). The AUC value for each parameter was calculated, and the diagnostic accuracy was compared. The AUC for all of these bio parameters are greater than 0.5, TNF-α (0.94), IFN-γ (0.97) and ADA (0.93), indicating that TNF-α, IFN-γ and ADA are reliable for the clinical diagnosis of TPE (Fig. 2).

Using the ideal cutoff point according to the ROC analysis, for pleural T cells subsets CD3+, CD4+ and T regs, the thresholds were found to be 75%, 38% and 13% respectively (Table 3). The AUC for all of these cells are greater than 0.5, CD3 (0.75), CD4 (0.69) and T reg (0. 68).

Combined-diagnostic value of IFN-γ, TNF-α, ADA and T cell subsets

We were interested in determining whether the combinations of two or three of the parameters would improve the diagnostic sufficiency for differentiating TPE from MPE. As a result, we further investigated the potential combined-diagnostic value of IFN-γ, TNF-α, ADA with T cells subsets. The measurement of TNF-α plus IFN-γ, TNF-α plus ADA, as well as TNF-α plus IFN-γ plus ADA, were 100% specific for discriminating TPE from MPE. However, sensitivities ranged from 65% to 80%, with the highest sensitivity seen with TNF-α plus IFN-γ (Table 4). Interestingly, CD3+ plus CD4+ as well as CD3+ plus CD4+ plus T reg combinations were all 100% specific for discriminating TPE from MPE (Table 4). Furthermore, the specificity of combined-diagnostic value of TNF-α, IFN-γ, ADA with T cells subsets was >95% (Table 4). These data suggest that combinations of biochemical parameters and T cells subsets are valuable markers for the differential diagnosis of TPE from MPE.

Discussion

Although, TPE is usually seen in the young age, it may be seen also in the old age and the differential diagnosis with MPE might be a problem. Hence many markers that may be helpful in the differential diagnosis were studied. TPE usually shows a lymphocytic preponderance, especially CD4+ T cells [8,9]. Studies ongoing for more than a decade have provided firm evidence for the existence of a unique CD4+CD25+ population of "professional" regulatory/suppressor T cells that actively and dominantly prevent both the activation and the effector function of autoreactive T cells that have escaped other mechanism of tolerance [10,11]. The substances in PEs accompanying the infiltrate of immune cells, such as cytokines, chemokines, growth factors, and other soluble mediators, have been proposed to be helpful in the diagnosis of PEs [12]. The current study investigated differential diagnostic efficiencies of T cells subsets with IFN-γ, TNF α and ADA in distinguishing tuberculous from MPEs.

As shown in this study, evaluation of the lymphocyte subsets showed that T-cell markers (CD3+ T-cells, CD4+ T-cells and T reg cells) were significantly higher in TPE than MPE (76.46% vs. 65.29%; *P* 0.004, 51.21% vs. 43.50%; *P* 0.044 and 14.60% vs 12.43%; *P* 0.032 respectively). Several recent investigations [13–15] as well as our study, demonstrated a significant increase in the frequency of CD4+, Treg cells from patients with TB compared to patients with cancer. We did not study the mechanisms by which T cells subsets were recruited into TPE in the present study. The primary aim of this study was to compare T cells subsets in TPE and MPE. An increased percentage of CD4+CD25+ T cells in TPE might be due to active recruitment or local differentiation. It has been demonstrated that human CD4CD25 T cells preferentially migrate to pleural space, and the chemokine CCL22 mediates trafficking of CD4CD25 T cells to pleural space [16,17].

Using the ideal cutoff point according to the ROC analysis, for pleural T cells subsets CD3, CD4 and T regs, the thresholds were found to be 75%, 38% and 13% respectively. The AUC for all of these cells are greater than 0.50, CD3 (0.75), CD4

Table 2 Pleural fluid T cell subsets, enzyme activity of ADA, concentration of TNF-α, and IFN-y.

Group	CD3 Mean (SD)	CD4 Mean (SD)	T reg Mean (SD)	ADA Mean (SD)	TNF-α Mean (SD)	IFN-y Mean (SD)
Tuberculosis	76.49 (11.62)%	51.20 (11.26)%	14.60 (2.92)%	84.22 (41.47)	122.45 (47.69)	2.26 (1.62)
Malignancy	65.29 (11.68)%	43.50 (12.09)%	12.43 (3.24)%	23.19 (17.93)	35.03 (31.88)	0.30 (0.20)
P value	0.004	0.04	0.032	<0.0001	<0.0001	<0.0001

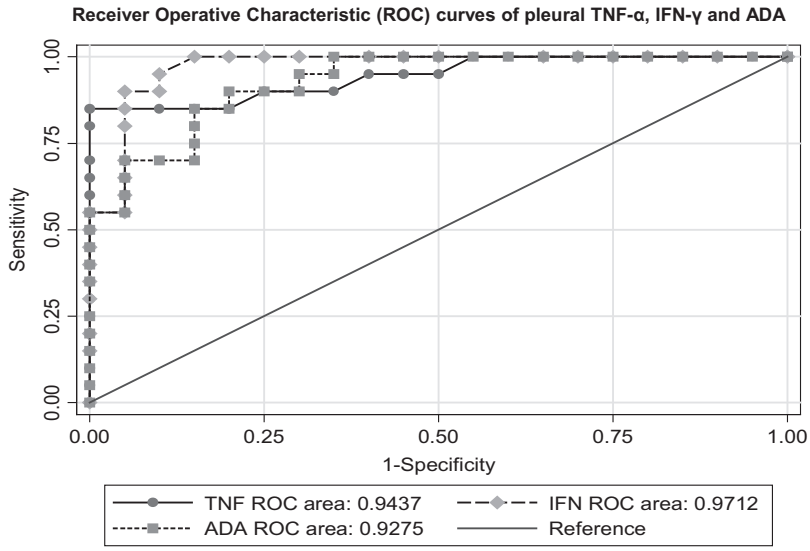

Figure 1 Receiver Operative Characteristic (ROC) curves of pleural TNF-α, IFN-γ and ADA. The cut-off values of TNF-α, IFN-γ and ADA were determined by the best sum of sensitivity and specificity on the ROC curve. Diagonal line indicates the line of no discrimination.

Table 3 Differential diagnostic significance of TNF-α, IFN-γ, ADA and T cell subset.

Bio-parameter	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	Accuracy (%)
TNF-α	0.94 (0.82–0.99)	⩾50	85.00	100.00	92.50
IFN-γ	0.97 (0.86–0.99)	⩾0.5	90.00	95.00	92.50
ADA	0.93 (0.80–0.99)	⩾35.5	85.00	85.00	85.00
CD3	0.75 (0.59–0.88)	⩾75.2	85.00	65.00	75.00
CD4	0.69 (0.53–0.83)	⩾38.2	40.00	95.00	67.50
T reg	0.68 (0.52–0.82)	⩾12.9	60.00	75.00	67.50

(0.69) and T reg (0.68). Interestingly, CD3 plus CD4 as well as CD3 plus CD4 plus T reg combinations were all 100% specific for discriminating TPE from MPE. Therefore we confirmed that all of these cells are of great reliability for the differential diagnosis of TPE from MPE.

Several studies have reported that adenosine deaminase (ADA), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ could serve as differential diagnosis biomarkers for pleural effusion caused by TB or malignant diseases [18–20]. The current study investigated the diagnostic value of TNF-α, IFN-γ and ADA in distinguishing TPE from pleural effusions of other etiologies. Previous literatures demonstrated significant accumulated TNF-α in TPE [18–20] compared to MPE. As expected, pleural level of TNF-α, ADA, and IFN- γ concentration were significantly higher in TPE than MPE (84.22 ± 41.47 vs. 23.19 ± 17.93 U/l: $P < 0.0001$, 122.45 ± 47.69 vs. 35.03 ± 31.88 pg/ml: $P < 0.0001$ and 2.26 ± 1.62 vs. 0.3 ± 0.20 IU/ml: $P < 0.0001$ respectively).

Using the receiver operating characteristic curve, we noted that the AUC for all of these bio parameters are greater than 0.5, TNF-α (0.94), IFN-γ (0.97) and ADA (0.93), indicating that TNF-α, IFN-γ and ADA are reliable for the clinical diagnosis of TPE. With the greatest AUC (0.97), the sensitivity, specificity and accuracy of IFN-γ are 90%, 95% and 92% respectively. TNF-α yielded comparable diagnostic values, AUC was 0.94, sensitivity was 85%, specificity was 100%, and accuracy was 92%. With the sensitivity of 85% and specificity of 85%, ADA was of less clinical accuracy than TNF-α and IFN-γ. The diagnostic accuracy of TNF-α and IFN-γ seemed to be the optimum marker in diagnosing TPE, though ADA had high precision. Therefore, we confirmed that all of these three bio parameters are of great reliability for the diagnosis of tuberculous pleural effusion.

To the best of our knowledge, the present study was the first attempt to combine all these methods for the diagnosis of pleural TB. In clinical practice (to avoid any misdiagnoses of diseases), the discriminating diagnosis is determined by taking many factors into account, rather than by testing any single method [21]. The combinations of two or more biomarkers are required to be positive for a diagnosis to be made, which increased the specificity at the expense of sensitivity. The measurement of TNF-α plus IFN-γ, TNF-α plus ADA, as well as TNF-α plus IFN-γ plus ADA, were 100% specific for discriminating TPE from MPE. Interestingly, CD3+ plus CD4+ as well as CD3+ plus CD4+ plus T reg combinations were all 100% specific for discriminating TPE from MPE. Furthermore, the specificity of combined-diagnostic value of IFN-γ, TNF-α, ADA with T cells subsets was >95%. These data suggest that combinations of biochemical parameters

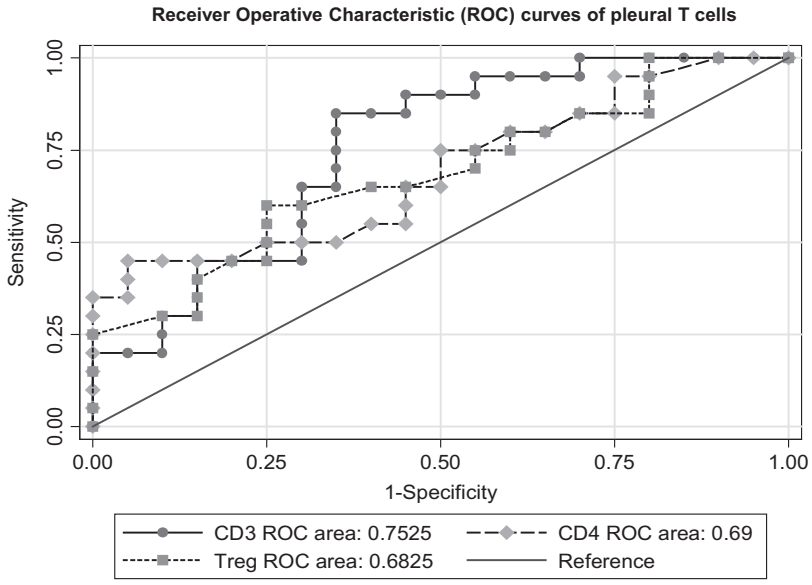

Figure 2 Receiver Operative Characteristic (ROC) curves of pleural T cells. The cut-off values of CD3+, CD4+ and T reg were determined by the best sum of sensitivity and specificity on the ROC curve. Diagonal line indicates the line of no discrimination.

Table 4 Combined-biochemical and T cell subset parameters' diagnostic value analysis.

Bio-parameter	Sensitivity (%)	Specificity (%)	Accuracy (%)
TNF-α & IFN-Y	80.00	100.00	90.00
TNF-α & ADA	70.00	100.00	85.00
IFN-γ & ADA	75.00	100.00	87.50
TNF-α, IFN-Y & ADA	65.00	100.00	82.50
CD3 & CD4	40.00	100.00	70.00
CD3 & T reg	30.00	85.00	57.50
CD4 & T reg	10.00	95.00	52.50
CD3, CD4 & T reg	10.00	100.00	55.00
TNF-α & CD3	75.00	100.00	87.50
TNF-α & CD4	35.00	100.00	67.50
TNF-α & T reg	55.00	100.00	77.50
ADA & CD3	70.00	100.00	85.00
ADA & CD4	30.00	100.00	65.00
ADA & T reg	45.00	95.00	70.00
IFN-Y & CD3	80.00	95.00	87.50
IFN-Y & CD4	35.00	100.00	67.50
IFN-Y & T reg	55.00	95.00	75.00

and T cells subsets are valuable markers for the differential diagnosis of TPE from MPE.

Conclusion

Our investigation suggested that when compared to malignant pleural effusion, T cells subsets, IFN-γ, TNF-α and ADA all increased in TPE. In addition, combinations yielded the optimal clinical accuracy on making differential diagnosis between TPE and MPE.

Conflicts of interest

The authors have no conflicts of interest to declare in relation to this article. The authors are responsible for the content and the writing of the paper.

References

[1] World Health Organization, Global Tuberculosis Control: Surveillance, Planning, Financing, WHO report, Geneva, 2011.
[2] M.P. Golden, H.R. Vikram, Extrapulmonary tuberculosis: an overview, Am. Fam. Physician 72 (9) (2005) 1761–1768.
[3] G.M. Winslow, A. Cooper, W. Reiley, M. Chatterjee, D.L. Woodland, Early T-cell responses in tuberculosis immunity, Immunol. Rev. 225 (2008) 284–299.
[4] Z.H. Tong, HZ. Subpopulations of helper T lymphocytes in tuberculous pleurisy, Tuberculosis 93 (3) (2013) 279–284.
[5] X.J. Qin, H.Z. Shi, Q.L. Liang, L.Y. Huang, H.B. Yang, CD4+CD25+ regulatory T lymphocytes in tuberculous pleural effusion, Chin. Med. J. 121 (2008) 581–586.
[6] Y.Q. Chen, H.Z. Shi, X.J. Qin, et al, CD4+CD25+ regulatory T lymphocytes in malignant pleural effusion, Am. J. Respir. Crit. Care Med. 172 (2005) 1434–1439.
[7] J. Ferrer, Pleural tuberculosis, Eur. Respir. J. 10 (4) (1997) 942–947.
[8] H. Fujiwara, I. Tsuyuguchi, Frequency of tuberculin-reactive T-lymphocytes in pleural fluid and blood from patients with tuberculous pleurisy, Chest 89 (1986) 530–532.
[9] H. Shiratsuchi, I. Tsuyuguchi, Analysis of T cell subsets by monoclonal antibodies in patients with tuberculosis after in vitro stimulation with purified protein derivative of tuberculin, Clin. Exp. Immunol. 57 (1984) 271–278.
[10] E.M. Shevach, CD4+CD25+ suppressor T cells: more questions than answers, Nat. Rev. Immunol. 2 (2002) 389–400.
[11] H.Z. Shi, X.J. Qin, CD4+CD25+ regulatory T lymphocytes in allergy and asthma, Allergy 60 (2005) 986–995.
[12] A. Trajman, M. Pai, K. Dheda, et al, Novel tests for diagnosing tuberculous pleural effusion: what works and what does not?, Eur Respir. J. 31 (2008) 1098–1106.

[13] L. de Aguiar, L. Antonangelo, F. Vargas, et al, Malignant and tuberculous pleural effusions: immunophenotypic cellular characterization, Clinics 63 (5) (2008) 637–644.
[14] H. Yang, H. Shi, T lymphocytes in pleural effusion, Chin. Med. J. 121 (2008) 579–580.
[15] J. Fontenot, A. Rudensky, A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3, Nat. Immunol. 4 (2005) 331–337.
[16] X. Qin, H. Shi, Q. Liang, L. Huang, H. Yang, CD4CD25 regulatory T lymphocytes in tuberculous pleural effusion, Chin. Med. J. 121 (7) (2008) 581–586.
[17] C. Wu, Q. Zhou, X. Qin, S. Qin, H. Shi, CCL22 is involved in the recruitment of CD4_CD25 high T cells into tuberculous pleural effusions, Respirology 15 (2010) 522–529.
[18] A. Ciledag, A. Kaya, S. Erol, et al, The comparison of pleural fluid TNF-alpha and IL-10 levels with ADA in tuberculous pleural effusion, Curr. Med. Chem. 17 (19) (2010) 2096–2100.
[19] Z.C. Gao, R.X. Tian, Clinical investigation on diagnostic value of interferon-gamma, interleukin-12 and adenosine deaminase isoenzyme for tuberculous pleurisy, Chin. Med. J. 118 (3) (2005) 234–237.
[20] L. Valdes, E. San Jose, L. Ferreiro, et al, Interleukin 27 could be useful in the diagnosis of tuberculous pleural effusions, Respir. Care 59 (3) (2014) 399–405.
[21] Y.B. Wu, Z.J. Ye, S.M. Qin, C. Wu, Y.Q. Chen, H.Z. Shi, Combined detections of interleukin 27, interferon-gamma, and adenosine deaminase in pleural effusion for diagnosis of tuberculous pleurisy, Chin. Med. J. (Engl.) 126 (17) (2013) 3215–3221.