Potential diagnostic value of pleural fluid cytokines levels for tuberculous pleural effusion

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Research Article

Keywords: Tuberculous, Pleural effusion, Diagnosis

Posted Date: September 2nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-68599/v1

Version of Record: A version of this preprint was published at Scientific Reports on January 12th, 2021. See the published version at https://doi.org/10.1038/s41598-020-79685-1.

Abstract

Background: Tuberculous pleural effusion (TPE) is one of the most common forms of extrapulmonary tuberculosis. Patients with tuberculous or malignant pleural effusions (MPE) frequently have similar clinical manifestations and pleural fluid profile. New biomarkers for the differential diagnosis of TPE are required.

Objective: We sought to determine of whether cytokine profiles in the pleural effusion of patients were suitable as tools for the differential diagnosis of TPE.

Methods*:* 30 patients with TPE, 30 patients with MPE, 14 patients with empyema and 14 patients with parapneumonic effusion were enrolled consecutively from the Masih Daneshvari Hospital, Tehran, Iran between Dec 2018-Dec 2019. The levels of interleukin (IL)-6, IL-18, IL-27, CXCL-8, CCL-1 and IP-10 were determined in pleural effusions by ELISA along with measurements of adenosine deaminase (ADA).

Results*:* The levels of all analytes measured except IL-18 were higher in TPE compared with non-TPE subjects (all $p < 0.01$). The best predictors of TPE were combined ADA.IL-27 (optimal cut-off value = 42.68 10^3.U.ng/L^2, sensitivity 100%, specificity 98.28%, $p \leq 0.0001$), ADA (optimal cut off value 27.5 IU/L, sensitivity 90%, specificity 96.5%, $p \leq 0.0001$) and IL-27 (optimal cut-off value = 2363 pg/ml, sensitivity 96.7%, specificity 98.3%, $p \leq 0.0001$). A high level of IL-6 (optimal cut-off value = 3260 pg/ml, sensitivity 100%, specificity 67.2%, $p \leq 0.0001$), CXCL-8 (optimal cut-off value = 144.5 pg/m, sensitivity 93.3%, specificity 58.6%, $p \leq 0.0001$), CCL-1 (optimal cut-off value = 54 pg/mL, sensitivity 100%, specificity 70.7%, $p \leq 0.0001$) and IP-10 (optimal cut-off value = 891.9 pg/mL, sensitivity 83.3%, specificity 48.3%, $p = 0.0001$) were also predictive of TPE.

Conclusion: High ADA.IL-27, ADA and IL-27 levels differentiate between TPE and non-TPE with improved specificity and diagnostic accuracy.

Introduction

Mycobacterium tuberculosis (*Mtb*) is one of the oldest and most important human pathogens and infection with *Mtb* has a high rate of mortality worldwide (1). Nearly one-third of the world's population is asymptomatically (latently) infected with tuberculosis, and about 3 to 10 percent of these people progress to active disease throughout their life. In 2018, ten million people became infected with tuberculosis and 1.5 million died which including 0.3 million people con-infected with HIV. (https://www.who.int/tb/global-report-2019).

Tuberculosis has two forms in human being based on affected organs, one pulmonary and second is extra-pulmonary tuberculosis. In extra-pulmonary form many organs may involve but importantly pleural involvement is an important manifestation of the disease (2). Pleura are divided into a parietal layer and visceral layer. The parietal layer lines the inner aspect of the chest wall whilst the visceral layer covers the interlobar fissures. These two layers are separated by a cavity that contains 1-10 ml of fluid (3). Pleural effusions (PEs) are an accumulation of fluid between the pleural layers (4) and are a clinical problem induced by several etiologies such as local diseases of the pleura and resulting from increased pressure to the lung, organ dysfunction, systemic diseases, pulmonary infections, pleural tumor metastasis and tuberculous pleurisy (5).

Differential diagnosis of TPE from other pleural effusions, especially malignant pleural effusion (MPE), is challenging clinically (6). TPE and MPE are both of lymphocytic source (2). The gold standard for differentiating TPE from other pleural effusions with different etiologies is the isolation *Mtb* from either pleural fluid or pleural biopsy (100% specificity) (7). Although culturing of sputum has a diagnostic value with 100% specificity, it is time consuming and delays the diagnosis. Manifestations of granuloma (~95%), provided that other causes of granulomatosis are discounted, is also used to diagnose tuberculous pleurisy but is considered as an invasive approach (8).

TPE is a delayed hypersensivity reaction to *Mtb* and mainly the result of pathological immune response associated with increasing in the cytokines, including interleukins (ILs) and chemokines (9-11). Based on previous studies (12-16) we hypothesized that cytokine and chemokine levels in TPE may differentiate this disease from other causes of PE. Thus, in the current study, we measured the levels of adenosine deaminase (ADA), IL-6, IL-18, IL-27, CXCL-8, CCL-1 and IP-10 in the pleural fluid and shown that ADA, IL-6, IL-27, combined ADA-IL-27, CXCL-8, CCL-1 and IP-10 levels are significantly higher in TPE than in other PE containing groups. In addition, we report that levels of ADA, IL-27 and combined ADA.IL-27 have the best sensitivity and specificity to predict the diagnosis of TPE.

Materials And Methods

Patient selection

The study protocol was approved by the Institutional Review Board for human studies of clinic center from Massih Daneshvari Hospital, Tehran, Iran. The study was carried out in accordance with the approved Ethics (Ethic code: IR.SBMU.MSP.REC.1397.584). From December 2018-December 2019, 190 consecutive patients with pleural effusions of unknown causes were enrolled. Patients were referred to the Infection wards of the Massih Daneshvari Hospital from across Iran.

Inclusion criteria included: a) no invasive procedures to the pleural cavity, b) not receiving anti-tuberculosis therapy, and c) not suffering from lung trauma for three months prior to hospitalization. At the time of sampling, none of the patients received antibiotic therapy, anti-tuberculosis drugs, anti-

malignancy treatments, corticosteroids, or non-steroidal anti-inflammatory drugs. Patients without a clear diagnosis and patients with more than one possible etiology of effusion, and with a pleural transudate, hemothorax or chylothorax were excluded from study.

Thirty HIV negative patients, aged 18-84 yr., with a positive *Mtb* test in biopsy specimens and pleural tissue granuloma were enrolled as TPE. 30 patients, aged 32-80 yr., newly diagnosed with malignant cells in their pleural fluid and/or on pleural biopsy specimen were enrolled in the MPE group. Based on histologically analysis, 15 cases were adenocarcinoma, 10 patients had squamous cell carcinoma (SCC) and 5 patients suffered from non-squamous cell carcinoma (NSCC).

Empyema (EMP) was confirmed in 14 patients, aged 20-75yr by the presence of frank pus in their pleural effusions or smear or positive bacterial or fungal culture of pleural fluid (except for *Mtb*). 14 patients with parapnemonia (PPE) were also included based on PE findings including glucose <60 mg/dl; pH<7, LDH>1000 and no organisms found in staining culture of effusion.

Sample collection and processing

The pleural fluid (5 mL) was collected in heparin containing tubes, by thoracocentesis within 24h of hospitalization and immediately placed in ice. Tubes were centrifuged at 1200 x g for 5 min and mononuclear cells isolated by Ficoll-Hypaque gradient (Pharmacia, Uppsala, Sweden) within 1h. Total and differential cell counts, protein, lactate dehydrogenase (LDH), ADA, glucose, cytology, and bacterial examination were evaluated in the biochemistry laboratory of the Masih Daneshvari Hospital. In addition; the cell-free supernatants of pleural fluid were frozen at –80 °C immediately after centrifuge for later determining concentrations of cytokines by ELISA.

Measurement of cytokines and chemokines

The concentrations of IL-6 and IL-27 were measured by enzyme linked immunosorbent assay (ELISA) (R&D SYSTEM, Minneapolis, MN, US). The concentrations of IL-18, CCL-1, and IP-10 were measured by ELISA (Invitrogen by Thermo Fisher Scientific, Vienna, Austria). CXCL-8 was measured by ELISA (BD Biosciences, CA, USA) according to the manufacturer's protocol.

Statistical analysis

Analysis was performed using SPSS version 16.0 (SPSS, Inc. Chicago, USA) and GraphPad Prism software (version 6; 07 GraphPad Software, Inc.). Non-parametric Mann-Whitney U test (Median, 95% confidence intervals (CI) was used for the non-normally distributed variables and a t-test (Mean ± SEM) used for normally distributed variables. Receiver operating characteristic (ROC) curve analyses were used to evaluate the capacity of ADA and other biomarkers to differentiate TPE from non-TPE. The area under the ROC curve (AUC) was calculated, and 95% confidence intervals (CIs) were used to test the hypothesis that the AUC is 0.5. An optimum cut-off value was established by using Receiver operating Curve (ROC). P-values < 0.05 were considered as statistically significant.

Results

190 patients were enrolled in this study with 145 subjects providing PE exudates and 45 subjects with PE transudate (**Fig.1A, 1B**). 102 samples were excluded due to failure to meet the diagnostic criteria (30), transudate effusions (45) and exudates with miscellaneous etiology (27) (**Fig. 1B**). A total of 88 patients were included in the current study and classified in 4 diagnostic groups: TPE, MPE, PPE and EMP. The demographic data of the patients and their biochemical characteristics are given in **Tables 1-3**. The distribution of the cytokines and chemokines in each group of subjects are summarized in **Table 4**.

ADA levels can discriminate between TPE and MPE

Patients with TPE show a significant elevation of pleural protein (P = 0.0055) and LDH (P ≤ 0.0001) (**Tables 2 & 3**). The levels of adenosine deaminase (ADA) in TPE (42.73 ± 1.71 IU/L) were also significantly higher than in non-TPE subjects (18.86 ± 0.7045 IU/L) (P ≤ 0.0001) (**Tables 2 & 3, Fig. 2A & 2D**). The area under curve (AUC) when ADA was used to differentiate TPE from non-TPEs (including malignant, empyema and parapneumonic) was 0.975 (95% confidence interval, 0.9471 to 1.005; p ≤ 0.0001) (**Fig. 2B**). With a cut-off value of 27.5 IU/L, we obtained a sensitivity of 90%, a specificity of 96.5%, together with positive likelihood ratios (PLR = 26.1), negative likelihood ratio (NLR = 0.1), positive predictive value (PPV = 93.1), negative predictive value (NPV = 94.9) and diagnostic accuracy of 94.3% (**Table 5**). The ROC analysis of ADA levels in TPE and MPE gives an AUC of 1.0 (95% CI: 1.0 to 1.0, p ≤ 0.0001) (**Fig. 2C**). With a cut-off value of 20.5 IU/L, we obtained a sensitivity of 100%, a specificity of 100%, together with PLR = 1/0 (-), NLR = 0, PPV = 100, NPV = 100 and a diagnostic accuracy of 100% (**Table 6**).

Cytokine levels in tuberculous and non-tuberculous pleural fluid.

The concentrations of ADA, IL-27, combined ADA.IL-27, IL-6, CXCL-8, CCL-1and IP-10 in the TPE and non-TPE (MPE, EMP, PPE) groups were detected in the pleural fluid. We found that these cytokines in the TPE group were higher than the respective levels in the non-TPE groups (**Table 4**). The

concentration of IL-18 in the TPE group was not significantly different from non- TPE groups (**Table 4**).

The median IL-27 concentration in the TPE group was 4725 pg/ml (25–75% percentile, 3993-7598 pg/ml), which was significantly higher than in non-TPE groups (978 pg/ml/ 25–75% percentile, 835.3-1401 pg/ml; P ≤ 0.0001) (**Table 4, Fig. 3A**). This gave a high AUC value of 0.9879 (95% CI: 0.9640 to 1.012; p ≤ 0.0001) (**Fig. 3B**). Furthermore we determined the optimal IL-27 cut-off value of 2363 pg/mL in the pleural fluid by ROC curve. With this cut-off value, a sensitivity of 96.67% (95% CI: 82.78 to 99.92%), a specificity of 98.28% (95% CI: 90.76 to 99.96%), together with PLR = 56.07, NLR = 0.03, PPV = 96.6 and NPV = 98.2 for TPE diagnosis compared with non-TPEs. Thus, the diagnostic accuracy of IL-27 levels in pleural effusion was 97.72 % (86/88) (**Table 5**). The comparison of IL-27 levels in the TPE group and the others non-TPEs are shown in **Table 4** and **Fig. 3C.** The capacity of IL-27 to differentiate TPE from MPE group was assessed with ROC curve analysis. The ROC analysis shown an AUC of 0.99 (95% CI: 0.9952 to 1.003) (**Fig. 3D**) and the optimal cut-off value 1028 pg/ml. Using this cut-off value, the sensitivity of 100% (95% CI: 88.43–100%) and specificity of 96% (95% CI: 82.87–99.92%) could be used for diagnosing of TPE. The PLR = 25, NLR = 0, PPV = 96.7, NPV = 100 and Diagnostic value = 98.3% (59/60) were calculated for IL-27 (**Table 6**).

Since IL-27 and ADA were the best individual predictors of TPE we determined whether combined IL-27.ADA would provide even greater predictive ability. The median ADA.IL-27 level in the TPE group was 208.5 10^3.U.ng/L^2 (25–75% percentile, 178.2-261.6 10^3.U.ng/L^2), was significantly higher than in the non-TPE group (19.23 10^3.U.ng/L^2/25–75% percentile, 13.3-25.42 10^3.U.ng/L^2; P ≤ 0.0001) (**Table 4, Fig. 4A**). This was associated with a high AUC of 0.9994 (95% CI; 0.9975 to 1.001; P ≤ 0.0001) (**Fig. 4B**). However, the ROC curve (**Fig. 4B**) show that ADA.IL-27 separated TPE from the non-TPE group better than did ADA (0.9471) or IL-27 (0.9879). Furthermore, we determined the optimal ADA.IL-27 cut-off value of 42.68 10^3.U.ng/L^2 in the pleural fluid by ROC curve. With this cut-off value, a sensitivity 100% (95% CI: 88.43 to 100.0%) a specificity of 98.28% (95% CI: 90.76 to 99.96%) together with PLR = 58, NLR = 0.0, PPV = 96.78 and NPV = 100 for TPE diagnosis compared with non-TPEs. Thus, the diagnostic accuracy of IL-27.ADA levels in pleural effusion was 98.86% (87/88) (**Table 5**). The comparison of ADA.IL-27 levels in the TPE group and non-TPE group are shown in **Table 4** and **Fig. 4C.** The capacity of ADA.IL-27 to differentiate TPE from MPE group was assessed with ROC curve analysis. The ROC analysis shown an AUC of 1.0 (95% CI: 1.0 to 1.0) (**Fig. 4D**) and the optimal cut-off value 31.14 10^3.U.ng/L^2 Using this cut-off value, the sensitivity of 100% (95% CI: 88.43–100%) and specificity of 100% (95% CI: 88.43–100%) could be used for diagnosing of TPE. The PLR = 1/0 (-), NLR = 0, PPV = 100, NPV = 100 and Diagnostic value = 100% (60/60) were calculated for ADA.IL-27 (**Table 6**).

The median IL-6 level in the TPE group was 5735 pg/ml (25–75% percentile, 4935-6302pg/ml) was significantly higher than in the non-TPE group (2273 pg/ml/ 25–75% percentile, 1121-3832 pg/ml; P ≤ 0.0001) (**Table 4, Fig. 5A**). This was associated with a high AUC value of 0.9342 (95% CI; 0.8854-0.9830; P ≤ 0.0001) (**Fig. 5B**). An optimal IL-6 cut-off value of 3260 pg/mL for PE was calculated using the ROC curve. A sensitivity of 100% (95% CI: 88.43-100.0%), a specificity of 67.24 (95% CI: 53.66-78.99%), PLR = 3.03, NLR = 0, PPV = 61.2 and NPV = 100 were obtained for differentiating of TPE from non-TPEs. The diagnostic accuracy this cytokine for TPE diagnose was 78.4% (69/88) (**Table 5**). The comparison of IL-6 levels in the TPE group and non-TPE subjects is shown in **Table 4** and **Fig. 5C**. The ROC analysis of IL-6 levels in the TPE and MPE groups gave an AUC of 0.99 (95% CI: 0.9737 to 1.006; P ≤ 0.0001) (**Fig. 5D**) and the optimal cut-off value of 3026 pg/ml. Using this cut-off value, a sensitivity of 100% (95% CI: 40.60-77.34%), a specificity of 90% (95% CI: 88.43-100.0%), PLR = 10, NLR = 0, PPV = 90.9, NPV = 100 and diagnostic accuracy for the differential diagnosis of TPE from MPE was 95% (57/60) (**Table 6**).

The median CXCL-8 concentration in the TPE group was 1038 pg/ml (25–75% percentile, 344.1-1853 pg/ml), which was significantly higher than with the non-TPEs (123 pg/ml/ 25–75% percentile, 67.75-485.6 pg/ml; P ≤ 0.0001) (**Table 4, Fig. 6A**). This was associated with a moderate AUC value of 0.7905 (95% CI: 0.6942 to 0.8868; P ≤ 0.0001) (**Fig. 6B**). The optimal CXCL-8 cut-off value of 144.5 pg/mL was determined by the ROC curve. With this cut-off value, a sensitivity of 93.3% (95% CI: 77.93 to 99.18%), a specificity of 58.6% (95% CI: 44.93 to 71.40%), PLR = 2.256, NLR = 0.11, PPV = 53.8 and NPV = 94.4 were calculated for the TPE diagnosis. The diagnostic accuracy of CXCL-8 level was 70.45% (62/88) for differentiating of TPE from non-TPEs (**Table 5**). The comparison of CXCL-8 levels in TPE with non-TPE groups is shown in **Table 4** and **Fig. 6C**. The ROC curve analysis of CXCL-8 level in the TPE and MPE groups shown an AUC of 0.7839 (95% CI: 0.6664 to 0.9013; P = 0.0002) (**Fig. 6D**) and an optimal cut-off value of 144.5 pg/ml. Using this cut-off value, a sensitivity of 93.3% (95% CI: 77.93 to 99.18%), a specificity of 60% (95% CI: 40.60 to 77.34%), PLR = 2.33, NLR = 0.11, PPV = 70, NPV = 90 and diagnostic accuracy of 76.6% (46/60) was obtained for diagnosing of TPE from MPE (**Table 6**).

The median CCL-1 levels in the TPE group was 565 pg/ml (25–75% percentile, 104.8-2353 pg/ml), which was significantly higher than in the non-TPE group (32.2 pg/ml/ 25–75% percentile, 12.2-61.98 pg/ml; P ≤ 0.0001) (**Table 4, Fig. 7A**). This was associated with an AUC value of 0.905 (95% CI; 0.8441-0.9662; P ≤ 0.0001) (**Fig. 7B**). The optimal CCL-1 cut-off value of 54 pg/ml was calculated from the ROC curve and gave a sensitivity of 100% (95% CI: 65.28 to 94.36%), a specificity of 70.69% (95% CI: 34.95 to 61.78%), PLR = 3.4, NLR = 0, PPV = 63.8 and NPV = 100 for diagnosing TPE. The diagnostic accuracy of CCL-1 levels for diagnosing of TPE from non-TPEs was 80.68% (71/88) (**Table 5**). The comparison of CCL-1 levels between TPE and non-TPEs groups is shown in **Table 4** and **Fig. 7C**. The ROC analysis of CCL-1 levels in the TPE and MPE gives an AUC of 0.9867 (95% CI: 0.9641 to 1.009; P ≤ 0.0001) (**Fig. 7D**) and an optimal cut-off value of 51.85 pg/ml. Using this cut-off value, a sensitivity of 100% (95% CI: 88.43% to 100%), a specificity of 93.3% (95% CI: 77.93% to 99.18%), PLR = 14.28, NLR = 0, PPV = 93.7, NPV = 100 and diagnostic accuracy of 96.6% (58/60) were obtained for the differential diagnosis of TPE from MPE (**Table 6**).

The median IP-10 level in the TPE group was 2833 pg/ml (25–75% percentile, 909-4184 pg/ml), was significantly higher than in the non-TPE group (944.4 pg/ml/ 25–75% percentile, 355.5-1970 pg/ml; P = 0.0001) (**Table 4, Fig. 8A**). This was associated with a moderate AUC of 0.746 (95% CI; 0.6350 to 0.8569; P = 0.0002) (**Fig.8B**). The optimal IP-10 cut-off value of 891.9 pg/ml for TPE was determined using the ROC curve. With this cut-off value, a

sensitivity of 83.33% (95% CI: 65.28 to 94.36%), a specificity of 48.28% (95% CI: 34.95 to 61.78%), PLR = 1.61, NLR = 0.35, PPV = 45.5 and NPV = 84.8 for TPE diagnosis was obtained. The diagnostic accuracy of IP-10 level for TPE differentiating was 60.2% (53/88) (**Table 5**). The comparison of IP-10 levels between TPE and non-TPE groups are shown in **Table 4** and **Fig. 8C**. The ROC analysis of IP-10 levels in TPE and MPE gave an AUC of 0.8244 (95% CI: 0.7197 to 0.9292; P ≤ 0.0001) (**Fig. 8D**) and an optimal cut-off value of 2072 pg/ml. Using this cut-off value, a sensitivity of 60% (95% CI: 40.60% to 77.34%), a specificity of 100% (95% CI: 88.43% to 100%), PLR = 1/0 (-), NLR = 0.4, PPV = 100, NPV = 84.8 and a diagnostic accuracy of 80% (48/60) were obtained for the differential diagnosis of TPE from MPE (**Table 6**).

The median IL-18 concentration in the TPE group was 2196 pg/ml (25-75%, 513.4-3035 pg/ml) was not significantly different from non-TPE groups (1003 pg/ml/ 25-75% percentile, 577.8-2291 pg/ml) (P = 0.1797) (**Table 4, Fig. 9A**). This was associated with a low AUC value of 0.5879 (95% CI: 0.454 to 0.7219; P = 0.178) (**Fig. 9B**). The optimal IL-18 cut-off value of 2285 pg/ml for differentiating TPE from non-TPE was obtained from the ROC curve. The cut-off value gave a sensitivity of 50% (95% CI: 31.3- 68.7%), a specificity of 75.9% (95% CI: 62.83–86.13%), PLR = 2.07, NLR = 0.66, PPV = 51.7 and NPV = 74.5 for diagnosing TPE. The diagnostic accuracy of IL-18 in PEs was 67.04% (59/88) (**Table 5**). IL-18 levels in TPE and non-TPE groups are shown in **Table 4** and **Fig. 9C** were shown. ROC curve analysis for IL-18 in TPE and MPE gave an AUC of 0.7778 (95% CI: 0.6537 to 0.9019; P = 0.0002) (**Fig. 9D**). The optimal cut-off value of 1200 pg/ml was for differentiating of TPE from MPE gave a sensitivity of 60% (95% CI: 40.6-77.34%), a specificity of 100% (95% CI: 88.43-100%), PLR = 1/0 (-), NLR = 0.4, PPV = 100, NPV = 71.4 and a diagnostic accuracy of 80% (48/60) for the differential diagnosis of TPE from MPE (**Table 6**).

Discussion

In this study, we demonstrated that the levels of ADA, IL-27, ADA.IL-27, IL-6, CXCL-8, CCL-1 and IP-10 in PE were elevated in patients with TPE compared to non-TPE subjects including empyema, parapneumonia, and malignancy. In contrast, IL-18 levels were similar between TPE and non-TPE subjects. We also described the predicted value of the former cytokines and chemokines for differentiating between TPE and non-TPE diagnosis. We identified specific cut-off levels that gave good-excellent discrimination between causes of PE and among the markers studied, ADA.IL-27 had the highest and IP-10 had the lowest diagnostic accuracy. The predictive ability of IL-27 was similar to that of ADA. The sensitivity and specificity of ADA.IL-27 was higher than that of IL-27 and ADA alone and improved the diagnostic capability.

TB, malignancy, cardiovascular disease and infections can also result in PE. Although several methods including biochemical tests, cytology, bacterial culture and biopsy examination are used to determine the cause of PE in many cases the etiology remains ill-defined (17). Current diagnostic tests for *Mtb* within pleural fluid have variable results. For example, the positive rate for a smear test for bacillus tuberculosis is about 10% but can be increased to 20-30% by cultivation. The diagnostic value increases further to 70-80% using pleural biopsies (8). Thus, developing a simple test that enables the differential diagnosis of PE-causing diseases.

Various analytes within PE are currently being studied as potential biomarkers of disease etiology including ADA, LDH, CRP and IFN-g. For example, 40 IU/ml ADA in PE has a sensitivity (81-100%) and specificity (83-100%) for TPE (18). Reducing the ADA cut-off value to >35 U/mL results in a lower sensitivity (93%) and specificity (90%) for diagnosing TPE (19). In addition, several studies have shown that IFN-γ levels are elevated in TB pleurisy (20-22). In a recent meta-analysis the sensitivity and specificity of IFN-γ for the diagnosis of TPE were 89% and 97% respectively (22). In addition, PCR methods have been used to track tubercle bacilli in pleural effusion at the early stage. However, the range reported for a positive test is between 12–100% (23). Due to these diverse results and the time taken to culture *Mtb* it is clear that new discriminatory biomarkers are needed.

IL-27 is a member of the IL-12 cytokine family. TB enhances the production and secretion of IL-27 from antigen presenting cells (APCs) increasing its local concentration (24). Previous studies have shown the high diagnostic value of IL-27 cytokine for the diagnosis of TPE (2, 16, 25) with higher levels of IL-27 in TPE than in non-TPE patients (16, 26). Wu and colleagues reported that the diagnostic accuracy of IL-27 was even higher than seen with IFN-γ or with ADA with a sensitivity of 95% and specificity of 97.6% (16). A meta-analysis of IL-27 studies in TPE gave a pooled sensitivity of 0.92 (95% CI, 0.90–0.95) with a specificity of 0.90 (95% CI, 088–0.92) (26). In the current study, we found an equally high sensitivity and specificity of IL-27 for the diagnosis of TPE.

IL-6 is a pleiotropic cytokine with broad-ranging immune effects (27). Importantly, IL-6 promotes IFN-γ production during TB infection in mice (28). Increased levels of IL-6 have previously been reported in TB pleurisy compared to non-TB pleurisy subjects (29) which supports our findings. In that earlier study the sensitivity and specificity of IL-6 in predicting TPE compared to non-TPE were 90.6% and 76.5% respectively (29). Furthermore, Xirouchaki and colleagues reported that IL-6 levels were significantly higher in TPE patients than in PPE subjects (30). In the current study, the level of IL-6 in TPE group was higher than in non-TPE groups, and sensitivity and specificity were 100% and 67.24% respectively.

IL-18 is an IFN-g factor (31) which, often acts in concert with IL-12 (32). Increased pleural concentrations of IL-18 have been reported previously in TPE (31). In a meta-analysis study, the sensitivity and specificity of IL-18 for the diagnosis of TPE were 87% and 92% respectively (14). However, in our study we found no difference in the levels of IL-18 in TPE and non-TPE patients and subsequently a much lower sensitivity and specificity which may suggest genetic differences between Chinese and Iranian subjects or the clinical definitions used. Interestingly, IL-18 was able to differentiate between TPE and MPE.

CXCL-8 causes chemotaxis and localization of neutrophils and lymphocytes within the pleural space in man (33). CXCL-8 was found previously to be higher in patients with empyema and parapneumonia compared to cases of non-infectious effusions (MPE and TPE) (34-36). For example, Ceyhan

and colleagues reported that CXCL-8 levels were significantly higher in empyema/parapneumonic effusions groups rather than TB (34). However, Dlugovitzky and co-workers showed statistically higher CXCL-8 levels in TPE patients compared with PPE patients (37). In the current study, CXCL-8 levels were statistically higher in the TPE group compared to non-TPE patients. This reflects the important role of CXCL-8 in stimulating T cells and participating in the granulomatous formation process (37) but also the need to get better quantification and validation in larger cohorts.

CCL1 or I-309 is a monocyte attractant (38) and has been implicated in the formation and maintenance of granuloma following Mtb infection (39). CCL-1 levels were significantly raised in the plasma of patients with pulmonary tuberculosis (PTB) (40). In this study, we show for the first time that this chemokine can be used for the diagnosis of TPE with a sensitivity and specificity of 100% and 70.69%.

IFN-γ inducible protein (IP-10) is produced by antigen presenting cells in response to Mtb-specific antigens in patients with active TB (41, 42). IP-10 levels in TPE patients were reported previously to be significantly higher than in non-TPE patients groups with a sensitivity (80%) and specificity (82%) for the diagnose of TPE using a cut-off point of 28170pg/ml (43). We report similar findings in the current study with IP-10 levels in TPE patients being higher than those in non-TPE subjects.

This study shows for the first time, that CCL-1 was a good predictor of TPE compared to non-TPE subjects with 100% sensitivity and a specificity of 70.7%. However, its diagnostic value was less than that of ADA, IL-27 and combined ADA.IL-27. There were some limitations to this study including the restricted number of patient samples and the analysis of only four PE disease groups. Our study indicates an excellent predictive value for IL-27 (97.72%) and ADA (94.3%), a high sensitivity and specificity for the differential diagnosis of TPE. In addition, we show an even greater accuracy of ADA.IL-27 as a predictive marker of TPE (98.86%). Our data demonstrated that soluble mediators obtained from PE samples may provide high levels of sensitivity and specificity for pleural diseases which may be applicable for the development of a rapid and non-invasive diagnostic test.

In summary, we have demonstrated that CCL-1 is significantly higher in TPE patients compared to non-TPE patients and is a good differentiator between clinical groups. Furthermore, combining ADA.IL-27 improves the predictive value of ADA and IL-27 on their own and could be useful as a supporting biomarker in the differential diagnosis of TPE.

Declarations

Ethics approval and consent to participate:

The study was approved by the Ethics Committee of the Dr. Masih Daneshvari Hospital, and all patients gave signed informed consent (ethic code: IR.SBMU.MSP.REC.1397.584).

Consent for publication:

All authors have read the manuscript and consent to publication in the Journal

Availability of data and material:

Not applicable

Competing interests:

The authors confirm that there are no competing interests.

Funding:

This study was supported by internal funding.

Tables

Table 1: Demographic data of the study population.

	Patients	TPE	MPE	EMP	PPE	P value†
N	88	30	30	14	14	
Age, yr.	56.6± 1.9	52.3 ± 3.9	59.5 ± 2.8	56.0 ± 4.2	59.9 ± 3.2	0.3676
Sex (F/M), n	43/45	13/17	19/11	6/8	5/9	

Values are presented as mean ± standard error of the mean (SEM). TPE = tuberculous pleural effusion, MPE = malignant pleural effusion, EMP = Empyema, PPE = Parapneumonic.

†Comparisons of data between TPE, MPE, EMP and PPE effusions were performed using one-way analysis of variance.

Table 2: Biochemical and cytological characteristics of pleural effusions *

	TPE	MPE	EMP	PPE
LDH (IU/L)	631.9 ± 19.7†	382.8 ± 10.6	657.4 ± 18.2	413.2 ± 24.4
ADA (IU/L)	42.7 ± 1.7†	14.5 ± 0.6	22.2 ± 1.2	24.1 ± 0.4
Protein (g/L)	39.2 ± 1.8†	33.0 ± 1.5	35.9 ± 2.3	31.8 ± 2.6
Differential cell counts, %				
Lymphocytes cells	72.8 ± 1.07†	53.5 ± 0.7	14.4 ± 0.8†	19.3 ± 0.9†
Neutrophils cells	8 ± 0.4	5.9 ± 0.3	78.4 ± 0.9†	53.7 ± 1.1
Macrophage cells	12.5 ± 0.4†	31.2 ± 0.4†	5.7 ± 0.5†	8.6 ± 0.5
Mesothelial cells	3.1 ± 0.2†	6.6 ± 0.3†	5.7 ± 0.3†	1.6 ± 0.3†
Malignant cells	-	3.5 ± 0.3	-	-

Values are presented as mean ± standard error of the mean (SEM). TPE = tuberculous pleural effusion, MPE = malignant pleural effusion, EMP = Empyema, PPE = Parapneumonic.

Table 3: Comparison of biochemical variables between tuberculous pleural effusion (TPE) and non-TPE subjects

	TPE	Non-TPE	P value
LDH (IU/L)	631 ± 19.7*	456.4 ± 17.5	P≤0.0001
Protein (g/L)	39.2 ± 1.78	33.40 ± 1.14	0.0055
ADA (IU/L)	42.7 ± 1.71	18.9 ± 0.7	P≤0.0001

*Values are shown as mean ±SEM.

Table 4: The concentrations of the cytokines in pleural effusions*.

	TPE	MPE	P value	EMP	P value	PPE	P value	Non-TPE	P value¥
IL-27 pg/ml	4725 (3993-7598)	838.5 (715.5-967.5)	≤ 0.0001	1403 (1163-1720)	≤ 0.0001	1409(1003-1699)	0.002	978 (835.3-1401)	≤ 0.0001
IL-6 pg/ml	5735 (4935-6302)	1240 (783.3-1955)	≤ 0.0001	4114 (3413-5150)	≤ 0.0001	2875 (1957-4167)	≤ 0.0001	2273 (1121-3832)	≤ 0.0001
IL-18 pg/ml	2196 (513.4-3035)	568.5 (395-800.3)	0.0008	2409 (1156-4595)	0.2522	2196 (1487-2663)	0.5539	1003 (577.8-2291)	0.1797
CXCL-8 pg/ml	1038 (344.1-1853)	125 (66.5-599.6)	≤ 0.0001	184.5 (114.1-1041)	0.0093	98.02 (50.25-326.5)	≤ 0.0001	123 (67.75-485.6)	≤ 0.0001
CCL-1 pg/ml	565 (104.8-2353)	12.21 (6.4-23.35)	≤ 0.0001	42.9 (30.18-61.98)	0.0195	126.3(42.65-392.3)	≤ 0.0001	32.2 (12.2-61.98)	≤ 0.0001
IP-10 pg/ml	2833 (909-4184)	662 (300.7-1524)	≤ 0.0001	1503 (1157-3323)	0.3892	823.4 (484.9-2286)	0.0101	944.4 (355.5-1970)	0.0001
ADA† IU/L	42.73±1.71	14.9±0.61	≤ 0.0001	25±1.11	≤ 0.0001	21.21±0.32	≤ 0.0001	18.9±0.07	≤ 0.0001†
ADA.IL-27 10^3.ng.IU/L^2	208.5 (178.2-548.8)	12.2(9.15-13.53)	≤ 0.0001	33.86 (26.46-4.52)	≤ 0.0001	30.25(21.57-35.23)	≤ 0.0001	19.23 (13.33-48.96)	≤ 0.0001†

*Values are presented as the median and 25–75% percentile.

¥Comparisons of data between TPE and the other groups were performed using Mann-Whitney U test.

† Values are shown as mean ±SEM. Comparisons of data between TPE and the other groups were performed using student *t-test.*

Table 5: The diagnostic accuracy of IL-27, IL-6, IL-18, CXCL-8, CCL-1, IP-10 and ADA in the differentiation of tuberculous from non-tuberculous effusions (Malignant, empyema and parapneumonis effusions). ADA: Adenosine deaminase, IL: Interleukin

Variables	Cut-off value	Area under curve(95% confidence interval)	P value	Sensitivity (%)	Specificity (%)	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value	Diagnostic accuracy (%)
IL-27	>2363pg/ml	0.9879	≤0.0001	96.67	98.28	56.07	0.03	96.6	98.2	97.72
IL-6	>3260pg/ml	0.9342	≤0.0001	100	67.24	3.03	0.0	61.2	100	74.4
IL-18	>2285pg/ml	0.5879	0.1797	50	75.9	2.07	0.66	51.7	74.5	67.04
CXCL-8	>144.5pg/ml	0.7905	≤0.0001	93.3	58.6	2.256	0.11	53.8	94.4	70.45
CCL-1	> 54pg/ml	0.905	≤0.0001	100	70.69	3.41	0.0	63.8	100	80.68
IP-10	>891.9pg/mL	0.746	0.0001	83.33	48.28	1.61	0.35	45.5	84.8	60.2
ADA	>27.5 IU/L	0.9759	≤0.0001	90	96.5	26.1	0.1	93.1	94.9	94.3
ADA.IL-27	>42.68 10^3.U.ng/L^2	0.9994	≤0.0001	100	98.28	58.13	0.0	96.78	100	98.86

Table 6: The diagnostic accuracy of IL-27, IL-6, IL-18, CXCL-8, CCL-1, IP-10 and ADA in the differentiation of tuberculous from malignant effusion.

Variables	Cut-off value	Area under curve(95% confidence interval)	P value	Sensitivity (%)	Specificity (%)	Positive likelihood ratio	Negative likelihood ratio	Positive predictive value	Negative predictive value	Diagnostic accuracy (%)
IL-27	1028 pg/ml	0.99	≤0.0001	100	96	25	0	96.7	100	98.3
IL-6	3026 pg/ml	0.99	≤0.0001	100	90	10	0	90.9	100	95
IL-18	1200 pg/ml	0.7778	0.0002	60	100	-	0.4	100	71.4	80
CXCL-8	144.5 pg/ml	0.7832	0.0002	93.3	60	2.33	0.11	70	90	76.6
CCL-1	51.85 pg/ml	0.9867	≤0.0001	100	93.3	14.28	0	93.7	100	96.6
IP-10	2072 pg/ml	0.8244	≤0.0001	60	100	-	0.4	100	71.4	80
ADA	20.5 IU/L	1.0	≤0.0001	100	100	-	0	100	100	100
ADA.IL-27	31.14 10^3.U.ng/L^2	1.0	≤0.0001	100	100	-	0	100	100	100

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Figures

Figure 1

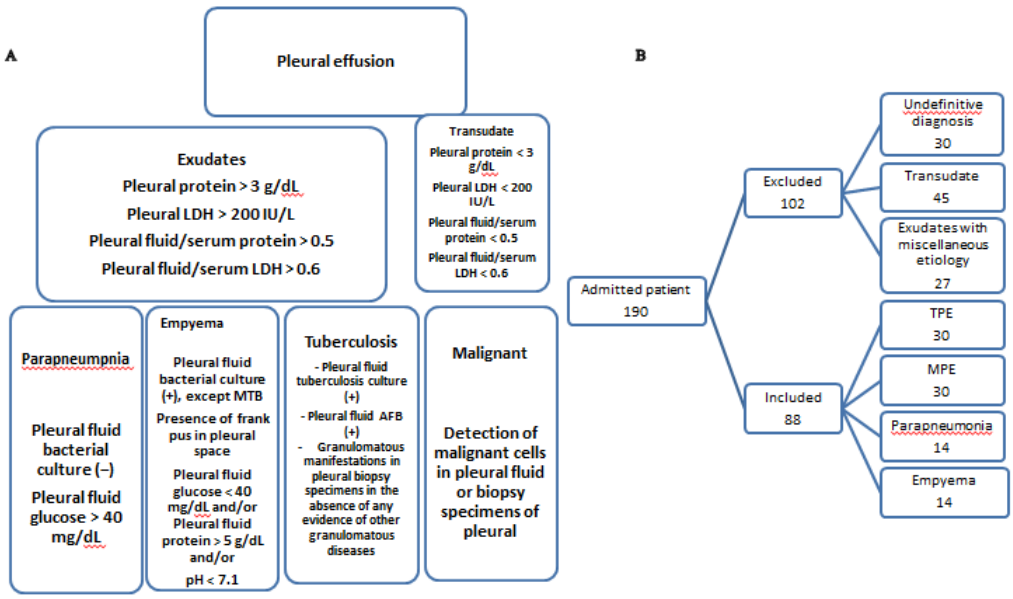

Figure 1

(A) The source and the type of pleural effusion samples. (B) Flow chart of study groups.

Fig. 2

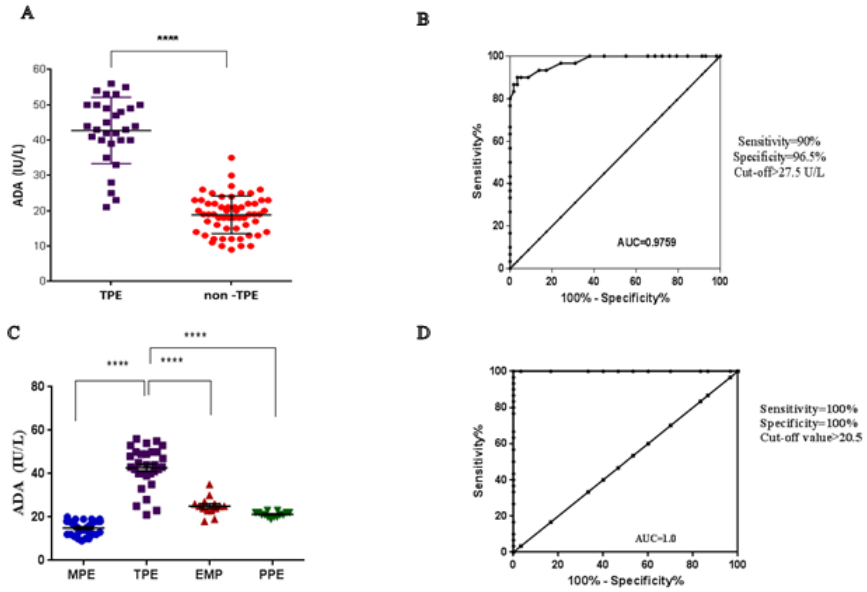

Figure 2

(A) Adenosine deaminase (ADA) Pleural fluid concentrations in TPE (n = 30) and non- TPE groups (n = 58). (B) ROC curves of ADA for differential diagnosis of tuberculous (n = 30) versus non-TPE (n = 58), (C) and TB (n = 30) versus MPE (n = 30). (D) ADA pleural fluid concentrations in TPE (n=30) and in 3 etiologies of pleural effusions: malignant (n = 30), empyema (n = 14) and parapneumonic (n = 14) pleural effusions. In each data bar, Horizontal bars indicate mean and the bottom and top of the bar represent the standard error of the mean (SEM).

Fig. 3

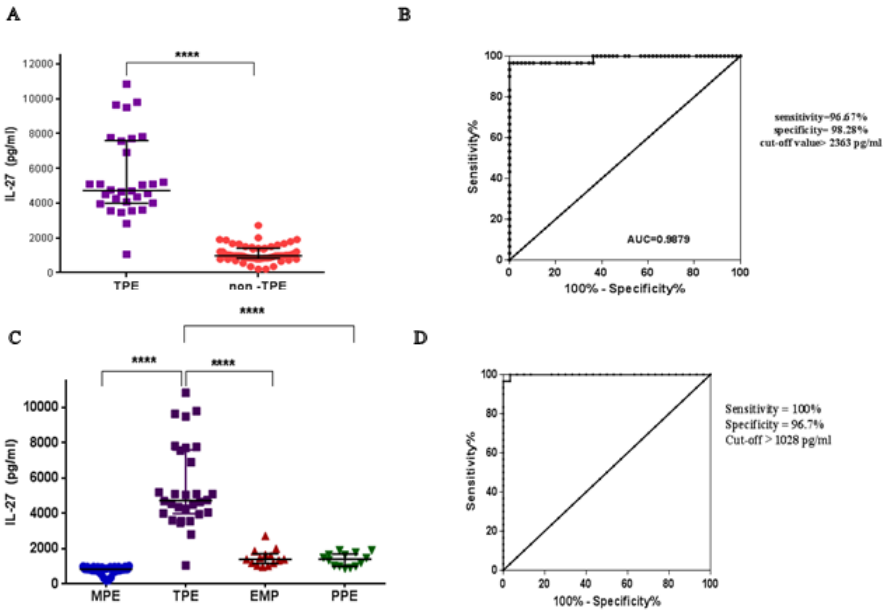

Figure 3

(A) IL-27 pleural fluid concentrations in TPE (n = 30) and non- TPE groups (58). (B) ROC curves of interleukin 27 for differential diagnosis of tuberculous (n = 30) versus non- TPE (n = 58), (C) and TB (n = 30) versus malignant pleural effusion (n = 30). (D) IL-27 pleural fluid concentrations in tuberculous pleural effusion (n=30) and in 3 etiologies of pleural effusions: malignant (n = 30), empyema (n = 14) and parapneumonic (n = 14) pleural effusions. In each data bar, Horizontal bars indicate median and the bottom and top of the bar represent the interquartile range.

Fig. 4

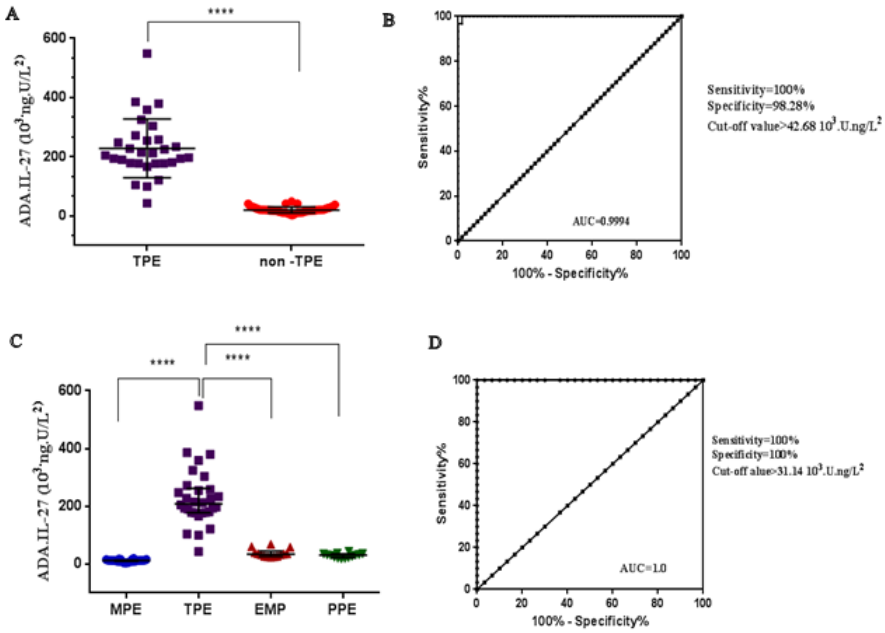

Figure 4

(A) ADA.IL-27 pleural fluid concentrations in TPE (n = 30) and non- TPE groups (n = 58). (B) ROC curves of ADA.IL-27 for differential diagnosis of tuberculous (n = 30) versus non-TPE (n = 58), (C) and TB (n = 30) versus MPE (n = 30). (D) IL-27 pleural fluid concentrations in TPE (n = 30) and in 3 etiologies of pleural effusions: malignant (n = 30), empyema (n = 14) and parapneumonic (n = 14) pleural effusions. In each data bar, Horizontal bars indicate median and the bottom and top of the bar represent the interquartile range.

Fig. 5

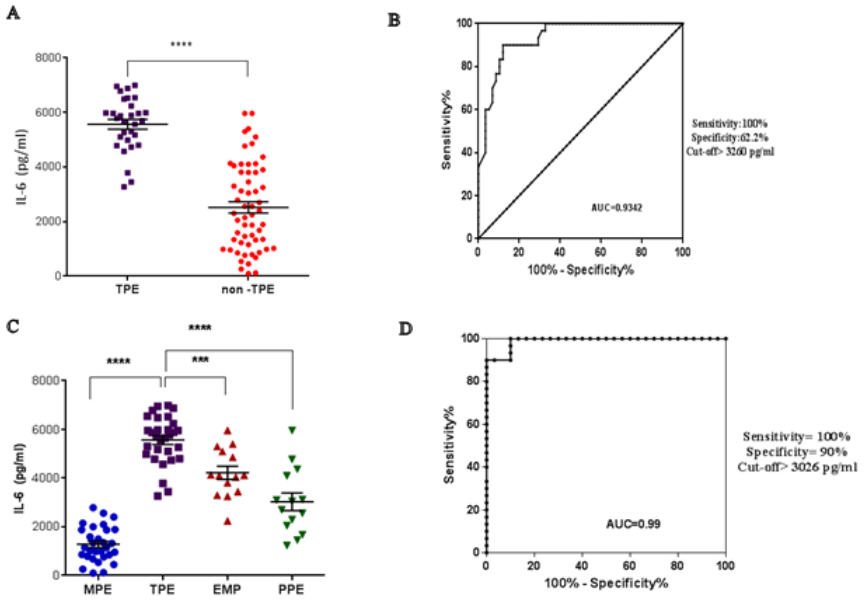

Figure 5

(A) IL-6 pleural fluid concentrations in TPE (n = 30) and non- TPE (n = 58) pleural effusions. (B) Receiver operating characteristic (ROC) curves of IL-6 for differential diagnosis of TB (n = 30) versus non-TPE (n = 58), (C) and TB (n = 30) versus MPE (n = 30). AUC = Area under curve. (D) IL-6 pleural fluid concentrations in TPE (n = 30) and in 3 etiologies of pleural effusions: malignant, empyema and parapneumonic pleural effusions. In each data bar, horizontal bars indicate median and the bottom and top of the bar represent the interquartile range.

Fig. 6

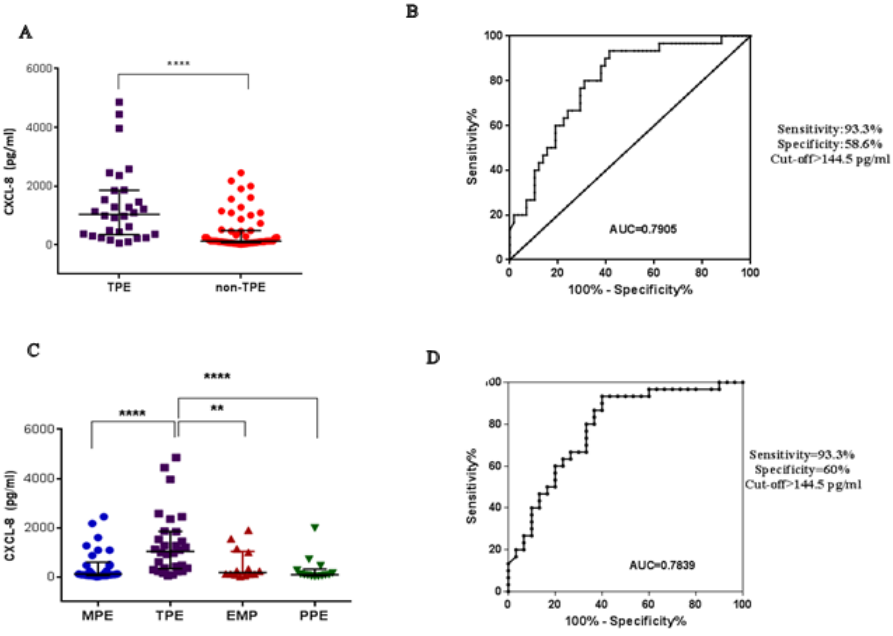

Figure 6

(A) CXCL-8 pleural fluid concentrations in TPE (n = 30) and non- TPE groups (n = 58). (B) ROC curves of CXCL-8 for differential diagnosis of TB (n = 30) versus non-TPE (n = 58), (C) and TB (n = 30) versus MPE (n = 30). (D) CXCL-8 pleural fluid concentrations in TPE (n = 30) and in 3 etiologies of pleural effusions: malignant (n = 30), empyema (n = 14) and parapneumonic (n =14) pleural effusions. In each data bar, Horizontal bars indicate median and the bottom and top of the bar represent the interquartile range.

Fig. 7

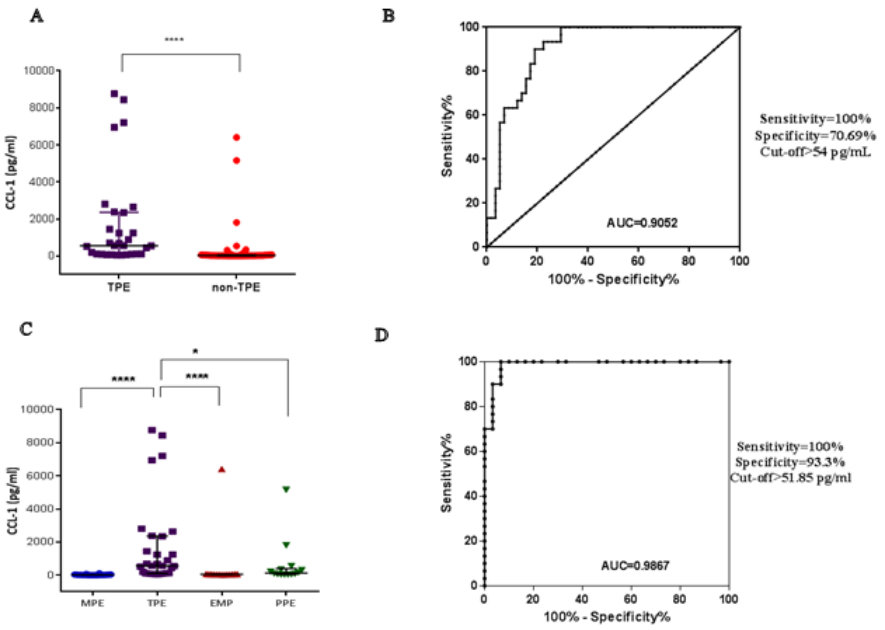

Figure 7

(A) CCL-1 pleural fluid concentrations in TPE (n = 30) and non- TPE (58). (B) ROC curves of CCL-1 for differential diagnosis of TB (n = 30) versus non-TPE (n = 58), (C) and TB (n = 30) versus MPE (n = 30). (D) CCL-1 pleural fluid concentrations in TPE (n = 30) and in 3 etiologies of pleural effusions: malignant (n = 30), empyema (n = 14) and parapneumonic (n = 14) pleural effusions. In each data bar, Horizontal bars indicate median and the bottom and top of the bar represent the interquartile range.

Fig. 8

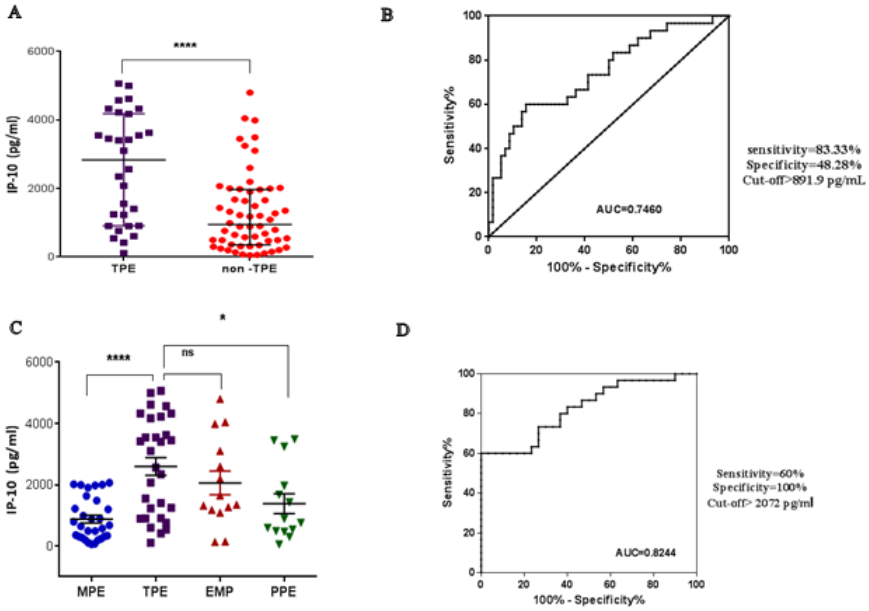

Figure 8

(A) IP-10 pleural fluid concentrations in TPE (n = 30) and non- TPE (58). (B) ROC curves of IP-10 for differential diagnosis of TB (n = 30) versus non-TPE (n = 58), (C) and TB (n = 30) versus MPE (n = 30). (D) IP-10 pleural fluid concentrations in TPE (n = 30) and in 3 etiologies of pleural effusions: malignant (n = 30), empyema (n = 14) and parapneumonic (n = 14) pleural effusions. In each data bar, Horizontal bars indicate median and the bottom and top of the bar represent the interquartile range.

Fig. 9

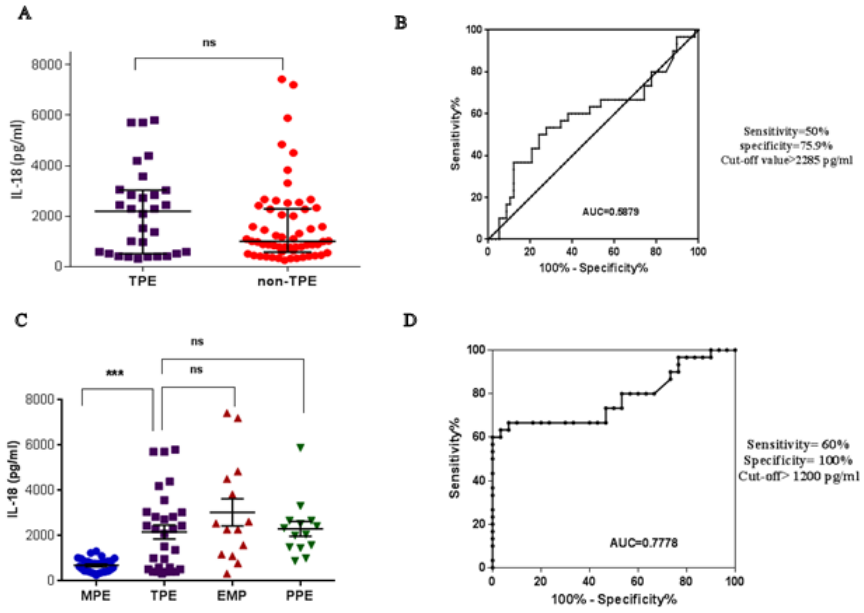

Figure 9

(A) IL-18 pleural fluid concentrations in TPE (n = 30) and non- TPE groups (n = 58). (B) ROC curves of IL-18 for differential diagnosis of TB (n = 30) versus non-TPE (n = 58), (C) and TB (n = 30) versus MPE (n = 30). (D) IL-18 pleural fluid concentrations in TPE (n = 30) and in 3 etiologies of pleural effusions: malignant (n = 30), empyema (n = 14) and parapneumonic (n = 14) pleural effusions. In each data bar, Horizontal bars indicate median and the bottom and top of the bar represent the interquartile range.