A novel interleukin 36 gamma for diagnosing and differentiating malignant from infectious pleural effusion

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Background
Pleural effusions can be caused by various diseases, making their diagnosis challenging. Light's criteria are often used to distinguish transudative and exudative effusions. Exudative effusions have a more complex etiology, including parapneumonic, tuberculous, and malignant effusion.
Objectives
The main objective of this study was to evaluate the effectiveness and sensitivity of Interleukin 36 gamma (IL-36γ) as a diagnostic marker for differentiating between malignant and infectious pleural effusions.
Methods
The study involved 100 patients with pleural effusion. Patients were categorized into five groups based on their final diagnosis. An ELISA technique was used to quantitatively measure the levels of pleural fluid IL-36γ.
Results
The results showed that pleural IL-36γ levels were higher in tuberculous pleural effusion compared with malignant, transudative, and uncomplicated pleural effusion. In contrast, pleural IL-36γ levels were higher in complicated pleural effusion compared with tuberculous, malignant, transudative, and uncomplicated pleural effusion.
Conclusion
Based on the study's findings, it was concluded that pleural IL-36γ is a novel biomarker that can be used to diagnose and differentiate malignant pleural effusion from infectious pleural effusion.

Keywords:
interleukin 36, pleural effusion, tuberculous pleural effusion

Introduction

Exudative pleural effusions are characterized by a complex etiology and are typically associated with conditions such as parapneumonic pleural effusion (PPE), tuberculous pleural effusion (TPE), and malignant pleural effusion (MPE) [1].

PPE is an exudative pleural effusion that is known by presence of fluid between the two pleural layers with underlying lung infection on the same side (ipsilateral) of the chest. PPE can be categorized into three stages: uncomplicated parapneumonic effusion (UPPE), complicated parapneumonic effusion (CPPE), and thoracic empyema [2].

Distinguishing between UPPE and CPPE/empyema is crucial in clinical practice because it significantly influences treatment decisions. UPPE can often be managed with appropriate antibiotics, while CPPE and empyema usually require more aggressive interventions like pleural drainage or surgery [3].

Clinically, there can be difficulty in differentiating between TPE and PPE due to similarities in both clinical presentation and laboratory findings. This highlights the need for effective diagnostic tools and markers, such as the interleukin 36 gamma (IL-36γ) biomarker to aid in the differentiation of these conditions [4].

The IL-36 cytokines are comprised of IL-36 receptor antagonist (IL-36Ra), anti-inflammatory cytokine, and three pro-inflammatory cytokines, IL-36α, IL-36β, and IL-36η. These cytokines are involved in the immune response in a number of ways. Minimal amounts of IL-36 cytokines are produced in a wide range of organs, and their expression patterns vary. Notably, among other organs, the lung is known to express and induce IL-36γ at the highest level [3].

It has been observed that WNT5A-induced nonclassical WNT signaling mediated by IL-36γ has a role in promoting the executing of Mycobacterium tuberculosis (Mtb) by macrophages. It implies that IL-36γ might play a part in modulating the immune response and host defense mechanisms against Mtb infection, potentially making it a significant factor in the context of tuberculosis [5].

Patients and Methods

The study was conducted following established ethical standards and received the necessary approvals (RC.19.3.2023). All participants were provided with information about the study's objectives and details, and they gave their informed consent to participate.

The research is described as a comparative cross-sectional study that included 100 patients chosen by convenient sampling technique according to a specific inclusion and exclusion with pleural effusion of different etiologies; 65 (65%) males and 35 (35%) females and they varied in age from 19 to79 years (mean; 51.85 ± 15.5) admitted at chest department Benha University Hospital in the period between March 2022 and May 2023.

Patients were divided into five groups according to their final diagnosis:

(a) Group 1: this group consisted of 20 (20%) cases with TPE. There were 10 males and 10 females in this group, and the ages of the patients ranged from 30 years to 60 years.
(b) Group 2: group 2 included 20 (20%) cases with MPE. In this group, there were 11 males and nine females, and the patients' ages ranged from 19 years to 73 years.
(c) Group 3: this group comprised 20 (20%) cases with UPPE. It had 13 males and 7 females, with patient ages ranging from 26 years to 75 years.
(d) Group 4: group 4 included 20 (20%) cases with CPPE. In this group, there were 17 males and three females, and the ages of the patients ranged from 23 years to 70 years.
(e) Group 5: the final group, group 5, was made up of 20 (20%) cases with transudative pleural effusion. There were 14 males and six females in this group, and the patients' ages ranged from 45 years to 79 years.

Infectious pleural effusion (IPE) included parapneumonic pleural effusion and tuberculous pleural effusion, while noninfectious pleural effusion (NIPE) included malignant pleural effusion and transudative pleural effusion.

Inclusion criteria
According to modified Light's criteria, an effusion was classified as an exudate if it met one or both of the following conditions: effusion protein/serum protein ratio greater than 0.5, Effusion lactate dehydrogenase (LDH)/serum LDH ratio greater than 0.6 [6].

The criteria for inclusion in group 1, which focuses on patients with TPE: (I and II Plus any of IV or V).

(a) Classification of effusion as exudative based on the modified Light's criteria.
(b) ADA level greater than 40 U/l [7].
(c) Tuberculin skin test proved to be positive [8].
(d) The presence of acid-fast bacilli in the pleural effusion examination.
(e) The presence of caseating granulomas in a biopsy obtained through thoracoscopy or Abram's needle pleural biopsy [9].

The inclusion criteria for group 2, which focuses on patients with MPE include: [10].

(a) Symptomatic pleural effusion that is rapidly accumulating. The effusion should be of a significant volume, typically occupying more than half of a hemithorax.
(b) The pleural effusion's chemical analysis should confirm that it is exudative in nature.
(c) Cytological examination of the pleural fluid may reveal the presence of exfoliative malignant cells and should be confirmed by histopathological examination of biopsies obtained through various biopsy methods. These biopsies help in identifying the presence of cancerous cells and the primary malignancy. The requirement for both cytological examination and histopathological examination of biopsies helps establish a robust diagnosis of malignancy in the pleural effusion.

The inclusion criteria for group 3, which focuses on patients with UPPE [11]:

(a) Exudative pleural effusion identified based on the modified light's criteria.
(b) Negative results on gram stain and culture.
(c) pH higher than 7.2.
(d) Glucose greater than 40 mg/dl.
(e) LDH less than 1,000 IU/l.

The inclusion criteria for group 4, which focuses on patients with (empyema) CPPE: [12].

(a) Exudative pleural effusion identified based on the modified Light's criteria.

(b) Pleural fluid pH less than 7.20.
(c) The glucose concentration in the pleural fluid should be less than 40 mg/dl.
(d) The concentration of LDH in the pleural fluid should be greater than 1000 IU/l.
(e) Gram stain and culture results may be positive.

Group 5 comprises patients with transudative pleural effusion

Transudative pleural effusion is identified by its chemical analysis as it does not meet any of modified light criteria [13].

The exclusion criteria for the study are essential for defining the specific patient population that will be included and ensuring that the study's results are not confounded by certain factors or treatments. Here are the exclusion criteria for the study:

(a) Treatment with anticancer drugs.
(b) Treatment with anti-tuberculous therapy.
(c) Using other anti-inflammatory therapies and glucocorticoids.
(d) Patients who refuse to participate.

The various diagnostic procedures and investigations that were performed on the patients as part of the study included:

(i) Full medical history.
(ii) Thorough physical examination.
(iii) Routine laboratory investigations
    (a) A battery of routine laboratory tests, including complete blood picture, erythrocyte sedimentation rate (ESR), and coagulation profile.
    (b) Liver function tests, focusing on liver enzymes and serum albumin.
    (c) Kidney function tests, measuring serum urea and serum creatinine.
    (d) Measurement of total serum protein and LDH levels to differentiate transudative from exudative pleural effusions.
(iv) Radiological Examination:
    (a) Chest imaging, including plain chest radiography.
    (b) When indicated, additional imaging such as chest computed tomography, abdominal and chest ultrasonography, and echocardiography were performed.
(v) Diagnostic thoracocentesis:
    (a) A diagnostic thoracocentesis was performed, likely to obtain pleural fluid for analysis and diagnosis [14].

(vi) Tuberculin skin test:
    (a) Tuberculin skin test was administered to support the diagnosis of TPE E; however, a negative result can be seen in approximately one third of patients [15].
(vii) Sputum Examination:
    (a) Sputum examination for acid-fast bacilli was conducted as a diagnostic test to identify the presence of mycobacterial infection.
(viii) Collection and processing of pleural fluid samples:

The diagnostic examinations and laboratory tests performed on the pleural fluid samples include:

(a) Physical examination of pleural fluid:
    (i) This examination includes assessing the color, aspect, turbidity, and specific gravity of the pleural fluid.
(b) Chemical examination of pleural fluid:
    (i) This examination includes analyzing proteins, glucose, and LDH in the pleural fluid. Exudative and transudative pleural effusions are classified based on modified Light's criteria, which include criteria related to protein/serum protein ratio, LDH/serum LDH ratio, and absolute LDH value.
(c) Bacteriological examination of pleural fluid:
    (i) This examination involves conducting culture and sensitivity tests on the pleural fluid to check for nonspecific infections. Additionally, a smear of pleural fluid is examined for acid-fast bacilli (AFB) using the Ziehl–Neelsen stain.
(d) Cytological examination of pleural fluid:
    (i) Cytological examination of the pleural fluid aspirate is performed to detect malignant cells and inflammatory cells, with a specific focus on lymphocytic count and mesothelial cells.
(e) Quantitative measurement of pleural fluid IL-36γ.

The study included the measurement of pleural fluid IL-36γ levels using the enzyme-linked immunosorbent assay (ELISA) technique (DuoSet ELISA, R and D Systems China Co., Ltd.) IL-36γ-specific antibody was bound to a microplate. Unbound capture antibody was washed away. Plates were blocked and washed. Samples were added and any IL-36γ present was bound by the immobilized antibody. Unbound materials were washed away. Streptavidin-horseradish peroxidase was used to bind to the detection antibody. Unbound streptavidin-horseradish peroxidase was washed away. Tetramethylbenzidine substrate solution was added to the wells and a blue color develop in proportion to the amount of IL-36γ present in the sample.

(ix) Pleural Biopsies:
 (a) Pleural biopsies were taken for patients in groups 1 (tuberculous pleural effusion) and 2 (malignant pleural effusion) using either closed pleural biopsies with Abram's needle or thoracoscopic biopsies. These biopsies provide histological information for diagnosis.

Data management

(a) The collected data were managed and statistically analyzed using the Statistical Package for Social Science, Released 2011. IBM SPSS Statistics for windows, version 20.0. Armonk, NY: IBM Crop. Data were assessed for normality of distribution using statistical tests.
(b) Categorical data were presented as numbers and percentages.
(c) Continuous data were presented as mean and standard deviation (SD) for normally distributed data and as median and interquartile range (IQR) for skewed data.
(d) Statistical tests, such as Mann–Whitney's test and Kruskal–Wallis test, were used for comparing data between groups.
(e) Correlation analysis was performed using Spearman's correlation coefficient.
(f) Sensitivity and specificity analyses were conducted using receiver operating characteristic curve analysis to evaluate diagnostic accuracy.

The study's data management and statistical analysis procedures aim to provide a comprehensive and rigorous assessment of the collected data and support the study's objectives related to diagnosing and differentiating pleural effusion types.

Results

100 patients were assessed in this study. The mean age of the studied group was 51.85 ± 15.5 and the age ranged from (19 to 79 years). There were 65% males and 35% females. 32 (32%) of them were hypertensive, 34 (34%) were diabetic and 56(56%) were smokers. The level of IL-36γ was compared using Mann Whitney test and revealed no statistically significant variation in relation to sex ($p = 0.463$), smoking state ($p = 0.290$) or being diabetic ($p = 0.660$) or hypertensive ($p = 0.230$) [Table 1] The median level of IL-36γ in the TPE group (n=20) was 774.89 pg/ml, in MPE (n=20) was 158.22 pg/ml, in UPPE (n=20)was 327.11 pg/ml, finally, it was 2920.30 pg/ml in CPPE group (n=20). Kruskall–Wallis comparison demonstrated that there was a highly significant difference ($P < 0.001$) in IL-36γ across the various study groups (Table 2).

The IL-36γ in infectious and noninfectious pleural effusions differed significantly using Mann–Whitney test ($P < 0.001$). The pleural IL-36 γ median value was higher in infectious pleural effusions(n=60) (774.89 pg/ml) than in noninfectious ones(n=40) (122 pg/ml) (Table 3).

Table 1 Characteristic data of the studied patients and IL36 γ level among them

All patients	Frequency (*n*=100)	IL36 γ pg/ml (in pleural fluid) Median (IQR) (*n*=100)	Mann–Whitney test (Z)	*P*
Age (Mean±SD)	51.85±15.5(min–max) 19–79			
HTN, *n* (%)				
Yes	32 (32)	301.01 (684.0)	1.20	0.230
No	68 (68)	343.0 (2179.0)		
DM, *n* (%)				
No	66 (66)	323.50 (682.45)	0.440	0.660
Yes	34 (34)	327.11 (2020.0)		
Smoking, *n* (%)				
No	44 (44)	301.01 (669.0)	1.059	0.290
Yes	56 (56)	343.0 (1685.0)		
Sex, *n* (%)				
Female	35 (35)	288.03 (620.0)	0.734	0.463
Male	65 (65)	334.0 (2028)		

Table 2 Comparison between pleural interleukin 36 gamma level in different studied groups

Cause	IL36 γ pg/ml (in pleural fluid) Median (IQR)	Kruskal–Wallis	*P*
TPE (*n*=20)	774.89 (193.88)	94.677	<0.001 (HS)
MPE (*n*=20)	158.22 (32.97)		
UPPE (*n*=20)	327.11 (84.13)		
CPPE (*n*=20)	2920.30 (612.30)		
Transudative pleural effusion (*n*=20)	47.87 (23.43)		

*CPPE, complicated pleural effusion; *IL: interleukin; *MPE, malignant pleural effusion; TPE, Tuberculous pleural effusion; *UPPE, uncomplicated parapneumonic pleural effusion.

Table 3 Comparison between pleural interleukin 36 gamma level in infectious and non-infectious pleural effusion

Cause	IL36 γ pg/ml(in pleural fluid) Median (IQR)	Mann–Whitney test (Z)	P
Infectious pleural effusion (*n*=60)	774.89 (2042.25)	8.429	<0.001
Non-infectious pleural effusion (*n*=40)	122 (112.12)		

Table 4 Correlation between pleural interleukin 36 gamma (pg/ml) and pleural ADA(IU/L), tuberculin skin test and pleural LDH in different groups

Pleural IL36 γ (pg/ml)	All groups(*n*=100)		Infectious group (*n*=60)		Noninfectious group (*n*=40)		TPE (*n*=20)		MPE (*n*=20)	
	(ρ)*	P value	(ρ)*	P	(ρ)*	P	(ρ)*	P	(ρ)*	P
Pleural ADA(IU/l)	0.871	<0.001	0.781	<0.001	0.524	<0.001	0.405	0.076	0.165	0.488
Tuberculin skin test	–	–	0.005	0.969	–	–	–	–	–	–
Pleural LDH	0.900	<0.001	0.709	<0.001	0.739	<0.001	0.339	0.143	0.129	0.588

*(ρ) Spearman correlation coefficient.

Table 5 Diagnostic performance of interleukin 36 gamma in diagnosing tuberculous effusion based on receiver operating characteristic curve analysis

	AUC	P	Cut off value) pg/ml	Sensitivity, *n* (%)	Specificity, *n* (%)	95% confidence interval
TPE vs MPE	1.0	0.0001	≥192.06	100	95	1.0–1.0
TPE vs Transudate effusion	1.0	0.0001	≥71.50	100	90	1.0–1.0
TPE vs UPPE	1.0	0.0001	≥391.09	100	95	1.0–1.0
TPE vs Non TPE	0.750	0.001	≥457.58	100	75	0.655–0.845
IPE vs NIPE	0.999	0.0001	≥186.95 pg/ml	100	98	0.997–1.0

*CPPE, complicated pleural effusion; *IL, Interleukin; *IPE, infectious pleural effusion; *MPE, malignant pleural effusion; *NIPE, noninfectious pleural efusion; TPE, tuberculous pleural effusion; *UPPE, uncomplicated pleural effusion.

Spearman correlation coefficient analysis demonstrated that in the infectious group, pleural ADA (ρ=0.781, *P* <0.001) and pleural LDH (ρ=0.709, *P* <0.001) had a highly significant positive correlation to pleural IL-36 γ, while tuberculin skin test (ρ= 0.005, *P* =0.969) had very week correlation to pleural IL-36 γ. However, in noninfectious group pleural ADA (ρ=0.524, *P* <0.001) and pleural LDH (ρ=0.739, *P* <0.001) had a highly significant positive correlation to pleural IL-36 γ (Table 4).

The diagnostic cutoff value for diagnosing TPE vs non-TPE was greater than or equal to 457.58 (*P*=0.001), with 100% sensitivity, 75% specificity, 95% CI= 0.655–0.845 and AUC=0.750, that of TPE verses UPPE was greater than or equal to 391.09 with 100% sensitivity and 95% specificity, 95% CI= 1.0 –1.0 and AUC=1.0 and the cutoff point of IL-36 γ for differentiating infectious from noninfectious pleural effusion was greater than or equal to 186.95 pg/ml (*P*=0.000) with 100% sensitivity and 98% specificity 95% CI= 0.997–1.0 and AUC= 0.999 (Table 5).

Discussion

In clinical practice, pleural effusion is a frequently observed complication that can result from a variety of disorders. Even though there are several markers for the etiological diagnosis of PE, finding additional biomarkers to increase diagnostic precision is still a major research goal and difficulty [16].

Numerous studies have demonstrated the correlation between IL-36 and the development of several illnesses, such as tuberculosis, pneumonia, malignant tumors, and infections. These findings point to a higher possibility that IL-36 contributes to the pathogenic pathways of pleural effusion and may have additional diagnostic ramifications [17].

This study demonstrated that there was a highly significant difference (*P* <0.001) in IL-36γ across the various study groups; pleural IL-36γ was higher in tuberculous pleural effusion (774.89 pg/ml) than in uncomplicated pleural effusion (327.11 pg/ml), malignant (158.22 pg/ml) and transudative (47.87 pg/ml); on the other hand, pleural IL-36γ was higher in complicated pleural effusion (2920.30 pg/ml) than in tuberculous, uncomplicated pleural effusion, malignant and transudative pleural effusion (Table 2).

Guo and colleagues study supported this, finding that the pleural concentrations of IL-36γ in people with PPE, TPE, MPE, and transudate were estimated to be 368.0 (210.0–1521.0) pg/ml, 818.5 (569.0, 1157.0) pg/ml, 185.3 (155.9–269.0) pg/ml, and 121.9 (46.3–196.5) pg/ml, respectively. When compared with MPE and transudative effusion, the pleural level of IL-36γ was

considerably higher in PPE and TPE. Nevertheless, no statistically significant differences ($P>0.05$) were seen between the PPE and TPE groups [18].

Additionally, Song and colleagues' investigation revealed that the pleural fluid concentrations of IL-36γ in patients with transudate, MPE, TPE, and PPE were 82.8 ± 15.0 pg/ml, 180.6 ± 11.7 pg/ml, 964.7 ± 121.7 pg/ml, and 1186.1 ± 269.1 pg/ml, respectively. Compared with those with transudate and MPE, those with TPE and PPE diagnoses had significantly greater IL-36γ concentrations. Furthermore, compared with the UPPE subgroup, the CPPE/empyema subgroup showed noticeably higher IL-36γ levels ($P<0.0001$) [19].

This makes sense because lung infections caused by a variety of pathogenic bacteria have been associated with the inflammatory cytokine IL-36γ. Macrophages secrete IL-36γ into the extracellular space by encasing it in particles and exosomes, which they make quickly in the lung [20].

Mtb enters the pleural cavity in modest amounts in TPE, which causes a localized immune response. A substantial T-helper type 1 lymphocyte response happens after neutrophil inflow and monocyte migration. IL-36γ production has been found to be caused by Mtb infection, which activates a host-dependent pathway including the Toll-like receptor and myeloid differentiation primary response gene 88 (MyD88) [21].

This study demonstrated that in infectious group, pleural ADA ($\rho=0.781$, $P <0.001$) and pleural LDH ($\rho=0.709$, $P <0.001$) had a highly significant positive correlation to pleural IL36 γ, while tuberculin skin test ($\rho= 0.005$, $P =0.969$) had very week correlation to pleural IL36 γ. However, in noninfectious group pleural ADA ($\rho=0.524$, $P <0.001$) and pleural LDH ($\rho=0.739$, $P <0.001$) had a highly significant positive correlation to pleural IL36 γ (Table 4).

Guo and colleagues data, which indicated that IL-36γ levels in the pleural fluid were favorably correlated with LDH ($r=0.7189$, $P<0.0001$) in PPE, were in agreement with this. In TPE patients, there is a positive correlation between pleural IL-36γ and pleural ADA ($r=0.4022$, $P=0.0375$) [18].

The diagnostic cutoff value for diagnosing TPE vs Non TPE was greater than or equal to 457.58 ($P=0.001$), with 100% sensitivity, 75% specificity, 95% CI= 0.655–0.845 and AUC=0.750, that of TPE versus UPPE was greater than or equal to 391.09 with 100% sensitivity and 95% specificity, 95% CI= 1.0–1.0 and AUC=1.0

and the cutoff point of IL-36 γ for differentiating infectious from noninfectious pleural effusion was greater than or equal to 186.95 pg/ml with 100% sensitivity and 98% specificity ($P=0.000$) 95% CI= 0.997–1.0 and AUC= 0.999 (Table 5).

Depending on the previous data, IL-36 γ was better to differentiate infectious from non-infectious pleural effusion as sensitivity was 100%, specificity was 98%, *P* value was 0.000), 95% CI was 0.997–1.0 and AUC was 0.999.

This was in line with Guo *et al.*'s assessment of the usefulness of pleural fluid IL-36γ in using receiver operating characteristic curves to distinguish UPPE from CPPE/empyema, TPE from UPPE, and IPE (PPE and TPE) from NIPE (MPE and transudate). The AUCs were 0.904 ($P=0.0278$), 0.904 ($P<0.0001$), and 1 ($P=0.0001$), respectively. For the purpose of discriminating between IPE and NIPE, TPE and UPPE, and UPPE and CPPE/empyema, the ideal cut-off values for IL-36γ were 293.0 pg/ml, 657.5 pg/ml, and 736.0 pg/ml, respectively [17].

Conflicts of interest
There are no conflicts of interest.

References

1 McCauley L, Dean N. Pneumonia and empyema: causal, casual or unknown. J thoracic dis 2015; 7:992.

2 Shehta M, Fathy AM, Ibrahim MA. Could pleural fluid lactate level have an added diagnostic value in para pneumonic effusion? Egypt J Chest Dis Tuberc 2023; 72:340–345##2023.

3 Ferreiro L, Porcel JM, Bielsa S, Toubes ME, Álvarez-Dobaño JM, Valdés L. Management of pleural infections. Expert Rev Respir Med 2018; 12:521–535.

4 Anara C, Yavuzb MY, Alıcıc İO, Güldavald F, Büyüksirind M. Diagnostic value of pleural fluid lactate dehydrogenase/ adenosine deaminase ratio in differentiating parapneumonic effusion from tuberculous pleurisy. Egypt J Chest Dis Tuberc 2021; 70:547–553##2021.

5 Gao Y, Wen Q, Hu S, Zhou X, Xiong W, Du X, *et al*. IL-36γ promotes killing of Mycobacterium tuberculosis by macrophages via WNT5A-induced noncanonical WNT signaling. J Immunol 2019; 203:922–935.

6 Light RW, Erozan YS, Ball WC. Cells in pleural fluid: their value in differential diagnosis. Arch intern med 1973; 132:854–860.

7 Burgess LJ, Maritz FJ, Le Roux I, Taljaard JJ. Use of adenosine deaminase as a diagnostic tool for tuberculous pleurisy. Thorax 1995; 50:672–674.

8 Valdés L, Alvarez D, San José E, Penela P, Valle JM, García-Pazos JM, *et al*. Tuberculous pleurisy: a study of 254 patients. Arch intern med 1998; 158:2017–2021.

9 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; 22:589–591.

10 Beauchamp HD, Kundra NK, Aranson R, Chong F, MacDonnell KF. The role of closed pleural needle biopsy in the diagnosis of malignant mesothelioma of the pleura. Chest 1992; 102:1110–1112.

11 Light RW. Pleural Diseases. 5th ed. Baltimore, MD: Lippincott, Williams and Wilkins; 2007.

12 Light RW. Parapneumonic effusions and empyema. In: Light RW. Pleural diseases. 3ª ed. Baltimore: Willianms & Wilkin; 1995. 129–53.

13 Porcel JM, Light RW. Diagnostic approach to pleural effusion in adults. American family physician 2006; 73:1211–1220.

14 Saguil A, Wyrick K, Hallgren J. MD Diagnostic.,(2014): Approach to Pleural Effusion. Am Fam Physician 2014; 90:99–104.

15 Valdés L, Alvarez D, San José E, *et al*. Tuberculous pleurisy: a study of 254 patients. Arch Intern Med 1998; 158:2017–21.

16 Rosa MP, Silvia B, Aureli E, Montserrat MA, José MP. Diagnostic accuracy of pleural fluid adenosine deaminase for diagnosing tuberculosis. Meta-analysis of Spanish Studies 2019; 55:23–30.

17 Neurath MF. IL-36 in chronic inflammation and cancer. Cytokine & Growth Factor Reviews 2020; 55:70–79.

18 Guo L, Zhang Q, Chengna L, Xudan M, SonG X, Huang J, *et al*. A novel biomarker for pleural effusion diagnosis:Interleukin-36γ in pleural fluid. J Clin Lab Anal 2022; 37:e24799.

19 Song X, Guo L, Zhang Q, Chen W, Fan W, Chengna LV, *et al*. The diagnostic value of interleukin-36 cytokines in pleural effusions of varying etiologies. Clinica Chimica Acta 2023; 549:117533.

20 Kovach MA, Singer BH, Newstead MW, *et al*. IL-36gamma is secreted in microparticles and exosomes by lung macrophages in response to bacteria and bacterial components. J Leukoc Biol 2016; 100:413–421.

21 Porcel JM. Advances in the diagnosis of tuberculous pleuritis. Ann Transl Med 2016; 4:282.