Diagnostic values of adenosine deaminase cytokines in fluid contributed to the detection of extrapulmonary tuberculosis

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Key words: ADA, IL-1β, IL-2, TNF-α, IFN-γ, cytokines, tuberculosis pleura, peritoneum, meninges.

Contributions: BTTH, sample collection, biomedical data compilation and analysis; HHY, manuscript writing and review; NMH, study design, final data compilation and analysis, and manuscript writing and review; LTMH, biomedical data compilation and analysis; LCT, LVD, NVK, manuscript review; TKC, contributed to final data compilation and machine learning methods; NTD, project leader. All authors read and approved the final manuscript.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethics approval and consent to participate: the study was approved by the Ethics Committee of Thanh Nhan Hospital (Decision No. 88/BVTN-CĐT).

Patient consent for publication: patients agreed to participate in the study.

Funding: none.

Availability of data and materials: data are available upon request. Please contact the respective authors for further information.

Received: 24 September 2024.
Accepted: 25 September 2024.

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Licensee PAGEPress, Italy
Italian Journal of Medicine 2024; 18:1810
doi:10.4081/itjm.2024.1810

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ABSTRACT

Tuberculosis (TB) is a serious global public health problem. Detecting the changes of adenosine deaminase (ADA), interleukin (IL)-1β, IL-2, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) released from macrophages and monocytes is greatly valuable in the diagnosis of TB. Hence, this study was designed to determine the optimal cut-off points of ADA, IL-1β, IL-2, TNF-α, and IFN-γ, allowing to differentiate pleural TB (PLTB), peritoneal TB (PTB), and meningeal TB (MTB). The study included 386 patients with pleural effusion, 47 patients with peritoneal effusion, and 134 patients with encephalitis and meningoencephalitis suspect of TB. ADA was determined by an enzyme kinetics method; cytokine concentration was measured by a test based on the competitive enzyme-linked immunosorbent assay principle. Sensitivity, specificity, and positive and negative predictive value were calculated and described. In the aspiration fluid, ADA activity was greatly different between PLTB, PTB, and MTB groups with values of 37.5 U/L, 30.5 U/L, and 8.1 U/L and sensitivity and specificity of 87.7% and 83.4%; 100% and 88.9%; 97.3% and 98.6%, respectively. Similar, IFN-γ concentrations were distinctively different between PLTB, PTB, and MTB patients (with values of 120 pg/mL, 200 pg/mL, and 30 pg/mL with sensitivity and specificity of 80.3% and 80.9%; 80.0% and 92.6%; 98.6% and 61.7%, respectively). The determination of ADA and IFN-γ levels in the aspiration fluid is potentially considered a highly sensitive and specific test for differentiating PLTB, PTB, and MTB.

Introduction

Tuberculosis (TB) remains a global health problem. Human immunodeficiency virus (HIV) co-infected and multidrug-resistant (MDR)-TB are great challenges for humanity by exacerbating the disease and increasing the incidence of extrapulmonary TB patients. The global number of deaths officially caused by TB in 2021 (1.4 million) was more than double the number caused by HIV/AIDS (0.65 million), and this mortality has been much more severely affected by the COVID-19 pandemic. In contrast, deaths from HIV/AIDS continued to decline between 2019 and 2021. Vietnam is one of the 30 countries with the highest burden of MDR-TB in the world. More than 172,000 people became ill, and 10,400 died from TB.[1] In the United States, the incidence of extra-

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pulmonary TB elevated from 15.7% in 1993 to 21% in 2006, among which the incidence of pleural TB (PLTB) ranked 2nd (19.8%), and peritoneal TB (PTB) ranked 6th (9%).[2] In Brazil, extrapulmonary TB accounts for 16.8% of cases, and PLTB also ranked 2nd (18.8%).[3]

As the initial symptoms of TB are fairly insidious, TB patients were often diagnosed in the later stage. In addition, current diagnostic methods still have certain limitations, leading to a great challenge in the diagnosis of extrapulmonary TB. Microbial culture is considered the gold standard, but the sensitivity is not high and requires minimally 20 days to obtain results. The first line of defense against *Mycobacterium tuberculosis* involves the activation of macrophages, especially activated T lymphocytes. The release of proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor (TNF), IL-2 or interferon-γ (IFN-γ) from macrophages and monocytes is important for the killing and elimination of microorganisms. Adenosine deaminase (ADA) is found in monocytes and macrophages. Previous studies have shown that ADA is secreted by antigen-presenting cells and acts as a cytokine, activating and increasing the number of monocytes, macrophages and T-CD4+ lymphocytes. Understanding the activity of cytokines and substances involved in the TB immune response, such as ADA, is helpful for the diagnosis and monitoring of TB treatment.[4-6]

This study aimed to determine the optimal cut-off points of ADA, IL-1β, IL-2, TNF-α, and IFN-γ to differentiate PLTB, PTB, and meningeal TB (MTB).

Materials and Methods

Research ethics

The study was approved by the Ethics Committee of Thanh Nhan Hospital (Decision No. 88/BVTN-CĐT).

Study settings

A cross-sectional study was conducted from January 2018 to January 2021 on 386 patients with pleural effusion, 47 patients with peritoneal effusion, and 134 patients with meningitis and encephalitis suspect TB. Those patients were examined and treated at Central Lung Hospital, Hanoi Lung Hospital, Bach Mai Hospital, and Thanh Nhan Hospital, Hanoi, Vietnam. Selection criteria included: i) patients over 15 years of age who agreed to participate in the study; ii) patients diagnosed with TB according to the guidelines of the Ministry of Health of Vietnam. Exclusion criteria included: i) pregnant women; ii) patients with medical conditions such as blood disease; patients with autoimmune diseases, immunodeficiency; iii) exhausted patients.

Sample collection

A 3-mL blood sample was taken from each patient to measure ADA and cytokine levels. Samples can be stored at room temperature (25°C) for 2 hours for the most accurate results, or at -18°C to -35°C for 30 days with minor effects on the test.

Adenosine deaminase activity measurement

ADA activity was measured by the decrease in nicotinamide adenine dinucleotide + hydrogen concentration at 340 nm absorbance over time. The calibration was performed, and the calibration curve was built before running the sample. Tests were performed by the automated biochemistry system AU680 (Beckman Coulter, Brea, CA, USA).

Cytokine level measurement

Cytokine enzyme-linked immunosorbent assay (ELISA) was designed based on the competitive ELISA principle and performed with an ELISA Biotek reader (Biotek, Winooski, VT, USA). IL-1β quantification using human IL-1β SimpleStep ELISA® Kit of AbCAM company (Cambridge, UK).

Statistical analysis

Data were analyzed using SPSS 16.0 software (IBM, Armonk, NY, USA). χ^2 test, one-way analysis of variance, Kruskal-Wallis and Mann-Whitney test were used to compare characteristics among three patient groups. The sensitivity and specificity of different biomarkers were computed for differentiating irritable bowel syndrome associated with diarrhea and inflammatory bowel disease. The receiver operating characteristic (ROC) chart evaluating the discriminant value of ADA, IL1-β, IL-2, TNF-α, IFN-γ in the diagnosis of PLTB, PTB, or MTB is shown in Figure 1. A p-value of less than 0.05 was used for detecting statistical significance.

Results

There was a significant difference in age ($p<0.05$) between TB and non-TB patients in the pleural effusion group but no significant difference ($p>0.05$) between TB and non-TB patients in the peritoneal effusion and meningitis and encephalitis groups. Differences among the three patient groups regarding age are presented in Table 1.

Table 2 shows that in all three groups of pleural effusion, peritoneal effusion, and meningitis, the difference in genders between TB and non-TB patients was not significant ($p>0.05$).

Table 3 shows, in the TB group, the rate of TB patients with symptoms of fever and more-than-2-week cough was higher than the non-TB significant ($p<0.05$). Meanwhile, the rate difference between TB and non-TB patients with symptoms of fever, weight loss, bloating, and abdominal pain was not significant in the ascites ($p>0.05$). In the meningitis group, the rate of TB patients with symptoms of fever, vomiting, weight loss, and headache was not greatly different from the non-TB patients. However, a significantly higher rate of TB patients with neck pain was recorded in the comparison with non-TB patients ($p<0.05$).

Table 4 is a comparison between different TB diagnostic methods; ADA and INF-γ had higher sensitivity and specificity, and the combination of both tests, ADA and INF-γ increased the diagnostic sensitivity. When both tests were negative, the negative predictive value (NPV) was 84%, higher than that of the polymerase chain reaction test.

Table 5 shows that in our study, at a cut-off threshold of 30.2 U/L of ADA peritoneal fluid, there were three false positive cases. They were all metastatic peritoneal cancer with ADA results of 38.3 U/L, 68.1 U/L, and 120.8 U/L, respectively.

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Table 6 shows that the confirmation of the presence of TB bacteria by tests is the gold standard of TB diagnosis. In research, these tests had a high specificity and positive predictive value (PPV) (100%), but a great low sensitivity. Meanwhile, the ADA and IFN-γ tests have high sensitivity and NPV.

Discussion

One of the reasons for TB's rapid development is late detection, which creates the basis for community transmission. It was mentioned that among all cases presenting with pleural effusion, 25% are unable to be attributed to a spe-

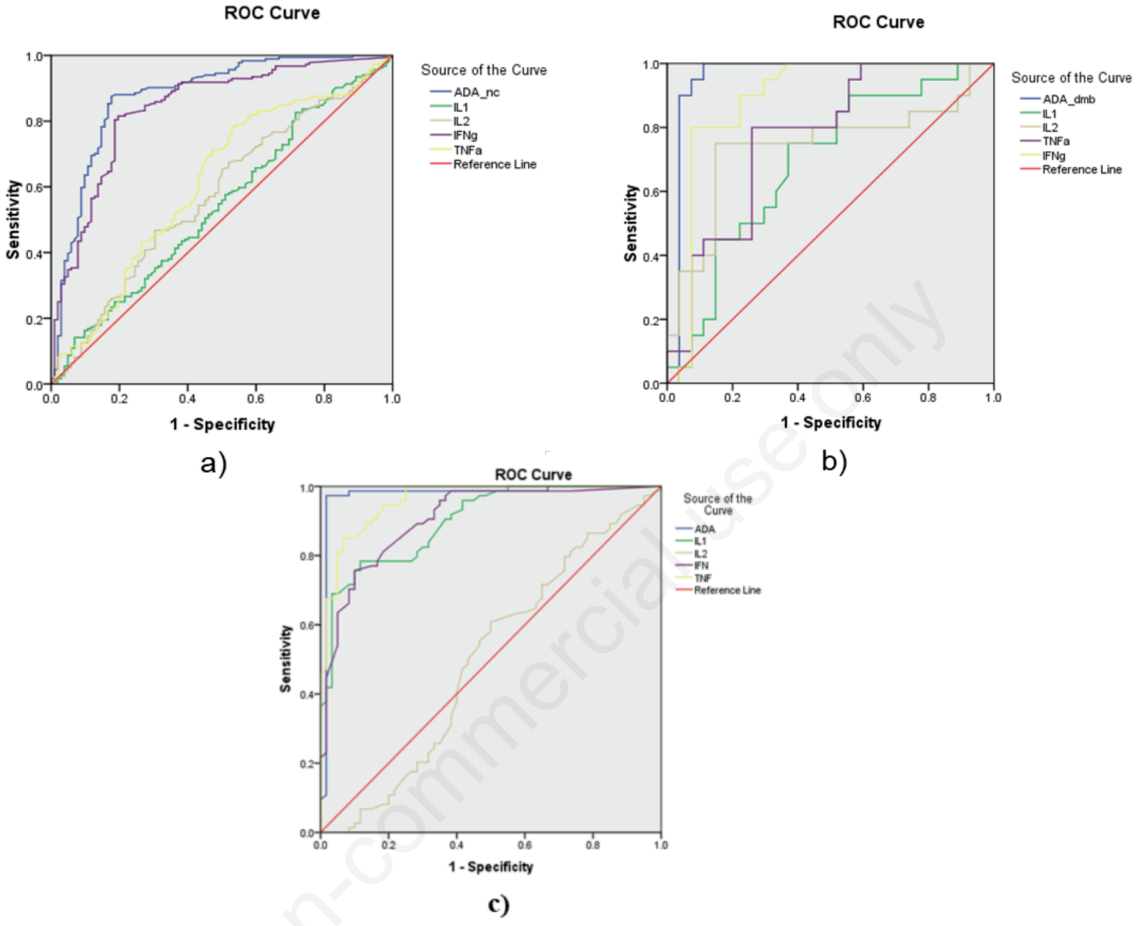

Figure 1. Receiver operating characteristic (ROC) chart evaluating the discriminant value of adenosine deaminase (ADA), interleukin (IL)-1-β, IL-2, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) in the diagnosis of pleural tuberculosis (a), peritoneal tuberculosis (b), and meningococcal tuberculosis (c).

Table 1. Age characteristics.

Groups		Tuberculosis	Non-tuberculosis	Total
Pleural effusion	n (%)	234 (60.6)	152 (39.4)	386 (100)
	Mean±SD	47.4±20.3	63.7±16.6	53.4±20.6
	Min÷Max	16÷91	34÷91	16÷94
	p (*t*-test)	0.004		
Peritoneal effusion	n (%)	20 (42.6)	27 (57.4)	47 (100)
	Mean±SD	50.5±16.3	54.3±13.6	52.6±14.8
	Min÷Max	21÷79	28÷78	21÷79
	p (*t*-test)	0.2		
Meningitis and encephalitis	n (%)	74 (55.2)	60 (44.8)	134 (100)
	Mean±SD	47.15±18.4	52.62±18.8	47.6±18.7
	Min÷Max	16÷90	16÷92	16÷92
	p (*t*-test)	0.09		

SD, standard deviation; Min, minimum; Max, maximum.

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cific diagnosis, even after thoracentesis and closed pleural biopsy. As many as 50% of the patients in this undiagnosed group will eventually be diagnosed with a malignancy. Other diagnostic possibilities include TB, fungal disease, connective tissue disease-related pleuritis, pulmonary infarction, rib fractures, asbestos-related pleural effusion, and nonspecific pleuritis.[7]

This study provides scientific evidence for the application of ADA, IL-1β, IL-2, TNF-α, and IFN-γ to differentiate PLTB, PTB, and MTB.

The optimal cut-off point of ADA pleural fluid was 37.5 U/L with a sensitivity of 87.7%, a specificity of 83.4%, a positive diagnostic value of 89.2%, and negative diagnostic value of 81.3%, showing a noticeable value of the ADA test in the diagnosis of PLTB. A study conducted in Spain (2010) used a cut-off threshold of 35 U/L with a sensitivity of 93% and a specificity of 90% for the diagnosis of TB in cases of exudative effusion.[8] A previous study carried out in England on 345 patients with pleural effusion with a rate of PLTB of 2% also used a cut-off threshold of 35 U/L which yielded high sensitivity, specificity, and NPV (85.7%; 90.9%; and 99.7%), but very low PPV (16.7%).[9]

Studies on ADA pleural fluid showed that the area under the curve (AUC) values of the ROC curve were above 0.9,[10-12] and the cut-off threshold employed was 35-40 U/L.[13] In countries with low TB incidence, the diagnostic use of ADA should be further considered, but the NPV of this test was high, which means when ADA pleural fluid concentration was under 35 U/L, the risk of the patient acquiring TB was low.[8,9]

In PTB, the ADA threshold was 30.5 U/L with a sensitivity of 100%, a specificity of 88.9%, a PPV of 86.9%, and an NPV of 100%. A meta-analysis of 16 published studies implicating ADA in the diagnosis of PTB was performed. ADA cut-off threshold of greater than 30 (30-40) U/L for peritoneal fluid was utilized in 14 studies, and less than 30 U/L was used in 2 studies. Sensitivity and specificity were determined as 93% [95% confidence interval (CI): 89-95%) and 96% (95% CI: 94-97%), respectively.[14] However, an American study showed that the ADA test in ascites had poor sensitivity and limited specificity in non-TB endemic areas.[15]

Table 2. Gender characteristics.

Group	Gender	Tuberculosis	Non-tuberculosis	Total
Pleural effusion	Male (%)	148 (63.2)	98 (64.5)	246
	Female (%)	86 (36.8)	54 (35.5)	140
	Total (%)	234 (100)	152 (100)	386
	p (χ^2)	0.5		
Peritoneal effusion	Male (%)	14 (70)	17 (62.9)	31
	Female (%)	6 (30)	10 (37.0)	16
	Total (%)	20	27	47
	p (χ^2)	0.5		
Meningitis and encephalitis	Male (%)	47 (63.5)	45 (75)	92
	Female (%)	27 (36.5)	15 (25)	42
	Total (%)	74	60	134
	p (χ^2)	0.5		

Table 3. Common symptoms.

Clinical symptoms	TB-caused, n (%)	Non-TB caused, n (%)	p
Hydrothorax			
n (patients)	234	152	
Fever*	156 (66.7)	46 (30.3)	<0.001
Weight loss**	76 (32.5)	36 (23.6)	0.269
Cough	203 (86.6)	127 (83.6)	0.448
Persistent cough >2 weeks	103 (44.1)	47 (30.9)	0.034
Chest pain	196 (83.8)	113 (74.3)	0.185
Dyspnea	142 (60.5)	95 (62.5)	0.07
Ascites			
n (patients)	20	27	
Fever*	10 (50.0)	6 (22.2)	0.06
Weight loss**	5 (40.0)	4 (18.5)	0.3
Bloating	12 (60.0)	21 (77.8)	0.2
Abdominal pain	6 (30.0)	8 (29.6)	0.6
Meningitis			
n (patients)	74	60	
Fever*	5 (6.76)	1 (1.67)	0.224
Vomiting	43 (58.1)	25 (41.6)	0.058
Weight loss**	10 (13.5)	16 (26.7)	0.056
Headache	64 (86.5)	46 (76.7)	0.14
Neck pain	32 (43.24)	16 (26.67)	0.047

TB, tuberculosis; *fever: longer than 1 week; **weight loss: losing 5% body weight within 3 months without diet change.

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Table 4. Comparison of diagnostic tests in pleural tuberculosis.

	Specimen	n	Sens%	Spec%	PPV%	NPV%
ADA (cut-off point ≥37.5 u/L)	Pleural	386	87.7	83.4	89.2	81.3
IL-1β (cut-off point ≥ 56.0 pg/mL)	Pleural	386	82.1	28.4	64.4	46.8
IL-2 (cut-off point ≥58.0 pg/mL)	Pleural	386	53.4	69.7	73.1	49.3
TNF-α (cut-off point 78.0 pg/mL)	Pleural	386	77.8	47.4	69.5	58.1
IFN-γ (cut-off point 120 pg/mL)	Pleural	386	80.3	80.9	86.6	72.8
ADA+INF-γ*	Pleural	386	93.5	61.8	81.5	84.0
AFB	Sputum	257	7.5	100	100	40.0
MGIT culture	Pleural	386	19.7	100	100	44.7
	All	386	30.3	100	100	48.3
TRC ready MTB	Pleural	63	2.8	100	100	43.5
	All	72	13.6	100	100	42.4
Tuberculosis PCR	Pleural	87	39.1	81.3	75.0	48.1
	All	102	43.4	79.4	76.7	47.4
Anatomy	Biopsied tissue	138	63.3	100	100	67.0

PVV, positive predictive value; NPV, negative predictive value; Sens, sensitivity; Spec, specificity; ADA, adenosine deaminase; IL, interleukin; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; AFB, acid-fast bacillus; MGIT, mycobacteria growth indicator tube; TRC ready MTB, Mycobacterium tuberculosis reverse transcription concerted reaction; PCR, polymerase chain reaction; *positive when at least one out of two tests have values greater or equal to cut-off value and negative when both tests have values less than cut-off value.

Table 5. Values of some tests in the diagnosis of peritoneal tuberculosis.

	Specimen	n	Se %	Sp %	PPV %	NPV %
ADA (cut-off point ≥ 30.5 u/L)	Peritoneal effusion	47	100	88.9	86.9	100
IL-1β (cut-off point 112.8 pg/mL)	Peritoneal effusion	47	75.0	62.9	60.0	77.3
IL-2 (cut-off point 86.0 pg/mL)	Peritoneal effusion	47	75.0	85.2	78.9	82.1
TNF-α (cut-off point ≥ 56.8 pg/mL)	Peritoneal effusion	47	80.0	74.1	69.6	83.3
IFN-γ (cut-off point 200.0 pg/mL)	Peritoneal effusion	47	80.0	92.6	88.9	86.2
ADA+IFN-γ*	Peritoneal effusion	47	100	85.2	83.3	100
AFB staining	Sputum	25	7.6	91.7	50.0	47.8
MGIT culture	Peritoneal effusion	47	35.0	100	100	67.5
	All**	47	40.0	96.3	88.9	64.4
TRC ready MTB	Peritoneal effusion	8	0	100	0	50.0
	All	10	0	100	0	50.0
Anatomy	Biopsied tissue	17	88.9	100	100	88.9

PVV, positive predictive value; NPV, negative predictive value; Se, sensitivity; Sp, specificity; ADA, adenosine deaminase; IL, interleukin; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; AFB, acid-fast bacillus; MGIT, mycobacteria growth indicator tube; TRC ready MTB, Mycobacterium tuberculosis reverse transcription concerted reaction; PCR, polymerase chain reaction; *positive when at least one out of two tests have values greater or equal to cut-off value and negative when both tests have values less than cut-off value; **1 case was pulmonary tuberculosis with positive (+) AFB and MGIT. However, this patient was classified as peritoneal effusion due to cirrhosis because the background disease was cirrhosis, peritoneal fluid protein was 7.71g/L, MGIT culture of peritoneal fluid (-). This case has ADA peritoneal fluid of 7.6u/L.

Table 6. Values of some cerebrospinal fluid tests in diagnosis of meningococcal tuberculosis.

	n	Se%	Spe%	PPV%	NPV%
ADA (8.1 u/L)	134	97.3	98.3	98.6	96.7
IL-1β (≥12.3 pg/mL)	134	78.4	88.3	89.2	76.8
IL-2 (18.0 pg/mL)	134	86.5	21.7	57.7	56.5
IFN-γ (77.1 pg/mL)	134	98.6	61.7	76.0	97.4
TNF-α (30.0 pg/mL)	134	85.1	93.3	94.0	83.5
ADA+IFN-γ*	134	100	61.7	76.3	100
AFB staining	34	0	100	0	41.2
MGIT culture	93	5.8	100	100	45.6
TRC ready MTB	45	21.4	100	100	43.6
Gene Xpert	93	15.1	100	100	47.1

PVV, positive predictive value; NPV, negative predictive value; Se, sensitivity; Spe, specificity; ADA, adenosine deaminase; IL, interleukin; TNF-α, tumor necrosis factor-α; IFN-γ, interferon-γ; AFB, acid-fast bacillus; MGIT, mycobacteria growth indicator tube; TRC ready MTB, Mycobacterium tuberculosis reverse transcription concerted reaction; *positive when at least one out of two tests have values greater or equal to cut-off value. Negative when both tests have values less than cut-off value.

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In tuberculous meningitis, the AUC in the diagnosis of MTB was 0.975 (95% CI: 0.942-1,000), meaning that ADA is valuable in the diagnosis of tuberculous meningitis. In a previous study, the most common cut-off threshold ranged from 3.4-10 U/L.[16] Another study published in 2020 on 54 meningitis patients, including 8 MTB, showed that the optimal clinical cut-off level was 5.1 U/L for the diagnosis of meningitis with a sensitivity of 100%, and a specificity of 91%. In some cases, a false increase of ADA might be observed in individuals with specific conditions such as central nervous system lymphoma and fungal infections.[17] In a meta-analysis of 3548 patients with meningitis, 1490 suspect TB cases had both cerebrospinal fluid ADA and mycobacteria cultures. The optimal cut-off level was calculated as 2.0 U/L (AUC=0.86; 95% CI: 0.82-0.89; p<0.01; sensitivity of 85.9% and specificity of 77.7%, with a ratio for a positive test of 3.85 and for a negative test of 0.18). These results suggest that a negative ADA test result should not be used solely as a reason to exclude or discontinue the anti-TB treatment. In our study, 60 patients with clinical non-tuberculous meningitis had symptoms quite similar to meningococcal disease's symptoms. Among 74 patients diagnosed with tuberculous meningitis, evidence of TB bacteria was found in 17 cases (22.9%) and was not identified in 57 (77.1%) cases. Globally, this rate varies between different studies. Some authors confirmed that the analysis of cerebrospinal fluid was an important step in the diagnosis of tuberculous meningitis. However, this finding has limitations in some cases, such as meningitis in children, HIV co-infection with MTB, or other pathogens with similar clinical symptoms, such as cryptococcal infections.[18]

For INF-γ, according to the ROC curve chart, the AUC of INF-γ pleural fluid in the diagnosis of PLTB was 84% with p<0.05. An INF-γ value of 120 pg/mL had the highest Youden index, which was the differential value for PLTB. A meta-analysis of 22 studies, measuring INF-γ in a total of 2101 patients with pleural effusion, showed a sensitivity of 89%, a specificity of 97%, and an AUC of 99% for the diagnosis of TB.[19] Khan *et al*. suggested that the optimal cut-off values for IFN-γ and ADA were 0.5 pg/mL and 16.65 U/L, respectively. Results for sensitivity and specificity of IFN-γ and ADA were 100% and 100%, 86% and 74%, respectively. IFN-γ PPV was 100%, and NPV was 100%.[20]

In PTB, with a threshold of IFN-γ in peritoneal fluid of 200 pg/mL, sensitivity was 80%, specificity was 92.6%, NPV was 86.2%, PPV was 88.9% and accuracy was 87.2%. According to Oliver *et al.*, in 50 patients with ascites, 17 cases (34%) were infected with TB after bacterial culture. An IFN-γ threshold of 26 pg/mL in peritoneal fluid allowed a sensitivity of 81%, and a specificity of 100%.[19] In their study, Zhou *et al.* also showed that the value of IFN-γ in TB diagnosis was high, with sensitivity of 91.7%, specificity of 76.5%, and PPV of 84.6%.[20]

In tuberculous meningitis, the AUC of cerebrospinal fluid IFN-γ in the diagnosis of MTB was 0.906 with p<0.001. The cut-off of 30 pg/mL provided a diagnostic value for meningoencephalitis with a sensitivity of 98.6% and a specificity of 61.7%. In a study on tuberculous meningitis in children, the author showed that IFN-γ, when used in combination with other biomarkers, could achieve sensitivity and specificity up to 100%.[21]

Although IL-1β, IL-2, TNF-α are the primary cytokines involved in the body's inflammatory response against TB bacteria, the role of these tests in aspiration fluid is limited in distinguishing extrapulmonary TB from other infectious diseases. We suggested that vaccination with TB might play a role in the increase of these cytokines. Many studies around the world showed that, despite playing a central role in regulating and activating the immune response, the sensitivity and specificity of IL-2 in the diagnosis of pleural were fairly poor. In addition, many studies recognized that IL-2 was valuable in differentiating latent and active TB. Hence, IL-2 was considered a valuable biomarker for detecting active TB. According to Tural Onur *et al*., IL-2 in diagnosing PLTB had a sensitivity and specificity of 82.69% and 52.9%, respectively; thus, IL-1β and IL-2 were considered valuable tests for discriminating membranous TB in non-TB endemic countries, which were comparable to ADA levels.[21] It was found that Gram (-) bacteria increased levels of proinflammatory cytokines (especially IL-1β) in comparison with Gram (+). The usage of antibiotics cefuroxime and gentamicin accelerated the production of IL-1β and IL-6, whereas killing the bacteria with ciprofloxacin and imipenem-cilastatin diminished the cytokine production.[22]

Antibiotic overuse is probably a confounding factor as many patients received different antibiotics before admission. In addition, the usage of some anti-inflammatory drugs also caused a decline of proinflammatory cytokines' (IL-6, macrophage inflammatory protein-1α, and IL-1β) levels by the inhibition of nuclear factor κ B.[23]

Compared with other TB diagnostic methods, the acid-fast bacillus (AFB) test allows for quick results but with a low sensitivity. Culture of TB bacilli normally requires at least 2-6 weeks and a large volume of fluid specimen. This requirement was unresponsive to cerebrospinal fluid in some cases, especially in children. Another test, such as anatomy, can be less time-consuming and relatively specific, but patients may be at risk of organ damage, bleeding, and some other complications, and the biopsy procedure may be impossible for some patients. Therefore, diagnostic biopsy is usually only recommended for difficult-to-diagnose or cancer-diagnostic cases. Molecular bioassays that amplify a specific nucleotide fragment of TB can provide quick results with limited sensitivity, especially in cerebrospinal fluid or peritoneal fluid, and the accuracy of results depends on the quality of the specimen. A study conducted in Spain on 214 PLTB patients reported that bacterial culture was positive in 30/198 cases of pleural fluid (15%), in 44/157 cases of sputum (28%), and the AFB test was positive in 22/158 cases of sputum (14%).[24]

This study had several limitations. Specimens of patients involved in this study were collected from healthcare centers specializing in TB; thus, the incidence of TB-positive (+) samples may be high and may not be representative of the population. In some cases, the usage of antibiotics and anti-inflammatory drugs could not be exploited. The study did not exploit patients who used any antibiotics and anti-inflammatory drugs before because this issue was not mentioned in the medical records.

Conclusions

In the aspiration fluid, ADA activity was greatly different between PLTB, PTB and MTB groups with values of 37.5 U/L, 30.5 U/L, and 8.1 U/L and sensitivity and speci-

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ficity of 87.7% and 83.4%; 100% and 88.9%; 97.3% and 98.6%, respectively. Similarly, IFN-γ concentrations were distinctively different between PLTB, PTB, and MTB patients (with values of 120 pg/mL, 200 pg/mL, and 30 pg/mL with sensitivity and specificity of 80.3% and 80.9%; 80.0% and 92.6%; 98.6% and 61.7%, respectively). Determination of ADA and IFN-γ in aspiration fluid is a highly sensitive and specific test to differentiate PLTB, PTB, and MTB.

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