Application of adenosine deaminase combined with γ-interferon release test in the diagnosis of tuberculous pleural effusion in patients over 40 years old

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Research Article

Keywords: adenosine deaminase, interferon gamma releasing assays,aged,diagnosis,pleural effusion, tuberculosis

Posted Date: May 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1647917/v1

Abstract

Background: The diagnostic accuracy of pleural biomarkers in patients with tuberculous pleural effusion (TPE) of different ages is inconsistent, but specific studies in different age groups are lacking. Therefore, we investigated the cut-off value of adenosine deaminase (ADA) and its combined gamma interferon release assay (IGRA) in the diagnosis of TBE patients of different ages in aged≥40 years.

Methods: A retrospective analysis of 176 patients who were hospitalized from 2015 to 2020 and had pleural effusion with or without fever,night sweats, fatigue,cough and other clinical manifestations and were highly suspected of tuberculous pleural effusion. The results of medical thoracoscopy and pleural effusion ADA and IGRA were analyzed. The patients were divided into 40-59-year-old group and≥60-year-old group.

Results: Among the 176 patients, 85 were aged 40-59 years, and 91 were aged≥60 years. The cut-off value of ADA in ROC analysis is 31.5 U/L, the accuracy is higher than 40 U/L (85.80%vs.82.95%), but the diagnostic sensitivity in patients aged≥60 years is lower than that in the group of 40-59 years old (71%vs.83%).The sensitivity of IGRA detection in patients aged≥60 years was higher than that of ADA (93% vs.71%). The detection sensitivity of ADA combined with IGRA in pleural effusion was the highest in the 40-59-year-old group, reaching 100%. The specificity of ADA united IGRA detection in pleural effusion was the highest in the age≥60 years group, reaching 100%.

Conclusions: The detection of ADA and IGRA in pleural effusion has good diagnostic value for patients with suspected TBE aged≥40 years. ADA unites IGRA detection is beneficial to the detection rate of TBE in patients with exudative pleural effusion aged 40-59 years, and it is beneficial to the differential diagnosis of TBE in patients aged≥60 years.

1 Background

According to the latest WHO report, pulmonary tuberculosis is a serious global public health problem, especially in developing countries. In recent years, the decline in the number of cases and incidence rates has slowed down, and the mortality rate has increased. China is one of the countries with a high burden of tuberculosis and given much attention. Tuberculous pleural effusion (TPE) is the most common extrapulmonary tuberculosis[1]. TPE is an exudative pleural effusion caused by Mycobacterium tuberculosis infection. Early diagnosis and treatment can reduce its morbidity and mortality. If untreated, 43%-65% of patients can develop pulmonary tuberculosis[2].

Adenosine deaminase (ADA) is an important enzyme in the metabolism of purine nucleosides. It is widely present in body tissues. The content of ADA in lymphocytes is high. When TPE occurs, the host responds to pleural lymphocytes through cellular immunity. The number of cells increases significantly and the activity is enhanced, so the content of ADA in pleural effusion is significantly increased, and it is widely used in the diagnosis of TPE because of its economical detection, convenience and availability[3–5]. However, ADA can also increase in lung abscess, lymphoma, malignant mesothelioma and other diseases. It is easily affected by age factors and the diagnostic accuracy is affected significantly with the increase of age.Therefore, we chose the combination of interferon-gamma release assay (IGRA), which is not easily affected by age factors, for the diagnosis of suspected TPE[6–11]. At present, the critical value of ADA for TPE diagnosis is generally 40U/L, but due to the influence of tuberculosis prevalence, age, organ failure, critical condition, etc., the critical value of various reports is different and the guidance is different. For example, the Turkish guideline recommends The diagnosis of pleural effusion disease at age ≥ 40 years should still be confirmed by biopsy even if ADA > 40 U/L[12].Elderly patients with pleural effusion have complex etiologies, multiple comorbidities, and high risk of invasive procedures. With the intensification of the aging population, the diagnostic methods of TPE in the elderly still need to be continuously optimized. Based on this, we focused on TPE patients aged

40–59 years and ≥ 60 years old, and explored the optimal cut-off value of ADA in this population and the diagnosis of ADA combined with interferon-gamma release assay (IGRA) in TBE patients of different ages.

2 Objectives And Methods

2.1 Subjects, demographics, and informed consent

Inclusion criteria: Patients aged ≥ 40 years with exudative pleural effusion were diagnosed based on the Light definition criteria. Exclusion criteria: Combined with medical thoracoscopy contraindications: lung and chest wall adhesion in the entire thoracic cavity; hypercapnia and severe respiratory distress; uncontrollable cough; for patients with clear consciousness and judgment but not sign the informed consent form. The data of 199 patients were initially observed. After excluding those who did not meet the inclusion criteria, we finally included 176 patients. All patients underwent pleural effusion ADA, IGRA, and medical thoracoscopy biopsy, of which 90 were TPE and 86 were non-TPE. There were 85 cases in the 40-59-year-old group and 91 cases in the ≥ 60-year-old group.(Fig. 1)

The diagnostic criteria for TPE are as follows: Thoracoscopic pleural biopsy showed tuberculosis and/or acid-fast mycobacteria; Pleural effusion caused by pleurisy caused by other causes has been excluded clinically. There were 52 cases of malignant pleural effusion in non-TPE, 4 cases of empyema, 3 cases of systemic lupus erythematosus (SLE), 2 cases of hepatic pleural effusion, 3 cases of eosinophilic pleural effusion, and 1 case of heart failure. Pleural cytology or pleural biopsy after pleural effusion examination is consistent with the diagnosis of malignant disease even if a malignant pleural effusion is diagnosed. There was no macroscopic empyema in the pleura, and the biochemical and clinical effusion examinations were consistent with parapneumonic effusion, and the diagnosis of parapneumonic effusion was confirmed by the observation of physiological and clinical improvement after antibiotic treatment. If there is a complicated pleural effusion caused by a clear disease, the diagnosis should be made after the medical thoracoscopy and pathological biopsy are completed to exclude other causes. All participants were followed up for 12 months to confirm the accuracy of the final diagnosis.

Demographic data (age and gender),address,smoking index, comorbidities(hypertension,diabetes,chronic obstructive pulmonary disease,tumor,nephrotic syndrome,liver cirrhosis, heart failure), diagnosis, diagnostic method, recording relevant biochemical indicators information. This study was approved by the Guangyuan Central Hospital Ethics Committee,which waived the need for informed consent. We confirm that all methods were carried out in accordance with relevant guidelines and regulations.

2.2 ADA detection

ADA activity was detected by colorimetric assay using a commercial kit (Adenosine Deaminase Detection Kit; Beijing Strong Biotechnology Co., Ltd.Beijing,China) at 37°C. One unit of ADA is defined as the amount of enzyme that produces 1 micromolar inosine per minute from adenosine at 37°C, and the result is expressed in International Units (IU/L) per liter of pleural fluid (PF).

2.3 IGRA detection

All subjects collected 45 mL of PF and tested within 6 h. PF samples were centrifuged at 500 g for 10 min. Discarding the sample supernatant for TB T-SPOT detection. Tuberculosis testing was performed according to the manufacturer's instructions (Oxford Immunotec Ltd. Oxford, UK). Microspheres were resuspended in 8 mL of AIM-V medium (GIBCO, Rockville,MD,USA). Mononuclear cells were isolated using Ficolle-Hypaque Lymphocyte Separator, washed, resuspended and counted. Empty wells were used as negative controls, T lymphocyte mitogen lectin was used as positive controls, and ESAT-6 and CFP-10 polypeptides were in different wells, respectively. Pleural fluid mononuclear

cells were added to wells pre-coated with anti-IFN-γ monoclonal antibody (2.5×10^5 cells per well) and incubated at 37°C for 16–20 hours.Spot-forming cells (SFCs) were read using an automated enzyme-linked immunosorbent spot reader (CTL-ImmunoSpotS5 Versa analyzer). The assay was considered valid when the positive control was > 20 SFCs/10^6 monocytes and the negative control was < 6 SFCs/10^6 monocytes. Final SFCs of ESAT-6 or CFP-10 were defined as ESAT-6 or CFP-10 sfc minus negative control SFCs. The largest SFCs in the T-SPOT assay were defined as larger SFCs in the final ESAT-6 and CFP-10[13].

2.4 Data Analysis

The obtained data were analyzed by SPSS19.0 statistical software, measurement data were expressed as($\bar{x} \pm s$), and t test was used. Enumeration data were expressed as rate (%) using χ2 test. IGRA and ADA used ROC curve analysis to evaluate the optimal critical value of TPE, and $P < 0.05$ was considered statistically significant.

3 Results

3.1 The included patients were divided into 40-59-year-old group and age ≥ 60-year-old group. TPE accounted for 56.47% and 46.15% of the group, respectively, with no statistical significance ($P > 0.05$). However, there were significant differences in age, smoking index and proportion of comorbidities between the two groups ($P < 0.05$). There was no significant difference in ADA, ESR and IGRA between the two groups ($P > 0.05$). (Table 1)

Table 1 Comparison of clinical features between two groups of patients with exudative pleurisy

Cases	40≤Age<60	Age≥60	Statistics	p value
	85 cases	91 cases		
Diseases[TPE/non-TPE(TPE%)]	48/37(56.47%)	42/49(46.15%)	χ^2=1.87	0.17
Gender[male/female(male%)]	53/32(62.35%)	61/30(67.03%)	χ^2=0.42	0.52
Age(years)	49.72±5.37	69.62±6.31	t=-22.46	0.00
Smoking index	174.18±236.89	294.73±333.93	t=-2.78	0.01
Underlying diseas cases(percentage)	25(29.41%)	65(71.43%)	χ^2=31.05	0.00
ADA level in pleuralfluid(U/L)	35.21±16.83	31.97±16.56	t=1.29	0.20
ESR(mm/h)	26.80±10.37	26.48±10.89	t=0.20	0.84
IGRA[positive/total(positive%)]	49/85(57.65%)	46/91(50.55%)	χ^2=0.89	0.35

3.2 ROC curve analysis was performed for all ages ≥ 40 years old, and the optimal critical value was 31.5U/L, and the AUC was 0.94(95%CI, 0.90–0.97). When the cutoff value was 26U/L, the sensitivity was 92%, the specificity was 78%, and the accuracy was 85.23%. When the cutoff value was 40U/L, the sensitivity was 70%, the specificity was 97%, and the accuracy was 82.95. The highest accuracy (85.8%) was achieved with a cutoff value of 31.5U/L. (Table 2)

Table 2 Sensitivity, specificity,LR+/−, PPV, NPV, and ACC values according to ADA cutoff values in patients aged 40 years and older

ADA U/L	ADA 26	ADA 31.5	ADA 40
Sensitivity,(95%CI)	0.92(0.84-0.97)	0.82(0.72-0.89)	0.70(0.59-0.79)
Specificity,(95%CI)	0.78(0.67-0.86)	0.90(0.81-0.95)	0.97(0.89-0.99)
LR+(95%CI)	4.17(2.79-6.24)	7.86(4.20-14.69)	20.07(6.55-61.49)
LR−(95%CI)	0.10(0.05-0.20)	0.20(0.13-0.31)	0.31(0.23-0.43)
PPV,(95%CI)	0.81(0.72-0.88)	0.89(0.80-0.95)	0.95(0.86-0.99)
NPV,(95%CI)	0.91(0.81-0.96)	0.83(0.73-0.90)	0.75(0.66-0.83)
Acc%,(95%CI)	85.23(0.79-0.90)	85.80(0.80-0.90)	82.95(0.77-0.88)

Abbreviations: 95% CI, 95% confidence interval; Acc, accuracy; ADA, adenosine deaminase enzyme; LR+,positive likelihood ratio; LR−, negative likelihood ratio; PPV, positive predictive value;NPV, negativepredictive value.

3.3A retrospective pooled analysis of previous studies on optimal cutoff values for ADA in different age groups is presented in Table 3.

Table 3
Previous studies on the best cut-off values for ADA in different age groups

ADA source	Diagnostic Optimal Cutoff(U/L)	Suitable age	References
Pleural effusion	72	< 55years	[7]
Pleural effusion	26	> 55 years	[7]
Pleural effusion	43	> 45 years	[8]
Pleural effusion	29	< 45 years	[8]
Pleural effusion	35.9	> 45 years	[10]
Pleural effusion	23.3	< 45 years	[10]
Pleural effusion	21.4	≤ 40 years	[11]

3.4 IGRA has a sensitivity of 90%, a specificity of 84%, and an accuracy of 87% in the overall TPE patients. The diagnostic sensitivity and specificity for patient aged ≥ 60 years are slightly higher than those of patients aged 40–59 years (93%vs.88%, 86%vs.81%). ADA was more specific (98%vs.81%) but less sensitive (71%vs.83%) than the 40-59-year-old group for the diagnosis of TPE aged ≥ 60years. The sensitivity of IGRA combined with ADA for the diagnosis of TPE was 100% in the 40-59-year-old group, which was higher than that in the other group (98%), but the specificity was higher in those aged ≥ 60 years (82%vs.73%), There was no significant difference in accuracy (89%vs. 88%). The specificity of IGRA combined with ADA was 100% in the age ≥ 60 years group, higher than that in the other group (97%), and the diagnostic accuracy was also higher than that in the 40-59-year-old group (89%vs.85%).There was no significant difference in sensitivity (76%vs. 75%) between the two groups. (Table 4)

Table 4
Diagnostic utility of pleural IGRA, ADA, CEA and their integrations for the discriminating diagnosis of TPE and No-TPE

Assays	Sensitivity (95%CI)	Specificity (95%CI)	LR+ (95%CI)	LR− (95%CI)	PPV (97%CI)	NPV (98%CI)	Acc%, (95%CI)
IGRA							
All patients	0.90(0.81–0.95)	0.84(0.74–0.90)	5.53(3.41–8.97)	0.12(0.06–0.22)	0.85(0.76–0.91)	0.89(0.79–0.94)	0.87(0.81–0.91)
40-59years	0.88(0.74–0.95)	0.81(0.64–0.91)	4.63(2.35–9.09)	0.15(0.07–0.33)	0.86(0.72–0.94)	0.83(0.67–0.93)	0.85(0.75–0.91)
≥60	0.93(0.79–0.98)	0.86(0.72–0.94)	6.50(3.26–12.97)	0.08(0.03–0.25)	0.85(0.71–0.93)	0.93(0.81–0.98)	0.89(0.81–0.94)
ADA							
40-59years	0.83(0.69–0.92)	0.81(0.64–0.91)	4.40(2.23–8.69)	0.21(0.21 – 0.11)	0.85(0.71–0.93)	0.80(0.62–0.90)	0.82(0.73–0.89)
≥60	0.71(0.57–0.82)	0.98(0.88-1.00)	19.83(5.06–77.68)	0.20(0.11–0.37)	0.98(0.85-1.00)	0.75(0.62–0.85)	0.89(0.81–0.94)
Pleural IGRA or ADA							
All patients	0.99(0.93-1.00)	0.78(0.67–0.86)	4.48(3.01–6.66)	0.01(0.00-0.10)	0.82(0.74–0.89)	0.99(0.91-1.00)	0.89(0.83–0.93)
40-59years	1.00(0.91-1.00)	0.73(0.56–0.86)	3.70(2.18–6.28)	0	0.83(0.70–0.91)	1.00(0.84-1.00)	0.88(0.79–0.94)
≥60	0.98(0.86-1.00)	0.82(0.67–0.91)	5.31(2.94–9.61)	0.03(0.00-0.20)	0.82(0.68–0.91)	0.98(0.86-1.00)	0.89(0.81–0.94)
PleuralIGRA and ADA							
All patients	0.76(0.65–0.84)	0.99(0.93-1.00)	64.98(9.23-457.66)	0.25(0.17–0.36)	0.99(0.91-1.00)	0.79(0.70–0.86)	0.87(0.81–0.91)
40-59years	0.75(0.60–0.86)	0.97(0.84-1.00)	27.75(3.99-193.14)	0.26(0.16–0.42)	0.97(0.84-1.00)	0.75(0.60–0.86)	0.85(0.75–0.91)
≥60	0.76(0.60–0.87)	1.00(0.91-1.00)	0	0.24(0.14–0.41)	1.00(0.87-1.00)	0.83(0.71–0.91)	0.89(0.81–0.94)

3.5 The ROC curve analysis was performed for the 40-59-year-old group and the age ≥ 60 years, respectively, and the best cutoff value was 31.5U/L, AUC was 0.93 (95%CI, 0.88–0.99) and 19.5U/L, AUC was 0.57 (95% CI, 0.45–0.70). (Fig. 2)

4 Discussion

The etiology of pleural effusion is complex, especially in the middle-aged and older people, and it is difficult to distinguish it from TPE, which is often a difficult problem in the process of clinical diagnosis. Combining different causative factors, TPE is still the most common cause of exudative pleural effusion in developing countries[11, 14]. TPE is caused by Mycobacterium tuberculosis (MTB) infection. MTB detection is the gold standard for the diagnosis of

TPE, but the detection rate of MTB in pleural effusion is very low and has a high false-positive rate, which is easy to be missed and the culture time is long[15, 16]. In particular, TPE patients aged ≥ 40 years face diagnostic challenges in worldwide, but there is no consensus in the literature on diagnosis in this age group, so further studies are needed[17, 18]. As the population ages, the number of people over the age of 60 is increasing. Previous studies have shown that advanced age, disease severity, and organ failure will affect the accuracy of ADA in diagnosing TPE. Based on this, we included patients with exudative pleural effusion age ≥ 40 years and divided them into 40-59-year-old group and age ≥ 60-year-old group for separate research, hoping to provide some help for the diagnosis of TPE patients of different ages.

A large number of previous studies have shown that ADA 40U/L is a widely accepted cut-off value for the diagnosis of TPE, but in recent years, there has been a lot of controversy over the optimal cut-off value of ADA in a series of studies, especially regarding the greater influence of age and local tuberculosis prevalence on it[10, 14, 19, 20]. We analyzed the included patients with exudative pleural effusion aged ≥ 40 years and obtained the optimal cut-off value of TPE is 31.5 U/L and AUC is 0.94 (95% CI, 0.90–0.97), which has a good clinical diagnostic value. Burcu Arpinar Yigitbas[3] found that ADA 26U/L was 84.3% sensitive, 80.4% specific, and 82.35% accurate for TPE diagnosis in people aged ≥ 40 years. We also used ADA 26U/L as the cut-off value analysis and found that the sensitivity of this group of patients was 92%, the specificity was 78%, and the accuracy was 85.23%, which was close to the results reported above but the specificity and accuracy were lower than ADA31. 5 U/L.It can be seen that there are certain differences in the optimal ADA thresholds for different groups of people in different regions of the same age. Tay et al[7] found that ADA 26 IU/L was the best for the diagnosis of TPE in people older than 55 years, with a sensitivity of 94.7% and a specificity of 80%. However, Abrao et al[8] and other studies believe that ADA 43 IU/L is the best for the diagnosis of TPE when the age is older than 45 years, with a sensitivity of 82.7 and a specificity of 72.4%.

The optimal ADA 29 IU/L for the age of less than 45 years had a sensitivity of 88.6 and a specificity of 91.5%. A recent study found that only 4.65% of elderly TPE patients had an ADA greater than 40 IU/L[14, 21].In order to explain the above differences, we included ADA40IU/L in the age group ≥ 40 years old and found that the sensitivity was 70%, the specificity was 97%, and the accuracy was 82.95%. It can be seen that using ADA40IU/L as the best cut-off value for the diagnosis of TPE has great differences in different research samples, populations, and regions, and ADA can also be elevated by other diseases in the body, but this serious influencing factor has not been considered in many studies. Therefore, there are some differences in the results of different samples. How to accurately correct the optimal threshold of ADA or seek more accurate diagnostic indicators needs further research.

The stimulatory antigens ESAT-6 and CFP10 used in the IGRA test are unique to MTB and are not affected by BCG and body immunity, which improves the diagnostic specificity and avoids the influence of non-tuberculous bacilli and BCG on the results, and has good diagnostic value for TPE[22, 23]. A recent study reported significant differences in IGRA between TPE and non-IGRA groups due to antigen-specific responses to MTB, including malignant pleural effusion, pneumonia, and cirrhosis[21]. It has been reported that IGRA has a high diagnostic accuracy of more than 90.2% for patients with ADA uncertain TPE[24].

The data analysis of this group found that IGRA has a greater diagnostic advantage in the age ≥ 60 years old group than the group of 40–59 years old, with sensitivity(93%vs.88%), specificity(86%vs.81%) and diagnostic accuracy rates (89% vs. 85%) are both high.The diagnostic accuracy rate of the whole population is 87%, which is close to the results of Mollo B[25] study (sensitivity is 80%, specificity is 72%), but lower than some other related reports, which may be related to the high comorbidity and age of this group[26].Unfortunately, we did not perform immune function tests such as lymphocytes and their subsets in peripheral blood and pleural effusion to judge the function of peripheral and local

lymphocytes in the pleural cavity, so as to conduct a more in-depth analysis of the influencing factors of IGRA and ADA. Previous studies have found that ADA detection has low diagnostic sensitivity for patients aged ≥ 60 years. We also performed ROC curve analysis on ADA alone for patients aged ≥ 60 years and found that the optimal cut-off value was 19.5 U/L, and the AUC was 0.57 (95% CI, 0.45–0.70), which shows that its diagnostic value is limited, but this may be related to the age(average age was 69.62 ± 6.31 years), which is basically consistent with previous reports[7, 24, 27]. Therefore, we analyzed ADA combined with IGRA and found that the sensitivity of ADA combined with IGRA in pleural effusion was 100% in the 40-59-year-old group. The detection rate of TPE has good application value and is worthy of clinical promotion. However, its specificity is 73%, so exudative pleural effusion in this population should be combined with other more specific biomarkers or biopsy to diagnose TPE. The combined detection and analysis of IGRA and ADA found that the specificity was 100% in the age ≥ 60 years old group, which has great clinical application for excluding TPE in patients with exudative pleural effusion who are elderly, unwilling to biopsy, and difficult to diagnose,which is consistent with the results of some Chinese related studies[28]. Although the above analysis has obtained promising results, the biomarkers of TPE have their own limitations, and more in-depth research is needed for more economical, non-invasive and accurate diagnostic methods[14, 22].In conclusion, ADA combined with IGRA has good diagnostic and differential diagnostic value in patients with exudative pleural effusion over the age of 40, especially for elderly patients with suspected TBE who are unwilling to undergo medical thoracoscopy or pleural biopsy, and those medical units that do not have thoracoscopy equipment.Moreover, the application of ADA combined with IGRA detection can eliminate the pain and risk caused by invasive operations, reduce the medical burden, and promote more accurate diagnosis of TBE.

5 Conclusion

The detection methods of ADA and IGRA in pleural effusion are simple, economical and reliable, and have good diagnostic and differential diagnostic value for suspected TBE and aged ≥ 40 years. In patients with exudative pleural effusion aged 40 to 59 years, the use of pleural effusion ADA combined with IGRA detection is beneficial to the detection rate of TBE. For patients with exudative pleural effusion aged ≥ 60 years, the sensitivity of IGRA detection is higher than that of ADA, and ADA combined with IGRA detection is helpful for differential diagnosis of TBE.

Abbreviations

TPE: tuberculous pleural effusion; ADA:Adenosine deaminase; IGRA:interferon gamma release test; PF:pleural fluid; SFCs:Spot-forming cells; MTB:Mycobacterium tuberculosis; SPSS: Statistical package for social sciences;CI: Confidence interval; Acc:accuracy; LR+:positive likelihood ratio; LR−:negative likelihood ratio; PPV: positive predictive value;NPV: negativepredictive value.

Declarations

Ethics approval and consent to participate

The authors confirm that all methods were carried out in accordance with relevant guidelines and regulations.The need for informed consent was waived by the ethics committee full name:Guangyuan Central Hospital Ethics Committee because of the retrospective nature of the study. We confirm that all experimental protocols were approved by the Guangyuan Central Hospital Ethics Committee.The study was conducted according to the guidelines of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed in the study are available from the corresponding author on reasonable request. (Dong Lixia,Email: shuzhili@tmu.edu.cn)

Competing interests

The authors declare no conflict of interest.

Funding

This work was supported by the key R&D projects of the Science and Technology Bureau of Guangyuan City, Sichuan Province[20ZDYF0007]

Author contributions

Contributed to conception and design: Li ZS and Dong LX. Contributed to acquisition of data: Li ZS and Zeng J. Contributed to analyses of data: Li ZS. Contributed to interpretation of data: Li ZS, Wang M, Zhao R and Zheng D. Drafting the: Li ZS and Chen JY. Revising the paper for important intellectual content: all authors. Final approval of the version submitted: all authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of anypart of the work are appropriately investigated and resolved: all authors. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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References

1. Porcel J: **Tuberculous pleural effusion**. Lung 2009, **187**(5):263–270.
2. Gopi A, MadhavConclusionan SM, Sharma SK, Sahn SA: **Diagnosis and treatment of tuberculous pleural effusion in 2006**. Chest 2007, **131**(3):880–889.
3. Arpinar Yigitbas B, Satici C, Kosar AF: **Adenosine deaminase cutoff value when diagnosing tuberculous pleurisy in patients aged 40 years and older**. The clinical respiratory journal 2021, **15**(1):109–115.
4. Klimiuk J, Krenke R: **[Role of biomarkers in making the diagnosis of tuberculous pleurisy]**. Pneumonologia i alergologia polska 2011, **79**(4):288–297.
5. Zhang M, Li D, Hu ZD, Huang YL: **The diagnostic utility of pleural markers for tuberculosis pleural effusion**. Annals of translational medicine 2020, **8**(9):607.

6. Solari L, Soto A, Van der Stuyft P: **Performance of clinical prediction rules for diagnosis of pleural tuberculosis in a high-incidence setting**. Tropical medicine & international health: TM & IH 2017, **22**(10):1283–1292.
7. Tay TR, Tee A: **Factors affecting pleural fluid adenosine deaminase level and the implication on the diagnosis of tuberculous pleural effusion: a retrospective cohort study**. BMC infectious diseases 2013, **13**:546.
8. Abrao FC, de Abreu IR, Miyake DH, Busico MA, Younes RN: **Role of adenosine deaminase and the influence of age on the diagnosis of pleural tuberculosis**. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease 2014, **18**(11):1363–1369.
9. Lee SJ, Kim HS, Lee SH, Lee TW, Lee HR, Cho YJ, Jeong YY, Kim HC, Lee JD, Hwang YS: **Factors influencing pleural adenosine deaminase level in patients with tuberculous pleurisy**. The American journal of the medical sciences 2014, **348**(5):362–365.
10. Korczynski P, Klimiuk J, Safianowska A, Krenke R: **Impact of age on the diagnostic yield of four different biomarkers of tuberculous pleural effusion**. Tuberculosis (Edinburgh, Scotland) 2019, **114**:24–29.
11. Jiang CG, Wang W, Zhou Q, Wu XZ, Wang XJ, Wang Z, Zhai K, Shi HZ: **Influence of age on the diagnostic accuracy of soluble biomarkers for tuberculous pleural effusion: a post hoc analysis**. BMC pulmonary medicine 2020, **20**(1):178.
12. Kim SB, Shin B, Lee JH, Lee SJ, Lee MK, Lee WY, Yong SJ, Kim SH: **Pleural fluid ADA activity in tuberculous pleurisy can be low in elderly, critically ill patients with multi-organ failure**. BMC pulmonary medicine 2020, **20**(1):13.
13. Losi M, Bossink A, Codecasa L, Jafari C, Ernst M, Thijsen S, Cirillo D, Ferrarese M, Greinert U, Fabbri LM *et al*: **Use of a T-cell interferon-gamma release assay for the diagnosis of tuberculous pleurisy**. The European respiratory journal 2007, **30**(6):1173–1179.
14. Skouras VS, Kalomenidis I: **Pleural fluid tests to diagnose tuberculous pleuritis**. Current opinion in pulmonary medicine 2016, **22**(4):367–377.
15. Lei X, Wang J, Yang Z, Zhou S, Xu Z: **Diagnostic Value of Pleural Effusion Mononuclear Cells Count and Adenosine Deaminase for Tuberculous Pleurisy Patients in China: A Case-Control Study**. Frontiers in medicine 2019, **6**:301.
16. Ling DI, Flores LL, Riley LW, Pai M: **Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens**: **meta-analysis and meta-regression**. *PloS one* 2008, **3**(2):e1536.
17. Demirer E, Miller AC, Kunter E, Kartaloglu Z, Barnett SD, Elamin EM: **Predictive models for tuberculous pleural effusions in a high tuberculosis prevalence region**. Lung 2012, **190**(2):239–248.
18. Xu HY, Li CY, Su SS, Yang L, Ye M, Ye JR, Ke PP, Chen CS, Xie YP, Li YP: **Diagnosis of tuberculous pleurisy with combination of adenosine deaminase and interferon-γ immunospot assay in a tuberculosis-endemic population: A prospective cohort study**. Medicine 2017, **96**(47):e8412.
19. Chang KC, Chan MC, Leung WM, Kong FY, Mak CM, Chen SP, Yu WC: **Optimising the utility of pleural fluid adenosine deaminase for the diagnosis of adult tuberculous pleural effusion in Hong Kong**. *Hong Kong medical journal = Xianggang yi xue za zhi* 2018, **24**(1):38–47.
20. Hamada T, Sanaka M, Hata E, Hasegawa T: **[Pleural adenosine deaminase levels in tuberculous pleurisy–its diagnostic performance under the different prevalences in the different age of population]**. The Japanese journal of thoracic and cardiovascular surgery: official publication of the Japanese Association for Thoracic Surgery = Nihon Kyobu Geka Gakkai zasshi 1998, **46**(1):51–57.
21. Liao M, Yang Q, Zhang J, Zhang M, Deng Q, Liu H, Graner MW, Kornfeld H, Zhou B, Chen X: **Gamma interferon immunospot assay of pleural effusion mononuclear cells for diagnosis of tuberculous pleurisy**. Clinical and

vaccine immunology: CVI 2014, **21**(3):347–353.

22. Zhang Q, Zhou C: **Comparison of laboratory testing methods for the diagnosis of tuberculous pleurisy in China**. Scientific reports 2017, **7**(1):4549.
23. Krenke R, Safianowska A, Paplinska M, Nasilowski J, Dmowska-Sobstyl B, Bogacka-Zatorska E, Jaworski A, Chazan R: **Pleural fluid adenosine deaminase and interferon gamma as diagnostic tools in tuberculosis pleurisy**. Journal of physiology and pharmacology: an official journal of the Polish Physiological Society 2008, **59 Suppl 6**:349–360.
24. Yang X, Zhang J, Liang Q, Pan L, Duan H, Yang Y, Li H, Guo C, Sun Q, Jia H *et al*: **Use of T-SPOT.TB for the diagnosis of unconventional pleural tuberculosis is superior to ADA in high prevalence areas: a prospective analysis of 601 cases**. BMC infectious diseases 2021, **21**(1):4.
25. Mollo B, Jouveshomme S, Philippart F, Pilmis B: **Biological markers in the diagnosis of tuberculous pleural effusion**. Annales de biologie clinique 2017, **75**(1):19–27.
26. Feng M, Sun F, Wang F, Cao G: **The diagnostic effect of sequential detection of ADA screening and T-SPOT assay in pleural effusion patients**. Artificial cells, nanomedicine, and biotechnology 2019, **47**(1):3272–3277.
27. Santos AP, Corrêa RDS, Ribeiro-Alves M, Soares da Silva ACO, Mafort TT, Leung J, Pereira GMB, Rodrigues LS, Rufino R: **Application of Venn's diagram in the diagnosis of pleural tuberculosis using IFN-γ, IP-10 and adenosine deaminase**. PloS one 2018, **13**(8):e0202481.
28. Li Y, Xu H, Su S, Ye J, Chen J, Jin X, Lin Q, Zhang D, Ye C, Chen C: **Clinical validation of a highly sensitive assay to detect EGFR mutations in plasma cell-free DNA from patients with advanced lung adenocarcinoma**. PloS one 2017, **12**(8):e0183331.

Figures

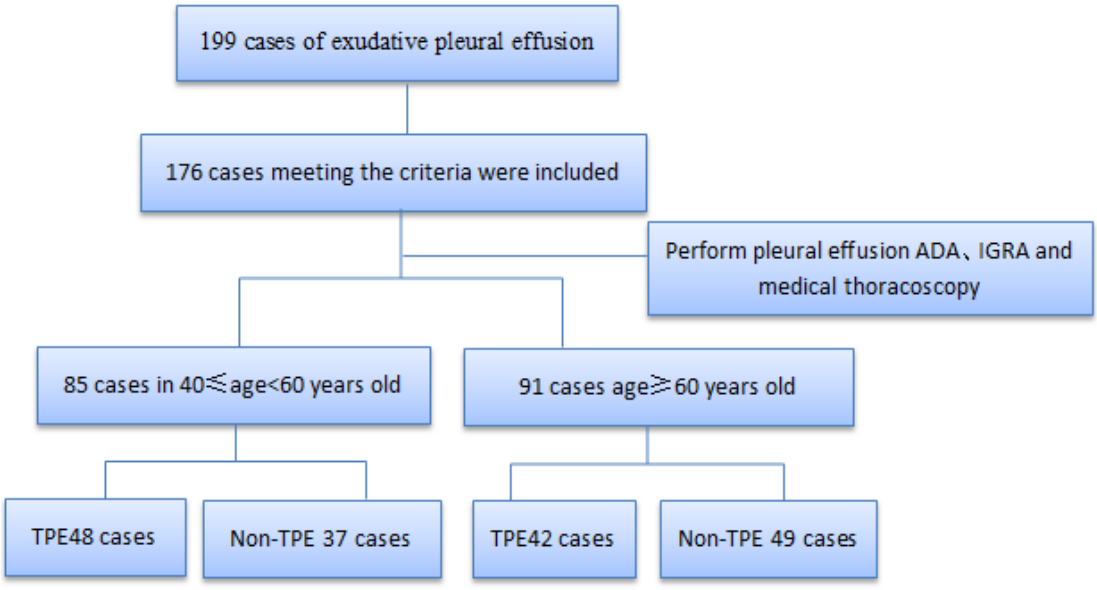

Figure 1

Case selection process and grouping

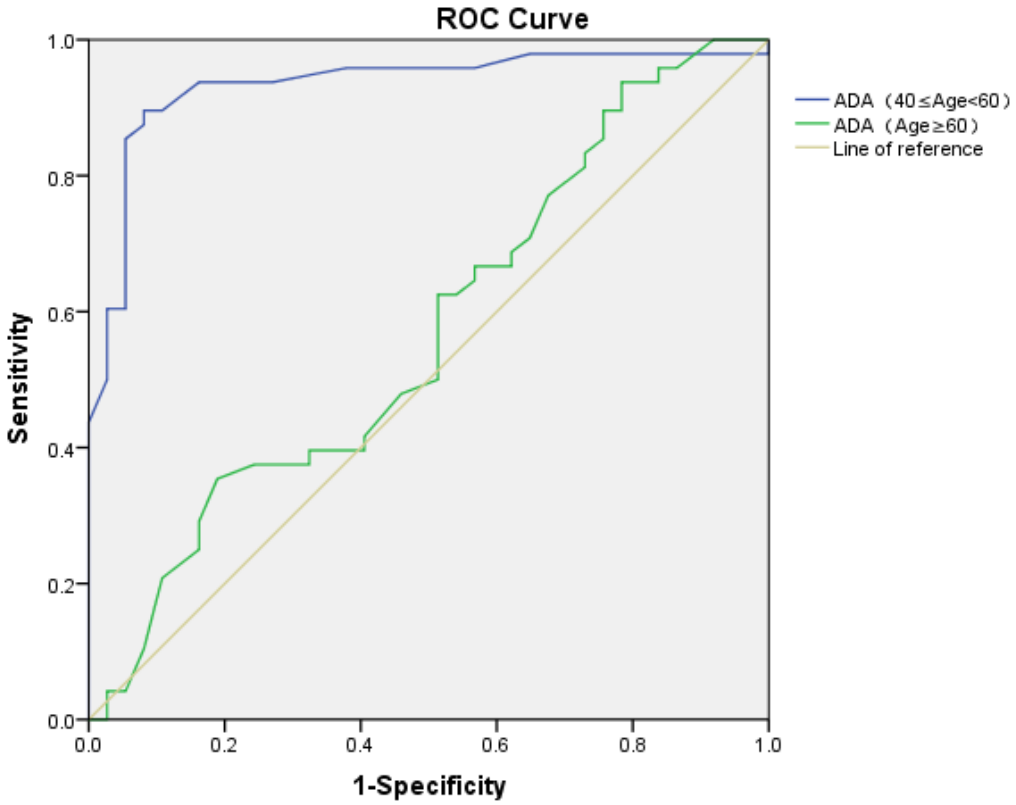

Figure 2

ROC analysis between different ages