Original Article

Pleural fluid soluble Fas ligand and tuberculous pleural effusion: a prospective diagnostic test accuracy study

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Background: The diagnosis of tuberculous pleural effusion (TPE) is challenging for pulmonologists. Adenosine deaminase (ADA), interferon-gamma (IFN-γ), and interleukin-27 (IL-27) have some limitations for diagnosing TPE. Soluble Fas ligand (sFasL) had a high diagnostic value for TPE. However, it remains unknown: (I) whether sFasL has an additional diagnostic value to the traditional markers (e.g., ADA); (II) whether sFasL provides a net benefit in patients with undiagnosed pleural effusion; (III) factors affecting the diagnostic accuracy of sFasL for TPE. This study aimed to evaluate the additional diagnostic value and benefit of pleural fluid sFasL for TPE.

Methods: We prospectively enrolled 211 patients with undiagnosed pleural effusion. The concentration of sFasL in pleural fluid was measured by an enzyme-linked immunosorbent assay (ELISA). The diagnostic accuracy and net benefit of sFasL and ADA for TPE were analyzed by a receiver operating characteristic (ROC) curve, decision curve analysis (DCA), net reclassification improvement (NRI), and integrated discriminant improvement (IDI).

Results: The area under the ROC curves (AUCs) of sFasL and ADA were 0.74 (95% CI: 0.65–0.83) and 0.80 (95% CI: 0.71–0.90), respectively. The decision curve of sFasL revealed net benefit. The continuous NRI and IDI of sFasL were 0.36 (0.00–0.72, P=0.05) and 0.02 (–0.01–0.06, P=0.18), respectively.

Conclusions: Pleural fluid sFasL has moderate diagnostic accuracy for TPE.

Keywords: Tuberculous pleural effusion (TPE); soluble Fas ligand (sFasL); diagnostic accuracy; sensitivity; specificity

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Introduction

Background

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis* (*M.tb*), has a high prevalence and annual incidence worldwide (1). According to the report released by the World Health Organization (WHO), the number of newly diagnosed TB was 6.4 million worldwide in 2021, and approximately 1.6 million people died from TB (2). TB can be classified as pulmonary TB (PTB) and extrapulmonary TB (EPTB), and the latter accounts for 16% of TB cases (3). The common types of EPTB are tuberculous lymphadenitis, pleuritis, and spondylitis (4). A multicenter cross-sectional study showed that tuberculous pleuritis accounts for about 50% of EPTB in China (5). Pleural effusion is a common sign of tuberculous pleuritis, and the pleural effusion caused by tuberculous pleuritis is thus termed tuberculous pleural effusion (TPE). Diagnosing TPE is challenging for pulmonologists because pleural effusion is not a specific sign of TPE. It can also be caused by malignancy, pneumonia, and heart failure (HF) (6). The gold standards for TPE are pleural fluid *M.tb* culture, Ziehl-Neelsen staining, or pleural biopsy (7). However, these tools have some limitations. *M.tb* culture has a long turn-around time and thus does not facilitate early diagnosis. In addition, the sensitivity of *M.tb* culture is <20% (8). Ziehl-Neelsen staining is an easy-to-perform tool with a short turn-around time; however, its sensitivity is <3% (8). Ultrasound- or CT-guided percutaneous pleural biopsy and medical thoracoscopy have high diagnostic accuracy for TPE, but they are invasive tools that can cause operating-related complications (9,10). In addition, medical thoracoscopy needs special training, which limits its clinical application in remote areas. The nucleic acid amplification test (NAAT) has high specificity for TPE, but its sensitivity is only around 50% (11-13). By contrast, pleural fluid or serum biomarkers are of clinical value because of low cost, feasibility, minimal invasiveness, and short turn-around time (14,15).

Some pleural biomarkers have been reported to be useful diagnostic tools for TPE, such as adenosine deaminase (ADA), interferon-gamma (IFN-γ), and interleukin-27 (IL-27) (14). The sensitivities and specificities of these biomarkers are >90%, as indicated by meta-analyses (16-18). However, these biomarkers have limitations. For example, the diagnostic performance of ADA for TPE is influenced by many factors, such as age (19). Pleural IFN-γ and IL-27 are not standardized, and the comparability between different commercial kits is poor (14). Therefore, developing novel pleural biomarkers to improve the diagnostic accuracy of available markers or replace them remains valuable.

Rationale and knowledge gap

Fas ligand (FasL, CD95) is a type II transmembrane protein of approximately 40 kDa. It belongs to the tumor necrosis factor (TNF) family (20). The binding of Fas receptor and FasL can induce cellular apoptosis via caspase-8 (21). There are two types of FasL, named membrane-bound Fas ligand (mFasL) and the soluble Fas ligand (sFasL), respectively. The mFasL is cleaved by the activated metalloproteinases (MMPs) to form the sFasL (22). sFasL can competitively bind to Fas and thus has a protective effect on apoptosis (20).

Preliminary studies revealed that pleural sFasL had a high diagnostic value for TPE (14,23-25). However, it remains unknown: (I) whether sFasL has an additional diagnostic value to the traditional markers (e.g., ADA); (II) whether sFasL provides a net benefit in patients with undiagnosed pleural effusion; (III) factors affecting the diagnostic accuracy of sFasL for TPE. This study aimed to investigate the additional diagnostic accuracy and net benefit of pleural fluid sFasL for TPE. We present this article in accordance with the STARD reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1076/rc) (26).

Highlight box

Key findings

- Pleural fluid soluble FasL ligand (sFasL) has moderate diagnostic accuracy for tuberculous pleural effusion (TPE).

What is known and what is new?

- Preliminary studies revealed pleural sFasL had a high diagnostic value for TPE. It remains unknown whether sFasL provides additional diagnostic value beyond adenosine deaminase (ADA).
- This study indicates that sFasL does not provide added diagnostic value beyond ADA. Age may affect the diagnostic accuracy of sFasL for TPE.

What is the implication, and what should change now?

- Age should be considered when interpreting the diagnostic value of sFasL for TPE. sFasL is an alternative diagnostic tool for TPE.

Methods

Participants

Participants in this study were from a prospective, pre-registered, and double-blind study termed SIMPLE (Chinese Clinical Trial Registry: ChiCTR1800017449) (27). The design details of the SIMPLE study have been introduced previously (27,28). Briefly, we prospectively enrolled the patients with undiagnosed pleural effusion who visited the Affiliated Hospital of Inner Mongolia Medical University (AHIMMU, 2018–2021) and the Affiliated Changshu Hospital of Nantong University (ACHNU, 2020–2021).

The inclusion criteria for participant enrollment were: (I) patients with undiagnosed pleural effusion; (II) diagnostic thoracentesis was performed, and the pleural fluid specimen was obtained. The presence of pleural effusion was confirmed by chest X-ray, CT, or ultrasound. The exclusion criteria were: (I) patients who had a history of pleural effusion with known etiology in the last three months at the point of diagnosis; (II) age <18 years old; (III) pregnant woman; (IV) patients with insufficient pleural fluid specimen; (V) patients who developed pleural effusion during hospitalization; and (VI) patients with pleural effusion caused by trauma or surgery.

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committees of the AHIMMU (No. 2018011) and the ACHNU (No. KY2021014). Informed consent was obtained from all participants.

Diagnostic criteria

TPE was diagnosed with pleural fluid *M.tb* culture, Ziehl-Neelsen staining, and pleural biopsy. Positive biopsy was defined as the presence of pleural granuloma with the exclusion of other granulomatous diseases. In some patients who has a high probability of TPE and other types of pleural effusion can be excluded, TPE was diagnosed by treatment response to antituberculosis therapies and follow-up. Parapneumonic pleural effusion (PPE) was diagnosed by pleural fluid bacterial culture, imaging (loculated effusions), and response to antibiotic therapy. Malignant pleural effusion (MPE) was diagnosed by effusion cytology, pleural biopsy, or evidence of primary cancer with the exclusion of benign pleural effusion. HF was diagnosed by history, laboratory findings, and response to diuretics. The concentration of sFasL was masked to the clinicians who made the diagnosis.

Routine biomarkers

The pleural fluid specimen of the participants was obtained at the time of admission. After centrifugation at 2,000 rpm for 10 minutes, the pleural fluid supernatant was aliquoted and stored at –80 to –70 °C for further analysis.

The sFasL concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems) following the manufacturer's instructions. Concentrations of white blood cells (WBC), protein, lactate dehydrogenase (LDH), and ADA in the pleural fluid were collected from the participant's medical records. ADA and LDH were measured by Beckman AU5831 (AHIMMU) and ADVIA 2400 (ACHNU). All tests were performed by technicians who were unaware of the clinical diagnosis.

Statistical analysis

Continuous variables were expressed as the median and interquartile range (IQR). We used the Mann-Whitney U test to compare continuous variables between the TPE and the non-TPE groups. The Kruskal-Wallis H tests were used to compare continuous data in more than two groups. Categorical variables were compared with the Chi-squared test. A receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic accuracy of sFasL. A decision curve analysis (DCA) was used to assess the net benefit of sFasL. The added diagnostic value of sFasL was estimated using net reclassification improvement (NRI) and integrated discriminant improvement (IDI) (29). The mathematical basis of NRI and IDI has been introduced in an easy-to-understand way in a previous publication (30). All statistical analyses and graphs were performed using SPSS 25.0, GraphPad Prism 8.3.0, Stata 15.1, and R 4.2.2. $P<0.05$ was considered statistically significant.

Results

Basic characteristics of the participants

Figure 1 is a flowchart of participant selection. A total of 232 participants were recruited, including 62 in ACHNU and 170 in AHIMMU. Twenty-one participants were excluded, and 211 were used for data analysis. *Table 1* lists the baseline characteristics of the participants. There were 33 TPEs (15.7%) and 178 non-TPEs (84.3%). The non-TPEs comprised 92 patients with MPE, 42 patients with PPE, 28 patients with HF, and 16 other types of pleural effusion.

The median [IQR] ages of patients with TPE and non-TPE were 72 [64–80] and 73 [65–79] years, respectively.

sFasL concentration and etiologies of pleural effusion

As shown in *Figure 2A*, the median concentration of sFasL in TPE patients (147.7 pg/mL) was significantly higher than that in non-TPE (98.2 pg/mL) (P<0.001 by Mann-Whitney U test). A subgroup analysis of non-TPE patients showed that the sFasL concentration significantly differed among PPE, MPE, HF, and other pleural effusion (*Figure 2B*, P<0.001 by Kruskal-Wallis H test).

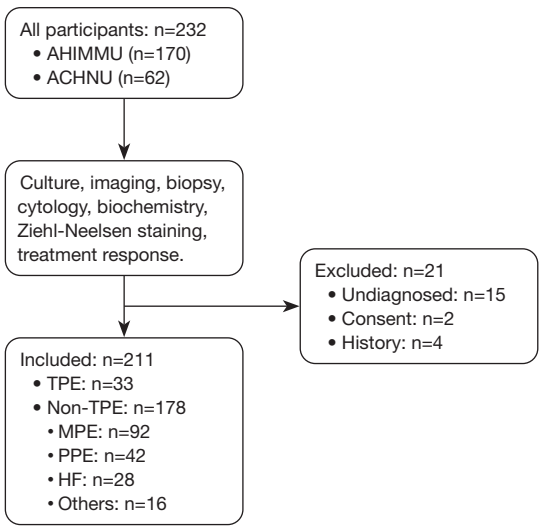

Figure 1 A flowchart of the participant selection. AHIMMU, the Affiliated Hospital of Inner Mongolia Medical University; ACHNU, Affiliated Changshu Hospital of Nantong University; TPE, tuberculous pleural effusion; MPE, malignant pleural effusion; PPE, parapneumonic pleural effusion; HF, heart failure.

Diagnostic performance of sFasL and ADA

Figure 3 shows the ROC curves of ADA, sFasL, and their combination. The area under the ROC curve (AUC) was 0.74 (95% CI: 0.65–0.83) and 0.80 (95% CI: 0.71–0.90) for sFasL and ADA, respectively (P=0.17 by Delong's test). A logistic model combining ADA and sFasL had an AUC of 0.80 (95% CI: 0.72–0.89), but its AUC did not significantly differ from that of ADA (P=0.97 by Delong's test). The prespecified thresholds and their corresponding sensitivities and specificities were summarized in *Table 2*. At the 110 pg/mL threshold, sFasL had a sensitivity of 0.73 (95% CI: 0.58–0.88) and specificity of 0.60 (95% CI: 0.53–0.67). In addition, we analyzed the AUCs of sFasL in the AHIMMU and ACHNU separately and found that their AUCs were 0.72 (95% CI: 0.61–0.85) and 0.74 (95% CI: 0.57–0.90), respectively.

We performed NRI and IDI analysis to examine whether sFasL provides added diagnostic value beyond ADA. The continuous NRI (95% CI) and IDI (95% CI) were 0.36 (0.00–0.72, P=0.05) and 0.02 (–0.01–0.06, P=0.18), respectively.

The net benefit of sFasL in patients with undiagnosed pleural effusion

Figure 4 shows the decision curves of pleural fluid sFasL, ADA,

Table 1 Comparison of clinical and laboratory variables in patients with TPE and non-TPE patients

Variables	TPE (n=33)	Non-TPE (n=178)	P
Age (years)	72 [64–80]	73 [65–79]	0.579
Sex (F/M)	14/19	62/116	0.404
Pleural fluid WBC (×10^6/mL)	1,574.0 [876.5–2,849.0]	851.5 [411.8–1,833.5]	0.001
Pleural fluid protein concentration (g/L)	44.0 [36.4–47.6]	35.8 [23.4–43.1]	0.001
Serum protein (g/L)	65.1±8.3	61.6±8.1	0.027
Pleural fluid/serum protein ratio	0.66 [0.61–0.74]	0.55 [0.38–0.69]	0.005
Pleural fluid glucose (mmol/L)	5.3 [4.7–6.4]	6.0 [4.8–6.9]	0.182
Pleural fluid LDH (U/L)	283.0 [176.0–394.0]	232.00 [141.0–486.0]	0.468
Pleural fluid ADA (U/L)	42.2 [14.6–53.5]	9.2 [5.3–16.1]	<0.001

Continuous data are presented as the median and interquartile range, or mean and standard deviation, and compared with Mann-Whitney or Student's *t*-test. TPE, tuberculous pleural effusion; WBC, white blood cell; LDH, lactate dehydrogenase; ADA, adenosine deaminase.

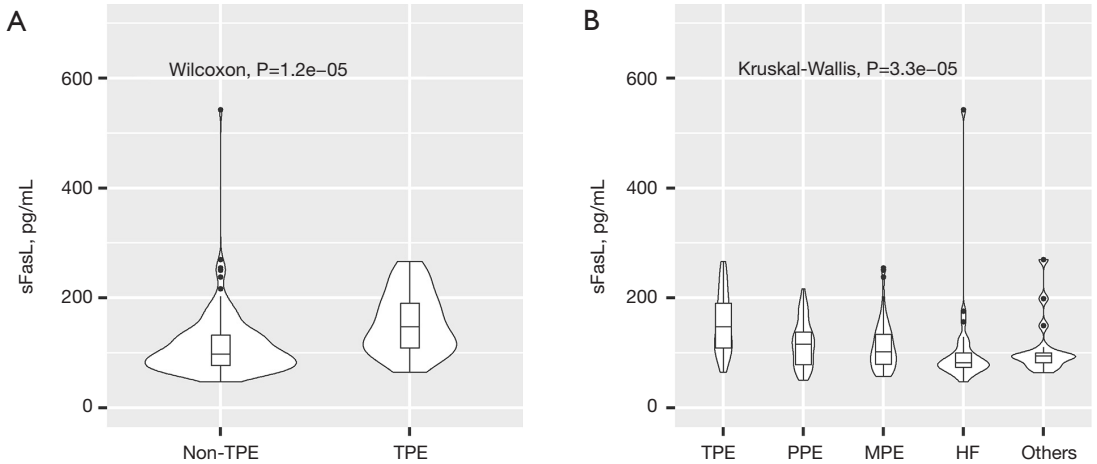

Figure 2 sFasL and etiologies of pleural effusion. (A) sFasL levels between TPE and non-TPE patients. (B) sFasL levels among etiologies of non-TPE subgroups. sFasL, soluble Fas ligand; TPE, tuberculous pleural effusion; PPE, parapneumonic pleural effusion; MPE, malignant pleural effusion; HF, heart failure.

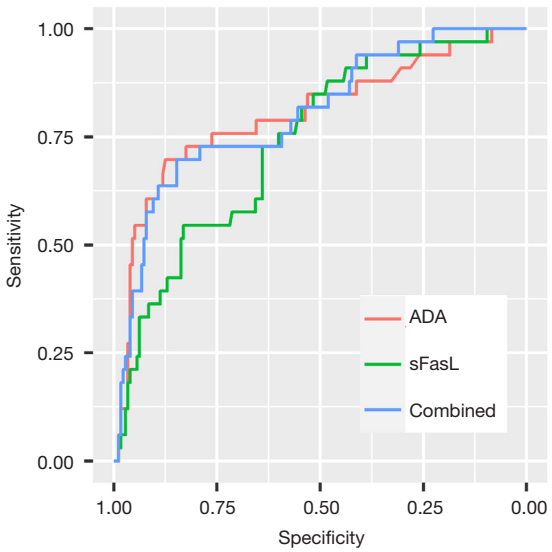

Figure 3 ROC curves of sFasL, ADA, and their combination. ADA, adenosine deaminase; sFasL, soluble Fas ligand; ROC, receiver operating characteristic.

and their combination. Compared with ADA, the decision curve of sFasL was closer to two reference lines, indicating that the net benefit of sFasL is inferior to that of ADA.

Discussion

Key findings

The primary findings of this study can be summarized as follows: (I) pleural fluid sFasL concentration was significantly higher in TPE than in non-TPE, which is consistent with previous studies (31-33); (II) pleural fluid sFasL had moderate diagnostic accuracy for TPE; (III) measurement of sFasL in pleural effusion patients provides no additional diagnostic value beyond ADA, despite its net benefit. These results indicate that pleural sFasL is an alternative test for TPE.

Table 2 Diagnostic accuracy of sFasL, ADA, and their combination for TPE

Biomarkers	AUC (95% CI)	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)
sFasL	0.74 (0.65–0.83)	110 pg/mL	0.73 (0.58–0.88)	0.60 (0.53–0.67)
ADA	0.80 (0.71–0.90)	35 U/L	0.61 (0.42–0.76)	0.92 (0.88–0.95)
sFasL + ADA	0.80 (0.72–0.89)	0.20	0.64 (0.45–0.79)	0.85 (0.80–0.90)

sFasL, soluble Fas ligand; ADA, adenosine deaminase; TPE, tuberculous pleural effusion; AUC, area under the ROC curve; CI, confidence interval.

Strengths and limitations

Compared with previous studies, our study has some strengths. First, we analyzed the distribution of sFasL in non-TPE patients and found PPE and MPE patients had higher sFasL than HF patients (P<0.001). By contrast, previous studies did not investigate the distribution of sFasL in non-TPE patients. This result indicates that in clinical settings with a high prevalence of MPE and PPE, the diagnostic accuracy of sFasL may decrease. Second, this is the first study investigating the added diagnostic value of sFasL on ADA. Investigating the added value of sFasL is of high clinical relevance. ADA is a guideline-endorsed biomarker for TPE (34). If sFasL could not provide added diagnostic value beyond ADA, measuring sFasL and ADA together is meaningless. Third, we performed a DCA and revealed the net benefit of sFasL.

Our study has some limitations. First, the sample size of our study is small, and only 33 TPEs were included. The small sample size may decrease the precision of our study. Second, we used the stored pleural fluid specimens to determine sFasL, and the long-term stability of sFasL remains unknown. Third, we did not investigate the cellular origin of sFasL. However, this study provides a novel insight into the diagnostic accuracy of sFasL for TPE.

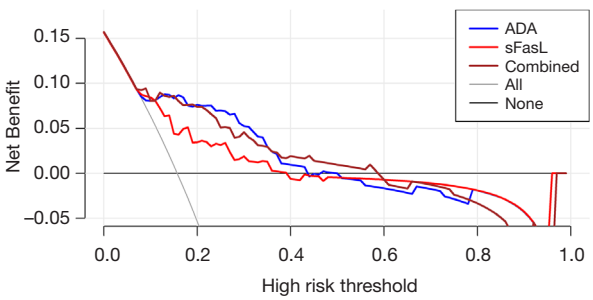

Figure 4 Decision curves of sFasL, ADA, and their combination. ADA, adenosine deaminase; sFasL, soluble Fas ligand.

Comparison with similar research

We searched the PubMed database and found that three studies have investigated the diagnostic accuracy of sFasL for TPE (23-25). The characteristics and primary findings of these studies are summarized in *Table 3*. They concluded that the diagnostic accuracy of sFasL had sensitivities and specificities of >90% (23-25). We found that sFasL had an AUC of approximately 0.74, which is lower than that in previous studies. We speculate that the inconsistency between previous studies and our work is partially due to differences in the study population. Notably, the average age of TPE patients was higher in our study, while the age of TPE was lower in previous studies (23-25). Indeed, age can affect the diagnostic accuracy of pleural biomarkers for TPE (19,23,35). Notably, a previous study and our work found that age was negatively correlated with sFasL (23). Therefore, we conclude that in clinical settings where TPE patients are younger than non-TPE patients, sFasL has high diagnostic accuracy. In contrast, in settings where TPE patients' ages are comparable to those of non-TPE patients, the diagnostic accuracy of sFasL decreases. Notably, the participants' mean age in a study is around 76 years, but the AUC in that study was 0.88 (24), indicating that age can only partially explain the heterogeneity across previous studies.

We found that sFasL in non-TPE patients varied according to their diagnosis. MPE and PPE patients had higher sFasL than HF patients. Therefore, the composition of non-TPE is a potential source of inconsistency between previous studies and our study. When interpreting the diagnostic value of sFasL for TPE, it is crucial to consider the underlying causes of pleural effusion in a clinical setting.

Table 3 Characteristics of previous research investigating the diagnostic accuracy of sFasL for TPE

Study	Year	Country	TPE/non-TPE	Diagnostic criteria	Mean or medium age (years)		AUC
					TPE	Non-TPE	
Wu (24)	2010	China	23/56	CRS	76	76	0.88
Klimiuk (25)	2015	Poland	44/159	CRS	52	68	0.95
Korczynski (23)	2019	Poland	60/162	CRS	54	NR	~0.93
This study	2023	China	33/178	CRS	72	73	0.74

sFasL, soluble Fas ligand; TPE, tuberculous pleural effusion; AUC, area under the ROC curve; CRS, complex reference standard; NR, not reported.

We found that the diagnostic accuracy of sFasL decreased in elderly patients. This finding is biologically plausible. The Fas/FasL system plays a regulatory role in immune cell apoptosis (36) and has thus been involved in the pathogenesis of TB. sFasL, the soluble form of FasL, is released after being cleaved from its membrane partner by metalloproteinases (37). FasL expression has been detected in activated T cells and natural killer (NK) cells (37,38). T cells and NK cells are enriched in the pleural fluid of TPE patients (39). Therefore, we hypothesized that the sFasL in the pleural fluid of TPE patients were cleaved from the lymphocyte. The expression of FasL in lymphocytes is regulated by several transcription factors, such as c-JUN, c-FOS, Y-box binding protein 1 (YB-1), nuclear factor of activated T cells (NFAT), NF-κB, specificity protein-1 (SP1) (37). Previous studies indicated aging decreased the activity of these transcription factors (37,40-42). Animal studies also revealed that old mice had fewer $Fas^{+}FasL^{+}CD8^{+}$ T cells than young mice (43).

Conclusions

In conclusion, we find the diagnostic accuracy of sFasL is moderate and comparable to that of ADA. sFasL has net benefits in patients with pleural fluid. Therefore, sFasL is an alternative test for diagnosing TPE. Due to the limitations of this study, further studies are needed to evaluate the diagnostic value of sFasL for TPE in different clinical settings.

Acknowledgments

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1076/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1076/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1076/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1076/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was performed following the Declaration of Helsinki (as revised in 2013). This study was approved by the Ethics Committees of the Affiliated Hospital of Inner Mongolia Medical University (No: 2018011) and the Affiliated Changshu Hospital of Nantong University (No. KY2021014). Informed consent was obtained from all participants.

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