Clinical significance of serum transforming growth factor-β1 and procollagen type I N-propeptide in post-tuberculosis tracheobronchial stenosis

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Received October 23, 2020; Accepted February 25, 2021

DOI: 10.3892/etm.2021.10002

Abstract. Non-invasive strategies for monitoring post-tuberculosis (TB) tracheobronchial stenosis (PTTS) are clinically important but currently lacking. Transforming growth factor-β1 (TGF-β1) and procollagen type I N-propeptide (PINP) have been identified as markers of fibrosis. The present study aimed to investigate the clinical significance of serum TGF-β1 and PINP in PTTS. Serum samples were collected from 119 patients with tracheobronchial TB after the condition was treated for at least 6 months (59 patients with airway stenosis and 60 patients with no stenosis). Serum TGF-β1 and PINP levels were measured using ELISA and compared between the groups. Relationships between serum TGF-β1 and PINP levels and clinical characteristics, interventional bronchoscopy and outcomes of airway stenosis were analysed. The correlation between TGF-β1 and PINP, and their diagnostic efficacy for airway stenosis were also analysed. The TGF-β1 and PINP levels in the airway stenosis group were higher than those in the non-stenosis group. Furthermore, airway stenosis with atelectasis or mucus plugging was associated with higher TGF-β1 levels, and airway stenosis with atelectasis, mucus plugging, right main bronchus stenosis or severe airway tracheal stenosis was associated with higher PINP levels. In addition, TGF-β1 and PINP levels increased after interventional bronchoscopy therapy and airway stenosis with recurrent stenosis was associated with higher baseline levels of both markers. Finally, TGF-β1 levels were positively correlated with PINP levels in patients with airway stenosis. The area under the receiver operating characteristic curve of TGF-β1 and PINP for distinguishing airway stenosis from non-stenosis cases was 0.824 (95% CI: 0.748-0.900) and 0.863 (95% CI: 0.796-0.930), respectively. Therefore, TGF-β1 and PINP are potential biomarkers that may be useful for diagnosing and monitoring PTTS.

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Abbreviations: TB, tuberculosis; TBTB, tracheobronchial TB; PTTS, post-TB tracheobronchial stenosis; TGF-β1, transforming growth factor-β1; PINP, procollagen type I N-propeptide; HRCT, high-resolution computed tomography; H&E staining, haematoxylin and eosin staining; ROC, receiver operating characteristic; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; CI, confidence interval

Key words: tracheobronchial tuberculosis, fibrosis, biomarker, transforming growth factor-β1, procollagen type I N-propeptide

Introduction

Tuberculosis (TB) is a major global health problem associated with significant morbidity and potential mortality (1,2). Diagnostic bronchoscopy studies have suggested that 10-50% of patients with pulmonary TB have tracheobronchial TB (TBTB) (3,4). Airway stenosis is the most common complication of TBTB. Despite adequate anti-TB therapy, patients with TBTB develop different degrees of airway stenosis, resulting in marked narrowing of the bronchial lumen, dyspnoea on exertion and obstructive pneumonia (5,6). In the long term, >90% of patients develop stenosis (6). The damage after TBTB is residual and long-term (7,8). Therefore, in addition to anti-TB treatment, monitoring and determining the degree of post-TB tracheobronchial stenosis (PTTS) are important for early diagnosis and treatment, and for improving the survival of patients and their quality of life. In the early stages, the disease is difficult to diagnose based on clinical presentation alone and is frequently missed (6). Bronchoscopy is thus far the major strategy for diagnosing and monitoring PTTS (9-11). In general, bronchoscopy is selectively performed when PTTS is suspected in patients with severe cough, wheezing or haemoptysis. Furthermore, bronchoscopy is invasive and tends to increase the risk of occupational exposure among medical staff, and unvaccinated patients with TB may try to avoid hospital visits during the time of the COVID-19 pandemic (12-17). These limitations have essentially forced the early diagnosis and non-invasive monitoring program

to shift from in person to remote follow-up, which involves telemedicine and remote laboratory monitoring (18-20). These challenges in diagnosing and monitoring the stenosis status have created an urgent need for reliable biomarkers to predict the progression of PTTS (20,21).

The exact pathogenesis of PTTS has remained to be fully elucidated; one possible underlying mechanism involves diffuse fibrosis in the trachea and bronchus (6,22-25). Molecular changes usually require less time to manifest than morphological changes. Fibrosis is frequently accompanied by changes in serum levels of the markers (26). Thus, circulating biomarkers that allow the non-invasive assessment of the existence and degree of PTTS have immense clinical value. However, only a few biomarkers have been proposed for predicting PTTS. An isoform of transforming growth factor-β (TGF-β), TGF-β1, is a master regulator of tissue repair, inflammation and fibrosis (27). Current pathological evidence suggests that TGF-β1 is a central mediator driving the fibrotic process induced by multiple profibrotic stimuli (27,28). Previous studies suggested that as a non-invasive biomarker, serum TGF-β1 is a marker of fibrotic involvement in systemic sclerosis (29) and is related to the development of moderate to severe radiation-induced fibrosis (30). Furthermore, serum TGF-β1 is an independent predictor of recurrence of atrial fibrillation in patients with paroxysmal atrial fibrillation (31). Procollagen type I N-propeptide (PINP) is cleaved from type I pro-collagen during its extracellular processing (32). As a profibrotic biomarker, serum PINP has been used to reflect the healing of Achilles' tendon rupture (33). High serum PINP levels have been reported in pathological fibrotic conditions. One study suggested that changes in serum PINP levels in patients with liver diseases may reflect the severity of liver cirrhosis (34). Furthermore, the baseline serum PINP levels in patients with chronic heart failure are higher than those in the controls and are decreased after therapy (26).

Although serum TGF-β1 and PINP are implicated in the development of multiple fibrosis, their utility in PTTS is not well established. Hence, in the present study, the potential of utilising serum TGF-β1 and PINP levels as diagnosing/monitoring biomarkers for PTTS was assessed.

Materials and methods

Study design and population. A total of 119 patients with TBTB after the condition was treated for at least 6 months (59 patients with airway stenosis and 60 patients with no stenosis) from the Department of Respiratory and Critical Care Medicine at The First Affiliated Hospital of Chongqing Medical University (Chongqing, China) between March and September 2020 were included in the study. All of these patients had no positive acid-fast bacilli, positive *Mycobacterium tuberculosis* culture or pathological diagnosis of TB. In addition, bone disease, cardiovascular disease, liver fibrosis, kidney fibrosis, evidence of other systemic diseases causing fibrosis, malignancy and other infectious diseases were also excluded. For the airway stenosis group, data regarding clinical characteristics, such as demographic information, symptoms at presentation, chest high-resolution computed tomography (HRCT) and bronchoscopic findings were collected. These clinical characteristics and serum TGF-β1 and PINP levels in paired blood samples were analysed. All subjects provided written informed consent for participation in the study. The study was performed in accordance with the Declaration of Helsinki and the Uniform Requirements for Manuscripts Submitted to Biomedical Journals. The protocol was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (approval no. 2020-147).

Measurement of serum TGF-β1 and PINP levels. Venous blood samples (2.0 ml) were collected from each subject in tubes without anticoagulant. Serum was separated by centrifugation of blood samples at room temperature for 10 min at 2,000 x g, and samples to be used for biomarker assays were refrigerated at 4˚C and transferred to the Department of Central Laborarory at The First Affiliated Hospital of Chongqing Medical University (Chongqing, China), where they were stored at -80˚C until further use. This process was performed within 1 h of blood collection. Commercially available ELISA kits were used for TGF-β1 (cat. no. EK0513; Wuhan Boster Biological Technology, Ltd.) and PINP (cat. no. EH1092; Wuhan Fine Biotech Co., Ltd.) assays as per the manufacturers' protocols. The lower limits of detection were 15.6 pg/ml for TGF-β1 and 1.563 ng/ml for PINP. The absorbance (optical density) was measured at 450 nm using a microplate reader (Infinite M Plex; Tecan Trading AG).

Chest HRCT. Chest HRCT images were obtained with shallow breathing using a 64-slice helical HRCT system (Siemens AG). Two radiologists blinded to the clinical data interpreted all HRCT findings by consensus. Atelectasis and mucus plugging on chest HRCT were used to assess PTTS.

Diagnostic criteria for PTTS and interventional bronchoscopy. PTTS was diagnosed based on the detection of lesions using a bronchoscope and microbiological testing for TB bacilli. The types of PTTS are described as inflammatory infiltration, ulceration necrosis, granulation hyperplasia, cicatrices stricture, bronchomalacia and lymph fistula (4). The degree of stenosis was classified into five grades according to the cross-sectional area of the trachea (35). The patients were divided into two groups: Mild-to-moderate stenosis group (<75%) and severe stenosis group (≥75%). All patients with PTTS were treated with interventional bronchoscopy according to treatment guidelines (9) using a bronchoscope (CV-290; Olympus Corp.), an electrocautery needle knife (VIO 300S; Erbe Elektromedizin GmbH), a multi-use cryosurgery system (Erbokryo CA; Erbe Elektromedizin GmbH) and a dilatation balloon (Endo-Flex GmbH). An airway stent (Micro-Tech Europe GmbH) was considered to relieve symptoms of patients with dyspnoea only if the conventional airway interventional therapy was not effective. Patients were followed up for 1 month after the bronchoscopic intervention treatment.

Histological analysis. During interventional bronchoscopy an electrocautery needle knife was first used to release airway cicatrix tissues and subsequently, cryotherapy or forceps biopsy was performed to obtain the cicatrix tissues. Tissue sections (5 μm in thickness) were subjected to haematoxylin and eosin (H&E) and Masson staining to enable the histological

evaluation of airway cicatrix tissue fibrosis. Morphometric analysis was performed using five measurements randomly taken in five different fields independently by two pathologists blinded to the clinical data. The scores of the two pathologists were combined in an average score and the final results were obtained.

Statistical analysis. The normality of distribution of continuous variables was assessed using the Shapiro-Wilk test, which indicated that they were not normally distributed. The data were then expressed as the median (interquartile range). Differences between any two groups were evaluated using the Mann-Whitney U-test, while differences between two related samples were assessed using the Wilcoxon matched-pair signed-rank test. Correlations were tested for significance by calculating Spearman's rank correlation coefficient. Categorical data were expressed as n (%) and comparisons for testing statistically significant differences were made using the χ^2-test (minimum expected values ≥5) or Fisher's exact test (minimum expected values <5). Receiver operating characteristic (ROC) curves generated by plotting sensitivity against 1-specificity were used to assess the diagnostic performances of serum TGF-β1 and PINP levels for distinguishing between airway stenosis and non-stenosis. For each ideal cut-off value (at the point of the highest Youden index), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were reported. Statistical analyses were performed using SPSS version 20.0 software (IBM Corp.) and P<0.05 was considered to indicate statistical significance (all P-values are from two-sided tests). Graphical representation was performed using GraphPad Prism 6.05 software (GraphPad Software, Inc.).

Results

Clinical information. The relevant clinical characteristics of the subjects are summarised in Table I. Of all the subjects (14 male and 105 female participants), the median (interquartile range) age was 27 (23-32) years. The significant clinical characteristics of all patients included age, sex, smoking, symptoms at presentation and chest HRCT features. Compared to patients with non-stenosis, those with stenosis more frequently presented with the symptoms of cough, dyspnoea and wheezing at presentation, as well as chest HRCT features of atelectasis and mucus plugging (all P<0.05; Table I). There were no significant differences in age, sex and smoking between patients with stenosis and those with non-stenosis (P>0.05).

The bronchoscopic features of stenosis are also summarised in Table I. All of the patients with airway stenosis enrolled in the present study had cicatrices stricture and 33 (55.93%) of them also had bronchomalacia; however, no other types of stenosis were detected. The chest HRCT and bronchoscopy features of representative cases of patients with PTTS are presented in Fig. 1. Chest HRCT indicated left lung atelectasis (A) and left main bronchus mucus plugging (B), and diagnostic bronchoscopy revelaed moderate (C) and severe (D) airway stenosis due to TBTB, respectively.

Confirmation of hyperplasia of fibrous tissue using histopathology. The airway cicatrix tissue was evaluated using H&E and Masson staining. The principal histological finding was squamous epithelialisation of the bronchial epithelial cells (Fig. 2A) and fibrotic lesions with thickened submucosal layers (Fig. 2A). Furthermore, electrocautery needle knife therapy resulted in marked cell coagulative necrosis (Fig. 2B). In addition, the presence of fibrosis was verified by Masson staining (Fig. 2C and D).

Serum TGF-β1 and PINP levels in the airway stenosis and non-stenosis groups. As presented in Fig. 3A, the serum TGF-β1 levels in the airway stenosis group were significantly higher than those in non-stenosis group [2,518.98 (2,302.58-3,080.27) vs. 1,839.62 (1,616.82-2,141.38) pg/ml, Z=-6.830, P<0.001]. As presented in Fig. 3B, the serum PINP levels in the airway stenosis group were significantly higher than those in the non-stenosis group [19.39 (12.37-26.14) vs. 10.63 (9.86-12.85) ng/ml, Z=-6.102, P<0.001].

Serum TGF-β1 and PINP levels in patients with airway stenosis and different clinical characteristics. Patients with atelectasis exhibited significantly higher serum TGF-β1 [2,954.85 (2,462.82-3,265.12) vs. 2,430.92 (2,262.60-2,766.27) pg/ml, Z=-2.746, P=0.038; Fig. 4A] and PINP [25.43 (19.81-33.35) vs. 17.53 (11.38-21.07) ng/ml, Z=-3.691, P<0.001; Fig. 4B] levels than those without atelectasis. Patients with mucus plugging had significantly higher TGF-β1 [3,075.18 (2,367.28-3,265.59) vs. 2,438.27 (2,271.83-2,851.15) pg/ml, Z=-2.106, P=0.035; Fig. 4C] and PINP [26.65 (18.74-34.54) vs. 18.65 (11.55-22.91) ng/ml, Z=-3.060, P=0.002; Fig. 4D] levels than those without mucus plugging.

Patients with left main bronchus stenosis and those with right main bronchus stenosis had comparable TGF-β1 levels (Z=-0.533, P=0.594, P>0.05; Fig. 4E), while patients with right main bronchus stenosis [22.71 (17.18-33.32) vs. 18.29 (11.92-21.15) ng/ml, Z=-2.404, P=0.016; Fig. 4F] had higher serum PINP levels than those with left main bronchus stenosis. Serum TGF-β1 levels were comparable between severe airway tracheal stenosis and mild-to-moderate airway tracheal stenosis (Z=-0.793, P=0.428, P>0.05; Fig. 4G), while serum PINP levels were higher in severe airway tracheal stenosis than those in mild-to-moderate airway tracheal stenosis [21.07 (17.18-31.55) vs. 15.33 (10.94-20.96) ng/ml, Z=-3.060, P=0.003; Fig. 4H]. Serum TGF-β1 and PINP levels did not vary significantly with age, sex, smoking, symptoms at presentation or bronchomalacia (P>0.05).

Changes in serum TGF-β1 and PINP levels after interventional bronchoscopy. Considering the invasive nature of interventional bronchoscopy therapy, samples from only 10 patients with airway stenosis were used to study the effect of interventional bronchoscopy therapy on serum TGF-β1 and PINP levels. As presented in Fig. 5, the levels of serum TGF-β1 (Z=-2.293, P=0.022; Fig. 5A) and PINP (Z=-2.803, P=0.005; Fig. 5B) increased significantly at 1 week after the interventional bronchoscopy therapy. As not all patients had been subjected to routine TGF-β1 and PINP examinations after each bronchoscopy, the data of the 10 patients were used to study the correlation between the degree of stenosis at baseline and biomarker levels after interventional bronchoscopy. In the post-interventional bronchoscopy period,

Table I. Clinical characteristics of study subjects.

Characteristic	Stenosis (n=59)	Non-stenosis (n=60)	Z/χ^2	P-value
Age (years)	26.0 (22.0-32.0)	28.0 (24.0-32.8)	-0.477	0.634[a]
Sex			0.287	0.592[b]
Male	6 (10.17)	8 (13.33)		
Female	53 (89.83)	52 (86.67)		
Smoking			NA	0.679[c]
Yes	3 (5.08)	2 (3.33)		
No	56 (94.92)	58 (96.67)		
Symptoms at presentation (overlapped, certain patients exhibited two or three symptoms)				
Cough			11.773	0.001[b]
Yes	23 (38.98)	7 (11.67)		
No	36 (61.02)	53 (88.33)		
Dyspnoea			19.855	<0.001[b]
Yes	21 (35.59)	2 (3.33)		
No	38 (64.41)	58 (96.67)		
Wheezing			20.169	<0.001[b]
Yes	17 (28.81)	0 (0)		
No	42 (71.19)	60 (100)		
Chest HRCT features (overlapped)				
Atelectasis			18.442	<0.001[b]
Yes	20 (33.90)	2 (3.33)		
No	39 (66.10)	58 (96.67)		
Mucus plugging			10.847	0.001[b]
Yes	16 (27.12)	3 (5.00)		
No	43 (72.88)	57 (95.00)		
Bronchoscopic features (overlapped)				
Tracheal stenosis			NA	NA
Yes	15 (25.42)	NA		
No	44 (74.58)	NA		
Main bronchus stenosis[d]			NA	NA
Left	32 (54.24)	NA		
Right	27 (45.76)	NA		
Stenosis degree			NA	NA
Mild-to-moderate	20 (33.90)	NA		
Severe	39 (66.10)	NA		
Bronchomalacia			NA	NA
Yes	33 (55.93)	NA		
No	26 (44.07)	NA		

Age is expressed as the median (interquartile range) and the other values are expressed as n (%). Differences between the two groups were assessed by the following corresponding statistical tests: [a]Age was analyzed by the Mann-Whitney U-test for not normally distributed continuous variables; [b]sex, cough, dyspnoea, wheezing, atelectasis and mucus plugging were analyzed by the χ^2-test (minimum expected values ≥5); [c]smoking was analyzed by Fisher's exact test (minimum expected values <5). [d]Among the stenosis group, there were 4 (12.50%) patients with left main bronchus stenosis combined with tracheal stenosis and 11 (40.74%) patients with right main bronchus stenosis combined with tracheal stenosis (P=0.018). HRCT, high-resolution computed tomography; NA, not available.

the serum TGF-β1 (Z=-2.132, P=0.033; Fig. 5C) and PINP (Z=-1.706, P=0.088, not statistically significant; Fig. 5D) levels in the severe stenosis group (n=6) at the baseline were also higher than those in the mild-to-moderate stenosis group (n=4).

The relative changes in the post- vs. pre-interventional bronchoscopy levels of serum TGF-β1 and PINP are presented in Table II. The variables included the number of biopsies taken and the duration of the bronchoscopy procedure per patient. When comparison of post- and pre-interventional

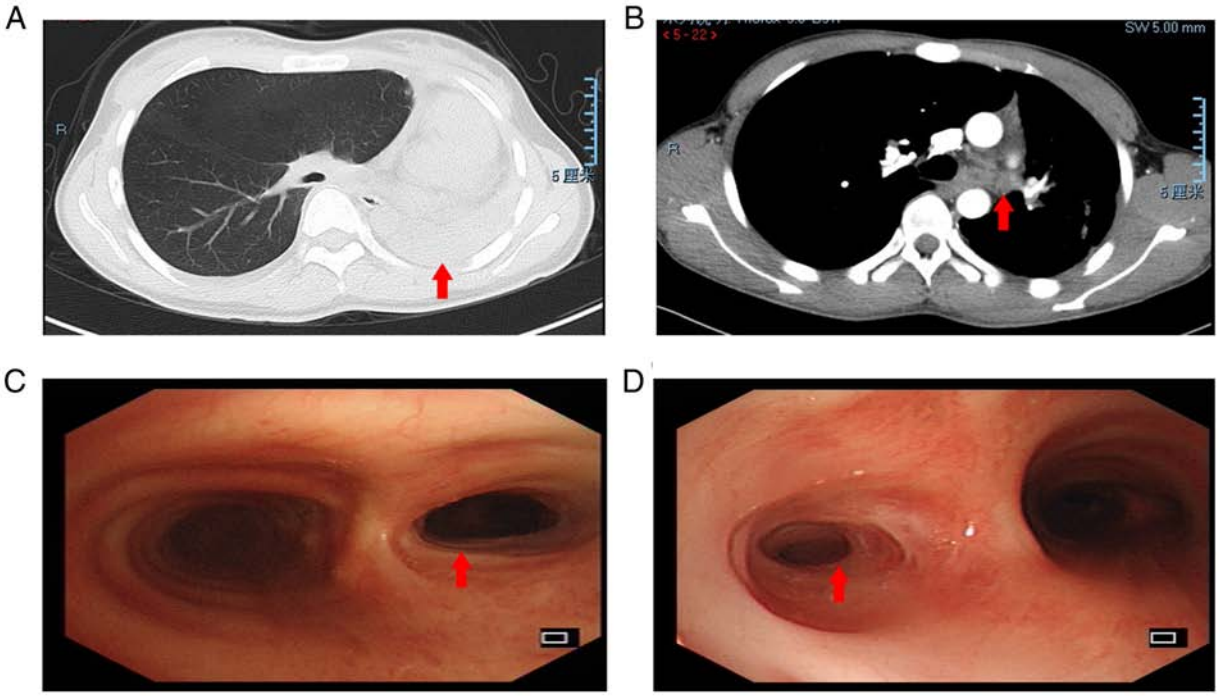

Figure 1. Chest HRCT and bronchoscopy features of patients with PTTS. (A and B) Chest HRCT indicating (A; 19 years old; female; chest routine scan; pulmonary window) left lung atelectasis (red arrow) and (B; 21 years old; male; chest enhanced scan; mediastinal window) left main bronchus mucus plugging (red arrow) due to TBTB (scale bars, 5 cm). (C and D) Diagnostic bronchoscopic appearance of PTTS; (C; 30 years old; female) moderate (red arrow) and (D; 24 years old; female) severe (red arrow) airway stenosis may be observed in the right and left main bronchi, respectively. TBTB, tracheobronchial tuberculosis; PTTS, post-tuberculosis tracheobronchial stenosis; HRCT, high-resolution computed tomography.

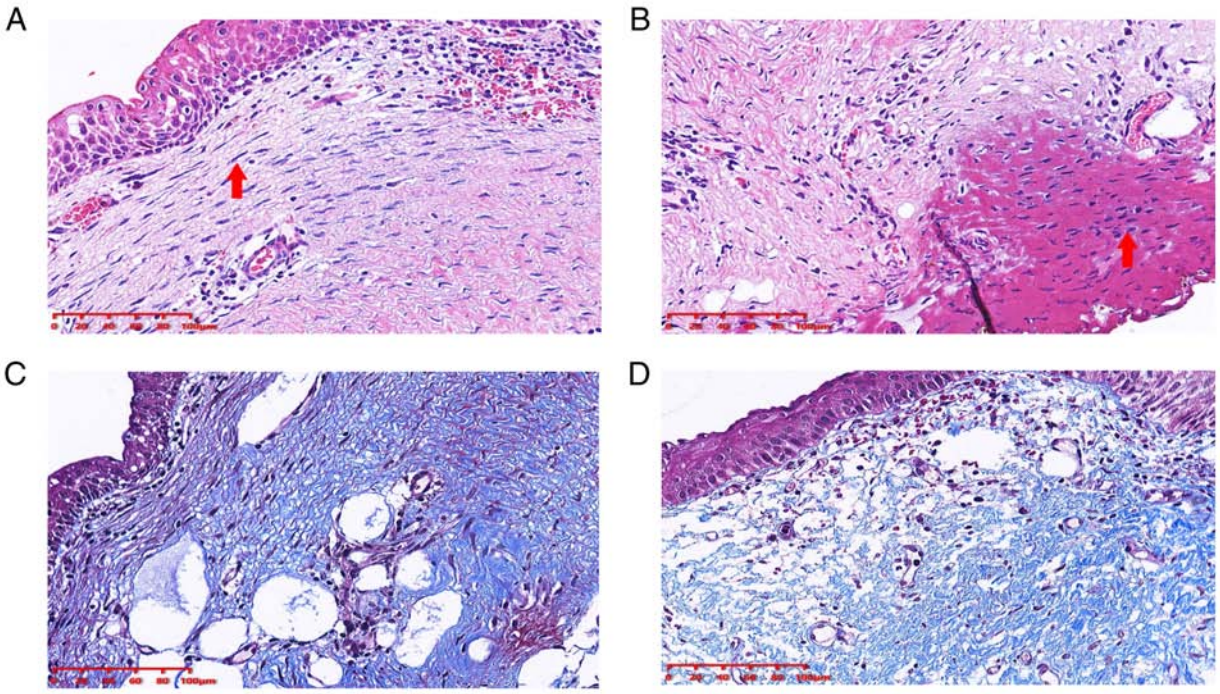

Figure 2. Histopathological assessment of biopsies of hyperplasia of fibrous tissue. (A and B) Histopathological examination with H&E staining demonstrating (A) squamous epithelialisation of the bronchial epithelial cells and proliferation of fibroblasts in the submucosa (red arrow), and (B) airway cicatrix tissue and coagulative necrosis due to electrocautery needle knife therapy (red arrow). (C and D) Masson staining. The blue colour reflects staining of collagen fibres (scale bars, 100 μm; magnification, x200).

bronchoscopy data was performed in each group, the increase in TGF-β1 and PINP levels was revealed to be greater in patients with≥3 biopsies than in those with<3 biopsies (Z=-1.358, P=0.175 and Z=-1.567, P=0.117, respectively). It was also greater when the median duration of the bronchoscopy procedure per patient was ≥60 min as compared to <60 min

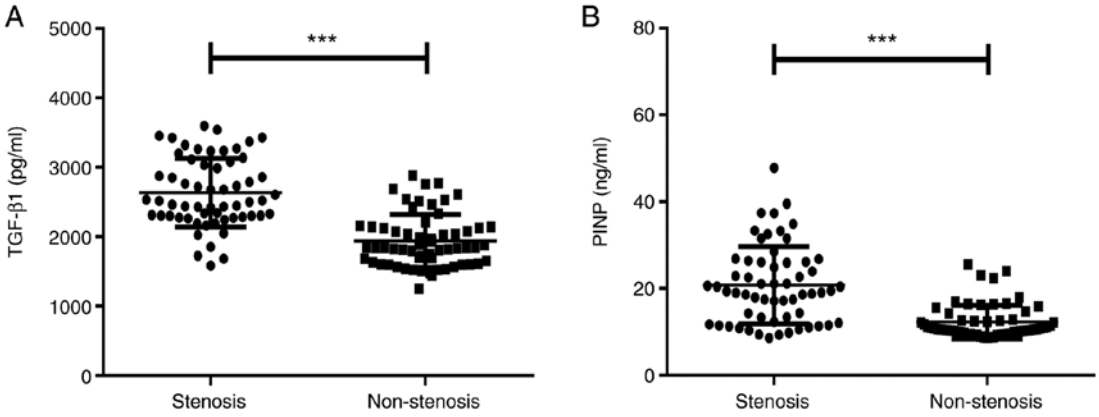

Figure 3. Comparisons of serum (A) TGF-β1 and (B) PINP levels between the airway stenosis and non-stenosis groups. Data are presented as the median (interquartile range). Differences between the two groups were assessed using the Mann-Whitney U-test. ***P<0.001. TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide.

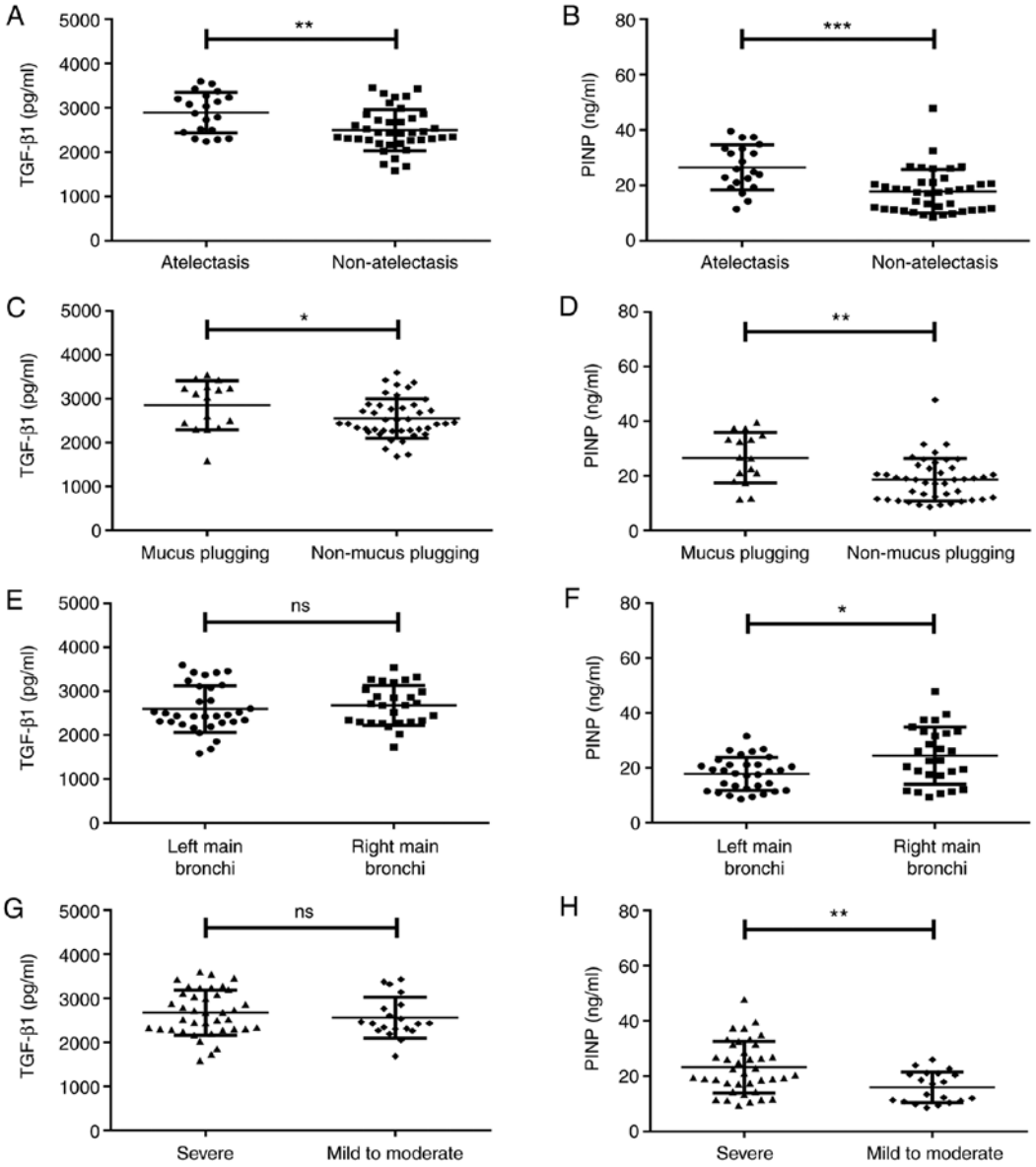

Figure 4. Relationships between serum TGF-β1 and PINP levels and different clinical characteristics in patients with airway stenosis. Relationships between (A) serum TGF-β1 and atelectasis and (B) PINP levels and atelectasis, (C) serum TGF-β1 and mucus plugging and (D) PINP levels and mucus plugging, (E) serum TGF-β1 and stenosis sites and (F) PINP levels and stenosis sites, and (G) serum TGF-β1 and the degree of stenosis and (H) PINP levels and the degree of stenosis. Data are presented as the median (interquartile range). Differences between the two groups were tested by the Mann-Whitney U-test. ns, not significant; *P<0.05, **P<0.01, ***P<0.001. TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide.

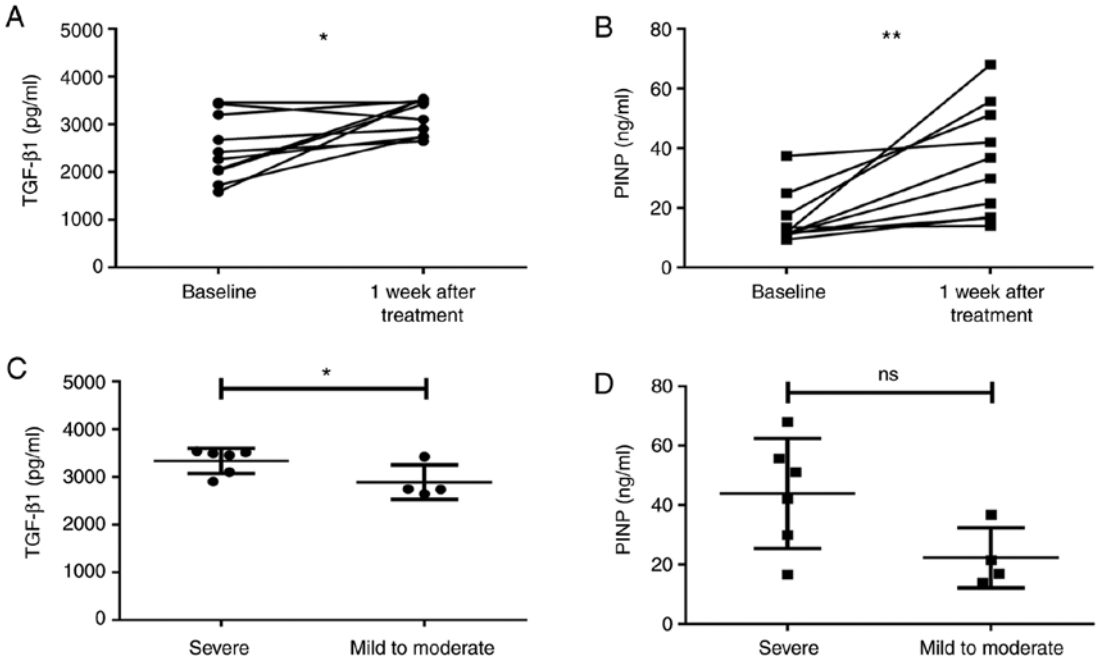

Figure 5. Changes in serum TGF-β1 and PINP levels in patients with airway stenosis during follow-up. Changes in serum (A) TGF-β1 and (B) PINP levels in 10 airway stenosis patients 1 week after interventional bronchoscopy therapy. Differences between the two groups were tested by Wilcoxon matched-pair signed-rank test. Relationships between serum (C) TGF-β1 and (D) PINP levels and degree of stenosis at the baseline. Data are presented as the median (interquartile range); differences between the two groups were assessed by the Mann-Whitney U-test. ns, not significant; *P<0.05, **P<0.01. TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide.

(Z=-1.919, P=0.055 and Z=-1.706, P=0.088, respectively; none of these was statistically significant).

Relationships between baseline levels of serum TGF-β1 and PINP and recurrence of stenosis. All of the patients with airway stenosis were treated using interventional bronchoscopy and longitudinally followed up for 1 month. As presented in Fig. 6, 32 (54.24%) patients were confirmed to have recurrent stenosis using bronchoscopy. The baseline levels of serum TGF-β1 [2,947.29 (2,371.74-3,272.32) vs. 2,339.71 (2,194.94-2,536.76) pg/ml, Z=-3.743, P<0.001; Fig. 6A] and PINP [21.13 (17.30-30.37) vs. 17.47 (11.27-22.56) ng/ml, Z=-2.419, P=0.016; Fig. 6B] in the subgroup with recurrence were significantly higher than those in the non-recurrent subgroup. Only two patients in the present study underwent serum TGF-β1 and PINP examinations while receiving repeated interventional bronchoscopy therapy. The serum TGF-β1 and PINP levels in the two patients fluctuated prior to each therapy, as presented in Fig. S1.

Correlation between serum TGF-β1 and PINP levels in the stenosis and non-stenosis groups. The serum PINP levels were positively correlated with TGF-β1 levels in patients with airway stenosis (P<0.001; Fig. 7A), whereas the correlations were not significant in the non-stenosis group (P=0.101; Fig. 7B).

Performance of serum TGF-β1 and PINP levels in distinguishing between airway stenosis and non-stenosis. The area under the ROC curve for serum TGF-β1 levels to distinguish patients with airway stenosis from those without stenosis was 0.824 (95% CI: 0.748-0.900) and that for PINP was 0.863 (95% CI: 0.796-0.930; Fig. 8). Thus, TGF-β1 and PINP levels performed well in distinguishing airway stenosis from non-stenosis. The cut-off value, 95% CI, P-value, sensitivity, specificity, PPV, NPV and accuracy associated with the highest Youden index are also presented in Table III. Serum TGF-β1 levels exhibited a sensitivity of 89.83% and specificity of 79.32%, while serum PINP levels exhibited a sensitivity of 67.80% and specificity of 91.77%. These results were all acceptable.

Discussion

TBTB is frequently followed by the development of airway stenosis, which is usually detected via invasive methods, such as bronchoscopy. However, bronchoscopy is performed selectively, particularly owing to the COVID-19 pandemic, and is associated with side effects (10,11,15). Hence, the present study aimed to assess the feasibility of using serum TGF-β1 and PINP levels as biomarkers for distinguishing between airway stenosis and non-stenosis and for monitoring the degree of PTTS. To the best of our knowledge, the present study was the first to demonstrate the clinical significance of serum TGF-β1 and PINP levels in PTTS.

TGF-β has been indicated to have an important function in local immunity in bronchial airway stenosis in active TBTB (27). However, the relationship between TGF-β and fibrosis post-TBTB had so far remained elusive. TGF-β1 has been suggested as a biomarker for fibrosis (28-31). In laboratory studies, TGF-β1 is used to promote collagen expression and fibrosis (36-42). In the present study, the serum levels of TGF-β1 were significantly higher in patients with airway stenosis than in those who did not have stenosis. This indicates

Table II. Changes in serum TGF-β1 and PINP levels between the post- and pre-interventional bronchoscopy.

Variable	TGF-β1 (pg/ml)	Z	P-value	PINP (ng/ml)	Z	P-value
Number of biopsies per patient		-1.358	0.175		-1.567	0.117
<3 (n=5)	231.10 (-49.80-748.49)			7.51 (2.81-17.81)		
≥3 (n=5)	1,400.41 (145.61-1,708.64)			25.50 (11.56-47.21)		
Duration of biopsies per patient (min)		-1.919	0.055		-1.706	0.088
<60 (n=4)	227.55 (-186.70-275.75)			4.85 (1.54-20.96)		
≥60 (n=6)	1,210.70 (357.14-1,585.24)			22.01 (9.66-42.69)		

Values are expressed as the median (interquartile range). Differences between the two groups were assessed by the Mann-Whitney U-test. TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide.

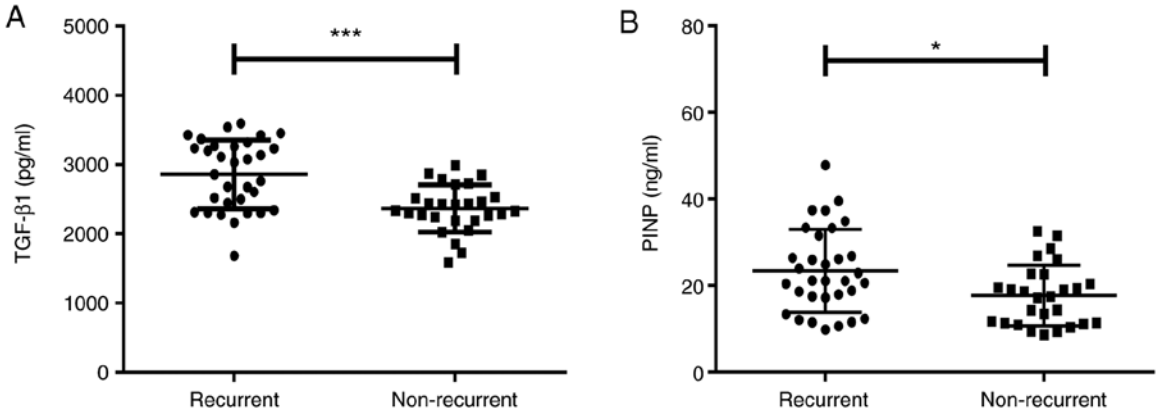

Figure 6. Relationships between baseline levels of serum TGF-β1 and PINP and recurrence of stenosis. Baseline serum (A) TGF-β1 and (B) PINP levels in recurrent and non-recurrent subgroups of airway stenosis. Data are presented as the median (interquartile range). Differences between the two groups were assessed using the Mann-Whitney U-test. *P<0.05; ***P<0.001. TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide.

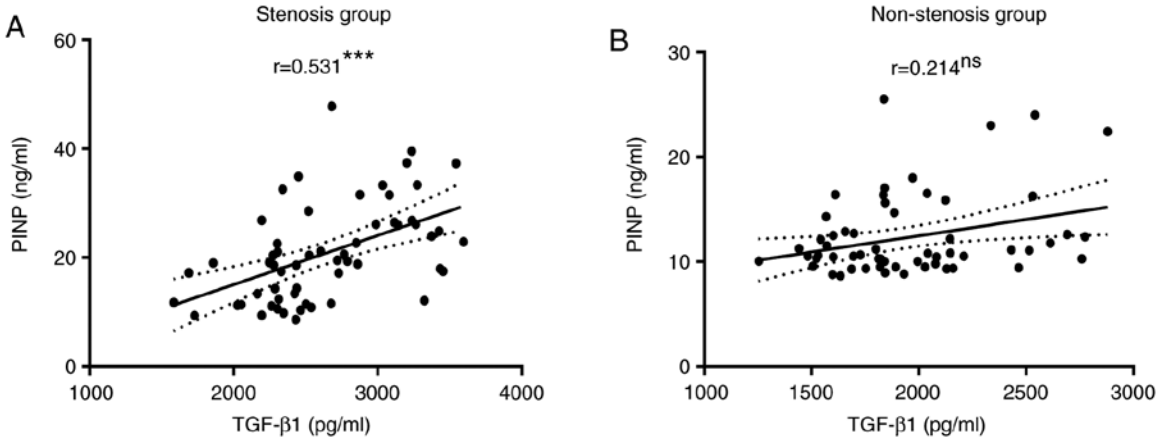

Figure 7. Correlation between serum TGF-β1 and PINP levels in different groups. Correlation between serum TGF-β1 and PINP levels in the (A) airway stenosis and (B) non-stenosis groups. Correlations were calculated by determining Spearman's rank correlation coefficients. The dotted lines indicate the 95% confidence band of the best-fit line. ns, not significant; ***P<0.001. TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide.

that TGF-β1 may be involved in the development and/or consequences of fibrosis after TBTB. The clinical characteristics of mucus plugging and atelectasis are also indicators of airway stenosis. In airway stenosis, the tracheal scar may cause partial or whole lung atelectasis and may even destroy the lung (3-6,7,43-44). In the present study, it was observed that patients with atelectasis or mucus plugging had significantly higher serum TGF-β1 levels than those in the control group. Biomarkers should reliably predict not only the extent of airway stenosis but also the response to therapy. The present results suggested that serum TGF-β1 levels higher than the baseline were related to worse response and prognosis for patients with

Table III. Summary of the results of the receiver operating characteristic curve analysis.

Parameter	Cut-off point	95% CI	P-value	HYI	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
TGF-β1	>2,162.16 pg/ml	0.748-0.900	<0.001	0.698	89.83	79.32	81.54	88.89	84.87
PINP	>17.10 ng/ml	0.796-0.930	<0.001	0.595	67.80	91.77	86.96	74.32	79.83

TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide; CI, confidence interval; HYI, highest Youden index; PPV, positive predictive value; NPV, negative predictive value.

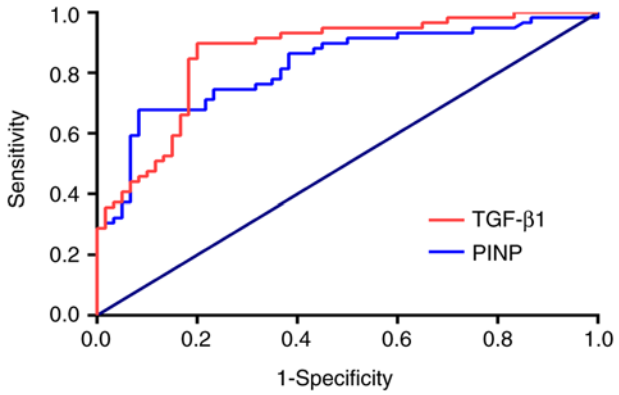

Figure 8. Comparison of receiver operating characteristic curves for airway stenosis thresholds. Diagnostic performance of serum TGF-β1 and PINP levels for distinguishing between airway stenosis and non-stenosis. TGF-β1, transforming growth factor β1; PINP, procollagen type I N-propeptide.

airway stenosis treated using interventional bronchoscopy during the follow-up. Consistent with these results, previous studies have reported that a high level of serum TGF-β1 is associated with the development of moderate-to-severe radiation-induced fibrosis due to intracavitary accelerated partial breast irradiation (30) and is an independent predictor of atrial fibrillation recurrence after catheter ablation (31). All of the above results suggest that TGF-β1 contributes considerably to airway scar hyperplasia and that its serum levels may be used to monitor the exacerbation rate of airway scarring for PTTS.

The serum PINP level is a biomarker reflecting collagen synthesis in pathological fibrotic processes (26,34); it not only indicates the severity of liver cirrhosis (34) but may also be used to monitor therapeutic efficacy for chronic heart failure (26). Therefore, serum PINP levels may be clinically valuable for the non-invasive assessment of the presence and extent of the profibrotic state of PTTS. In the present study, histopathological examination demonstrated the proliferation of fibroblasts in the bronchial submucosa and airway scar tissue. Furthermore, serum PINP levels were significantly higher in the airway stenosis than in the non-stenosis group. In addition, all patients with airway stenosis accompanied by atelectasis, mucus plugging or severe main bronchus stenosis had higher serum PINP levels. Of note, serum PINP levels were higher in patients with right main bronchus stenosis than in those with left main bronchus stenosis, possibly owing to the higher proportion of tracheal stenosis in patients with right main bronchus stenosis. This difference may be due to the different diameter and angle of the right main bronchus, which is conducive to the excretion of sputum containing TB bacilli to the trachea. The present results also suggested that serum PINP levels higher than the baseline were related to worse response and prognosis. Collectively, these results suggest that collagen synthesis increases in airway stenosis and that serum PINP levels are able to predict the severity of PTTS.

The present study also demonstrated the side effects of interventional bronchoscopy in terms of histopathological studies and serum biomarkers. Cell coagulative necrosis was induced by interventional bronchoscopy therapy, as evidenced by histopathological examinations and confirmed by the increased serum levels of TGF-β1 and PINP 1 week after interventional bronchoscopy. These results indicated that interventional bronchoscopy may induce the secretion of TGF-β1 by destroying the local microenvironment and may thus accelerate the exacerbation of scarring, which may explain the rapid post-therapy exacerbation of the scarring and airway restenosis. Statistical analysis revealed a positive correlation between the serum TGF-β1 and PINP levels of patients with airway stenosis, indicating that serum TGF-β1 and PINP may be used as complementary markers for evaluating airway stenosis in patients with PTTS. Furthermore, serum TGF-β1 and PINP levels had good diagnostic potential for distinguishing between airway stenosis and non-stenosis. Taken together, these results indicated that the serum TGF-β1 and PINP levels are potential clinical markers for diagnosing and real-time monitoring of PTTS.

The present study has several limitations. First, PTTS is mostly observed among females owing to the smaller bronchial lumen size and less expectorated sputum than in males, resulting in prolonged exposure to TB bacilli in the bronchi (3-5,45); therefore, the patients enrolled in this study were predominantly females. Further studies should be performed in males. Furthermore, the small number of patients reduced the power of the statistical calculations and increased the risk for type 2 statistical errors. However, the patients were selected from a larger cohort, the clinical characteristics reflecting airway stenosis were comprehensive, and cases of PTTS were closely monitored during the treatment. In addition, serum biomarker levels may be affected by post-TB pulmonary fibrosis (46). As another limitation, serum TGF-β1 and PINP measurements were not performed prior to each bronchoscopy and a further study should be performed using a larger sample size to obtain more representative results. Finally, longer periods of observation and measurement of serum biomarker levels after interventional bronchoscopy are

required to evaluate the predictive value of TGF-β1 and PINP for the recurrence of airway stenosis.

In conclusion, non-invasive strategies for monitoring PTTS are clinically important but lacking. The present study was the first to report that increased serum TGF-β1 and PINP levels have the potential to act as biomarkers for diagnosing PTTS. In future studies, more patients will be enrolled in a multi-centre study to provide sufficient data to confirm the results of the present study. These practically measurable markers may assist in monitoring the treatment response. At present, a prospective clinical study involving the use of a combination of drugs and interventional bronchoscopy for airway scar stenosis is underway at our hospital (registration no. ChiCTR1900024441). As this clinical trial was influenced by the COVID-19 pandemic, telemedicine and remote laboratory monitoring will be used to validate the utility of serum TGF-β1 and PINP levels as biomarkers in patients with PTTS. This approach is convenient with the advantage of reducing the risk of staff being infected and the financial costs, and may be used for the entire management of patients with TBTB in the future.

Acknowledgements

Not applicable.

Funding

This work was supported by the National Major Science and Technology Projects of China (grant no. 2018ZX10302302003).

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SG contributed to the conception and design of the study and the manuscript preparation. YW acquired serum samples, analysed the demographic data and prepared the manuscript. YSL and YB performed the interventional bronchoscopy, analysed the measurement results and drafted components of the manuscript. JJ and XHW acquired and analysed the chest HRCT data and drafted components of the manuscript. YC, XW and GH assisted in the experiments, analysed the experimental data and drafted components of the manuscript. YG and YL contributed to the design of the study, confirmed and approved the authenticity of the raw data and drafted components of the manuscript. All authors read and approved the final manuscript. All authors agreed to be 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.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (Chongqing, China). Informed consent was obtained from all the subjects whose sera were used in this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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