Clinical and Experimental Medicine
https://doi.org/10.1007/s10238-023-01214-x

RESEARCH

Plasma LTBP2 as a potential biomarker in differential diagnosis of connective tissue disease-associated interstitial lung disease and idiopathic pulmonary fibrosis: a pilot study

Menglin Zou[1,2] · Xingxing Hu[1] · Weiwei Song[1] · Han Gao[1] · Changrong Wu[1] · Weishuai Zheng[3] · Zhenshun Cheng[1,4]

Received: 9 September 2023 / Accepted: 2 October 2023

Abstract

Few biomarkers distinguish connective tissue disease-associated interstitial lung disease (CTD-ILD) from idiopathic pulmonary fibrosis (IPF). Latent transforming growth factor-β binding protein-2 (LTBP2), a secreted extracellular matrix protein, is involved in pulmonary fibrosis. However, the role of LTBP2 in differentially diagnosing CTD-ILD and IPF is unclear. In this study, enzyme-linked immunosorbent assays quantified plasma LTBP2 concentrations in 200 individuals (35 healthy controls, 42 CTD patients without ILD, 89 CTD-ILD patients, and 34 IPF patients). CTD-ILD and IPF were further classified based on chest imaging pattern and pulmonary function test results. Plasma LTBP2 levels were significantly elevated in the IPF group compared with the CTD-ILD group. ROC analysis further suggested the possible value of LTBP2 in differentially diagnosing CTD-ILD and IPF. Additionally, CTD-ILD patients with progressive lung fibrosis had higher plasma LTBP2 concentrations than those who did not. Similarly, patients with IPF developing acute exacerbation showed higher plasma LTBP2 levels than those with stable IPF. This is the first study showing that LTBP2 was closely associated with the usual interstitial pneumonia (UIP) pattern in rheumatoid arthritis-associated ILD (RA-ILD). Moreover, the optimal cutoff values of LTBP2 for distinguishing IPF from CTD-UIP/RA-UIP were 33.75 and 38.33 ng/mL with an AUC of 0.682 and 0.681, respectively. Our findings suggest that plasma LTBP2 levels may differentially diagnose CTD-ILD and IPF, and assess their fibrotic activity. Additionally, clinical LTBP2 evaluation may be a great aid to identifying the presence of the UIP pattern in RA-ILD and to discriminating IPF from CTD-UIP, particularly RA-UIP.

Keywords LTBP2 · Interstitial lung diseases · Pulmonary fibrosis · Usual interstitial pneumonia · Biomarker

Introduction

Interstitial lung diseases (ILDs) are a heterogeneous set of disorders that range from idiopathic pulmonary fibrosis (IPF) to secondary variants, including the exposure-related ILD and connective tissue disease-associated ILD (CTD-ILD) [1, 2]. Currently, pulmonary function testing (PFT) and high-resolution computed tomography (HRCT) are the two main methods for diagnosing and assessing ILDs [3–5]. However, performing PFT properly can be difficult to achieve in certain condition, such as in patients with poor general health. HRCT, a frontline diagnostic tool for ILDs, verifies pulmonary fibrosis (PF) through typical scarred tissues, which can easily cause delayed diagnosis [3, 6]. Additionally, the clinical application of the histopathological examination is limited by various factors, including the complexity and risk of developing acute exacerbation (AE) [7]. The aforementioned diagnostic

Menglin Zou, Xingxing Hu and Weiwei Song have contributed equally to this work and share first authorship.

✉ Changrong Wu
35834454@qq.com

✉ Weishuai Zheng
569424042@qq.com

✉ Zhenshun Cheng
zhenshun_cheng@126.com

1 Department of Respiratory and Critical Care Medicine, Zhongnan Hospital of Wuhan University, Wuhan, China

2 Fourth Ward of Medical Care Center, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou, China

3 Department of Respiratory and Critical Care Medicine, Hainan General Hospital, Hainan Affiliated Hospital of Hainan Medical University, Haikou, China

4 Wuhan Research Center for Infectious Diseases and Cancer, Chinese Academy of Medical Sciences, Wuhan, China

Published online: 21 October 2023

dilemmas in ILDs highlight the necessity of validating noninvasive biomarkers that are easy to evaluate. IPF is characterized by the histological manifestation of usual interstitial pneumonia (UIP) [8]. However, the UIP pattern can also occur in CTD-ILD [4]. In clinical practice, it may be difficult to distinguish between the IPF and CTD-ILD when the UIP pattern presents before extrapulmonary manifestations of the underlying CTD, particularly underlying rheumatoid arthritis (RA) [5]. Current treatment paradigms for CTD-ILD and IPF differ. Thus, it is clinically important to distinguish these diseases.

Latent transforming growth factor-β binding protein-2 (LTBP2) is a secreted extracellular matrix (ECM) protein [9, 10]. Experimental evidence from two studies proposed the relationship between LTBP2 and ILDs. A previous study reported that LTBP2 was a prognostic blood biomarker in IPF [11]. Our previous study showed that LTBP2 was a profibrotic cytokine in PF and its silencing suppressed lung fibroblast-to-myofibroblast differentiation [12]. To date, only a few biomarkers reflecting the pivotal fibrotic process of lung fibroblasts differentiation were identified. Although the potential utility of LTBP2 in the field of PF is emerging, to the best of our knowledge, the role of LTBP2 in differentially diagnosing CTD-ILD and IPF remains unclear.

This study comprehensively investigated the clinical relevance of LTBP2 in the plasma of ILDs (including CTD-ILD and IPF). The potential role of LTBP2 as a discriminating biomarker for CTD-ILD and IPF was also evaluated.

Materials and methods

Subjects

A study comprising 35 healthy controls (HCs), 42 CTD patients without ILD, 89 patients with CTD-ILD, and 34 patients with IPF, was conducted at the Zhongnan Hospital of Wuhan University. The American College of Rheumatology criteria were used to diagnose and classify the CTD-ILD [13]. Progressive lung fibrosis in CTD-ILD (F-CTD-ILD), the radiological UIP pattern in CTD-ILD and RA-ILD, and IPF were diagnosed according to the ATS/ERS consensus criteria [14]. Healthy volunteers with no sign of comorbidities were included in the HCs group.

Enzyme-linked immunosorbent assay (ELISA)

Human plasma samples were centrifugated and stored at − 80 °C for further detection. Plasma LTBP2 concentrations in blood samples were detected using ELISA kits (ELK Biotechnology, Wuhan, China).

Statistical analysis

IBM SPSS Version 23.0 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Data were expressed as the mean ± standard deviation (SD) or number of individuals (*n*) and percentages (%). A chi-square test was used for the count data between IPF and CTD-ILD. Statistical significance between different groups was estimated by the Mann–Whitney *U*-test, Student's *t*-test, or one-way ANOVA, where applicable. Correlation analysis was tested by estimating Pearson's correlation coefficient. The optimal cut-off levels for LTBP2 were determined using receiver operating characteristic (ROC) curve analysis.

Results

Differences in plasma LTBP2 concentrations between CTD-ILD and IPF

To determine whether LTBP2 correlates with various types of ILD activity, plasma LTBP2 concentrations were evaluated in ILDs which arose from distinct etiologies. The clinical details of the study population are presented in Table 1. Plasma LTBP2 concentrations were significantly higher in the CTD-ILD group and IPF group compared with the HC group. In particular, patients with CTD-ILD showed higher plasma LTBP2 concentrations than those with CTD. However, plasma LTBP2 levels were not significantly different between patients with CTD and HCs. Additionally, plasma LTBP2 levels were significantly elevated in patients with IPF compared to those with CTD-ILD (Fig. 1A).

This study included 36 patients with RA-ILD, 11 with SSc-ILD, 24 with IM-ILD and 18 with SS-ILD. We compared the plasma LTBP2 levels in various CTD-ILD subgroups. The RA-ILD, SSc-ILD, IM-ILD, and SS-ILD groups showed higher plasma LTBP2 concentrations than HCs. In particular, there were significant differences in plasma LTBP2 concentrations between patients with IPF and those in the four subgroups of patients with CTD-ILD. However, no significant difference in plasma LTBP2 levels was found between the four subgroups of patients with CTD-ILD (Fig. 1B).

Differences in plasma LTBP2 concentrations between non-F-CTD-ILD and F-CTD-ILD

In many CTDs, progressive pulmonary fibrosis (PPF) is described. CTD-ILD with evidence of PPF tends to have a worse prognosis [15]. Thus, we further explored whether LTBP2 could evaluate the progression of lung fibrosis in

Table 1 Demographic and clinical characteristics of healthy controls and patients

	Healthy Controls ($n=35$)	CTD patients without ILD ($n=42$)	CTD-ILD patients ($n=89$)	IPF patients ($n=34$)	*p
Sex (male), n (%)	17 (48.57)	9 (21.43)	23 (25.84)	24 (70.59)	0.001
Age, years, mean ± SD	67.09 ± 10.00	64.38 ± 9.50	69.49 ± 13.15	71.06 ± 8.94	0.524
Current/ever-smoker, n (%)	–	5 (11.90)	11 (12.36)	14 (41.18)	0.001
RA-ILD	–	–	36 (40.45)	–	–
SSc-ILD	–	–	11 (12.36)	–	–
IM-ILD	–	–	24 (26.97)	–	–
SS-ILD	–	–	18 (20.22)	–	–
CRP (mg/L), mean ± SD	–	–	24.39 ± 36.83	–	–
ESR (mm/h), mean ± SD	–	–	25.67 ± 24.65	–	–
Pulmonary function					
FVC (% predicted), mean ± SD	–	–	73.96 ± 19.76	77.14 ± 24.77	0.556
DLCO (% predicted), mean ± SD	–	–	57.85 ± 14.61	60.74 ± 16.22	0.445
CT/HRCT pattern, n (%)					
UIP pattern	–	–	33 (37.08)	34 (100)	0.001
Non-UIP pattern	–	–	56 (62.92)	–	–

RA—rheumatoid arthritis, *SSc*—systemic sclerosis, I*M*—inflammatory myositis, *SS*—Sjogren syndrome, *CRP*—C-reactive protein, *ESR*—erythrocyte sedimentation rate, *FVC*—forced vital capacity, *DLCO*—diffusing capacity of the lung for carbon monoxide

*p, p-value obtained after comparison between patients with IPF and those with CTD-ILD

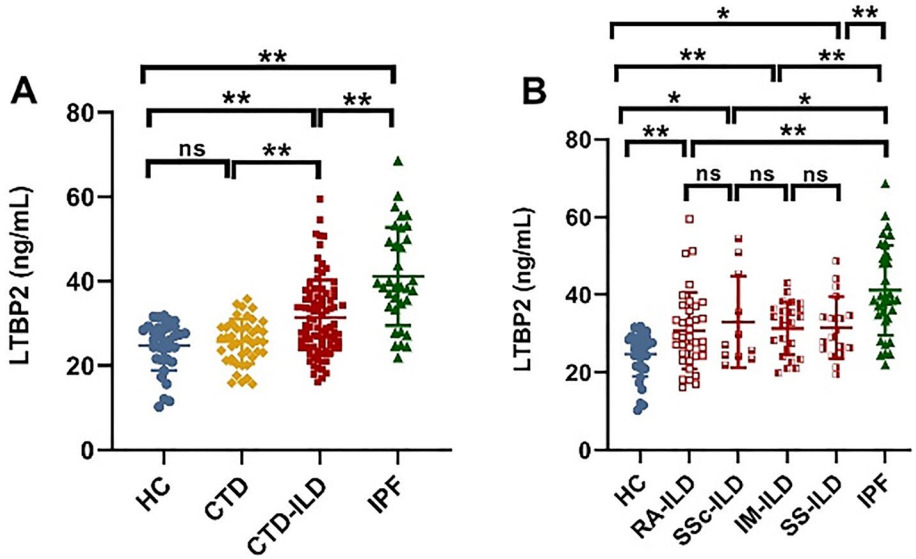

Fig. 1 Plasma LTBP2 concentrations of the study population in the study **A** Plasma LTBP2 concentrations in HCs and patients with CTD, CTD-ILD, and IPF. **B** Plasma LTBP2 concentrations in HCs, RA-ILD, SSc-ILD, IM-ILD, SS-ILD, and IPF. ns—not significant. *$p<0.05$, **$p<0.01$

CTD-ILD. In this study, a significant increase in plasma LTBP2 levels was found in patients with F-CTD-ILD (Fig. 2A, Table 2).

Differences in plasma LTBP2 concentrations between S-IPF and AE-IPF

This study also included 23 stable period of IPF (S-IPF) and 11 AE-IPF. Next, we compared LTBP2 levels between S-IPF and AE-IPF to determine whether LTBP2 could identify patients with IPF at risk of developing AE. Compared to HCs, both the S-IPF and AE-IPF groups showed varying degrees of impaired pulmonary function, whereas patients with AE-IPF manifested a severe decline (Table 3). Additionally, patients with IPF developing AE had higher plasma LTBP2 levels (Fig. 2B).

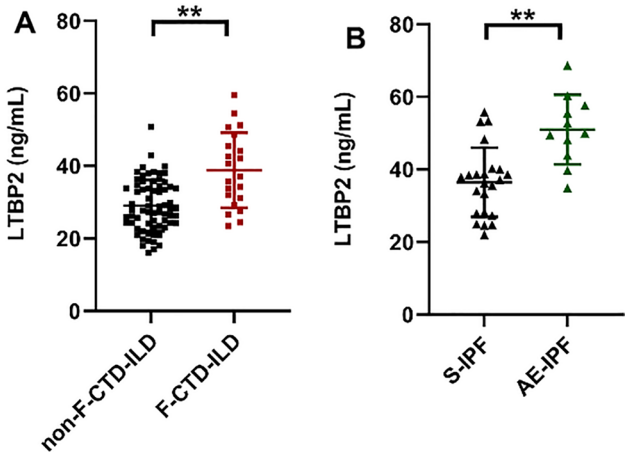

Fig. 2 Plasma LTBP2 concentrations in subgroups of CTD-ILD and IPF **A** Comparison of plasma LTBP2 concentrations in non-F-CTD-ILD and F-CTD-ILD. **B** Comparison of plasma LTBP2 concentrations in S-IPF and AE-IPF. $^{**}p < 0.01$

Plasma LTBP2 concentrations in ILDs with the UIP pattern

UIP is a radiological pattern that is an important risk factor for PPF in CTD-ILD [16]. In addition, the UIP subtype of RA-ILD has several clinical and histopathological characteristics with IPF [17, 18]. Thus, we assessed whether differences in plasma LTBP2 expressions correlated with the UIP pattern in ILDs with different etiologies. Plasma LTBP2 levels were significantly higher in patients with CTD-UIP than in those without UIP (Fig. 3A). Similarly, a significant increase in plasma LTBP2 concentrations was found in patients with RA-UIP compared with those without UIP (Fig. 3B). Interestingly, patients with IPF also showed higher plasma LTBP2 concentrations compared with those in the CTD-UIP group, particularly the RA-UIP group (Fig. 3C).

Relationships of plasma LTBP2 concentrations with clinical features

There was a significant negative correlation of plasma LTBP2 concentrations with FVC% in IPF and RA-UIP. Additionally, plasma LTBP2 levels significantly negatively correlated with DLCO% in IPF and RA-UIP (Tables 4 and 5). There were no correlation of plasma LTBP2 levels with the duration of RA disease, CRP, ESR, FVC%, or DLCO% in patients with RA-non-UIP or in those with RA-ILD (Table 5).

Diagnostic value of plasma LTBP2 levels in ILDs

We evaluated the diagnostic value of LTBP2 in ILDs of different etiologies using ROC curves. The optimal cutoff value of LTBP2 for distinguishing CTD-ILD from CTD/IPF was 32.43 and 34.75 ng/mL with an area under the curve (AUC) of 0.684 and 0.754, respectively (Fig. 4A, B). The ROC analysis revealed an AUC of 0.771 for distinguishing F-CTD-ILD from non-F-CTD-ILD, and the best cutoff value was 40.26 ng/mL. Additionally, the AUC for plasma LTBP2 in predicting AE in patients with IPF was 0.850 (Fig. 4C).

For ILDs with a UIP pattern, the area under the ROC curve for LTBP2 in distinguishing RA-UIP from RA-non-UIP was 0.737. The optimal cutoff values of LTBP2 for distinguishing IPF from CTD-UIP/RA-UIP were 33.75 and 38.33 ng/mL with an AUC of 0.682 and 0.681, respectively (Fig. 4D). A detailed ROC curve analysis is presented in Table 6.

Discussion

This study demonstrates the clinical relevance and differential diagnostic value of LTBP2 in ILDs which arise from distinct etiologies. In addition, the preclinical results from our previous study [12] and the clinical data in the

Table 2 Demographic and clinical characteristics of patients with CTD-ILD

	non-F-CTD-ILD ($n=68$)	F-CTD-ILD ($n=21$)	*p
Sex (male), n (%)	15 (22.06)	8 (38.10)	0.142
Age, years, mean ± SD	70.75 ± 11.07	65.43 ± 18.10	0.105
Current/ever-smoker, n (%)	10 (14.71)	1 (4.76)	0.226
CRP (mg/L), mean ± SD	23.79 ± 38.87	26.04 ± 31.43	0.826
ESR (mm/h), mean ± SD	22.72 ± 21.35	34.81 ± 31.74	0.050
FVC (% predicted), mean ± SD	74.17 ± 19.79	73.17 ± 20.57	0.883
DLCO (% predicted), mean ± SD	58.61 ± 14.22	54.91 ± 16.72	0.530
UIP pattern, n (%)	23 (33.82)	10 (47.62)	0.253

CRP—C-reactive protein, *ESR*—erythrocyte sedimentation rate, *FVC*—forced vital capacity, *DLCO* diffusing capacity of the lung for carbon monoxide

Table 3 Demographic and clinical characteristics of patients with IPF

	S-IPF ($n=23$)	AE-IPF ($n=11$)	*p
Sex (male), n (%)	17 (73.91)	7 (63.64)	0.538
Age, years, mean ± SD	70.09 ± 9.02	73.90 ± 8.83	0.367
Current/ever-smoker, n (%)	10 (43.48)	4 (36.36)	0.693
FVC (% predicted), mean ± SD	83.33 ± 23.05	49.25 ± 13.86	**0.009**
DLCO (% predicted), mean ± SD	63.69 ± 14.22	45.00 ± 10.22	**0.040**

Statistically significant results are highlighted in bold

FVC—forced vital capacity, *DLCO*—diffusing capacity of the lung for carbon monoxide

present study collectively suggest that LTBP2 may be a reliable blood biomarker for reporting the fibrotic activity of IPF and CTD-ILD.

In the pathogenesis of ILD, activated fibroblasts and myofibroblasts are core components of ILD progression [19]. LTBP2 participates in organ fibrosis, including that of the heart and skin [20, 21]. Our previous study showed

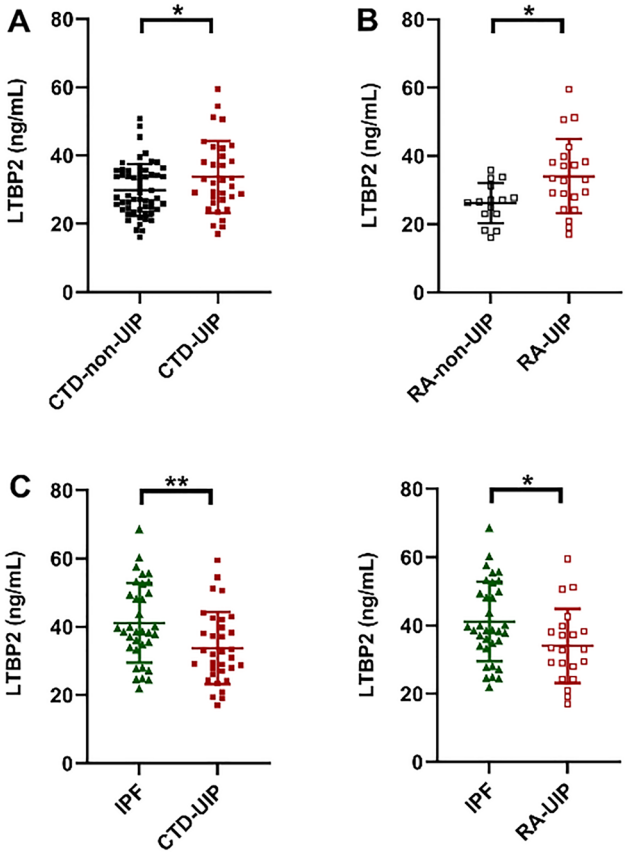

Fig. 3 Plasma LTBP2 levels in ILDs with the UIP pattern **A** Plasma LTBP2 concentrations in CTD-non-UIP and CTD-UIP. **B** Plasma LTBP2 concentrations in RA-non-UIP and RA-UIP. **C** Plasma LTBP2 concentrations in patients with IPF, CTD-UIP, and RA-UIP. *$p<0.05$, **$p<0.01$

Table 4 Correlation of plasma LTBP2 levels with pulmonary function tests in patients with CTD-ILD and in those with IPF

Variable	CTD-ILD		IPF	
	r	*P*	r	*p*
FVC (% predicted)	−0.135	0.332	−0.676	**0.001**
DLCO (% predicted)	−0.222	0.174	−0.696	**0.001**

Statistically significant results are highlighted in bold

r—Pearson's correlation coefficient; FVC—forced vital capacity; DLCO—diffusing capacity of the lung for carbon monoxide

that LTBP2 was expressed in lung-activated fibroblasts/myofibroblasts, and regulated fibroblasts differentiation into myofibroblasts in PF [12]. This study evaluated LTBP2 as a blood biomarker of ILDs with distinct etiologies. We found that plasma LTBP2 levels were significantly elevated in CTD-ILD and IPF compared with that of HCs. In particular, patients with CTD-ILD showed higher plasma LTBP2 concentrations than those with CTD. Further classification of patients with CTD-ILD showed that the RA-ILD, SSc-ILD, IM-ILD, and SS-ILD groups had higher plasma LTBP2 concentrations than the HCs. Taken together, these data indicated that LTBP2 levels were elevated in various types of ILDs.

To the best of our knowledge, this is the first study showing that LTBP2 supported the differential diagnosis of IPF and CTD-ILD. To discriminate ILDs of different etiologies is complex because they have analogical morphological, clinical, and radiological features. However, combined immunosuppressive treatments have been shown to have negative effects in patients with IPF [22]. The therapeutic approach is complicated by the fact that some patients develop ILD years before a diagnosis of CTD or may primarily manifest as (or are limited to) the lung manifestations of autoimmune diseases [5, 6, 23]. In the present study, our data suggested that there was a difference in plasma LTBP2 levels between IPF and CTD-ILD, which was crucial importance considering its therapeutic significance. LTBP2, secreted from lung-activated fibroblasts or myofibroblasts [11, 12], plays a vital role in regulating the ECM [24]. However, IPF is characterized by abnormal ECM remodeling [14, 19]. This may explain why LTBP2 can differentiate between CTD-ILD and IPF. However, future multicentral studies are essential to further understand and verify the role of LTBP2 in differential diagnosis.

Interestingly, we also found that LTBP2 identified IPF at risk of developing AE, but also predicted PPF in CTD-ILD. To date, AE is a devastating complication of IPF [25]. Lung fibroblast-to-myofibroblast differentiation is often observed in patients with diffuse alveolar injury,

Table 5 Correlation of plasma LTBP2 levels with continuous variables related to disease features in patients with RA-ILD

Variable	RA-non-UIP		RA-UIP		RA-ILD	
	r	*p*	r	*p*	r	*P*
Duration of RA disease (years)	0.529	0.178	−0.337	0.201	−0.099	0.644
CRP (mg/L)	0.076	0.858	−0.013	0.967	0.034	0.884
ESR (mm/h)	0.345	0.248	0.138	0.563	0.225	0.207
FVC (% predicted)	0.266	0.457	−0.781	**0.008**	−0.313	0.179
DLCO (% predicted)	0.243	0.643	−0.787	**0.021**	−0.412	0.143

Statistically significant results are highlighted in bold

r—Pearson's correlation coefficient; CRP—C-reactive protein; ESR—erythrocyte sedimentation rate; FVC—forced vital capacity; DLCO—diffusing capacity of the lung for carbon monoxide

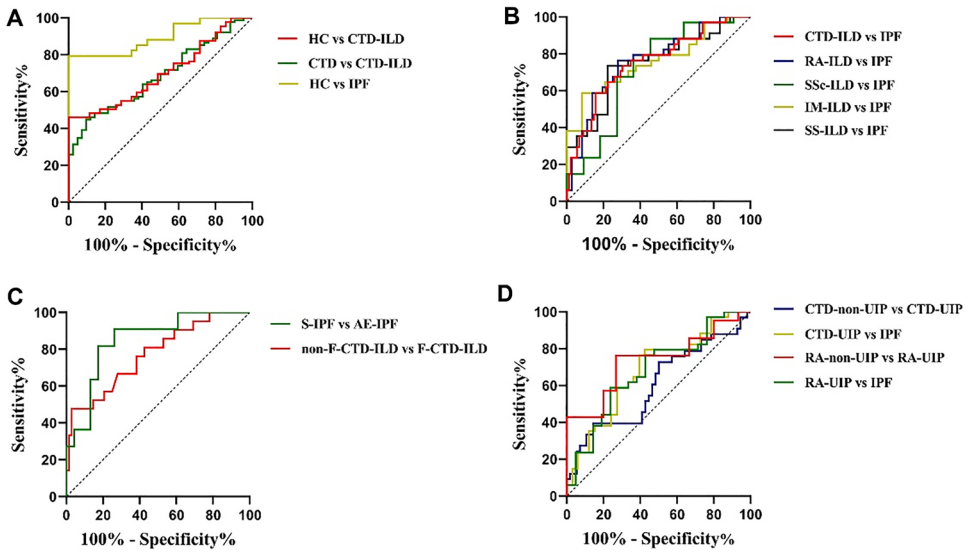

Fig. 4 Diagnostic value of plasma LTBP2 concentrations for patients with ILDs

regardless of the underlying disease [26]. The above findings support our present data that patients with AE-IPF typically had diffuse alveolar damage combined with potential fibrosis with a UIP pattern, and showed higher plasma LTBP2 levels than those who did not. Additionally, patients with F-CTD-ILD showed higher plasma LTBP2 levels than those without PPF. The ROC curves suggested the potential application of LTBP2 as a reliable blood biomarker for identifying PPF in CTD-ILD. Therefore, unlike other biomarkers that can only indirectly reflect initial or obsolete fibrosis, LTBP2 may be particularly suitable as a blood biomarker for accurately describing the acute phase and ongoing fibrogenesis. Therefore, evaluating LTBP2 concentrations in patients with IPF and CTD-ILD may contribute to the selection of appropriate therapeutic windows. First, early detection of AE in patients with IPF may be beneficial for the positive intervention with corticosteroids. Second, identification of the actively fibrotic phase in patients with CTD-ILD was conducive to timely conducted intervention with antifibrotic drugs and reduce the blind use of antifibrotic agents.

In this study, other major findings were that differences in plasma LTBP2 levels were related to the UIP pattern in RA-ILD. In addition, LTBP2 expression levels were higher in IPF than in CTD-UIP, particularly in RA-UIP. Characteristic of UIP include the alternating appearance of subpleural fibrosis and patchy areas of normal lung morphology. At this interface, there is a fibroblast lesion which is the site where myofibroblasts and ECM accumulate [27]. The above findings support our present data that RA-UIP showed higher plasma LTBP2 levels than those without UIP. One of hallmarks in IPF is UIP; this is also observed in CTD and exposure-related ILDs [14, 27]. A UIP pattern in certain ILDs is generally related to more rapid disease progression [15]. Moreover, RA-UIP has an analogous phenotype and mortality as IPF [5, 28]. Although IPF and RA-ILD may overlap, their treatment paradigms differ. Therefore, it is crucial to distinguish these diseases considering the therapeutic implications.

We acknowledge that the present study has some limitations. Firstly, all patients in the study were from the same hospital. Thus, to validate our preliminary results, a multicenter study comprising more types of patients would be

Table 6 Diagnostic performance of LTBP2 based on the ROC analysis

Variables	Cut-off value (ng/mL)	Sensitivity (%)	Specificity (%)	AUC	*p*
HC versus CTD-ILD	31.91	46.07	100	0.698	**0.0006**
HC versus RA-ILD	32.33	41.67	100	0.671	**0.0130**
HC versus SSc-ILD	33.76	36.36	100	0.652	0.1319
HC versus IM-ILD	31.91	58.33	100	0.745	**0.0015**
HC versus SS-ILD	32.84	44.44	100	0.718	**0.0101**
HC versus IPF	32.57	79.41	100	0.895	**0.0001**
CTD versus CTD-ILD	32.43	44.94	90.48	0.684	**0.0007**
CTD-ILD versus IPF	34.75	73.53	69.66	0.754	**0.0001**
RA-ILD versus IPF	33.95	76.47	72.22	0.764	**0.0001**
SSc-ILD versus IPF	31.55	79.41	63.64	0.717	**0.0324**
IM-ILD versus IPF	38.34	58.82	91.67	0.761	**0.0008**
SS-ILD versus IPF	34.75	73.53	77.78	0.747	**0.0037**
S-IPF versus AE-IPF	39.21	90.91	73.91	0.850	**0.0011**
non-F-CTD-ILD versus F-CTD-ILD	40.26	47.62	97.06	0.771	**0.0002**
CTD-UIP versus IPF	33.75	76.47	60.61	0.682	**0.0105**
CTD-non-UIP versus CTD-UIP	36.73	39.39	85.71	0.603	0.1047
RA-UIP versus IPF	38.33	58.82	76.19	0.681	**0.0254**
RA-non-UIP versus RA-UIP	27.83	76.19	73.33	0.737	**0.0168**

Statistically significant results are highlighted in bold

ROC—receiver operating characteristic, *AUC*—area under the curve, *RA*—rheumatoid arthritis, *SSc*—systemic sclerosis, *IM*—inflammatory myositis, *SS*—Sjogren syndrome, *F-CTD-ILD*—progressive pulmonary fibrosis in patients with CTD-ILD

conducive to offering additional evidence regarding the clinical relevance of LTBP2 in ILDs. Moreover, we had no data on plasma LTBP2 concentrations from the same patient under multiple clinical phases (before, during, and after PPF or AE). Such dynamic changes may explain why several patients showed relatively low plasma LTBP2 levels, even during the phase of AE-IPF and F-CTD-ILD.

Despite these limitations, this study demonstrates the possible value of LTBP2 as a discriminating diagnostic biomarker between IPF and CTD-ILD. Notably, our findings further suggest that LTBP2 may be suitable for describing acute-phase and ongoing fibrogenesis and may potentially predict the activity of fibrotic reactions in IPF and CTD-ILD. More importantly, plasma LTBP2 concentrations are closely correlated with the UIP pattern in RA-ILD. In this regard, clinical LTBP2 evaluation may be a great aid to identifying the existence of the UIP pattern in RA-ILD and to differentiating between IPF and CTD-UIP, particularly RA-UIP.

Acknowledgments Not applicable.

Author contributions ZC, WZ and CW designed this study. MZ, XH, WS, HG, CW, WZ, and ZC collected data and human blood samples. MZ, XH, WS and WZ performed experiments and analyzed the data. MZ and WZ wrote the original manuscript. MZ, XH and WS contributed equally to this study and shared first authorship. ZC, WZ and CW contributed equally to this study.

Funding This work was supported by funds from the National Natural Science Foundation of China (Grant No: 82070062) and the Climbing Project for Medical Talent of Zhongnan Hospital, Wuhan University (Grant No. PDJH202205).

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Ethical approval Human blood samples were obtained under the auspices of the Medical Ethics Committee of Zhongnan Hospital of Wuhan University-approved protocol.

Consent to participate Informed consent was obtained from all individual participants included in the study.

References

1. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit

Care Med. 2013;188(6):733–48. https://doi.org/10.1164/rccm.201308-1483ST.
2. Wong AW, Ryerson CJ, Guler SA. Progression of fibrosing interstitial lung disease. Respir Res. 2020;21(1):32. https://doi.org/10.1186/s12931-020-1296-3.
3. Ryerson CJ, Collard HR. Update on the diagnosis and classification of ILD. Curr Opin Pulm Med. 2013;19(5):453–9. https://doi.org/10.1097/MCP.0b013e328363f48d.
4. Yoo H, Hino T, Hwang J, et al. Connective tissue disease-related interstitial lung disease (CTD-ILD) and interstitial lung abnormality (ILA): evolving concept of CT findings, pathology and management. Eur J Radiol Open. 2022;9: 100419. https://doi.org/10.1016/j.ejro.2022.100419.
5. Matson S, Lee J, Eickelberg O. Two sides of the same coin? A review of the similarities and differences between idiopathic pulmonary fibrosis and rheumatoid arthritis-associated interstitial lung disease. Eur Respir J. 2021;57(5):2002533. https://doi.org/10.1183/13993003.02533-2020.
6. Vij R, Strek ME. Diagnosis and treatment of connective tissue disease-associated interstitial lung disease. Chest. 2013;143(3):814–24. https://doi.org/10.1378/chest.12-0741.
7. Cottin V. Lung biopsy in interstitial lung disease: balancing the risk of surgery and diagnostic uncertainty. Eur Respir J. 2016;48(5):1274–7. https://doi.org/10.1183/13993003.01633-2016.
8. Martinez FJ, Collard HR, Pardo A, et al. Idiopathic pulmonary fibrosis. Nat Rev Dis Primers. 2017;3:17074. https://doi.org/10.1038/nrdp.2017.74.
9. Robertson IB, Horiguchi M, Zilberberg L, Dabovic B, Hadjiolova K, Rifkin DB. Latent TGF-β-binding proteins. Matrix Biol. 2015;47:44–53. https://doi.org/10.1016/j.matbio.2015.05.005.
10. Gibson MA, Hatzinikolas G, Davis EC, Baker E, Sutherland GR, Mecham RP. Bovine latent transforming growth factor beta 1-binding protein 2: molecular cloning, identification of tissue isoforms, and immunolocalization to elastin-associated microfibrils. Mol Cell Biol. 1995;15(12):6932–42. https://doi.org/10.1128/MCB.15.12.6932.
11. Enomoto Y, Matsushima S, Shibata K, et al. LTBP2 is secreted from lung myofibroblasts and is a potential biomarker for idiopathic pulmonary fibrosis. Clin Sci (Lond). 2018;132(14):1565–80. https://doi.org/10.1042/CS20180435.
12. Zou M, Zou J, Hu X, Zheng W, Zhang M, Cheng Z. Latent transforming growth factor-β binding protein-2 regulates lung fibroblast-to-myofibroblast differentiation in pulmonary fibrosis via NF-KB signaling. Front Pharmacol. 2021;12:788714. https://doi.org/10.3389/fphar.2021.788714.
13. Khanna D, Mittoo S, Aggarwal R, et al. Connective tissue disease-associated interstitial lung diseases (CTD-ILD)-report from OMERACT CTD-ILD working group. J Rheumatol. 2015;42(11):2168–71. https://doi.org/10.3899/jrheum.141182.
14. Raghu G, Remy-Jardin M, Richeldi L, et al. Idiopathic pulmonary fibrosis (an update) and progressive pulmonary fibrosis in adults: an official ATS/ERS/JRS/ALAT clinical practice guideline. Am J Respir Crit Care Med. 2022;205(9):e18–47. https://doi.org/10.1164/rccm.202202-0399ST.
15. Spagnolo P, Distler O, Ryerson CJ, et al. Mechanisms of progressive fibrosis in connective tissue disease (CTD)-associated interstitial lung diseases (ILDs). Ann Rheum Dis. 2021;80(2):143–50. https://doi.org/10.1136/annrheumdis-2020-217230.
16. Rajan SK, Cottin V, Dhar R, et al. Progressive pulmonary fibrosis: an expert group consensus statement. Eur Respir J. 2023;61(3):2103187. https://doi.org/10.1183/13993003.03187-2021.
17. Juge PA, Crestani B, Dieudé P. Recent advances in rheumatoid arthritis-associated interstitial lung disease. Curr Opin Pulm Med. 2020;26(5):477–86. https://doi.org/10.1097/MCP.0000000000000710.
18. Spagnolo P, Lee JS, Sverzellati N, Rossi G, Cottin V. The lung in rheumatoid arthritis: focus on interstitial lung disease. Arthr Rheumatol. 2018;70(10):1544–54. https://doi.org/10.1002/art.40574.
19. Mei Q, Liu Z, Zuo H, Yang Z, Qu J. Idiopathic pulmonary fibrosis: an update on pathogenesis. Front Pharmacol. 2021;12:797292. https://doi.org/10.3389/fphar.2021.797292.
20. Pang XF, Lin X, Du JJ, Zeng DY. LTBP2 knockdown by siRNA reverses myocardial oxidative stress injury, fibrosis and remodelling during dilated cardiomyopathy. Acta Physiol (Oxf). 2020;228(3):e13377. https://doi.org/10.1111/apha.13377.
21. Sideek MA, Teia A, Kopecki Z, Cowin AJ, Gibson MA. Co-localization of LTBP-2 with FGF-2 in fibrotic human keloid and hypertrophic scar. J Mol Histol. 2016;47(1):35–45. https://doi.org/10.1007/s10735-015-9645-0.
22. Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012;366(21):1968–77. https://doi.org/10.1056/NEJMoa1113354.
23. Fischer A, Du Bois R. Interstitial lung disease in connective tissue disorders. Lancet. 2012;380(9842):689–98. https://doi.org/10.1016/S0140-6736(12)61079-4.
24. Miyazono K, Olofsson A, Colosetti P, Heldin CH. A role of the latent TGF-beta 1-binding protein in the assembly and secretion of TGF-beta 1. EMBO J. 1991;10(5):1091–101. https://doi.org/10.1002/j.1460-2075.1991.tb08049.x.
25. Collard HR, Ryerson CJ, Corte TJ, et al. Acute exacerbation of idiopathic pulmonary fibrosis: an international working group report. Am J Respir Crit Care Med. 2016;194(3):265–75. https://doi.org/10.1164/rccm.201604-0801CI.
26. Pache JC, Christakos PG, Gannon DE, Mitchell JJ, Low RB, Leslie KO. Myofibroblasts in diffuse alveolar damage of the lung. Mod Pathol. 1998;11(11):1064–70.
27. Raghu G, Remy-Jardin M, Myers JL, et al. Diagnosis of idiopathic pulmonary fibrosis: an official ATS/ERS/JRSALAT clinical practice guideline. Am J Respir Crit Care Med. 2018;198:44–68. https://doi.org/10.1164/rccm.201807-1255ST.
28. Samara KD, Trachalaki A, Tsitoura E, et al. Upregulation of citrullination pathway: from autoimmune to idiopathic lung fibrosis. Respir Res. 2017;18(1):218. https://doi.org/10.1186/s12931-017-0692-9.