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Research article

Transbronchial lung cryobiopsy and pulmonary fibrosis: A never-ending story?

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ABSTRACT

Background: The diagnostic process of pulmonary fibrosis (PF) is often challenging, requires a collaborative effort of several experts, and often requires bioptic material, which can be difficult to obtain, both in terms of quality and technique. The main procedures available to obtain such samples are transbronchial lung cryobiopsy (TBLC) and surgical lung biopsy (SLB).
Objective: The purpose of this paper is to review the evidence for the role of TBLC in the diagnostic-therapeutic process of PF.
Methods: A comprehensive review was performed to identify articles to date that addressed the role of TBLC in the diagnostic-therapeutic process of PF using the PubMed® database.
Results: The reasoned search identified 206 papers, including 21 manuscripts (three reviews, one systematic review, two guidelines, two prospective studies, three retrospective studies, one cross-sectional study, one original article, three editorials, three clinical trials, and two unclassifiable studies), which were included in the final review.
Conclusions: TBLC is gaining increasing efficacy and improving safety profile; however, there are currently no clear data demonstrating its superiority over SLB. Therefore, the two techniques should be considered with careful rationalization on a case-by-case basis. Further research is needed to further optimize and standardize the procedure and to thoroughly study the histological and molecular characteristics of PF.

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1. Introduction

1.1. Interstitial lung diseases and idiopathic pulmonary fibrosis

Interstitial lung diseases (ILDs) comprise a heterogeneous group of diseases in terms of aetiology, course, prognosis, and treatment, as well as specific clinical, functional, and radiological features [1,2]. A peculiar form of interstitial lung disease is pulmonary fibrosis (PF), which includes more than 200 different subtypes, but all share one characteristic: scarring of lung tissue. The most important categories of PF are:

1. Idiopathic pulmonary fibrosis (IPF) is the most common type of PF, with about 50,000 new cases diagnosed each year. The term "idiopathic" indicates that the ultimate cause is unknown. The male population is most frequently affected. Environmental factors, such as cigarette smoking and/or air pollution, appear to play a major role in the onset and progression of IPF (Fig. 1) [3–9]. Regarding genetic predisposition, some genes have been linked to IPF and a familial form has been described, although extremely rare, but our knowledge in this field is still limited. The typical onset of symptoms (e.g., exertional dyspnea, chronic nonproductive cough) is typically between 50 and 70 years of age [10–16];

Central panel, histology of an IPF lung showing the fibroblast foci bordering the cystic and dysfunctional areas (honeycomb cysts). Note that the vasculature is less represented because of extensive parenchymal remodeling.

Right panel, the low-magnification section of an IPF lung showing the abrupt transition between normal alveoli and pathological parenchyma, characteristic of the UIP.

2. ILDs secondary to other diseases, such as autoimmune diseases (e.g. rheumatoid arthritis, systemic sclerosis or Sjogren's syndrome), viral infections (e.g. COVID-19) and gastroesophageal reflux disease (GERD) [4–7];
3. ILDs secondary to environmental exposure to a wide range of chemicals or compounds, including naturally occurring (e.g. bird or animal droppings) and occupational (e.g. asbestos or silica). Furthermore, radiation treatments, and certain drugs (e.g. amphotericin B, bleomycin, methotrexate, nitrofurantoin) can be addressed as the cause of certain cases of ILD [15].

The diagnostic process for ILDs can be very challenging, requiring a thorough medical history, physical examination, lung function tests, high resolution computed tomography (HRCT) of the lung (Fig. 2), bronchoalveolar lavage and, if necessary, lung tissue examination [16–22].

Moreover, as stated in the most recent guideline, the role of multidisciplinary discussion (MDD) is recognized as crucial for the correct interpretation of different HRCT patterns of the chest, the decision of the optimal approach to obtain a bioptic specimen, and the reasoned interpretation of histological findings [1,2]. In the latest "European Respiratory Society guidelines on transbronchial lung cryobiopsy (TBLC) in the diagnosis of interstitial lung diseases," the task force suggests for patients with undiagnosed ILD and first uninformative TBLC, even after multidisciplinary discussion (MDD), the performance of a step-up surgical lung biopsy (SLB), if obtaining histopathological data is indicated (conditional recommendation, the certainty of evidence "very low") [1,2]. In contrast, in the case of uninformative TBLC, experts do not recommend performing a second TBLC [1,2]. TBLC has high diagnostic accuracy and an excellent safety profile [1,2]; however, it should be performed in centres with a high level of expertise [1,2]. In some studies, the diagnostic yield was greater than or equal to 70%, with a low adverse event rate (20%) and negligible mortality (0.1%) [1,2]. Furthermore, knowledge about the molecular characterization of IPF is advancing: a recent meta-analysis concluded that genomic classification tests, which are not widely available, predict histopathologic UIP in patients with ILD with high specificity (92%), improving diagnostic confidence, but low sensitivity (68%) [1,2,23–29]. Nevertheless, TBLC may be contraindicated in some patients because of its potential risk of major adverse events (pneumothorax and/or hemorrhage), which are significantly increased when performed on patients with certain conditions (e.g., severe emphysema, prevalence of lesions in the upper lobes, and pulmonary

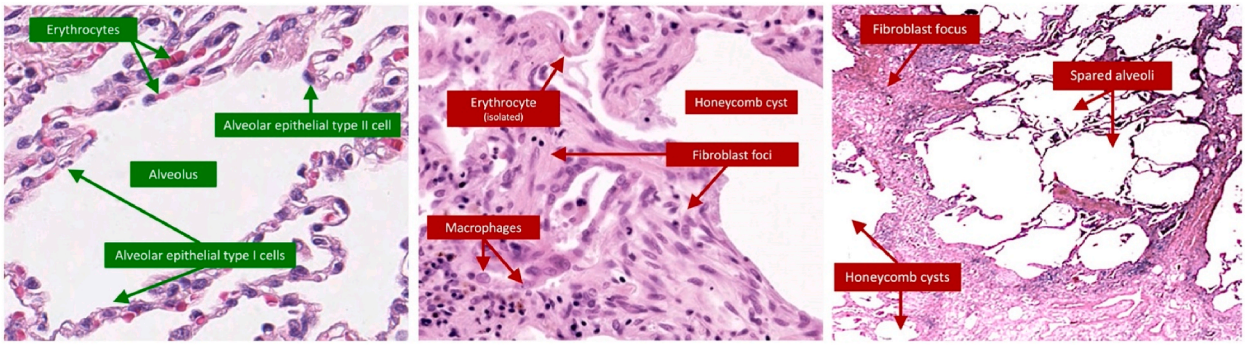

Fig. 1. Left panel, histology of a normal lung showing alveolar spaces separated by a single layer of epithelial cells (type I and type II). Multiple erythrocytes are visible in the lumen of the small vessels surrounding the alveolus.

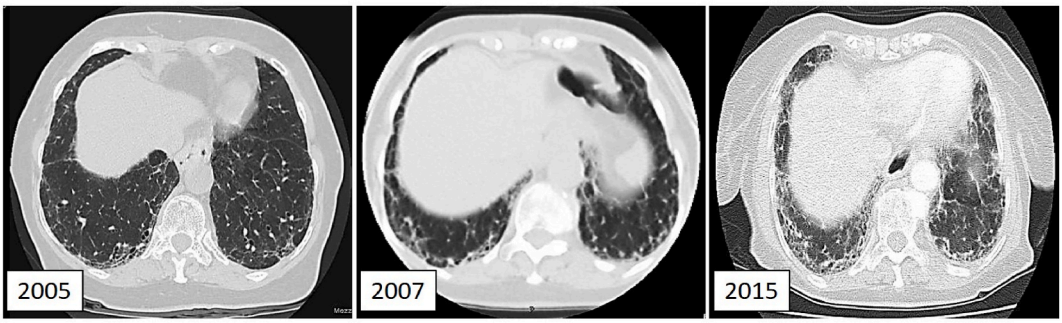

Fig. 2. CT scans of a 72-year-old female. Her brother died from IPF when aged 67 years. She was found to have a MUC5B mutation. The cryobiopsy made in 2015 found typical features of UIP-IPF (fibroblastic foci, initial honeycombing, bronchiolization). This case represent the early appearance of slowly progressive IPF in a never smoker patient with a familial mutation.

hypertension) [1,2,30–35]. SLB is burdened with significant costs and risks [1,2,36–41]. In addition, many subjects, given the stage of disease and/or advanced age and/or presence of comorbidities, are not eligible for a surgical procedure [1,2,42–49]. Several studies have reported the safety and feasibility of non-intubated video-assisted thoracic surgery (VATS) biopsy in patients with undetermined ILD [3,9–11]. Non-intubated VATS is an emerging technique with the goal of reducing postoperative complications. With the evolution of the VATS technique and anesthetic technology, the use of non-intubated VATS has become widely accepted in thoracic surgery. Non-intubated VATS is known to have fewer postoperative complications and faster recovery times than typical VATS because it does not use muscle relaxants, double-lumen tube intubation, and positive pressure ventilation injuries [9–11]. However, the incidence of postoperative pulmonary complications (PPC), including pneumonia, atelectasis, and acute respiratory distress syndrome, has been estimated to be up to 37.5% after thoracic surgery, increasing postoperative morbidity and mortality; moreover, the diagnostic yield is between 65 and 70% [7–9]. Despite the lack of established indications, recent literature offers evidence for the safety and efficacy of non-intubated VATS. However, further studies are needed to validate the short- and long-term results of this technique. The latest guidelines also emphasize the importance of multidisciplinary discussion (MDD) regarding the choice of biopsy technique and evaluation of the biopsy sample [2] (Fig. 3). Interestingly, two studies reported agreement between the diagnostic inter-ratings of TBLC and SLB ss [2,3,9–11]. The largest study demonstrated 70.8% agreement, which increased to 76.9% diagnostic agreement after MDD. In conclusion, a post hoc analysis suggested that the agreement between TBLC and SLB improves as more samples are taken, whereas

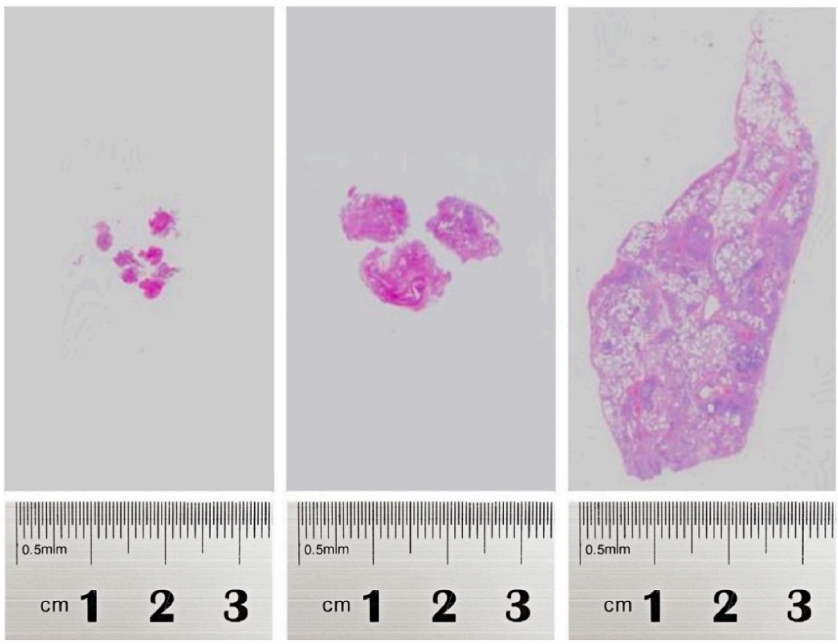

Fig. 3. Size comparison between transbronchial biopsies with standard forceps (left panel), lung cryobiopsies (middle panel) and surgical lung biopsies (right panel). Note that areas of advanced fibrosis with architectural distortion and fibrosis occur mainly at the periphery of the specimen with scattering of the centrilobular regions. The sharp demarcation between advanced fibrosis and normal-looking alveolar walls is also characteristic of the UIP pattern.

the smaller study reported only 38% diagnostic agreement [2,3,9–11].

1.2. Transbronchial lung cryobiopsy (TBLC)

Among the various ways to obtain a bioptic sample of the lung parenchyma, Transbronchial Lung Cryobiopsy (TBLC) has the peculiarity of using a cryoprobe (either 1,9 mm or 2,4 mm) to obtain lung tissue samples. This works due to the Joule-Thomson principle, which utilises compressed gas, which when released with a high-speed flow, expands rapidly, creating extremely low temperatures at the end of a probe, with consequent freezing (after 6–10 s) and adhesion of the tissue in contact with it [14–18]. The procedure is often aided by navigational guidance (e.g. r-EBUS – radial-Endobronchial Ultrasound –, fluoroscopy) [19]. The most common complications are bleeding and pneumothorax; however, their prevalence is still strongly debated by experts [20–22]. Likewise, its diagnostic yield, especially in comparison to SLB and Transbronchial Lung Biopsy (TLB), is still a matter of debate [23–25]. The common standardized procedure, that is widely accepted by most experts, requires that TBLC should be performed in intubated patients with deep sedation or general anaesthesia, using a prophylactic balloon blocker (Fogarty type) and under fluoroscopic guidance. Moreover, the whole procedure should be performed in an operating room with full anaesthesia support or in a dedicated bronchoscopy suite, and with emergency equipment immediately available. Finally, medications such as anticoagulants and antiplatelet agents should be withheld at the time of biopsy, where possible [23,26,57–59].

1.3. Safety profile and sampling procedure

Regarding safety, that is, procedural morbidity and mortality, there are data describing the different profiles of TBLC and SLB. Two observational studies [47,58] were able to compare these two techniques and obtained similar results: although the risk of severe bleeding was similar in both TBLC and SLB, the former, compared with the latter, seems to be safer in terms of mortality, acute exacerbation, and mean hospitalization time. Other studies, however, dealt with the safety profile of TBLC alone: four observational studies focused on the mortality rate, which was found to range from 0% to 4.1% with a median of 0.3%; seven observational studies focused on the pneumothorax rate, which occurs in a range from 1.4% to 20.2% of cases, with a median of 0.3%. 4% to 20.2% of cases, with a median of 9.5%; six observational studies focused on the rate of severe and moderate bleeding, which ranged from 0% to 6.3% (median of 1.1%) and 1.8% to 47%, respectively [1,6,8,47,58].

Regarding the sampling procedure, extrapolation of data from different histological studies conducted on SLB samples reasonably suggests that TBLC tissue samples should be taken from at least two different sites (different segments within the same lobe or different lobes) in order to increase the diagnostic yield of this procedure. In fact, ILDs are known to be characterized by histologic heterogeneity, so performing multiple cryobiopsies is a viable option to improve the diagnostic yield, at the cost of increasing the risk of pneumothorax (two sites: 24.6% VS. one site: 15.2%) [1,8,47,58]. This inference was confirmed by two observational studies, which emphasized the significant advantage of performing multiple sampling with TBLC (sampling two segments significantly increase the diagnostic yield to 92–96%). In addition, it is common practice to perform cryobiopsy by placing the cryoprobe tip 1 cm from the pleura in order to maximize the diagnostic yield, as the histological diagnosis of IPF requires a sample taken from the secondary lobule, while minimizing the risk of adverse events (e.g., bleeding and/or pneumothorax), as the pathologic changes in IPF are typically subpleural [1,6,8,47,58].

1.4. Molecular mechanisms involved in IPF

There is still controversy about the exact molecular mechanisms occurring in the pathophysiological process of IPF. However, it is a fact that the main trigger of this process is repetitive alveolar epithelial injury (e.g. cigarette smoking), which, combined with genetic predisposition (e.g. rs35705950 variant of the mucin 5b [MUC5B] gene promoter, mutations of genes involve in telomere maintenance or surfactant production) [27,28], leads to an abnormal reparative response that ultimately ends in fibrosis. A key role in this chain of events is played by the epithelial-to-mesenchymal transition (EMT) in alveolar epithelial type II (ATII) cells [29]. These cuboidal cells have the primary role of producing surfactant (a surface-active complex of phospholipids – such as dipalmitoylphosphatidylcholine – and proteins – named surfactant proteins C, B, A and D), but they are also involved in the regulation of innate immunity [30]. However, when lung parenchyma is damaged and fibrosis takes place, these cells are capable of losing the main characteristics of epithelial cells (contact adhesion and apical-basal polarity) while acquiring those of mesenchymal cells (invasion, migration and secretion of extracellular matrix components), thanks to many molecular mechanisms, such as Wnt, Sonic Hedgehog (SHH) and TGF- β pathways. This transition has been proved to be an important contributor to the early development of interstitial fibrosis, due to its role in recruiting the surrounding fibroblasts, which ultimately are activated into ECM-secreting myofibroblasts [4,29,31–33].

2. Materials and methods

2.1. Data source and search strategy

A reasoned search on the online database PubMed®, with the aim of detecting significant works that investigated the diagnostic and prognostic role of Transbronchial Lung Cryobiopsy in Idiopathic Pulmonary Fibrosis. The following keywords or synonyms were used to carry out the search: “IPF cryobiopsy”, “Idiopathic pulmonary fibrosis cryobiopsy”, “ILD Cryobiopsy”, without limitation of date of publication, up to the August 2, 2022.

Table 1
Electronic spreadsheet consisting of the following categories: author, title of the article, design, examined patients (number of patients and controls) and results.

Authors	Title of the article	Design	Examined patients	Results
Castillo D et al. (2020) [13]	A Multidisciplinary Proposal for a Diagnostic Algorithm in Idiopathic Pulmonary Fibrosis: The Role of Transbronchial Cryobiopsy	Systematic review		TBLC is useful for obtaining adequate lung tissue samples with minor adverse events, compared to SLB. This article also makes proposals for the use of TBLC in the diagnosis of IPF, listing pros and cons of both procedures.
Montufar F et al. (2018) [35]	Utilidad de la crio-tecnología para uso diagnóstico y terapéutico en neumología intervencionista: crio-biopsia pulmonar transbronquial y crioterapia [Transbronchial cryobiopsies and cryotherapy in lung diseases].	Review		TBLC should be the diagnostic method of choice in ILDs, since it has high diagnostic yield, low morbidity and mortality rate, low rate of complications and lower cost, but SLB remains the gold standard. It should be routine to evaluate a priori the feasibility of unilateral ventilation.
Raghu G et al. (2022) [2]	Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline.	Guideline		The updated guidelines for IPF suggest that TBLC be regarded as an acceptable alternative to SLB for making a histopathological diagnosis in patients with ILD of undetermined type in medical centres with experience performing and interpreting TBLC (conditional recommendation, very low quality evidence)
Behr J et al. (2021) [36]	S2K Guideline for Diagnosis of Idiopathic Pulmonary Fibrosis.	Guideline		When a histological diagnosis is required, TBLC should be preferred to SLB due to its non inferior diagnostic yield, similar rate of side effects, lower cost, higher patient's consent rate and less strict inclusion criteria. SLB should be reserved to patients who are fit and in whom a bronchoscopic diagnosis was inconclusive.
Han Q et al. (2021) [37]	The Application of Transbronchial Lung Cryobiopsy and Uniportal and Tubeless Video-Assisted Thoracic Surgery in the Multidisciplinary Diagnosis of Interstitial Lung disease-A Real-World Prospective Study.	Prospective study	137 patients without definite diagnosis, of whom 67 underwent UT-VATS and 70 TBLC.	The MDD considered UT-VATS more informative than TBLC in cases initially diagnosed as UIIP although they were equally valuable in patients initially diagnosed with IPAF/CTD-ILD. The two procedures showed a comparable safety profile, but TBLC was less expensive.
O'Mahony AM et al. (2021) [38]	Transbronchial lung cryobiopsy (TBLC) in the diagnosis of interstitial lung disease: experience of first 100 cases performed under conscious sedation with flexible bronchoscope.	Retrospective study	One hundred procedures on 85 patients, with a total of 272 TBLC.	TBLC may be an acceptable alternative to SLB with fewer complications. There appears to be a tendency towards improved diagnostic yield when sampling occurred in two lobes and when IPF was the diagnosis. Also, TBLC shows a potential role in distinguishing IPF from HP.
Ravaglia C et al. (2019) [25]	Diagnostic yield and risk/benefit analysis of *trans*-bronchial lung cryobiopsy in diffuse parenchymal lung diseases: a large cohort of 699 patients.	Retrospective study	699 patients with diffuse parenchymal lung diseases, specific pathological diagnosis in 614 cases, multidisciplinary diagnosis was obtained in 630 cases	TBLC seems to be associated with a higher diagnostic yield and a favorable risk/benefit ratio. It is noted that sampling at least two samples in different sites, using only 1.9 mm probe, intubating the patients and using bronchial blockers/catheters maximizes the diagnostic yield with a favorable risk/benefit ratio. Utility of TBLC in case of typical radiological UIP pattern is not clear.
Tomassetti S et al.	Bronchoscopic Lung Cryobiopsy Increases Diagnostic Confidence in	Cross-sectional study	117 patients with ILD, 58 underwent TBLC, 59 surgical lung biopsy	TBLC has a meaningful impact on diagnostic confidence in the

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Table 1 (*continued*)

Authors	Title of the article	Design	Examined patients	Results
(2016) [51]	the Multidisciplinary Diagnosis of Idiopathic Pulmonary Fibrosis			multidisciplinary diagnosis of ILD and may prove useful in the diagnosis of IPF.
Koslow M et al. (2020) [39]	Bronchoscopic Cryobiopsy and Forceps Biopsy for the Diagnostic Evaluation of Diffuse Parenchymal Lung Disease in Clinical Practice	Original article	The cohort included 120 cryobiopsy and 151 forceps biopsy cases	TBLC has higher diagnostic yield but also higher procedure-related complication than forceps biopsy. IPF may be a risk factor for complications.
Zaizen Y et al. (2019) [40]	Concordance between sequential transbronchial lung cryobiopsy and surgical lung biopsy in patients with diffuse interstitial lung disease		6 cases of UIP diagnosed with TBLC and SLB, one indeterminate UIP with TBLC and probable UIP with SLB	TBLC is useful for UIP diagnosis but not for other ILD. With a multidisciplinary approach, diagnosis of IPF may be determined by TBLC, whereas ILD other than IPF may require SLB.
Ronan N et al. (2018) [41]	Tissue and Bronchoalveolar Lavage Biomarkers in Idiopathic Pulmonary Fibrosis Patients on Pirfenidone		13 patients with IPF underwent TBLC before and after treatment with pirfenidone (6-months course)	TBLC offers a unique opportunity to potentially assess the course of disease activity and response to novel anti-fibrotic activity in IPF.
Poletti V et al. (2021) [42]	Time to Trust Transbronchial Cryobiopsy in Identification of Usual Interstitial Pneumonia Pattern?	Editorial		TBLC aids the diagnosis of IPF, but there are still questions about the criteria used to identify and grade the UIP pattern that need to be answered.
Maldonado F et al. (2019) [43]	POINT: Should Transbronchial Cryobiopsies Be Considered the Initial Biopsy of Choice in Patients With a Possible Interstitial Lung Disease?	Editorial		Given the importance of the histological diagnosis of ILDs, TBLC should be the first choice, provided that it is performed by expert personnel.
Yarmus L et al. (2019) [44]	COUNTER POINT: Should Transbronchial Cryobiopsies Be Considered the Initial Biopsy of Choice in Patients With a Possible Interstitial Lung Disease? No	Editorial		Given the increasing research on ILDs' pathogenesis, biomarkers, and therapy, lung tissue should be obtained with a conservative and proven approach, that is SLB.
Cooper WA et al. (2021) [45]	Cryobiopsy for Identification of Usual Interstitial Pneumonia and Other Interstitial Lung Disease Features. Further Lessons from COLDICE, a Prospective Multicenter Clinical Trial	Controlled clinical trial	65 patients	Subpleural and/or paraseptal fibrosis are not essential for diagnosing UIP in TBLC, provided that other guideline criteria features are present. The diagnostic accuracy of TBLC was strengthened when increased numbers of samples were taken.
Tomassetti S et al. (2020) [46]	Prognostic value of transbronchial lung cryobiopsy for the multidisciplinary diagnosis of idiopathic pulmonary fibrosis: a retrospective validation study	Single-centre, retrospective, investigator-initiated comparative study	500 consecutive cases, 426 of which were included: 266 had TBLC and 160 had SLB. 189 patients had idiopathic pulmonary fibrosis, 143 had other fibrotic interstitial lung diseases, and 94 had non-fibrotic interstitial lung diseases	TBLC makes an important diagnostic contribution in interstitial lung disease, on the basis of the prognostic distinction between idiopathic pulmonary fibrosis and other interstitial lung diseases when TBLC findings are included in multidisciplinary diagnosis.
Romagnoli M et al. (2019) [47]	Poor Concordance between Sequential Transbronchial Lung Cryobiopsy and Surgical Lung Biopsy in the Diagnosis of Diffuse Interstitial Lung Diseases	Two-center prospective study	21 patients	Pathological results from TBLC and SLB were poorly concordant in the assessment of ILD. SLBs were more frequently concordant with the final diagnosis retained at MDA.
Pajares V et al. (2020) [48]	MULTICRIO Group researchers. Transbronchial biopsy results according to diffuse interstitial lung disease classification. Cryobiopsy versus forceps: MULTICRIO study	Clinical trial	124 patients	Diagnostic yield for TBLC was higher than for TBLB, especially for two disease groups: IIPs and ILD of known cause or association. The increased risk of bleeding associated with TBLC confirms the need for safe airway management and prophylactic occlusion-balloon use.
Hetzel J et al. (2020) [23]	Transbronchial cryobiopsy increases diagnostic confidence in interstitial lung disease: a prospective multicentre trial	Prospective multicentre trial	128 patients	TBLC increases diagnostic confidence in the majority of ILD patients with an uncertain noninvasive diagnosis, with manageable side-effects. These data support the integration of TBLC into the diagnostic algorithm for ILD.

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Table 1 (*continued*)

Authors	Title of the article	Design	Examined patients	Results
Troy LK et al. (2020) [49]	Lung cryobiopsy and interstitial lung disease: What is its role in the era of multidisciplinary meetings and antifibrotics?	Review		TBLC has a diagnostic yield not as high as SLB, but the risk profile seems more acceptable and the accuracy appears to be good. There is increasing evidence for the utility of cryobiopsy in ILD diagnosis, particularly within the MDD.
Lynch DA et al. (2018) [50]	Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper.	Review		The role of transbronchial cryobiopsy in fibrotic ILDs remains unclear given the uneven experience around the world, the need for clearer standardization of the technique and the need to establish that the safety profile remains acceptable in less experienced hands. Surgical biopsy remains the gold standard for tissue diagnosis.

At first, two investigators (ST, BR) independently screened the title, abstract and full-text of the search records on the online database, focusing on those inherent the objective of the study. An article was considered eligible only when both the investigators agreed upon its inclusion in the study.

The criteria used to select the articles were:

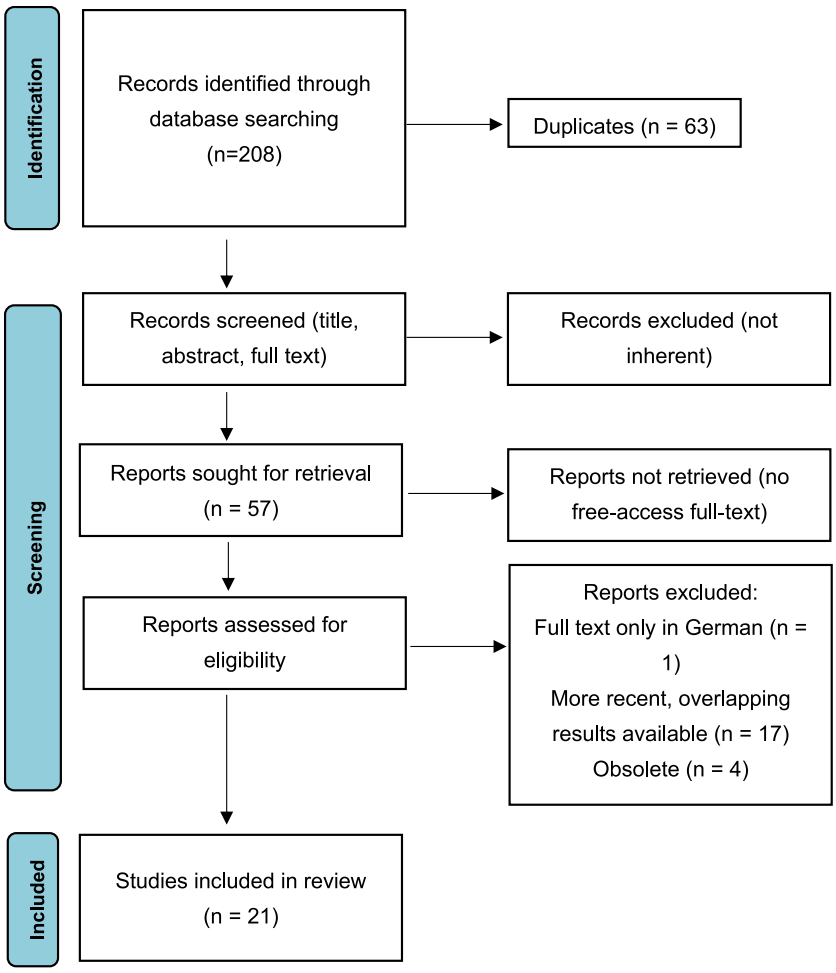

Fig. 4. Flowchart of reasoned review. Data are given as number of selected manuscripts categorized according to 4 different parts of the search process: identification, screening, eligibility and inclusion.

- high adherence to the research topic;
- recent and/or innovative and/or with alternative point of view.
- inclusion of all kinds of study designs;
- no distinction for language of the original text.

Then, in order to get the best selection of the records to include in the study, a third investigator (RP) screened the articles selected by ST and BR. Finally, the studies picked out after this process were analyzed by ST and BR in order to retrieve the most relevant data from each of them. These data were listed in an electronic spreadsheet designed for this study, consisting of the following categories: author, title of the article, design, examined patients (number of patients and controls) and results (see Table 1).

3. Results

3.1. Research and screening process

The reasoned search, conducted on MEDLINE (PubMed®), identified 206 records, which became 145 after removal of duplicates. After screening for title, abstract and full-text, 57 article underwent full-text review; however, 12 did not have a free access full-text, therefore 45 articles were assessed for eligibility were 45. After a final evaluation, 24 more articles were excluded, leaving 21 studies included in the review (see Fig. 4).

Table 1 contains the principal characteristics of the 21 studies included in this paper. Three of them were reviews, one a systematic review, two guidelines, two prospective studies, three retrospective studies, one cross-sectional study, one original article, three editorial, three clinical trials. Two studies had an unclassifiable design.

3.2. Quality appraisal

In the Supplementary File (available with the online version of this article) the methodological quality ratings are provided, according to the NIH. The average quality of the included articles was considered as “good”. No article was excluded solely based upon its quality.

3.3. Extrapolated data

3.3.1. Reviews

Montufar F et al. (2018) [35] clearly states that TBLC should be the diagnostic method of choice, when available, given its high diagnostic yield, low rate of adverse event and lower cost, compared to SLB, which remains however the gold standard. Also, as regards IPF in particular, it gives a practical advice for a safer technique, that is an evaluation a priori through blocking of the principal bronchus of the feasibility of unilateral ventilation in case of a severe adverse event (major bleeding and/or pneumothorax). The same response is given by Troy LK et al. (2020) [49], also underlining the utility of TBLC when applied in the context of MDD, helping clinicians to treat patients with antifibrotic drugs with a higher level of confidence. A different opinion is provided by Lynch DA et al. (2018) [50], remarking the superiority of SLB for tissue diagnosis in fibrotic ILDs, since TBLC still lacks standardization of the technique, and the safety profile remains to be determined in less expert hands.

3.3.2. Systematic reviews

The manuscript of Castillo D et al. (2020) [13] discussed the approach to decide between using SLB or TBLC to obtain a biopsy to confirm a UIP pattern, listing both the pros and cons of both the techniques, and recommending that the focus should not be to decide between two competing techniques in the diagnosis of patients with IPF, but to be able to recognize which is best suited to a particular situation.

3.3.3. Guidelines

The recently updated ERS guideline on IPF (Raghu et al., 2022) [2] make a conditional recommendation to consider TBLC an acceptable alternative to SLB for the histopathological diagnosis of ILDs of undetermined type, but only when performed in centres with enough experience. The results of Behr J et al. (2021) [36], expressed in the S2K guidelines for the diagnosis of IPF, express preference for TBLC over SLB due to its non-inferior diagnostic yield, similar rate of side effects, lower cost, higher patient’s consent rate and less strict inclusion criteria, leaving SLB only for fit patients with inconclusive bronchoscopic diagnosis.

3.3.4. Prospective studies

Han Q et al. (2021) [37] analyzed 137 patients without definite diagnosis, of whom 67 underwent UT-VATS and 70 TBLC. The authors concluded that Uniportal and Tubeless Video-Assisted Thoracic Surgery (UT-VATS) tends to be more informative than TBLC but only in cases initially diagnosed as UIIP (undefined idiopathic interstitial pneumonia), while the safety profile appears to be comparable and with the costs lower for TBLC. As regards the diagnostic yield, also the article of Romagnoli M et al. (2019) [47] on 21 patients states that SLB tends to be more effective than TBLC, having a more frequently concordant result with the final multi-disciplinary diagnosis.

3.3.5. Retrospective studies

Ravaglia C et al. (2019) [25], aimed to investigate the diagnostic yield and risk/benefit of TBLC, included in their study which included 699 patients with DPLDs. The analysis revealed the possible superiority of cryobiopsy over surgery, both for the diagnostic yield and risk/benefit ratio. Also, it made possible to make some technical recommendations to maximize both diagnostic yield and risk/benefit ratio, such as using only 1.9 mm probe, intubating the patients, and using bronchial blockers/catheters. However, it does not shed any light on the utility of TBLC in case of radiological definite UIP pattern. Tomassetti S et al. (2020) [46] as well analyzed a great number of cases (426), focusing of the prognostic value of cryobiopsy in the setting of multidisciplinary diagnosis of IPF, confirming its important contribution, given the prognostic distinction between IPF and other ILDs. Finally, O'Mahony et al. (2021) [38] studied 100 procedures on 85 patients, with a total of 272 cryobiopsies, performed under conscious sedation. In this case as well TBLC aids the diagnosis of ILD and can possibly be a valid alternative to SLB, when applied to the right patient. Its efficacy seems to improve even more when the sampling is done in two lobes and when the underlying diagnosis is idiopathic pulmonary fibrosis. Also, it shows a potential role in distinguishing IPF from HP (hypersensitivity pneumonia).

3.3.6. Cross-sectional study

In 2016, Tomassetti S et al. [51] performed an analysis on 117 patients with ILD, of whom 58 underwent TBLC and 59 surgical lung biopsy. It emerged that TBLC significantly aids the diagnostic confidence of MDD of ILDs and has the potential of specifically aiding the diagnosis of idiopathic pulmonary fibrosis.

3.3.7. Original article

The article of Koslow M et al. (2020) [39] compared TBLC and forceps biopsy used in the diagnostic process of DPLDs, stating the superiority of TBLC in terms of diagnostic yield but also its higher rate of procedure-related complications, with the diagnosis of IPF as a possible risk factor.

3.3.8. Editorials

The most recent editorial included in our study was by Poletti V et al. (2021) [42], which significantly contributes in supporting the role of TBLC in the diagnostic process of IPF but, at the same time, opens questions regarding the criteria used to identify and grade the usual interstitial pneumonia pattern. Two further editorials were also included. The first, by Maldonado F et al. (2019) [43], ultimately advocates for the use of cryobiopsy as the first step to obtain lung tissue to perform the histological diagnosis, but only in centres with trained interventional pneumologist. However, the second, by Yarmus L et al. (2019), disagrees and strongly advocates a more conservative and proven approach to the histological diagnosis of ILDs, due to the increasing need of lung specimens in order to carry on the growing research on the pathogenesis, biomarkers and therapy of ILDs [44].

3.3.9. Clinical trials

Research conducted by Pajares V et al. (2020) [48], which included 124 patients comparing transbronchial cryobiopsy and forceps lung biopsy, demonstrated the superiority, in terms of diagnostic yield, of TBLC (histopathological diagnosis 5 times more effective - 54.8% vs. 19.4% - and multidisciplinary diagnosis 4 times more effective - 47.6% vs. 19.4%). 4% - and multidisciplinary diagnosis 4 times more effective - 47.6% vs. 19.4%), especially for IIPs (Idiopathic Interstitial Pneumonias) and ILDs of known cause or association, while emphasizing its increased risk of bleeding, confirming the need for safe airway management and prophylactic use of occlusive balloons. Hetzel J et al. (2020) [23], on the other hand, worked on 128 patients with the aim of studying the rate of improvement in diagnostic confidence in interstitial lung disease using TBLC. The results show that this technique should be integrated into the diagnostic algorithm because it improves diagnostic confidence in most ILDs while ensuring manageable side effects. Finally, Cooper WA et al. (2021) [45] worked on 65 patients to evaluate the contribution of cryobiopsy in identifying UIP and other features of ILDs. It was found that regarding specimens obtained by transbronchial cryobiopsy, the presence of subpleural and/or paraseptal fibrosis is not essential to diagnose a UIP pattern, as the other features of the guideline-defined criteria are identifiable. In addition, the accuracy of diagnosis appears to be directly related to the number of samples taken. This evidence is the result of a follow-up study [45] of the previous COLDICE study [59]. Published in February 2020, this multicentered study addressed the concordance between TBLC and SLB in the diagnosis of ILD, through sequential TBLC and SLB performed on patients who needed a lung biopsy to confirm the diagnosis, previously assumed by MDD. The primary results had already given reason for the clinical utility of TBLC in the diagnostic pathway of ILDs, but without adequately addressing the safety profile.

3.3.10. Unclassified

The study of Ronan N et al. (2018) [41], in which 13 patients with IPF underwent TBLC before any treatment and after 6 months of treatment with pirfenidone, gives insight into the potential role of TBLC in assessing disease activity and response to anti-fibrotic therapy for idiopathic pulmonary fibrosis. As regards the research for the diagnostic process, Zaizen Y et al. (2019) [40] recruited 7 patient who underwent sequentially TBLC and SLB, 6 of whom got a histological diagnosis of UIP with both the techniques, while one cryobiopsy was indeterminate for UIP, but the surgical biopsy revealed a pattern UIP probable. These data suggest that TBLC is useful in case of an UIP pattern, making it possible to diagnose IPF, when put in the context of a multidisciplinary approach. On the contrary, it does not seem to be useful for the diagnosis of others ILDs, which may require SLB.

4. Discussion

There is a wide spectrum of ILD with significant heterogeneity, especially concerned determining the optimal approach to prognosis and treatment. Therefore, it is imperative to facilitate the diagnostic process, including through the availability of different modalities, which can be tailored on the patients' specific needs (e.g. general conditions, comorbidities, lung function). Such diagnostic tools need to be standardized, with clear inclusion/exclusion criteria, and well-studied risk profiles, while reducing the time delay for the final diagnosis.

The potential role of lung cryobiopsy in the diagnostic process of ILDs has been known for decades [52] and it has seen a growing interest amongst the scientific community, thanks to its low cost, high practicality and the physicians' steadily increasing experience, emerging as an effective alternative to other more conventional ways to obtain lung specimens (e.g., forceps biopsy, surgical lung biopsy). This fervour for TBLC is well expressed by the great number of scientific works crowding journals, databases, and the recent guidelines on TBLC in the diagnosis of interstitial lung diseases of the European Respiratory Society.

In reviewing the most significant and up-to-date literature on the role of TBLC in the diagnostic-therapeutic process of IPF, general opinion tends to be disposed toward incorporating this technique as a permanent and effective tool. Regarding lower-impact studies, such as reviews, systematic reviews, editorials, cross-sectional and retrospective studies, TBLC is generally considered a useful tool in the diagnostic process of IPF and a viable alternative to SLB. Several studies [25,35,43] support the use of TBLC as the first step in attempting to obtain a histological diagnosis. Some studies [25,35] also approach the field of risk profiling. Cryobiopsy carries an undeniable risk of adverse events, primarily pneumothorax and major bleeding, but also serious events such as acute exacerbation of IPF. Attempts to reduce the frequency and severity of such events have led to some common rules that, it must be said, still lack true standardization: maximize airway management, preferring intubation to spontaneous breathing; use of a 1.9-mm probe versus a 2.4-mm probe [25]; placement of prophylactic bronchus blockers to safely manage the eventuality of major bleeding [25,48]; a priori assessment of the feasibility of unilateral ventilation, in case of major bleeding and/or pneumothorax [35]. Other studies recognize the usefulness of TBLC, although they do not support its superiority over SLB [38,42,46,49,51].

In particular, this technique seems to be especially useful when implemented in MDD. This method requires the discussion of difficult to interpret cases by different experts (e.g., pulmonologists, radiologists, rheumatologists) to improve the possibility of correctly diagnosing ILD. This possibility is further improved by the availability of lung specimens for histological data, which can be acquired for this purpose with TBLC, which seems to provide a good diagnostic yield in this context, comparable to SLB, and an important aid in the prognostic stratification of various ILDs, especially IPF [40,46,49,51].

A more diplomatic response to this dispute is provided by the work of Castillo D et al. (2020) [13], who wisely approaches the issue differently, making sure that the goal of the study is not to didactically divide the two main bioptic techniques (e.g., best and worst), but rather to use the accumulated knowledge in this field to understand when one procedure is better than the other and vice versa. In particular, they highlight some useful factors to guide the choice: when there are central findings in the HRCT, the patients' lung function values are critically compromised and the bronchoscopy team is highly experienced, TBLC may be the first option; otherwise, when a higher diagnostic yield is required, lesions tend to be peripheral, lung function is fairly pre-served but the risk of pneumothorax and hemorrhage is high, SLB should be the first to be considered. Instead, two of the included articles came to an opposite result. The first [50], clearly states the superiority of SLB over TBLC, justifying this conclusion on the basis of an unclear safety profile and the lack of standardization of TBLC. However, this study, dated 2018, obviously did not consider subsequent research that further clarified the safety profile of TBLC [37], even though standardization has not yet been achieved. The second, a direct response to the manuscript by Maldonado F et al. (2019), argues in favor of SLB for a different reason, inherent to the scope of the research: given that the pathogenesis, molecular patterns, and therapeutic strategies of IPF have yet to be fully understood, SLB is the best way to obtain useful lung samples to pursue research on the above issues. Of course, this answer does not consider real-life clinical implications.

When larger impact studies such as prospective studies and clinical trials are considered, the conclusions are frankly not in favor of TBLC. In fact, two of the three prospective studies included in this article [37,47] state that SLB tends to be more informative about the final diagnosis, showing greater concordance with the multidisciplinary diagnosis. However, it is important to note that it has also been shown that the safety profile of TBLC is comparable to that of SLB, while the costs are lower [37]. Regarding clinical trials, those included in this paper did not compare TBLC and SLB. One [23] studied the potential of TBLC in increasing the diagnostic safety of ILDs, which was confirmed, giving further evidence of the effectiveness of this technique. Another [45] explored the histologic criteria needed to diagnose a UIP pattern in a lung specimen obtained by TBLC, revealing an important finding that subpleural and/or paraseptal fibrosis is not an essential criterion if the other criteria are present. This finding further validates the efficacy of cryobiopsy, because although it is not as effective as SLB in obtaining peripheral specimens, the biopsy could be as dirigens as that taken by surgery. The last one [48], on the other hand, focuses on the comparison of TBLC and forceps lung biopsy (TBB) and comes to the same conclusion as the original paper by Koslow M et al. (2020) [39]: the diagnostic yield of TBLC is higher, but so is its rate of adverse events (particularly bleeding), with IPF as a risk factor. Both papers clearly state that forceps lung biopsy is an obsolete procedure for the diagnosis of ILDs, although slightly safer.

Finally, it is worth mentioning the article by Ronan N et al. (2018) [41], as it succeeded in enrolling patients, even if only 13, who agreed to undergo TBLC both before starting antifibrotic treatment and after 6 months of pirfenidone, providing histological data of the effect of the said therapy on lung tissue.

Notably, an eminent guideline (S2K for IPF) [36], even openly preferred TBLC to SLB, addressing the usual arguments (similar diagnostic yield, lower rate and severity of adverse events, and lower cost), but also focusing on the patients' point of view, stating that they are usually more willing to undergo an endoscopic, rather than surgical, procedure, thus giving TBLC the added advantage of greater patient compliance.

In the latest "European Respiratory Society guidelines on transbronchial lung cryobiopsy (TBLC/TBC) in the diagnosis of interstitial lung disease," experts emphasize the role of TB as the first biopsy procedure. TB guidelines suggest that patients with undiagnosed ILD and an uninformative first TBLC perform a surgical lung biopsy (SLB), while researchers do not recommend performing a second TBLC [1]. In addition, the task force recommends discussing MDD before any type of procedure to plan the biopsy and afterwards to evaluate the sample to improve the accuracy of diagnosis. This question is even more important when it comes to the diagnosis of IPF, which is well known for its severe impact on the quality of life of affected patients, seriously impairing lung function, and disease extremely poor prognosis, with a median survival of 24–30 months, even worse than a diagnosis of non-small cell lung cancer [53]. The latest guidelines for IPF [2], published in May 2022, agree, with the guidelines for cryobiopsy [1], on many points such as MDD, imaging investigations, and regarding procedures to be performed to optimize the diagnostic-therapeutic management of patients with ILD. The guidelines on IPF provide a diagnostic algorithm for this disease, which first considers the radiological picture of high-resolution computed tomography (HRCT) of the chest [54,55]. The possible results are shown in Table 2.

According to the IPF guidelines [2], in the presence of a definite or probable UIP pattern, the diagnosis can be obtained by MDD; otherwise, in the case of an indeterminate radiologic pattern or one suggestive of an alternative diagnosis, a histologic diagnosis is required, according to the TBLC guidelines [1]. Further research is needed to improve knowledge about the usefulness and safety of TBLC, its role in the diagnostic algorithm of ILDs, and the impact of technical aspects of the procedure on diagnostic yield and safety.

5. Conclusions

The diagnosis of IPF still remains a significant challenge, and therefore there is a strong need for safe and practical approaches to obtain high quality lung tissue to improve the likelihood of making the correct diagnosis. This is essential to provide the affected patients a reliable prognosis – which, if confirmed, indelibly marks their life – and to offer the patients the chance of a therapeutic strategy. Also, as the research in the field of molecular characterization of IPF moves forward, it is likely that lung tissue will become even more important in the diagnostic and therapeutic process of this disease. Having said that, the best method to obtain such samples is yet to be determined and, perhaps, does not need to be. As a matter of fact, the evolution of medicine itself in something increasingly tailored on the patients' characteristics and needs automatically calls for tailored approaches to diagnosis and therapy. As regards to the debate SLB vs TBLC, the evidence-base is still conflicting. There is no perfect approach, and both TBLC and SLB have their pros and cons that need to be considered. However, it is indisputable that the growing diffusion of cryobiopsy and physicians' experience led to a progressive narrowing of the gap between the two techniques, TBLC is becoming more reliable and safer. This fact is clearly showed in the 2022 ERS guideline [2], in which, for the first time, TBLC made its way into the diagnostic process of IPF as a regular actor. Although, despite the choice of the right procedure for the single patient is crucial, it is overt that the expertise of the team (n° procedure/practitioner; n° procedures/year) and the technique's choice within the selected procedure are also pivotal. For instance, cryobiopsy can be carried out in extremely different ways, from rigid bronchoscopy under deep sedation or general anaesthesia [56] to flexible bronchoscopy under conscious sedation [38,57], which obviously bring along different risk profiles and highly differ in terms of feasibility. Admittedly, there is still large agenda for research, firstly to make cryobiopsy even safer, standardising the procedure and reducing both rate and severity of its main adverse events (pneumothorax, major bleeding, acute exacerbation), but also to clearly stratify patients eligible for TBLC rather than SLB and to make an even better use of the lung parenchyma specimens, expanding our knowledge of histology and molecular biology of IPF.

Institutional review board statement

Not applicable.

Table 2
HRCT features in IPF.

HRCT pattern	UIP pattern	Probable UIP pattern	Indeterminate for UIP	CT findings suggestive of an alternative diagnosis
Distribution	Often heterogeneous; occasionally diffuse; prevalently subpleural and basal; sometimes asymmetric	Prevalently subpleural and basal predominant; often heterogeneous	Diffuse; no subpleural predominance	CT findings suggestive for an alternative diagnosis (e.g. peribronchovascular with subpleural sparing, perilymphatic, upper or mid lung)
CT features	Honeycombing with or without traction bronchiectasis/ bronchiolectasis; irregular thickening of interlobular septa; usually superimposed with reticular pattern, mild ground-glass opacity; eventual pulmonary ossification.	Absence of honeycombing; reticular pattern with traction bronchiectasis/bronchiolectasis; eventual ground-glass opacities; absence of subpleural sparing	CT features of lung fibrosis that do not suggest any specific aetiology	Features suggestive for specific differential diagnosis (Non-Specific Interstitial Pneumonia, Sarcoidosis, Hypersensitivity Pneumonia, Connective Tissue Disease-ILD, smoke-related Interstitial Pneumonia, Lymphangioleiomyomatosis, Pulmonary Langerhans Cell Histiocytosis, Lymphocytic Interstitial Pneumonia, Desquamative Interstitial Pneumonia, drug toxicity, Organizing Pneumonia, asbestosis)

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Appendix A. Supplementary data

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