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Review

Bronchoalveolar lavage and lung biopsy in connective tissue diseases, to do or not to do?

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***Abstract*:** Bronchoalveolar lavage and lung biopsy (LBx) are helpful in patients with connective tissue diseases (CTD) and interstitial lung diseases (ILD) regardless of cause, including infectious, noninfectious, immunologic, or malignant. The decision whether to perform only bronchoalveolar lavage (BAL), and eventually a subsequent LBx in case of a nondiagnostic lavage, or one single bronchoscopy combining both sampling methods depends on the clinical suspicion, on patient's characteristics (e.g. increased biopsy risk) and preferences, and on the resources and biopsy techniques available locally (e.g. regular forceps *versus* cryobiopsy). In CTD-ILD, BAL has major clinical utility in excluding infections and in the diagnosis of specific patterns of acute lung damage (e.g. alveolar hemorrhage, diffuse alveolar damage, and organizing pneumonia). LBx is indicated to exclude neoplasm or diagnose lymphoproliferative lung disorders that in CTD patients are more common than in the general population. Defining BAL cellularity and characterizing the CTD-ILD histopathologic pattern by LBx can be helpful in the differential diagnosis of cases without established CTD [e.g. ILD preceding full-blown CTD, interstitial pneumonia with autoimmune features (IPAF)], but the prognostic and theragnostic role of those findings remains unclear. Few studies in the pretranscriptomics era have investigated the diagnostic and prognostic role of BAL and LBx in CTD-ILD, and it is reasonable to hypothesize that future studies conducted applying innovative techniques on BAL and LBx might open new and unexpected avenues in pathogenesis, diagnosis, and treatment approach to CTD-ILD. This is particularly desirable now that a new drug treatment era is emerging, in which we have more than one therapeutic choice (immunosuppressive agents, antifibrotic drugs, and biological agents). We hope that future research will pave the path toward precision medicine providing data for a more accurate ILD-CTD endotyping that will guide the physicians through targeted therapeutic choices, rather than to the approximative approach 'one drug fits them all'.

Keywords: autoimmune diseases, bronchoalveolar lavage, connective tissue diseases, interstitial lung diseases, lung biopsy

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Introduction

Connective tissue diseases (CTD) include a wide spectrum of systemic autoimmune diseases affecting different organs. The lungs can be affected either primarily or secondarily due to infections, drug toxicity, lymphoproliferative or neoplastic disorders that in patients with CTD are more frequent than in the immunocompetent population. The prevalence of interstitial lung disease (ILD) is around 15% and varies depending on the underlying CTD, as shown in Table 1.[1,2] Interstitial lung involvement in CTD can be severe and has major prognostic implications.

There are several clinical dilemmas that pulmonary physicians have to face when dealing with

Table 1. Prevalence of interstitial lung involvement in CTD.

Systemic sclerosis	up to 85%;
Rheumatoid arthritis	20–30%;
PM/DM	20–50%;
Sjogren's syndrome	up to 25%;
Systemic lupus erythematosus	2–8%;
Mixed connective tissue disease[a]	20–60%
IPAF[b]	100%

Source: Data are extracted from Antoniou *et al.*[1]
CTD, connective tissue diseases; DM, dermatomyositis; IPAF, interstitial pneumonia with autoimmune features; IR, incidence rate; PM, polymyositis.
[a]Mixed Connective Tissue Disease results from the overlap of features of the other CTD.
[b]IPAF is a research entity defined by the presence of interstitial lung disease and autoimmune features lacking definite criteria for CTD.

CTD-ILD, and both bronchoalveolar lavage (BAL) and lung biopsy (LBx) can provide useful insights. The three most difficult diagnostic challenges in patients presenting without prior history of CTD are represented by (1) ILD being the solely manifestation of CTD (e.g. lung-dominant antisynthetase syndrome), (2) ILD preceding the manifestation of CTD, and (3) ILD with autoimmune features, lacking specific criteria for CTD classification, recently identified as the distinct research category of IPAF (interstitial pneumonia with autoimmune features).[3]

Before attributing the diagnosis of ILD to the CTD itself, a careful differential diagnostic process and a review of the occupational, environmental, and ongoing medications are mandatory. The differential diagnosis between drug-related toxicity and CTD-ILD is challenging and relies on the temporal relationship between drug administration and ILD onset. BAL and LBx findings can be helpful but are not specific in this setting, and improvement after drug discontinuation will confirm the clinical suspect.

CTD patients experience immune dysregulation and are commonly treated with immunosuppressive drugs; therefore, infections represent another important differential and bronchoscopy with BAL and/or LBx represent the most useful diagnostic tools to detect lung infections. BAL and transbronchial biopsy are usually combined and performed during the same bronchoscopy, but BAL can be performed alone depending on the clinical suspicion (e.g. likelihood of infectious etiology) or the patient's underlying conditions (increased risks for LBx).

The need to perform BAL and LBx in CTD-ILD depends on the expected impact that the information will have on subsequent clinical decisions. The knowledge about how informative BAL and LBx relies on available published evidence and personal experience, and both can be biased: the first when the published evidence is dated, limited, or both and the second when one's personal experience is based on disappointing results of BAL and LBx that could be due to shortcomings of the local environment, rather than on inherent limits of the techniques itself.

Currently, the perceived value of BAL and LBx in the characterization of CTD-ILD is low, mainly related to the notion that in the majority of cases histopathology does not provide significant diagnostic or prognostic information. In addition, recent trials results are frequently interpreted as if antifibrotic therapy slows progressive ILD irrespective of underlying diagnosis, thus giving the wrong impression that making an accurate diagnosis is not any longer necessary in these disorders. The aim of this narrative review of the literature is to balance evidence in favor and against mini-invasive investigations in CTD-ILD with the objective to clarify what is the current and possible future role of BAL and LBx.

Table 2. Diagnostic BAL findings.

BAL finding	Diagnosis
Pneumocystis jirovecii, fungi, CMV transformed cells	Opportunistic infections
Milky effluent, PAS-positive noncellular corpuscles, amorphous debris, foamy macrophages	Alveolar proteinosis
Hemosiderin-laden macrophages, intracytoplasmic fragments of red blood cells in macrophages, free red blood cells	Alveolar hemorrhage syndrome
Malignant cells of solid tumors, lymphoma, leukemia	Malignant infiltrates
Dust particles in macrophages, quantifying asbestos bodies 'Oily' lipid vacuoles in macrophages	Dust exposure Lipoid pneumonia
Eosinophils > 25%	Eosinophilic lung disease
Positive lymphocyte transformation test to beryllium	Chronic beryllium disease
CD1-positive Langerhans cells increased	Langerhans cell histiocytosis
Atypical hyperplastic type II pneumocytes	Diffuse alveolar damage, drug toxicity

BAL, bronchoalveolar lavage; CMV, cytomegalovirus; PAS, Periodic acid-Schiff.

Methods

This review is based on previously published manuscripts that were identified through an MEDLINE search (1990 to 1 February 2021) of English-language literature. The literature search was limited to clinical journals with accessible full texts, and the key phrases used were 'bronchoalveolar lavage and connective tissue diseases' and 'lung biopsy and connective tissue diseases'. Pediatric cases were excluded. A total of 862 manuscripts were reviewed, but only a select number were chosen at the discretion of the authors. The literature search and the authors' clinical experiences were used to draft the manuscript and to give practical suggestions.

BAL in CTD-related ILD

BAL is useful in many ILD, including infectious, noninfectious, immunologic, or malignant disease,[4] but its utility in the assessment of disease activity has not been established. It remains unclear whether BAL could be better than other parameters to guide therapy and provide prognostic information; serial BAL to monitor disease course is not currently recommended.[4]

The utility of BAL to detect 'sub-clinical alveolitis' has been discredited and isolated lymphocytosis without overt lung involvement has not been shown to add value in predicting subsequent clinically significant disease. BAL can be useful in the diagnosis of specific CTD-ILD [e.g. lymphocytosis in high-resolution compute tomography (HRCT) typical for lymphocytic interstitial pneumonia (LIP) or organizing pneumonia (OP) can be sufficient to achieve a definite diagnosis] and to rule out complications, mainly infections and drug-related ILD. HRCT determines whether a CTD-ILD is fibrotic, but BAL may still be helpful in determining whether a disease is predominantly inflammatory or fibrotic. A very high lymphocyte count on BAL may well suggest a greater likelihood of reversible disease and drive management on this simple balance between reversible and irreversible disease, particularly in diseases in which information on specific ILD subgroups are scanty [e.g. systemic lupus erythematosus (SLE), Sjogren].

We will explore the utility of BAL in the differential diagnosis of CTD-ILD. BAL findings can be diagnostic *per se* or helpful to narrow the differential, as detailed in Tables 2 and 3.

The technique: technical and safety considerations

Through the instillation of saline in a specific lung segment, BAL harvests the secretions that coat

Table 3. BAL cellular patterns as an adjunct to diagnosis.

Lymphocytic
Extrinsic allergic alveolitis
Berylliosis
Sarcoidosis
Tuberculosis
NSIP (mainly cellular type)
LIP
Connective tissue disorders
Drug-induced pneumonitis
Malignant infiltrates
Silicosis
Crohn's disease
Primary biliary cirrhosis
HIV infection
Viral pneumonia
Neutrophilic (±eosinophilic)
Idiopathic pulmonary fibrosis
Desquamative interstitial pneumonia
Fibrotic NSIP
Acute interstitial pneumonia
Acute respiratory distress syndrome
Bacterial pneumonia
Connective tissue disorders
Asbestosis
Wegener's granulomatosis
Diffuse panbronchiolitis
Transplant bronchiolitis obliterans
Idiopathic bronchiolitis obliterans
Drug-induced reaction
Eosinophilic
Eosinophilic pneumonia
Churg-Strauss syndrome
Hypereosinophilic syndrome
Allergic bronchopulmonary aspergillosis
Desquamative interstitial pneumonia
Drug-induced reaction
Mixed cellularity
COP
Connective tissue disorders
NSIP
Drug-induced reaction
Inorganic dust disease

BAL, bronchoalveolar lavage; COP, cryptogenic organizing pneumonia; HIV, human immunodeficiency virus; LIP, lymphocytic interstitial pneumonia; NSIP, nonspecific interstitial pneumonia.

the surfaces of the bronchial and alveolar epithelium. The technique is extremely important for obtaining appropriate specimens because many technical factors can influence the amount and quality of fluid retrieved.

The BAL procedure is performed during standard flexible bronchoscopy, the tip of the bronchoscope is placed in a wedge position within the selected bronchial segment, and normal saline at room temperature is instilled and then retrieved. The total instilled volume needs to exceed 100 ml, and lesser volumes increase the proportion of bronchial cells and have shown a particularly low yield in extensive fibrotic diseases. Good sampling retrieves at least 30% of instilled volume. During suction, the negative pressure should be adjusted to avoid airway collapse. The total volume collected should be divided into three to five aliquots and the first 20–40 ml may be kept separated because it is more representative of the bronchial component and more suitable for microbiological evaluations than cellular analysis. BAL fluid should be either transported to the laboratory fresh at room temperature within 1 h after retrieval or transported on ice (4°C).

Safety of BAL is its major advantage compared with other more invasive techniques such as LBx. BAL is easily performed, well tolerated, and can be done in acutely ill patients [e.g. patients with

acute respiratory distress syndrome (ARDS) or intubated]. The most frequent sequelae are self-limiting fever and hypoxia, particularly following larger volume instillation. Serious adverse events are rare and usually observed in critically ill patients: persistent hypoxemia, hypotension, bradycardia, pneumothorax, bronchospasm, bleeding, and takotsubo.[5] BAL has rarely been reported to precipitate acute exacerbations of ILD, a very low prevalence has been reported in idiopathic pulmonary fibrosis (IPF) patients, and it remains unclear whether this applies to CTD-ILD.[6–8] The safety of BAL has been confirmed in a recent prospective cohort of 223 IPF patients who underwent bronchoscopy in the PROFILE study. All participants tolerated the procedure well, a cell differential could be obtained in all, and there were no immediate (<72 h) complications.[9] No absolute contraindications for BAL have been reported, but patients considered at particularly high risk of complications or BAL failure include respiratory failure ($PaO_2 < 60$ mmHg room air), severe asthma, chronic obstructive pulmonary disease (COPD) and emphysema (FEV1 < 60% pred or 1 L), coagulation disorders [platelet count (PLT) < 20.000], hemodynamic instability, acute myocardial infarction or life-threatening arrhythmias, severe renal insufficiency, and lack of compliance.[5,10]

BAL in CTD-ILD, indications and clinical utility

BAL has been used for decades in the evaluation of ILD, has a central role in excluding infections, is diagnostic in a number of rare disorders [e.g. diffuse alveolar hemorrhage (DAH), alveolar proteinosis], and has a central diagnostic role in the evaluation of CTD-ILD in the view of many experienced clinicians. A variety of studies may be performed on BAL fluid in patients with suspected CTD-ILD, and typical diagnostic studies include microbiological studies, cytopathology [to rule out neoplasm or to detect cells seen in specific conditions such as hyperplastic pneumocytes in diffuse alveolar damage (DAD), and virally infected cells], differential cell count, and cytofluorimetry to define the diagnosis and the disease activity.[11] Moreover, the acellular BAL component (supernatant) can be used for a number of investigations, including measurements of cytokines, chemokines, growth factors, antibodies, and immunoglobulins mainly for research purposes.[12,13]

The BAL utility in the evaluation of pulmonary infections in CTD-ILD. The classic scenario in which BAL is most useful is that of CTD-ILD with treatment that can cause drug-ILD and the concomitant risk of opportunistic infection. The management answer is either to increase immunosuppression or reduce it while treating infection. The answer can be very difficult to tease out, and BAL findings drive the pivotal management decision in the algorithm.

In the complex scenario of CTD-ILD (muscle weakness and esophageal dysmotility predisposing to aspiration and bronchopneumonia, immunosuppressive drug treatment predisposing to opportunistic infections), the exclusion of infections is one of the most common clinical indications for BAL. In CTD-ILD, Sun *et al.*[14] documented a BAL diagnostic yield for infection of 17.1%, with a sensitivity of 35.5% and a specificity of 97.4%. Positive predictive value is 91.7% and negative predictive value is 65.5%. The most common infectious agent isolated was *Aspergillus* sp. (four cases) and *Pneumocystis jirovecii* (three cases), mycobacterial infection was identified in one case, and the remaining were Gram-negative bacilli and Gram-positive cocci [*Klebsiella pneumoniae*, *Stenotrophomonas maltophilia*, *Corynebacterium*, one Methicillin-resistant *Staphylococcus aureus* (MRSA)]. Two factors increased the diagnostic yield: the presence of clinically relevant symptoms (symptomatic *versus* asymptomatic: 25.6% *versus* 3.7%, $p = 0.042$) and the presence of ground glass or consolidation (20.3% *versus* 0 with reticular pattern, $p < 0.001$). This study is limited by the absence of evaluation of several important pathogens, such as viruses.

COVID-19 may now be added to the list because it might mimic idiopathic or CTD ILD. BAL utility for the diagnosis of suspected COVID-19 is recognized in cases with negative nasal swabs, in which BAL can detect the virus in an additional third of patients (37.2%).[15] BAL might help to define an alternative diagnosis, among those CTD-ILD with acute onset (e.g. antisynthetase syndromes, lupus pneumonia, acute exacerbation of chronic CTD-ILD) that sometimes represent a challenging differential diagnosis with COVID-19 and can be useful in the rare scenario of not typical ILD with inconclusive testing. In selected patients with positive nasal swabs, BAL can be useful in detecting coexisting infections.

In conclusion, BAL is a safe technique for the diagnosis of pulmonary infections in CTD-ILD providing the clinician with accurate guidance for antibiotic or antiviral treatment. Possible limitations of this technique include the completeness of the available microbiology and virology panel and adequacy of the laboratory for testing strategies and adequate BAL specimen processing.[10,11]

In the diagnosis of ILD, BAL cellular analysis narrows the differential diagnosis with the identification or exclusion of a predominant inflammatory cellular pattern. This may support or exclude a specific type of ILD in the dynamic scenario of disease evolution.[16,17] The normal values of differential cytology in nonsmokers are macrophages $>80\%$, lymphocytes $<15\%$, neutrophils $<3\%$, eosinophils $<0.5\%$, and mast cells $<0.5\%$. Although no large, controlled trials have been conducted, several studies have shown that the prominence of specific immune cells in the BAL correlates with increased likelihood of certain types of ILD, as shown in Table 3. Pronounced eosinophilia is a feature of eosinophilic pneumonia and some drug reactions, and lymphocytosis suggests sarcoidosis, hypersensitivity pneumonitis (HP), cellular nonspecific interstitial pneumonia (NSIP), lymphocytic interstitial pneumonia (LIP), and some drug reactions. Bloody BAL fluid increasing in sequential aliquots is pathognomonic of DAH and milky appearance BAL characteristic of alveolar proteinosis. Macrophages with large 'oily' lipid vacuoles can be found in lipoid pneumonia. Pigmented macrophages are encountered in chronic hemorrhage, smokers, and in some occupational disease (e.g. coal workers).[11]

Neoplastic diseases can occasionally present with radiologic ILD appearance particularly in cases of lymphangitic carcinomatosis or lymphoproliferative lung disorders.[18] In some cases, areas of dense fibrosis on CT raise the problem of differential diagnosis with malignancy, which can be ruled out by BAL in combination with biopsy. Thus, BAL is instrumental for the diagnosis of adenocarcinoma with a sensitivity of 80% and lymphoma with a sensitivity of 50%.[19] Both CTD-ILD and drug-induced lung disorders may present with any type of patterns (lymphocytic, neutrophilic, eosinophilic, and mixed). Therefore, the differentiation between the two processes based on BAL alone is not possible though a pronounced eosinophilia and is rather suggestive of a drug reaction, in particular when lymphocytosis with foamy macrophages and plasma cells of HP are persent.[4]

When a clinical and serologic diagnosis of CTD-ILD is established, BAL is unlikely to change it.[20] No results have shown a superiority of BAL on HRCT and pulmonary function in the evaluation of disease severity, prognosis, and treatment response.

The role of BAL in Systemic Sclerosis (SSc) has been investigated in several studies, and fibrotic-related ILD neutrophilia resulted the most prevalent abnormality linked to greater pulmonary function impairment.[21,22] In earlier studies, BAL neutrophilia seemed related to SSc-ILD worse outcome, but Wells *et al.*,[21] in a large study, showed that after adjustment for disease severity, BAL neutrophilia had no independent prognostic significance. Bouros *et al.*[23] reported an increased mortality in SSc-NSIP with lower DLco and higher BAL eosinophils levels at baseline. Indeed, other trials showed no additional value of BAL compared with HRCT and pulmonary function tests in predicting disease progression or treatment response to cyclophosphamide.[22,24–26] When comparing IPF with SSc-ILD, after adjustment for disease severity, BAL neutrophilia did not differ between these two diseases, making difficult to find a link between BAL neutrophilia and diseases progression.[27] Thus, it appears that in SSc-ILD BAL neutrophilia is a marker of disease severity.[28] The study results are limited, and at present, there is insufficient evidence to support the routine use of BAL for SSc prognosis prediction or disease activity evaluation. Further larger collaborative trials, however, should address BAL usefulness in assessing SSc-ILD.

In rheumatoid arthritis (RA), the utility of BAL has been studied less extensively, but neutrophilia appears to be associated with more severe RA-ILD and with chronic fibrotic ILD pattern, more frequently the UIP pattern.[29] Lymphocytosis and eosinophilia are less frequent and do not seem to correlate with the presence of ILD or pulmonary function impairment.[30] In RA, Bronchiolitis obliterans is associated with an increase of neutrophils and lymphocytes in BAL. Among infections, *Aspergillus* can colonize cavitated rheumatoid nodules and tumor necrosis factor-α (TNFα) inhibitors increase the risk of infections, particularly mycobacterial infections. Methotrexate treatment is often used in RA and it can induce

acute/subacute lung toxicity with a BAL pattern characterized by striking lymphocytosis (>25% with low CD4/CD8 ratio).[12]

Despite the low prevalence of ILD in SLE (2–8% of cases), BAL can still be useful. The role of BAL in SLE is particularly relevant in the assessment of DAH and acute lupus pneumonitis that occurs in 1–4% of patients. Both are life-threatening conditions that should be promptly identified and differentiated from infections and aspiration pneumonia that in SLE are also significantly increased.

Polymyositis/dermatomyositis (PM/DM), antisynthetase syndrome and antiMDA-5 syndrome represent a large group of CTD-ILD that can have an acute onset of symptoms and that can be limited to the lungs (amyopathic variants). Patients with acute onset tend to progress rapidly to respiratory failure (due to a combination of aggressive organizing pneumonia and lung injury) and in selected cases BAL, in selected cases combined with LBx, may provide useful diagnostic information. Neutrophilia or lymphocytosis (with increased CD8+ cells) is found in the majority of cases; eosinophilia has also been reported.[12] Because of muscle weakness, these patients are prone to hypoventilation (22% of cases) and aspiration, thus are at increased risk for aspiration pneumonia (17% of cases) and pulmonary infections. Malignancy is another association (7% of cases)[31] and BAL may help in excluding malignancies and infections.

In Sjogren's syndrome (SS) lung fibrosis is uncommonly encountered, and BAL usually reveals a lymphocytosis (prevalence of CD8+ cells), whereas neutrophilia is uncommon. Lymphocytosis is usually an expression of follicular bronchiolitis, cellular NSIP, or OP, but given the high prevalence of lymphomas in this disease, bronchoscopy with BAL and LBx are recommended in suspicious cases.[32] In SS, neutrophilic alveolitis with an increase of CD8+ T lymphocytes is associated with lung function impairment.[33]

In summary, several BAL cytology profiles have been described in CTD-ILD with lymphocytosis also being observed in cases without lung involvement[34] and more frequently in cases with acute and subacute onset, correlating with cellular NSIP and OP patterns. Neutrophilia (usually mild to moderate) is present in more advanced chronic and fibrotic disease, whereas the DAD pattern in acute exacerbation of the underlying CTD-ILD is associated with marked neutrophilia and activated hyperplastic pneumocytes.[35] A recent study suggests that BALF lymphocyte and neutrophil count ⩾25% and <20%, respectively, predicted favorable survival after acute exacerbation.[36]

The therapeutic and prognostic value of BAL in CTD-ILD needs to be further elucidated in future studies. At present, BAL remains a valuable clinical tool mainly to exclude infections, detect acute exacerbations, confirm/differentiate HRCT features (e.g. to confirm OP when the typical HRCT appearance is present accompanied by a significant BAL lymphocytosis or distinguish DAH from alveolar proteinosis), and exclude rarer conditions such as malignancies and lymphoproliferative processes. Combination with transbronchial biopsy at the time of BAL may be useful to in the identification of an underlying histologic pattern and is recommended when there is a suspicion of malignancy.

Biomarkers in BAL and future directions

Many attempts have been made to find in BAL fluid valuable biomarkers for CTD-ILD. The discovery of a biomarker or a combination of biomarkers for the diagnosis, prognosis, treatment response and ILD endotyping would be of great clinical relevance. To date, large prospective studies are lacking and the current evidence comes from retrospective, single-center studies.

Autoantibodies play a crucial role in CTD-ILD as their presence is related to the pathogenesis of the lung tissue damage; nevertheless, their presence in BAL has been poorly investigated. A recent study by Salvador-Corres *et al.*[37] evaluated anti-ENAs in 155 BAL of patients with suspected ILD and found positive autoantibodies in 19 of them, including anti Ro52, anti-Ro60, CENP-B, anti La, Jo-1, Sm-RNP, anti SL70. Fourteen were diagnosed with CTD-ILD, three with IPAF, one with NSIP, and one with silicosis. In 90% of patients, the same autoantibodies were also detected in the serum. RA patients with ILD have significantly elevated levels of IgG RF in both serum and BAL, but the association with progression of RA-ILD remains to be elucidated.[38] The presence of anti-citrullinated antibodies has been detected in one study and may suggest that the lung can be the antigenic source of anti-CCP antibodies production with both healthy smokers

and RA-smokers with pulmonary involvement showing positive anti-citrullinated BAL cells (13.7% and 28.5% respectively).[39] Takeshita *et al.*[40] have recently shown that BAL of patients with RA, SS, and mixed CTD contains higher titers of disease-related autoantibodies. The authors produced monoclonal antibodies from BAL antibodies-secreting cells reverting somatic hypermutation into germline and found disease-specific antibodies selected against various modifications in lungs with some antibodies recognizing multiple targets indicating that epitope spreading may progress in the lung. These findings unveil the existence of active autoimmune process in the lungs of CTD-ILD. These preliminary findings confirm that autoantibody detection in BAL is feasible and holds great research utility.

Other biomarkers that are increased in BAL of CTD-ILD include KL-6,[41] CCL18 (in SSc),[42] CCL2 (in SSc),[43] matrix metalloprotease (MMP-9) (in SSc),[44] interleukin 6 (IL-6) (elevated in SSc; elevated in SLE but without correlation to the presence of ILD), endothelin-1 (in SSc), interferon-γ (INF-γ), and transforming growth factor-β (TGF-β) (lower in RA-UIP than in other subgroups).[45] In Table 4, several serum biomarkers and their clinical relevance are shown. The available studies are mainly of serum and a clear correlation with BAL and histopathology is lacking. Many questions about the true pathogenic role of those markers remain unanswered. A future direction for clinical and basic research would be to integrate serum biomarkers with BAL and histology findings, possibly applying innovative technologies such as transcriptomics and GWAS that can more accurately detect large numbers of pathogenetic pathways and genetic defects exploring cell derangement and microenvironment interaction.

LBx in CTD

Comparison between LBx techniques: safety and diagnostic yield considerations

The three techniques currently available to obtain lung tissue are transbronchial biopsy (TBBx), transbronchial lung cryobiopsy (TBLC), and surgical lung biopsy (SLB). SLB obtains the largest samples (optimal samples measures ~4 cm) *via* video-assisted thoracoscopic surgery and is the technique with the highest diagnostic yield (greater than 90%).[75,76] TBBx uses conventional forceps yielding small biopsies (around 3 mm) with significant crush artifacts and a low diagnostic yield in diffuse fibrotic ILD (at best 30%); when IPF is suspected, the specificity for UIP pattern is high, but is poor for other patterns.[77] Recently, TBLC has been shown to be a promising and less invasive alternative to SLB to diagnose ILD with a diagnostic yield significantly higher compared with TBBx and close to that of SLB (approximately 80%).[78–80] Mortality of SLB for the elective diagnosis of ILD has been shown in two large studies to be ~1.7%,[81,82] and for TBLC ranges from 0.1% to 0.5%.[78,79,83–85] TBLC has been shown to be a safer alternative to SLB and has been widely adopted for the diagnosis of ILD.[78,86] The most common complication of LBx procedures are pneumothorax and bleeding (severe bleeding pooled estimate of 0.3%, 95% CI = 0.1–0.7%, I = 0; moderate bleeding pooled estimate of 8.7%, 95% CI = 2.2–15.2%; I = 86.7%), chest pain is a common complication of VATS that can be avoided with TBLC, and both hospitalization lengths and costs of TBLC are significantly lower compared with SLB[84,87]

Among large observational studies that compared the diagnostic yield between SLB and TBLC, the robustly designed prospective study by Troy *et al.*[88] documented a good concordance between TBLC and SLB for both histologic pattern [weighted kappa = 0.78, 95% confidence interval (CI) = 0.55–0.86] and multidisciplinary diagnosis (weighted kappa = 0.62, 95% CI = 0.47–0.78). A recent study by Hetzel *et al.* confirmed our preliminary results showing in a large multicenter trial that TBLC provides information that significantly increase diagnostic confidence in the multidisciplinary diagnosis of ILD (high confidence diagnosis increased from 60% to 81.2%).[16,17] The prognostic prediction of multidisciplinary diagnosis of IPF compared with other ILD with better prognosis has been shown to be robust and comparable with that of SLB in a recent large retrospective cohort study.[89]

In conclusion, TBLC in comparison with SLB appears safer and useful to provide meaningful information in the context of the multidisciplinary discussion of cases. These conclusions are supported by data largely collected in patient cohorts enriched with idiopathic pulmonary fibrosis, other idiopathic ILD, or ILD other than CTD-ILD. Cases of CTD-ILD were variably included in TBLC studies, with appreciable small

Table 4. Serum biomarkers potentially useful in the diagnosis and prognosis prediction of CTD-ILD.

Disease	Biomarker	Diagnostic correlations (accuracy for ILD detection)	Correlation with disease severity at baseline	Prognostic correlations	Ref.
SSc	CXCL4	SE 100%, SPEC 94%	Lung fibrosis and pulmonary hypertension	Risk of progression	van Bon *et al.*[46]
	CC16	SE 52%, SPEC 89%	FVC and DLco	Disease activity	Hasegawa *et al.*[47]
	CCL18	NA	TLC, FVC, DLco, and HRCT	ILD progression and disease activity	Kodera *et al.*,[48] Prasse *et al.*,[42] Tiev *et al.*,[49] Elhaj *et al.*,[50]
	CCL2	SE 75%, SPEC 17%	TLC, FVC, DLco, and HRCT	ILD progression	Schmidt *et al.*,[43] Carulli *et al.*,[51] Hasegawa *et al.*[52]
	KL-6	SE 79–85%, SPEC 85–90%	FVC, DLco, and HRCT	Outcome	Yamane *et al.*,[53] Yanaba *et al.*,[54] Yanaba *et al.*,[55] Kodera *et al.*,[48] Hant *et al.*,[56] Bonella *et al.*[57]
	MMPs/ TIMPs	TIMP-1: SE 73%, SPEC 100%	MMP-7: DLco MMP-9: DLco, TLC MMP-12: FVC, HRCT ADAM12: FVC, HRCT TIMP-1: FVC, DLco	MMP-1, 8, 9: acute onset MMP-9, 12 and ADAM12-S and TIMP-1: ILD activity	Kim *et al.*,[58] Andersen *et al.*,[44] Moinzadeh *et al.*,[59] Manetti *et al.*[60] Oka *et al.*[61] Taniguchi *et al.*[62]
	Surfactant Proteins	SP-A: SE 33%, SPEC 100% SP-D: SE 68–89%, SPEC 73–89%	SP-D: FVC, DLco HRCT ggo	SP-D: ILD activity and treatment response	Takahashi *et al.*,[63] Asano *et al.*,[64] Kodera *et al.*,[48] Hant *et al.*,[56] Bonella *et al.*[57]
	YKL-40	SE 41%, SPEC 79%	FEV1 and DLco	Poor outcome	Nordenbaek *et al.*[65]
PM/DM	KL-6	SE 83–100%, SPEC 67–100%	FVC TLC FEV1 DLCO, HRCT	Disease progression	Bandoh *et al.*,[66] Kubo *et al.*,[67] Fathi *et al.*,[68] Arai *et al.*[69]
	SP-D	SB 73%, SPEC 93%	FVC, DLco	Poor outcome	Ihn *et al.*,[70] Arai *et al.*[69]
RA	KL-6	90% SPEC 97%	HRCT	FVC decline, ILD activity	Oyama *et al.*,[71] Kinoshita *et al.*[72]
	MMP-7, PARC, SP-D	[a]SE 0.87, SPEC 0.92	MMP-7 and SP-D	NA	Doyle *et al.*[73]

Source: Adapted from Bonella and Costabel.[74]
CTD, connective tissue diseases; CXC, chemokine; CXCL, chemokine ligand; DLCo, diffusing capacity carbon monoxide; FEV1, forced expiratory volume 1st second; FVC, forced vital capacity; ggo, ground glass opacity; HRCT, high-resolution computed tomography; ILD, interstitial lung diseases; KL, Krebs von den Lungen; MMP, matrix metalloproteases; PARC, pulmonary and activation-regulated chemokine; PM/DM, polymyositis/dermatomyositis; RA, rheumatoid arthritis; SE, sensitivity; SPEC, specificity; SP, surfactant protein; TIMP, metallopeptidase inhibitor; TLC, total lung capacity; YKL, chitinase-like-protein.
[a]Accuracy for ILD detection of the risk score = 0.38 × age − 6.4 × sex − 2.3 × ever-smoker − 0.0005 × RF + 0.0026 × CCP + 0.65 × MMP-7 + 0.15 × SP-D + 0.024 × PARC.

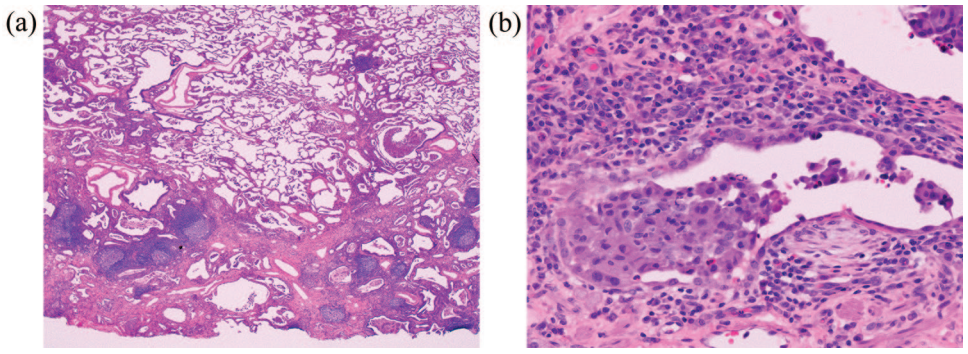

Figure 1. UIP in rheumatoid arthritis. The basic fibrotic pattern is UIP identical to that scene in IPF (patchy scarring and microscopic honeycombing) but pathologic clues that this is in RD are the lymphoid hyperplasia with germinal centers seen at low power (a, lower left) and the relative prominence of inflammation in general and plasma cells in particular (b, top left; note the fibroblast focus, lower right).

numbers in most of the studies not allowing separate analysis of data or any robust conclusion on the utility of TBLC in this setting.

The clinical utility of LBx in the management of CTD-ILD

Lung biopsies in CTD can show a variety of patterns, including a number of patterns of ILD. In general, the patterns have been labeled according to their counterparts among the idiopathic interstitial pneumonias (IIPs). While CTD-ILD show significant similarity to their IIP, there may be some distinctive differentiating features. CTD-ILD tend to show more prominent lymphoid hyperplasia and plasma cells and sometimes a paucity of fibroblast foci in the UIP pattern. Nevertheless, there is considerable overlap and there are often no histologic clues to CTD-ILD. Examples of distinctive features that may be encountered in CTD-ILD are shown in Figures 1–3.

Experts' opinion on the utility of LBx for the assessment of CTD-ILD diagnosis and diseases activity is heterogeneous, with some experts advocating the possible prognostic utility of differentiating UIP form other non-UIP patterns and other experts being more cautionary.[1,90] The decision whether to do a biopsy or not in CTD-ILD patients is currently tailored on patient's characteristics, multidisciplinary considerations, and local resources. In this review, we will address whether LBx information can be useful for the management of patients with CTD-ILD exploring the following: (1) current evidence suggesting that specific histologic patterns in CTD-ILD carry prognostic significance and (2) evidence supporting a different patients' treatment based on histologic patterns. Those evidence needs to be balanced against the risks and costs of invasive procedures that we have discussed above.

Preliminary findings in the 1990s suggested that the better prognosis of CTD-ILD (SSc, SS, and dermatomyositis) could be primarily linked to the high prevalence of NSIP compared with UIP.[91,92] Subsequent studies, however, showed appreciable differences between idiopathic and autoimmune ILD within the same histologic patterns (i.e. different appearance of myofibroblast, lower profusion of fibroblastic foci) and demonstrated also that the better prognosis of CTD-ILD compared with the idiopathic group was not linked solely to the presence of an NSIP pattern. In fact, CTD-UIP overall had a better prognosis compared with IPF.[92–94] Among those studies, the one with the largest UIP population was that published by Park *et al.*[94] in 2007 (203 IPF patients: 36 UIP-CTD, 66 idiopathic fibrotic NSIP, and 57 CTD-NSIP). Patients with UIP-CTD survived longer than IPF (mean 125.5 months compared with 66.9 months, $p = 0.001$), with CTD being an independent factor of better survival in UIP patients by multivariate Cox analysis, along with age and more preserved pulmonary function. On the contrary, no meaningful survival difference was observed neither between idiopathic and CTD ILD for NSIP (3 years survival rates of 77.6% and 88.9%, respectively; $p = 0.2$) nor between CTD-UIP and CTD-NSIP. A notable exception was represented by RA-UIP that showed a prognosis more similar to IPF. Survival of scleroderma was significantly better compared with IPF, but the prevalence of scleroderma-UIP is not reported, presumably too low to make a subanalysis. Among the deceased for CTD-ILD

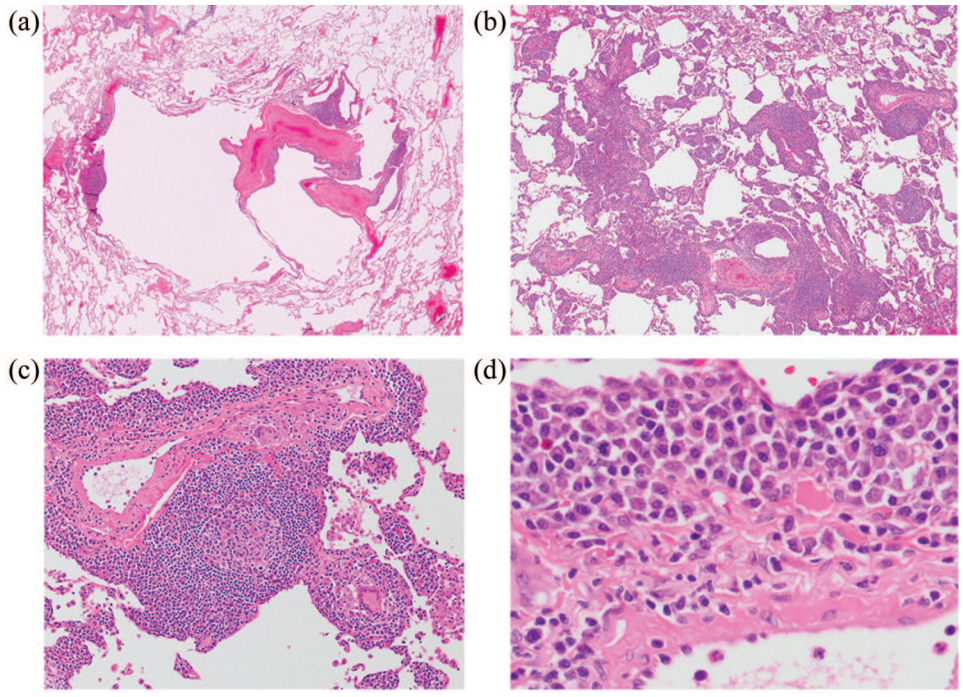

Figure 2. Sjogren's syndrome. (a) Sjogren's syndrome with the radiologic pattern of LIP (including radiologic cystic change) may show cystic change on biopsy. A small amount associated lymphoid hyperplasia is seen with the cyst but more prominent lymphoid tissue is seen in other fields, some along (b) bronchioles, including (c) germinal centers and (d) prominent plasma cells.

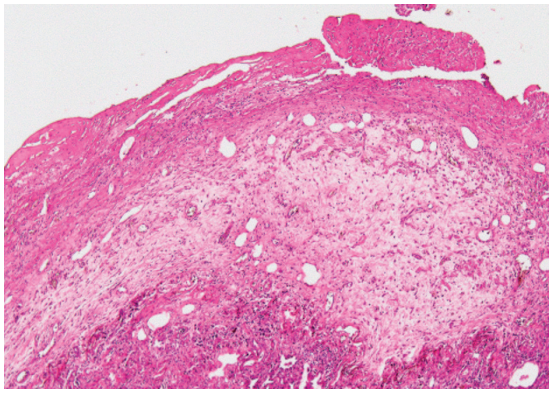

Figure 3. Rheumatoid pleuritis. While uncommon, the presence of active fibrinous pleuritis in the setting of interstitial lung disease that may be a clue in favor of CTD.

(eight CTD-ILD and 12 CTD-NSIP), more UIP patients compared with NSIP died of ILD progression (2/8, 25% compared with 1/12, 8.3%) or acute exacerbation (3/8, 37.5% compared with 1/12, 8.3%).

Other studies have focused on histopathologic subsets of SSc patients and the results of those studies, SSc patients (approximately 70% had limited cutaneous SSc in all subgroups) into NSIP ($N = 62$, of whom 47 were fibrotic) and UIP/end-stage lung (ESL) (total $N = 12$: UIP, $N = 6$/ESL, $N = 6$), found that the outcome was linked more strongly to disease severity at presentation and serial DLco trends rather than to histopathologic findings (5-year survival for NSIP was 90% and for UIP/ESL was 82%, $p = 0.33$). Even the survival between cellular and fibrotic NSIP did not differ. The authors caution against overinterpretation of their results that were limited by the small number of UIP, mixed with ESL patients, and by the mortality from lung cancer and severe systemic disease that considering overall mortality may have obscured the prognostic distinction between UIP and NSIP. The prognostic distinction between CTD-UIP and CTD-NSIP was found relevant in a study conducted by Fischer at al.[95] that on 22 scleroderma patients (all Scl-70 negative), 14 NSIP (13 fibrotic) and eight UIP, found a trend toward a significant survival difference, 15.3 years compared with 3 years, respectively, $p = 0.07$, but on multivariate Cox analysis, histopathologic subtypes failed to demonstrate any significant prognostic differences. The small populations and the

Table 5. Histopathologic features affecting the lung in CTD.

Disease	Lung histopathology patterns	Main differentials	Ref.
Rheumatoid arthritis (RA)	Follicular and constrictive bronchiolitis	Infections and smoking-related bronchiolitis	Spagnolo *et al.*[96] Lee *et al.*[105]
	Bronchiolocentric granulomatosis	Differential diagnosis with mycobacterial and fungal infections	
	Alveolar hemorrhage	Isolated pulmonary capillaritis, vasculitis	
	Rheumatoid nodules	Lung cancer and fungal and mycobacterial infections	
	ILD: UIP 56%, NSIP 33%, OP 11%, LIP, DIP, and diffuse lymphoid hyperplasia	Each of these entities should be differentiated from the idiopathic form. NSIP and OP can be drug related or related to infections, including COVID-19. Lymphoid hyperplasia with germinal centers, profusion of plasma cells, and presence of pleuritis favors RA-ILD.	
Systemic sclerosis (SSc)	Pulmonary hypertension in 40% of patients: pulmonary arteriopathy, with medial hypertrophy and concentric laminar intimal thickening. Plexiform lesions can be seen and occasionally occlusive venopathy (pulmonary veno-occlusive disease).		Perelas *et al.*[106]
	ILD: NSIP 80% (two-thirds are fibrotic), UIP, OP, and DAD	The histologic features are indistinguishable from those of idiopathic counterparts, except for the presence of pleural fibrosis. NSIP, OP and DAD differentials include drug toxicity or infections (including COVID-19) Coexistent findings may include the presence of microaspiration which is related to esophageal dysmotility.	
Systemic lupus erythematosus (SLE)	DAH: capillaritis manifesting as an infiltrate of necrotic neutrophils within the alveolar septa and destruction of alveolar walls and small vessel vasculitis with acute inflammation and necrosis of capillaries, arterioles and small muscular arteries	Drugs or illicit drugs toxicity, isolated capillaritis, vasculitis	Torre and Harari,[107] Myers and Katzenstein,[108] Cheema and Quismorio,[109] Schulte and Husain[110]
	Acute lupus pneumonitis: diffuse alveolar damage with hyaline membranes, interstitial edema, and arteriolar thrombosis	Drug toxicity or infection, including COVID-19	
	Antiphospholipid syndrome: pulmonary embolism and infarction, thromboembolic and nonthromboembolic pulmonary hypertension, pulmonary artery thrombosis and microthrombosis, intra-alveolar hemorrhage.		
	Chronic ILD (2–8%): NSIP, OP, LIP.	NSIP, OP can be idiopathic, related to drug toxicity, or infections (COVID-19) LIP should be differentiated by lymphoid nodular hyperplasia and lymphomas. SLE patients are at higher risk for lymphomas, lung cancer, and Kaposi sarcoma	

(Continued)

Table 5. (Continued)

Disease	Lung histopathology patterns	Main differentials	Ref.
Dermatomyositis/ polymyositis (DM/PM), *antisynthetase syndrome and anti MDA-5 syndrome*.	ILD: NSIP, OP, DAD (described in 50% of patients with anti Jo1 Ab) and one-third with UIP pattern	NSIP, OP and DAD can be idiopathic, related to drug toxicity, or infections (COVID-19) UIP can be idiopathic (IPF), less frequently drug related	Yousem *et al.*,[111] Mochimaru *et al.*[112]
Sjogren's syndrome (SS)	ILD: most frequent pattern is NSIP, others include follicular bronchiolitis, LIP, OP, and rarely UIP. Cystic disease on HRCT with lymphoid hyperplasia along airways on biopsy a clue to SS	Follicular bronchiolitis and LIP should be differentiated from a spectrum of other lymphoid disorders that include nodular lymphoid hyperplasia, lymphomatoid granulomatosis and malignant lymphomas (85% are MALT) for which SS patients have a highly increased risk (44-fold compared with the general population), 4–7% of all SS	

CTD, connective tissue diseases; DAD, diffuse alveolar damage; DAH, diffuse alveolar hemorrhage; DIP, desquamative interstitial pneumonia; HRCT, high resolution computed tomography; ILD, interstitial lung diseases; IPF, idiopathic pulmonary fibrosis; LIP, lymphocytic interstitial pneumonia; MALT, mucosal associated lymphoid tissue lymphomas; NSIP, nonspecific interstitial pneumonia; UIP, usual interstitial pneumonia.

retrospective design of those studies, along with the heterogeneity of SSc patients and the variable immunosuppressive drug regimens used, make very difficult to extrapolate solid conclusions on the prognostic utility of LBx in CTD-ILD. Taking into account those negative results and the risks of surgery, however, LBx in CTD-ILD has been abandoned and recent trials on drug treatment for CTD-ILD, including antifibrotic drugs, relied on the sole clinical-radiologic evaluation of cases.

In contrast to SSc patients, the most notable phenotypic distinction among RA-ILD patient is the presence of UIP pattern.[96] RA have the highest prevalence of UIP, and similar to IPF RA-UIP, patients tend to be elderly male and more frequently smokers with a significantly worse survival compared with non-UIP.[97–99] The natural history of RA-UIP can be punctuated by episodes of acute exacerbations and respiratory hospitalizations. Acute exacerbations are significantly more frequent in UIP-RA compared with non-UIP-RA (overall 1 year incidence 6.5% *versus* 1.7%) and have a prevalence and a prognosis similar to that of IPF (prevalence of 22% during a median follow-up of 8.5 years, with a mortality rate of 64%).[100] Survival separation and treatment decision (immunomodulation *versus* antifibrotic drugs) are currently driving the use of LBx in idiopathic disease, but surprisingly for RA, this has not been the case. Nowadays, with the availability of antifibrotic drug, this may change radically leading to a bioptic approach for RA-ILD and CTD-ILD more similar to that of idiopathic ILD.

Steps forward should guide the path through a more accurate definition and characterization of CTD-ILD with the intention to explore whether there are subgroups of patients for whom antifibrotics can be more beneficial. This idea comes from idiopathic ILD in which the distinction between UIP and not-UIP (defined either by HRCT or histologically) has clear prognostic and therapeutic implications.[89] Several studies have shown that in fibrotic ILD (e.g. HP and RA), the presence of UIP pattern guides the prognosis and that non-UIP cases may have a more favorable response to immunomodulation, whereas in IPF (idiopathic UIP), the use of immunosuppressive treatment is detrimental.[101–104]

In conclusion, with the notable exception of RA-UIP that seems to have a prognosis similar to IPF and worse compared with non-UIP-RA, for the other CTD-ILD, the distinction between NSIP and UIP does not seem to carry significant prognostic information. Those findings, however, have been extrapolated mainly from studies conducted in the pre-antifibrotic era, and mainly on SSc with a very small prevalence of UIP cases, and therefore should be interpreted with great caution. Whether histologic patterns impact CTD-ILD prognosis remains to be proven by larger prospective trials and we hope that the availability of TBLC in many referral centers as a much safer technique than SLB will allow in the future the design of innovative drug trials evaluating treatment response more precisely integrating HRCT findings with histology. To note that, even if the available evidence revolves mainly around the distinction between UIP and

not-UIP, histopathology is a precious font of information and the use of more sophisticated technologies rather than traditional hematoxylin-eosin staining could lead in the future to the development of much more accurate theragnostic biomarkers and to a deeper understanding of CTD-ILD pathogenesis.

The added value of lung histopathology in CTD

Few pulmonary lesions are pathognomonic of CTD (e.g. rheumatoid nodules). CTD-ILD can histologically simulate a variety of lung diseases, in particular the idiopathic ILD. Biopsy can also document other conditions that would change patient's management and prognosis such as neoplastic disorders, lymphoproliferative lung disorders, drug reactions, and some infections.

The successful diagnosis of CTD-ILD requires a dynamic integration of clinical and radiologic features, but when the scenario is unclear (i.e. cases lacking clear criteria for CTD), histology provides the most relevant piece of information. It is well known that some CTD-ILD (i.e. the majority of RA, a minority of SSc, mixed CTD, PM/DM, and SLE) can present with the UIP pattern observed in IPF. It is particularly important for the pathologist to suggest the possibility of a CTD if features suggest that possibility because the management of these condition is strikingly divergent, being immunosuppressive treatment detrimental in IPF, and beneficial in CTD-ILD. Patients with CTD-UIP compared with IPF-UIP have fewer fibroblastic foci and more prominent interstitial inflammatory infiltrate.[92] Conversely, the pathologist should be aware that CTD-ILD may be histologically identical to an IIP and the diagnosis of CTD ILD depends on serologic and/or clinical findings.

Table 5 focuses on histopathology features of most common CTD-ILD considering (SSc, RA, SLE, DM/PM antisynthetase and antiMDA5 syndromes, SS) and their most common differentials. Other lung histopathologic changes that can be challenging when evaluating a patient with CTD-ILD are represented by acute lung injury with DAD that can be superimposed to a fibrotic background as in acute exacerbations of the disease, and acute fibrinous and organizing pneumonia, which shows fibrin organized into intra-alveolar balls and has been reported in various CTD (SLE, SS, and DM). These forms of acute lung injury can also be secondary to drug or other toxic reaction or infection, and can be associated with hematologic malignancy.

The differential diagnosis most commonly encountered in the diagnosis of CTD-ILD, including IPAF, are infections and drug toxicity in which histopathology can sometimes provide useful hints.

IPAF is a research category that includes patients presenting with ILD and some autoimmune features, but lacking definitive criteria for CTD.[3] Histopathologic criteria are included in the morphologic domain; the other two diagnostic domains are the clinical domain and the serologic domain; and to fulfill the diagnosis, positive findings should be present in at least two domains. The histopathologic features are NSIP, OP, overlap of the two, LIP, interstitial lymphoid aggregates with germinal centers, and diffuse lymphoplasmacytic infiltration. Evidences are accumulating on this new research entity and histopathology seems to have a relevant role. We have recently revised a cohort of 360 IPF and found 22 cases (6%) of IPAF, six of them with the typical IPAF histopathologic features of lymphoid infiltrate superimposed to the UIP pattern. IPF/IPAF patients showed a significantly better prognosis and a significantly higher risk to evolve to full-blown CTD compared with non-IPAF/IPF (45% *versus* 0%) (Tomassetti *et al.*, unpublished data).

Infections are frequent in CTD and can present with clinical-radiologic features similar to CTD-ILD or drug toxicity. Frequent etiologic agents are *Pneumocystis jirovecii*, *Aspergillus*, Gram-negative bacilli, Gram-positive coccus and mycobacteria.[14] Routine microbiological tests and particularly BAL are of great utility when superinfection is suspected. In some, histopathology can be helpful to identify specific features (e.g. necrotizing granulomas of mycobacterial infection or *Aspergillus* hyphae) and allow treatment before culture results are available, in particular for slow-growing organisms (e.g. mycobacteria).

Drug toxicity represents another vast and complex field. There is no specific diagnostic feature on LBx that can help in the differential and clinical, imaging, BAL, and histology data all need to be integrated. The underlying primary disease, unclear temporal relations, multiple medications with known lung toxicity, and possible superinfections all make the differential diagnosis quite

complex. Histopathologic patterns of drug toxicity are many and may be indistinguishable from the underlying CTD-ILD. The list of medications implicated in lung toxicity and their reported histologic patterns can be found at *pneumotox.com*. Commonly observed patterns of drug-induced lung toxicity include chronic interstitial inflammation, NSIP, OP, eosinophilic pneumonia, pulmonary edema, DAD, HP, bronchiolitis (chronic or constrictive), pulmonary hemorrhage, and granulomatous diseases.[113]

Precision medicine in CTD-ILD, the added value of histology

CTD-ILD are characterized by a variety of lung changes that in a considerable proportion of patients extensively involve the lung parenchyma, progress, and ultimately lead to death. Despite recent progress, the pathogenesis of CTD-ILD remains poorly understood and reliable prognostic and therapeutic biomarkers are still lacking. CTD-ILD induce a profound derangement of the phenotype of all resident cells in the lung and there are emerging techniques to investigate those changes. After decades of formulating hypotheses based on animal models, clinical analogies between different diseases, or biological plausibility with limited validation in humans, lung research is now shifting toward the analysis of human lung. The increased availability of lung tissue through less invasive TBLC and the emergence of high profiling technologies are critical in this new era in which transcriptomics is becoming the golden opportunity for research and recent analysis on lung tissue of SSc patients detected previously unrecognized pathogenetic mechanisms driving myofibroblast differentiation and proliferation with microarray and single-cell analysis.[114,115]

The source of tissue together with sample size are the two key elements to consider when designing a transcriptomic study that can be conducted using BAL, blood, or lung tissue with different type of analyses, including bulk cells, single cell, or sorted cells. Fibrotic lungs, including the CTD-ILD lungs, are characterized by a dramatically altered architecture and cell phenotypes. Neither BAL nor homogenates of bulk tissue can properly capture the complexity of this process because they can't allow the understanding of how cells influence each other in the living fibrotic lung. Improving the cellular and spatial resolution of transcriptomics using single cell and tissue microenvironments is critical to decipher the fibrotic process.[116]

Pathogenetic studies on CTD-ILD have been critically limited by the shortage of lung tissue that in CTD-ILD is not routinely obtained due to the risks of surgery and to the perceived limited clinical utility of histologic information. With the contribution of novel mini-invasive techniques, however, we might overcome these obstacles and obtain from lung tissue the pivotal information we need to cross the line of precision medicine.

A jump in the future: HRCT, BAL, and LBx to draw the map of CTD-ILD

The history of clinical research in CTD-ILD revolves mainly around SSc and has been punctuated over time by interest in BAL analysis, few studies on histopathology (mainly limited by tissue availability), and the recent supremacy of HRCT and pulmonary function approach that is currently guiding clinicians in prognosis predictions, treatment decisions, and drug trial design. We recognize the unquestionable clinical utility of the HRCT functional approach that appears robust, easily reproducible, low costs for institutions, and low risks for patients, and we believe it will last for years. A new drug treatment era, however, is emerging; we already have more than one therapeutic choice, including immunosuppressive agents, antifibrotic drugs, and biological agents; and making the right choice appears already problematic. The HRCT functional approach has some limitations. In 2017, Poletti *et al.*[117] hypothesized the TBLC scenario in the upcoming 5 years shedding a light on the possibility that TBLC might appreciably change the clinical approach to ILD patients beyond that of HRCT, and we believe that this prediction largely applies to CTD-ILD. The possibility to obtain informative lung tissue with minimal side effects might broaden the indication to biopsy fragile patients for whom SLB is not an option, may increase our understanding of histologic patterns divergent from the HRCT features (e.g. radiologic NSIP may hide histopathologic UIP), may increase our ability to detect subsets of patients (e.g. coexisting areas of acute lung injury or organizing pneumonia in fibrotic ILD might identify a subgroup of patients that would benefit from steroids or immunosuppressive treatment added on antifibrotic treatment), and may implement research

on molecular markers increasing our understanding of the pathogenesis of CTD-ILD and propelling a personalized treatment approach that is currently lacking.[117]

Considering the paucity of studies that in the pre-transcriptomics era have investigated the diagnostic and prognostic role of BAL and LBx in CTD-ILD, one can hypothesize that future studies conducted applying innovative techniques on BAL and LBx might open new and unexpected scenery on CTD-ILD pathogenesis, diagnosis, and treatment approach. We hope that the innovative mini-invasive biopsy techniques and the new molecular techniques of analysis that are emerging will lead to the line of precision medicine in which functional, radiologic, serologic, and clinical data will be integrated with BAL analysis and histopathologic characterization of lesions to draw the transcriptomic map of the molecular signature of CTD-ILD, pointing toward a personalized therapeutic approach for each patient.

Author contributions

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