Research Article

Lung Fibrosis Conventional and Molecular Imaging Diagnosis Update

Youssef Ali[1#], Hongxia Chen[1#], Minjie Chen[1], Xicui Sun[1], Jingqing Liang[1], Qi Cao[1*], Liya Pi[2#], Li Yang[3], Bin Ren[4], Zhekang Ying[5], Shujing Li[1,6], Yamin Wan[1,7], Li Zhang[1,8] and Yingqi Zhang[9]

[1]Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine, United States

[2]Department of Pathology and Laboratory Medicine, Tulane University School of Medicine, United States

[3]Division of Gastroenterology and Hepatology, Tongji University, China

[4]Department of Surgery, University of Alabama at Birmingham School of Medicine, United States

[5]Department of Medicine, University of Maryland School of Medicine, United States

[6]Department of CT and MRI, The Children Hospital of Hebei Province, China

[7]Department of Radiology, The First Affiliated Hospital of Henan Medical University, China

[8]The School of Basic Medical Sciences, Wuhan University, China

[9]Department of Emergency, The First Affiliated Hospital of Hebei Medical University, China

[#]Equal contribution: Youssef Ali, Hongxia Chen, Liya Pi

*Corresponding author: Qi Cao, Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine, Baltimore, Maryland 20201, United States of America

Abstract

Interstitial lung diseases are one of life-threatening conditions with high morbidity and mortality. Lung biopsy is the "gold standard" to diagnose, grade, and stage lung fibrosis, but lung biopsy has limitations in the diagnosis of early-stage lung fibrosis because clinically neither used as the first choice nor as routine diagnosis technique. Lung biopsy is also limitations of invasiveness, cost, sampling variability, inter-observer variability, and the dynamic process of fibrosis. More and more evidence has demonstrated that stages of lung fibrosis are reversible if the injury and risk factors are removed before progression to end-stage lung fibrosis. Developing safe, effective, and reliable non-invasive assessment modalities is clinically required to detect early-stage lung fibrosis and to manage it precisely in personalized medicine. Conventional imaging methods including ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI) are useful in assessing advanced lung disease, including fibrotic scar in the lung by visualizing morphological and structural changes. Functional imaging techniques are useful for assessing moderate to advanced lung fibrosis using MR elastography (MRE), US elastography, and CT perfusion. MRE is considered the most accurate noninvasive imaging technique, and US elastography is currently the most widely used noninvasive means. However, these modalities are less accurate in early-stage lung fibrosis and some artifactual factors affect the accuracy of these techniques. Molecular imaging techniques of [18]Ffluoroproline and other radiotracers are target-specific imaging that has the potential to accurately diagnose early-stage liver and lung fibrosis. We provide an overview of recent advances in molecular imaging for the diagnosis and staging of lung fibrosis which will enable clinicians and researchers to detect early-stage lung fibrosis, monitor the progression of disease and potentially reverse lung fibrosis. We compare the promising technologies with conventional and functional imaging and assess the utility of molecular imaging in precision and personalized clinical medicine in the early stages of lung fibrosis.

Keywords: Early-stage lung fibrosis, Computed tomography, Magnetic resonance imaging, Ultrasound, Molecular imaging, [18]Ffluoroproline

Introduction

Interstitial lung diseases (ILDs) consist of a category of lung pathologies of similar clinical, radiological, and pathologic features. It emerges when fibrous tissues accumulate between the capillaries' endothelium and the alveolae epithelium, limiting gas diffusion and exchange [1,2]. Pulmonary fibrosis is a lifelong, debilitating lung disease that occurs when the lungs' upper or lower lobes suffer from fibrotic changes and microscopic injuries affect the lung parenchyma. It involves the respiratory system due to a group of serious lung diseases, including exposure to substances, autoimmune disorders, and viral or bacterial infections. However, Pulmonary fibrosis might sometimes develop for no apparent reason, which is called idiopathic pulmonary fibrosis (IPF). Exposure to occupational and environmental pollutants, particularly inorganic or organic dust, are the most prevalent recognized causes of ILD (Table 1). The prevalence of ILD is ranged from 25 to 74 per 100,000 population [3]. Gradual fibrous tissue deposition that replaces the normal lung parenchyma causes scarring and irreversible decrease in diffusion capacity of the lungs. This leads to stiffening and decreasing lung compliance which manifests as a restrictive lung disease pattern.

Lung tissue thickening and stiffening make it more difficult for the lungs to function correctly. As pulmonary fibrosis worsens, patients become increasingly short of breath. Current medications and therapies could help relieve symptoms which improve the patients' quality of life, unfortunately, no cure has developed for end-stage lung cirrhosis at the current moment. Lung transplantation may be a reasonable alternative in some situations. Recent advances in physiologic tests, lung radiography, and histopathological lung biopsy assessments have been developed [4-9]. High-resolution computed tomography (HRCT) advances helped dividing lung fibrosis into many distinct patterns. New lab techniques using the analysis of proteins, microarrays, mass spectrometry, and laser capture microdissection have built a good base for genetic medicine. Over the last years, using these new modalities and advances helped diagnose and differentiate various pulmonary fibrosis patterns. In this article, we will highlight the conventional and molecular imaging diagnoses used in the last years. We discuss recent advances in innovative imaging methods for ILD, and emphasize quantitative HRCT, new MRI, and positron emission tomography techniques (PET) (Table 1).

Table 1: Various etiologies causing diffuse interstitial lung diseases [4-9].	
1- Culprits Cause Inflammation and Fibrosis:	**2- Systemic Diseases Involve the Lungs and Cause Inflammation**
• Smoking	• Granulomatous diseases, e.g., sarcoidosis
• Organic dust	• Inflammatory vasculitis
• Inorganic dust (pneumoconiosis)	• Inherited diseases, e.g., tuberous sclerosis, hermansky-pudlak syndrome, neurofibromatosis
• Infection	• Autoimmune and collagen vascular diseases
• Drugs	• Rheumatoid arthritis
• Radiation	• Anti-Jo-1 antibody-positive interstitial lung disease
	• Systemic lupus erythematosus
	• Mixed connective tissue disease
	• Progressive systemic sclerosis
	• Ankylosing spondylitis
	• Sjögren syndrome
	• Psoriasis - pulmonary manifestations of psoriasis
	• Primary biliary cholangitis / primary biliary cirrhosis
	• Neoplasia
	• Primary, e.g., non-small cell lung cancer, lung adenocarcinoma, lymphoma
	• Secondary, e.g., pulmonary metastases
	• Miscellaneous, e.g., amyloidosis, alveolar proteinosis
3- Idiopathic (the cause is unknown) Patterns which Include:	
• The usual interstitial pneumonia (UIP) (idiopathic pulmonary fibrosis)	
• NON-UIP IIP	
• Cryptogenic organizing pneumonia	
• Nonspecific interstitial pneumonia (NSIP): non-smokers	

| • Acute interstitial pneumonitis (AIP) |
| • Respiratory bronchiolitis-interstitial lung disease (RB-ILD): smokers |
| • Lymphoid interstitial pneumonia (LIP) |
| • Desquamative interstitial pneumonia (DIP): end-state of RB-ILD |

IPF Genetics

Although the cause of IPF is not known, genetic findings in monogenic family variants have provided important perceptions to the role of hereditary mutations in disease pathology and intimate pathways of lung fibrosis. Genetic testing technologies are quickly improving, enabling the study of several genes at the same time. Each technique has its own set of benefits and drawbacks, from targeted next-generation sequencing (NGS) panels to whole- genome sequencing (WES). Inherited ILDs develop because of genetic mutations passed down from a parent or ancestor. Inherited ILDs are classified into systemic illnesses that affect many organs and disorders that predominantly affect the lungs. A detailed medical and family history provides crucial information regarding the ILD's specific nature for all these categories: First, the inheritance pattern offers vital information; for example, inborn metabolic abnormalities linked with ILDs have an autosomal recessive inheritance pattern. Second, some illnesses, such as lymphangioleiomyomatosis which occurs only in women. Another leading factor is the age at which the disease first emerges. In autosomal dominant kindreds with hereditary telomerase mutations and increasingly shorter telomere lengths, younger and more seriously afflicted individuals are found in subsequent generations, which point to genetic anticipation [10]. Although the preliminary studies show that genetic variations could aid in prognosis prediction, the relevance between genotype at different variants and survival is currently being studied and needs to be confirmed in prospective studies. Treatment trials in the future should consider phenotypic and genotypic diversity to know better how these traits may and should be factored in shared decision-making. Sequencing and genotyping patients are not now part of regular IPF or fibrotic IIP care due to a lack of precise data connecting genetic variations with tangible clinical outcomes or pharmacological responses. Regarding familial pulmonary fibrosis, cryptogenic pulmonary fibrosis, and short telomere syndrome, genetic analysis includes telomere-related gene and MUC5B sequencing, telomere length, and surfactant gene sequencing. While waiting for focused therapy, evidence of a pathogenic mutation should lead to genetic counseling [11].

Mechanisms of Fibrosis

Immune lung inflammation is a major pathogenesis of idiopathic pulmonary fibrosis and many other types of fibrotic ILD. Fibrous tissue deposition is caused by an initial inflammatory response to lung damage. The pathogenesis of pulmonary fibrosis involves genetic predisposition, age- related factors, and any trigger that causes epithelial injury, which can cause repetitive epithelial cell injury, cell stress and cell apoptosis and abnormal repair responses, as well as extracellular matrix accumulation. Imbalance of collagen accumulation and collagen degradation leads to progressive fibrosis and loss of lung function over time. Tobacco smoke, injuries from refluxed stomach contents, viral infections, occupational exposures, environmental exposures to both inorganic (metal, wood, and silica dust) and inorganic dust, and air pollution are all linked to interstitial fibrosis pathogenesis [12].

Injury of type I alveolar epithelial cells (AEC) and rupture of the AEC layer are seen as initiating events in IPF pathogenesis, and profibrotic cytokine release (e.g., chemokines, proteases, and transforming growth factor-beta [TGF-β]). AEC injury is gone with type II alveolar cell injury and dysfunction, activation of coagulation pathways, and abnormal wound repair mechanisms that steadily interrupt lung matrix when fibroblasts are activated as AECs go through epithelial-to-mesenchymal transition (EMT), causing myofibroblast activation and accumulation. The resulting tissue remodeling fails to restore normal epithelial barriers to stop myofibroblasts' abnormal activity and the inflow of blood myofibroblast precursors into the fibrosing lung. Type 2 AEC dysfunction could be one of major factors in the lungs' incapacity to heal after injury [13,14].

Interstitial Lung Disease Diagnosis

Early in the 20th century, ILD was described with its gross and microscopic pathology. In the 1960s, ILDs categorization appeared in the scientific community, and ILDs were categorized depending on the culprit cause (e.g., drugs, connective tissue diseases, environmental and occupational exposures, inheritance, and sarcoidosis). The absence of reliable, readily available biomarkers makes it difficult to monitor, treat, and investigate new treatments for these diseases. A vexing problem for clinicians is the presentation of a patent who exhibits the symptoms and signs of ILD that merit precise diagnosis and interventions. The advances in technology have promoted our detective accuracy and understanding of the ILDs. ILD is diagnosed by clinical, functional, radiological, and histological information. Chest X-ray is often the first imaging step,

then HRCT when the finding is uncertain after CXR and clinical assessment. Bronchoalveolar lavage, transbronchial lung biopsy, or surgical lung biopsy should be considered when the diagnosis is still unclear [2].

Clinical Presentation

Clinical presentation is a pillar in the diagnosis of ILDs, which can include the classic "3Cs": nonproductive cough over several months, clubbing of the nails, and coarse crackles on auscultation, and gradual onset of dyspnea on exertion plus the symptoms of the concurrent ongoing disease like fatigue, fever, myalgias, and arthralgias, if present. The clinical evaluation includes careful exploration of past medical history (comorbidities, medications, irradiation), potential exposures (occupational, avocational, environmental, infectious), and extrapulmonary evidence of a systemic illness.

Physiologic Testing

Ventilatory function and gas exchange testing is crucial in evaluating patients with ILD. Lung function studies are applied to monitor disease courses. However, these lung function testing has no rule in differentiating fibrosis from inflammation [15-17]. Generally, ILDs show an abnormal restrictive pattern and reduced diffusing capacity. But lung function studies have a minor role in predicting the prognosis and responsiveness to therapy.

Open Lung Biopsy

When the results of clinical evaluation, laboratory testing, pulmonary function testing, and imaging studies (for example, HRCT) cannot make a confident diagnosis of a specific type or stage of ILD, a lung biopsy is usually needed to carefully examine lung tissue. Surgical lung biopsy has the highest diagnostic sensitivity for ILDs. However, it carries surgical risks for the patients. The diagnostic yield of video-assisted thoracoscopic surgery was shown to be very high, with low morbidity and mortality [18]. In comparison with clinical data and HRCT, lung histopathology has the greatest impact on final diagnosis. Histopathology could guide therapy and determine the fibrosis patterns and remains the gold standard [19]. In order to provide the pathologist with adequate tissue to evaluate the pattern and distribution of disease, surgical biopsies should be greater than 4cm in the greatest dimension and include a depth from the pleural surface of 3 to 5cm.

Ideally, specimens are obtained from more than one lobe of the lung [20]. HRCT anatomical imaging plays an essential role in selecting the best location for biopsy [21].

Transbronchial Lung Biopsy

The flexible fiberoptic bronchoscope is a minimally invasive method to get the tissue needed for biopsy. The biopsy acquired from this method is usually adequate for diagnosing many lung diseases that cause lung fibrosis (e.g., infections, malignancy pneumoconiosis) [22]. Transbronchial lung biopsy is safer but gives acceptable samples in 78% of cases. This leads to a definite diagnosis in approximately 35% of patients [23].

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) is a minimally invasive procedure that obtains a sample of alveolar cells and evaluates cell counts, cytology, and culture via flexible bronchoscopy. It gives data about the immune status of the host, fibrogenesis, inflammation, and infections. BAL is used to rule out diffuse lung disorders other than RA-ILD in patients with an acute onset of respiratory symptoms or fever and radiographic abnormalities, such as alveolar hemorrhage, acute eosinophilic pneumonia, opportunistic or atypical infection, and malignancy. Because changes in cell and mediator content do not correspond with clinical parameters, pulmonary function assist in predicting prognosis, or identifying distinct kinds of ILD. In general, BAL is not used as a routine diagnostic approach to ILD.

Evaluation of Lung Fibrosis by Various Radiological Investigations Diffuse lung diseases are recognized as infiltrative opacification in the peripheral lung. Patterns vary among different causes. On the conventional chest radiograph, radiologists and pulmonologists can identify pathologic patterns of interstitial lung diseases [24]. Radiological investigations are critical in diagnosing and managing ILD; however, anatomical CT imaging in clinical practice is qualitative and subjective. Newer methods assist in resolving issues such as discerning peripheral reticulation of lung parenchyma from pleura and properly detecting the complicated density patterns of honeycombing. Quantitative evaluation can help determine the efficacy of antifibrotic therapy and detect early changes in pneumotoxic drugs, objectively assessing minor changes in the character or degree of the disease. Limitations to standardization of quantitative CT (e.g., lung density analysis, texture-based analysis, and machine learning metrics, and Vessel quantification) include the differences in scanner equipment and methods used in various centers.
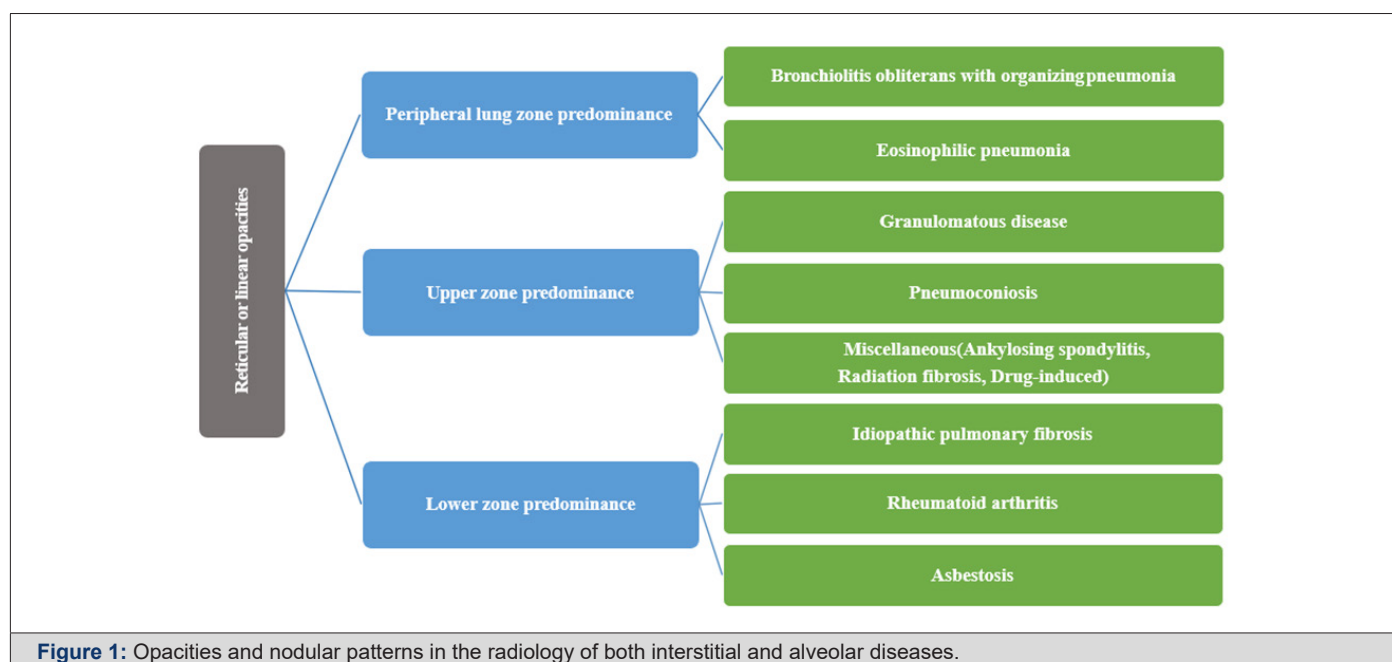
Chest Radiograph

A reticular pattern is the most frequent radiographic abnormality on regular chest radiographs; however, nodular, or mixed patterns are not uncommon [25]. Although the chest radiograph helps suggest the presence of ILD, the link between the radiographic pattern and the stage of disease (clinic or histopathology) is poor. Only the radiographic finding of honeycombing (small cystic spaces) correlates with pathologic findings, which indicates a poor prognosis. Chest X-ray can help determine the affected lobs and interstitial opacities vs. alveolar filling. In addition, chest X-ray

provides classifying the type of opacities into reticular, cystic, and nodular forms. Chest X-ray aids in excluding other differential diagnoses (e.g., lymphadenopathy, pleural affection, cardiomegaly). Chest X-ray is crucial as initial imaging but has low sensitivity and specificity to detect ILDs. In patients with mild ILD, the chest X-ray could be normal. Failure to properly evaluate such people may result in disease progression that is irreversible by the time the patient seeks medical help. Bibasilar reticular, nodular, and ground-glass opacities, and honeycombing are all possible findings.

HRCT Scanning

The definite diagnosis is more accurate with HRCT than with a chest X-ray. Chest CT is considered a critical tool to diagnose ILDs. However, it has a higher cost and higher radiation exposure to the patients. HRCT can identify a variety of parenchymal abnormalities and can detect problems earlier than chest radiography. Supine and prone images are taken to minimize misinterpretation of gravity-caused opacities in the dependent areas. Actually, ILD is a misnomer as it is challenging to find interstitium affection alone without the involvement of neighbor alveolar spaces. Accordingly,

there are some limitations to the pattern use of the conventional radiologic diagnostic modalities, which include the following: ground-glass opacities and nodular patterns appear in both interstitial and alveolar diseases (interstitial pneumonia) which usually comprises the alveolar compartment. In addition, most alveolar diseases involve the interstitium in the pathology process, and the air bronchogram, which is the ultimate sign for alveolar diseases, can be detected in ILD, such as sarcoidosis [26]. HRCT can differentiate a predominant ground glass pattern from reticular changes and honeycombing. HRCT also helps distinguish types of ILD. For example, ground-glass opacification is consistent with acute interstitial pneumonia, nonspecific interstitial pneumonia (NSIP), and desquamative interstitial pneumonia (DIP). Besides, HRCT imaging shows that traction bronchiectasis, reticular changes, and honeycombing are more typical of usual interstitial pneumonia (UIP) [27-30]. Infrequently, the HRCT may suggest UIP, but NSIP will be identified by biopsy [31]. Persistent areas of subpleural consolidation are more suggestive of organizing pneumonia [32], and the location of reticular or linear opacities helps in determining the cause (Figure 1).

Figure 1: Opacities and nodular patterns in the radiology of both interstitial and alveolar diseases.

A review of previously performed CT images should be helpful for identification of a pre-existing ILD. In addition, reviewing previous images can help determine the progression rate of ILD and whether the timing of changes in CT findings over time correlates with symptoms or medication usage.

Expert radiologists can find semi-quantitative CT scoring

analysis useful in giving prognostic insight, but there is no standard approach for assessing radiological extent or severity [33]. Advanced algorithms, such as unsupervised machine learning and deep learning approaches to image analysis, have the potential to standardize and develop the role of HRCT in ILD by quantifying overall lung histogram features, parenchymal texture features, regional CT density changes, and other assessments.

Lung Density Analysis

HRCT density data have been used to quantify lung architecture in a variety of respiratory diseases, most notably emphysema [34]. As the dense soft tissue in the lungs increases due to formation of fibrosis, the mean lung density (MLD) rises while the histogram kurtosis and skew decline [35]. Even though numerous studies have investigated the correlation between density histogram measures and ILD outcomes, reducing quantitative regional information from very sensitive imaging to global summary measures compromises the imaging data's richness. Other characteristics, for example, parenchymal destruction, air trapping in hypersensitivity pneumonitis (HP) in the combined pulmonary fibrosis and emphysema (CPFE) syndrome, can potentially confuse overall measures [36]. It's controversial if lung density measurement can distinguish between UIP pattern fibrosis/IPF and other forms of ILD. Do et al. found substantial variations in kurtosis and skewness in 28 patients with nonspecific interstitial pneumonia (NSIP) and 32 patients with the UIP fibrosis pattern [37]. However, Sverzellati et al. found no significant difference in skewness, kurtosis, or mean lung attenuation between patients with UIP pattern fibrosis/IPF and those with HP and/or unclassifiable idiopathic interstitial pneumonia in two trials [38,39].

Texture-Based Analysis and Machine Learning

Two-dimensional volumetric classification algorithms, computer vision-based method and texture-based analysis may be applied to characterize, analyze, and process image characteristics, emulating human visual perception and learning processes. Focal texture analysis has the capacity to quantify regional and global data. The textural characteristics may be used to determine severity (fine vs. coarse reticulation), extent of disease, and the type of abnormality (emphysema vs. honeycombing vs. cysts), which correlates with expert radiologist's evaluation [40]. The adaptive multiple feature method (AMFM) is a program for analysis of lung texture by recognizing HRCT patterns. AMFM quantifies the volume occupied by a range of radiological patterns combining 26 distinct mathematical characteristics of regional density patterns with a Bayesian classifier. This can show the difference between emphysema and fibrosis and identify ground glass opacification (GGO) and normal lung [41]. Another advancement of the AMFM provides for three- dimensional (3D) evaluation [42]. Honeycombing had a sensitivity and specificity of 100% for emphysema and consolidation and 95% and 97% for emphysema and consolidation, respectively [40]. Computer-Aided Lung Informatics for Pathology Evaluation and Rating (CALIPER) is an image analysis program that characterizes HRCT data using 3D histogram features within a regional voxel and morphological analysis. CALIPER-measured ILD changes of percentage ILD, total ILD volume, and total reticulation volume, were correlated with survival in a retrospective clinical study of 55 patients with IPF [43].

Vessel Quantification

The pulmonary vessel volume (PVV) is found correlation with visually and quantitatively measured disease severity, and with physiological data. PVV is better than the Age, Gender, and Physiology score in foreseeing the prognosis [44]. Because a radiologist's volumetric evaluation of the size and shape of branching vascular systems is exceedingly challenging, extra machine learning or deep learning approaches may reveal characteristics that humans cannot perceive or judge reliably.

Contrast-Enhanced CT

Iodine contrast has no major effect on the sensitivity or specificity by which ILD abnormalities are detected and classified. That is why HRCT is not routinely used in ILD standard diagnosis. On the other hand, there is a higher risk of radiation exposure in patients who receive contrast material [13].

Ultrasonography (US)

Transthoracic ultrasonography (TUS) beams cannot pass through air. So, the presence of air in the lungs generates certain artifacts. However, when the lung is diseased, the air-filled spaces fill with fluids or solid tissues, actual visualization of the diseased lungs is practical [45]. Subpleural abnormalities, pleural line irregularities, diffuse B-lines, reduction, or absence of lung sliding, and pleural thickening have been implied as possible findings in ILD [46,47]. The study for diagnosing connective tissue disease related ILD found TUS sensitivity and specificity are 91.5% and 81.3%, respectively which are comparable with HRCT [48].

Conventional Magnetic Resonance Imaging

In medicine, magnetic resonance imaging (MRI) is relatively new diagnostic technique. It is a radiation-free alternative that avoids the high radiation risk of the HRCT. MRI of the lungs to diagnose pulmonary fibrosis has limited clinical use. Steady-state free precession sequences, contrast-enhanced sequences, and non-contrast breath-holding sequences should all be included in an ideal chest MRI procedure for ILDs. Insufficient evidence in the literature supports the use of MRI in the diagnosis of ILD. Due to various challenges of low proton density of lung tissue, numerous air tissue interfaces producing magnetic field inhomogeneity, fast MR signal relaxation, and respiratory and cardiac motion, acquisition of lung MRI is difficult. Proton MRI methods such as ultrashort echo time (UTE) may provide for a better resolution image of ILD structural changes, and contrast-enhanced imaging can be used to measure

pulmonary perfusion. The major benefit of MRI is to measure functional and structural information in a single examination. New chest MRI methods have recently been studied that may offer information on ventilation, inflammation, perfusion, and structure, which could help with ILD progression assessment and treatment response prediction [49]. For morphological imaging, HRCT still outperforms MRI. Recent investigations using ultrashort echo time MRI found the picture quality to be comparable to HRCT. Several studies have shown that employing functional imaging to enhance chest MRI can help detect and quantify inflammatory changes [50].

A single shot rapid spin echo T2-weighted acquisition using Half-Fourier Single-shot Turbo Spin-Echo (HASTE) was the first sequence tested for morphological ILD imaging. Because HASTE image demonstrates high signal intensity in water-rich tissues, lung anomalies look bright and are surrounded by low-signal intensity air-filled parenchyma. *Hatabu, et al.* found that breath-hold HASTE sequences might be applied to diagnose a variety of lung interstitial abnormalities such as ground-glass opacity and bronchiectasis [50]. In individuals with cystic fibrosis, *Puderbach, et al.* performed HASTE before and after contrast agent delivery and found similarity to HRCT. T2-weighted HASTE is valuable to illustrate aspiratory penetrates, bodily fluid and liquid collection, and inflammatory bronchial thickening [51]. On T2-weighted images, air-space infiltrates appear as hyperintense zones that contrast with the appearance of the normal lung parenchyma [52]. The T1-3D-GRE can be used to assess consolidations, lung nodules, masses, and the mediastinum. T1-GRE images, on the other hand, have not enough information regarding infiltrative processes [53]. Although HRCT is still superior to MRI for assessing structural changes in ILD, many functional MRI methods may be useful in ILD. Ventilation techniques with and without gaseous contrasts might give vital information on ventilation mismatch in ILD patients [54]. T2-weighted sequences or diffusion-weighted imaging might be used to assess active lung inflammatory tissue [55]. The quantity of lung fibrosis might be measured using non-contrast and contrast-enhanced MRI methods [56]. Dynamic MRI may one day be used to assess diaphragm mechanics and ILD patients' progressive lung fibrosis [57].

Nuclear Imaging

Molecular imaging is a noninvasive imaging technique such as PET and SPECT, which use biomarkers to target the diseased lung. Definition of biomarker by The U.S. Food and Drug Administration is a characteristic that is measured as an indicator of normal biologic processes, pathogenic processes, or responses to an exposure or intervention, including therapeutic interventions [58]. For scintigraphic identification of ILD, 67Ga-citrate, 111In- octreotide,

and 99mTc-diethylene triamine pentaacetic acid (99mTcDTPA) aerosol have been proposed in nuclear medicine, but they have not established yet for diagnosis or follow-up [59]. The conventional clinical assessment reflects disease severity rather than disease activity. Here comes the importance of developing and studying quantitative biomarkers to help demonstrate the efficacy of various drugs on the diseases processes and select the appropriate therapeutic target.

FDG-PET Scanning

^{18}F-2-deoxy-2-fluoro-D-glucose (FDG) positron emission tomography (PET) is not routinely obtained in ILDs evaluation. Because ILDs have a concomitant inflammatory process and recruitment of cells that utilize more glucose, the FDG is believed to work as a promising biomarker in ILDs. ^{18}F-FDG PET imaging is used in studying and detecting lung fibrosis and pulmonary inflammation. Furthermore, FDG uptake can also be seen in sarcoidosis and pulmonary Langerhans cell histiocytosis while non-FDG uptake is seen in lung scar tissue, which is useful to differentiate fibrotic scar tissue from inflammatory lesion. A major drawback of ^{18}F-FDG PET scan is that it does not differentiate benign from malignant causes of ILDs. In a study on interstitial lung disease patients, pulmonary ^{18}F-FDG metabolism increased in all patients [60]. ^{18}F-FDG PET assessment can disentangle ILD from normal lung tissue in systemic sclerosis, suggesting that ^{18}F-FDG PET may be used as a new biomarker for systemic sclerosis-ILD disease activity [61]. SUVmax showed increase in ^{18}F-FDG uptake in the lungs of patients with IPF [62-65]. Findings confirm the potential clinical use of ^{18}F-FDG uptake (SUVmax) in this illness. Further research is required for more optimization and standardization of PET imaging in ILDs.

99mTc-MIBI Lung Scintigraphy

The 99mTc-methoxy-isobutyl-isonitrile (MIBI) is a lipophilic imaging agent frequently used to detect coronary artery disease via scintigraphy. This chemical become trapped in mitochondria. 99mTc-MIBI is taken up in vivo by tissues with abundant mitochondria, such as cardiac muscle and malignancies. Although cancerous tissues in the lungs have been shown to have abnormal 99mTc-MIBI activity, pulmonary absorption in non-neoplastic situations appears to be rare [66]. Despite the promising results from preclinical and pilot trials, this drug is not yet used in routine clinical practice.

Gallium-67 Scans

In fact, a gadolinium-based contrast agent is washed out from normal tissue but not from fibrotic tissue. Sarcoidosis and

lung infections could be diagnosed with 67Ga-citrate. However, the precise mechanism of gallium absorption and retention by inflammatory lung tissue is yet unknown. Gallium, bound to transferrin, has been demonstrated to be transported to the site of acute inflammation. Patients with severe pulmonary fibrosis have a significant absorption of 67Ga-citrate in the lungs, although this is unrelated to the disease's laboratory indicators. So, Gallium-67 Scans is not specific enough to be used in the standard diagnostic modalities for ILDs [67].

Evaluation of Early Stages of Idiopathic Pulmonary Fibrosis

Radiographic evidence of disease, even in the absence of symptoms, suggests the presence of "subclinical" stages of the illness that are poorly understood [68]. Early identification of IPF is becoming increasingly important due to the availability of treatments that can delay the illness's development. Providing therapy at the "window of opportunity" could lead to optimal outcomes [69]. Thin-slice chest HRCT screening for interstitial lung disease can be encouraged as a "spinoff " of lung cancer screening programs in smokers to get earlier diagnosis of IPF. Early detection of crackles on lung auscultation has been recommended as a critical component of early diagnosis of IPF. Various histologic subtypes of interstitial lung disease can be seen in lung biopsy histopathology

from people with early ILD. Jin et al. used low-dose CT to screen cigarette smokers for lung cancer, intending to assess the incidence of interstitial lung lesions at the initial CT examination and the incidence of development of interstitial lung lesions on 2-year follow-up CT scans. 37% of patients had fibrotic interstitial lung abnormalities developed to some typical IPF patterns at 2-year follow-up CT scan, and none had regressed at that time [70]. In diagnosing patients with asymptomatic ILD, HRCT scan is more sensitive than cardiopulmonary exercise and pulmonary function tests [71].

Activated pulmonary fibroblasts are major cellular sources of collagen synthesis. Activation of pulmonary fibroblasts presents in whole process of lung fibrosis from non-visual collagen deposit in the lung tissue to severe scar formation. Activated pulmonary fibroblasts uptake glucose as the energy of the cell activation, and the more numbers of activated pulmonary fibroblasts in the lung, the greater amount of glucose consumption of pulmonary fibroblasts. Thus FDG, an analog of glucose, may be used as a PET radiotracer of ^{18}F-FDG to quantify radiotracer uptake of the lung which may indicate activation grades of lung collagen synthesis in activated pulmonary fibroblasts in early-stage lung fibrosis (Figure 2B) compared to the healthy subject (Figure 2A). However, ^{18}F-FDG is a non-specific radiotracer which is uptaken by non-pulmonary fibroblasts including inflammatory cells (Figure 2C).
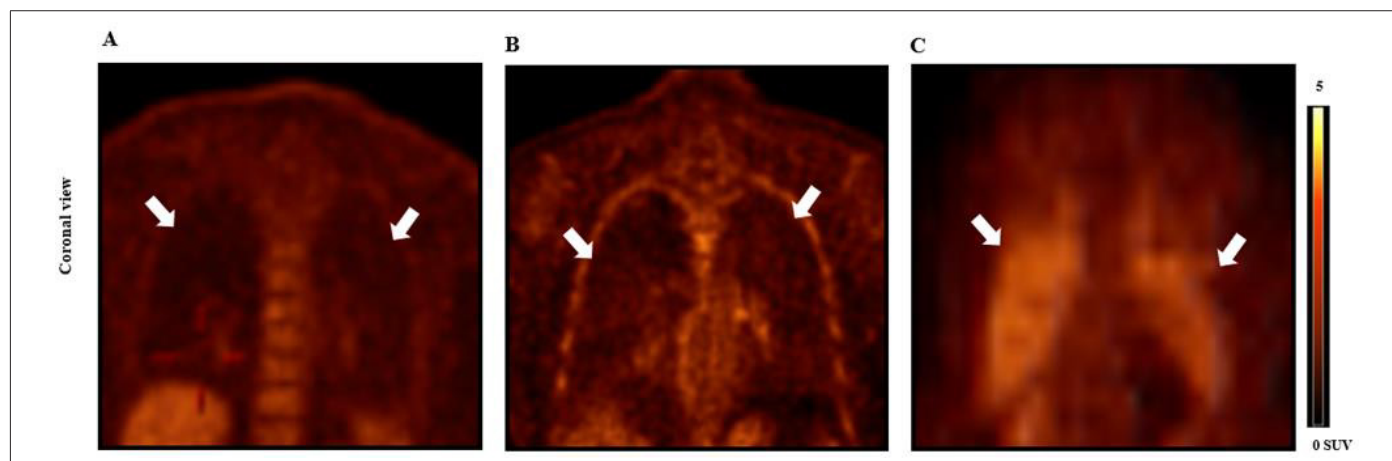

Figure 2: Human 18F-FDG PET imaging lung inflammatory and fibrotic cellular activation.

Note*: (A) ^{18}F-FDG PET lung imaging of healthy subject (SUVmax = 0.37, SUVmean = 0.17). (B) ^{18}F-FDG PET lung imaging of patient with early-stage interstitial lung fibrosis (SUVmax = 0.71, SUVmean = 0.46). (C) ^{18}F-FDG PET lung imaging of patient with lung radiation therapy and no evidence of pulmonary fibrosis was seen on CT (SUVmax = 1.47, SUVmean = 0.77). White arrows indicate lung fields.

In the ^{18}F-fluoroproline molecular imaging of collagenogenosis of hepatic stellate cells in experimental alcoholic steatohepatitis, unexpected findings showed increased uptake of 3H-proline specifically correspond to activation of pulmonary fibroblasts but not monocytes, pneumocytes, and endothelial cells in vitro isolated

from lung tissue of alcohol-fed mice (lower panel, Figure 3A) compared with control mice (top panel, Figure 3A). In treatment of LPS, PM 2.5 or Bleomycin, PFCs in vitro isolated from alcohol-fed mice increased uptake of 3H-proline (top panel, Figure 3B), which corresponds to increased lung collagen expression (lower panel,

Figure 3B). In vivo whole-body ^{18}F-fluoroproline PET imaging demonstrated increased standardized uptake values of both SUVmean and SUVmax in alcohol-fed mouse with status treatment of lipopolysaccharide (right column, Figure 3C). ^{18}F-fluoroproline uptake in the lung and other parts of the body was almost completely diminished in the same mouse with pre-administration of unlabeled fluoroproline (middle column, Figure 3C), indicating sharing proline transporter on cellular membranes of both ^{18}F-fluoroproline and unlabeled fluoroproline. These interesting findings of lung collagen synthesis activation by fibrotic producing cells in in vitro and in vivo experiments raise expectations that in vivo ^{18}F-fluoroproline PET imaging in application for early-stage lung collagenogenesis of activated pulmonary fibroblasts should be expanded into various experimental lung diseases. One unsurprised clinical study demonstrated no significance of ^{18}F-fluoroproline PET imaging lung fibrosis in patients [72].

In this study, subjects underwent to the imaging were at the end-stage "lung cirrhosis" (severe scarring lung), accepted long-term anti-fibrotic and anti-inflammatory therapy, and in very small numbers of only 5 patients with various complicative medical histories. In addition, subjects with early-stage lung fibrosis and normal healthy volunteers were not included. Our findings suggest that application of ^{18}F-fluoroproline PET imaging to early-stage lung fibrosis has significance because this non-invasive imaging technique may be useful for the detection of early-stage lung fibrosis (without lung scar and even no visualization of collagen deposits), monitoring progression of lung fibrosis, and assessment of therapeutic response in experimental lung diseases. In addition, our experimental lung fibrosis study of ^{18}F-fluoroproline PET imaging early-stage and reversible lung fibrosis overcomes human study's limitation [73-76]. For example, patients may have history of various environmental exposures to pathogens, accepting different treatment managements before performing the diagnostic imaging, presenting end-stage lung cirrhosis at the time of ^{18}F-fluoroproline PET imaging. Importantly, this non-invasive in vivo imaging technique omits invasive lung biopsy procedure-caused lung tissue damages and bleeding risk (Figure 3).
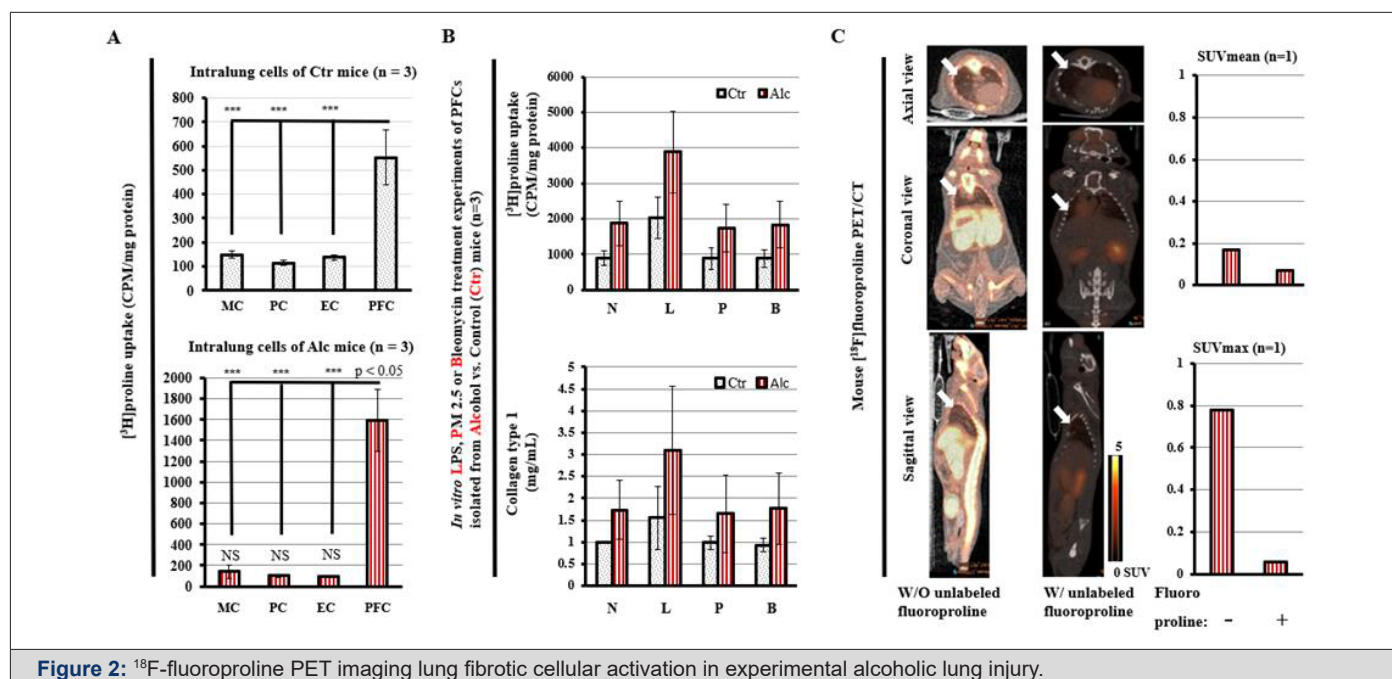

Figure 2: ^{18}F-fluoroproline PET imaging lung fibrotic cellular activation in experimental alcoholic lung injury.

(A) 3H-proline uptake by *in vitro* monocytes (MC), pneumocytes (PC), endothelial cells (EC), and PFCs isolated from lung tissues of alcohol-fed mice with status administration of lipopolysaccharides (lower panel) and controls (top panel). [3H]-proline uptake by culture activated PFCs was significantly higher in the alcohol-mediated lung injury mice treated with lipopolysaccharides compared to the control mice (p<0.05). ***, p<0.001 vs. PFCs in Ctr mice. NS, no significant difference of 3H-proline uptake by MC, PC and EC of alcohol-fed mice vs. control mice. (B) 3H-proline uptake (top panel) and collagen type 1 in culture media by PFCs isolated from alcohol-fed mice and control mice. 0.5x105 cells/mL were seeded in 1-mL culture dishes for overnight, and then treated for upto 60-min incubation with LPS at 10 mg/mL, PM2.5 at 50 mg/mL, or bleomycin at 20 mg/mL, respectively. N: no treatment, L: LPS, P: PM 2.5, B: Bleomycin, Alc: Alcohol, Ctr: Control. (C) ^{18}F-fluoroproline PET/CT images and SUVs. Axial, coronal, and sagittal views of

[18]F-fluoroproline PET/CT imaging in the same mouse without pre-administration of fluoroproline (left column) and with pre-administration of fluoroproline (5 mg/kg iv, middle column). Lung mean SUV (top panel, right column) and lung maximum SUV (lower panel, right column). The mouse was fed alcohol diet treated with status administration of LPS ip. injection and was imaged under the same imaging condition of 40-60 min after 1.0 MBq/g body weight ^{18}F-fluoroproline at two separated dates.

Summary

In the differential diagnosis of ILDs, HRCT is crucial. The ultimate diagnosis necessitates integrating radiological, clinical, and serological data, which is best achieved through an interdisciplinary approach. In many situations, the diagnosis made in this manner is so certain that histological confirmation isn't required. Lack of defined outcome measures to determine response to therapy handles clinical management of ILD patients. The utility of HRCT in evaluating ILD development and treatment response is still debatable. MRI imaging resolution does not match that of HRCT, but it might be helpful for functional imaging in ILD patients. Identification and quantification of MRI for inflammatory changes, using various tissue signal weighting, could be applied to track the efficacy of novel ILD medications. MRI may distinguish between active inflammatory and fibrotic changes in individuals with ILD, allowing physicians for more focused treatment. To establish whether MRI plays a role in the care of patients with ILD, further research will need to prove these assumptions. Functional MRI may fill the diagnostic gaps HRCT imaging of ILD patients leaves. HRCT has significant advancements in quantitative CT in semiautomated lung segmentation and HRCT has been improved success in the automated detection of honeycombing through texture analysis, as well as novel methods for evaluating the related pulmonary vascular alterations. While comorbidities such as emphysema can have an impact on physiological parameters of disease progression such as PFTs, quantitative CT should ideally be able to measure interstitial structural change precisely and objectively as an independent parameter, allowing for effective treatment response assessment and objective disease progression assessment. These approaches now require different degrees of manual input that should be automated before the technology can be applied to routine clinical practice. A few MRI measures, including conventional, hyperpolarized gas, dynamic contrast-enhanced MRI, and oxygen-enhanced, are being investigated.

There are several promising techniques available; however, testing the repeatability of derived metrics, and longitudinal observation in patients with ILD are necessary to determine accurate disease parameters. There is no standardized means for the lung US examination in ILD, but most studies included a small cohort of patients, which might cause bias due to changes in lung parenchyma. US has the necessary characteristics to improve the clinical practice in the detection of ILD. Application of the imaging technology is being researched, and more ongoing research methodologies are anticipated to occur, particularly in multicenter studies to verify its concurrent reliability, responsiveness, and validity. For phenotyping cellular metabolism and function of pulmonary lesions and fibrosis, evaluating the efficiency of novel targeted therapies and monitoring disease progression, researchers should look more into ^{18}F-FDG uptake and kinetic parameters in diffuse lung disorders. Regional differences in air, water, and blood fractions, on the other hand, can contribute to incorrect lung cell tracer concentration estimations. So, more standardization studies should be held. The repeatability and reliability of these outcome techniques will also be tested in patients with a variety of diffuse lung illnesses as well as healthy controls. Finally, combining PET quantification parameters with clinical data from lung tissue samples, pulmonary function tests, or CT scans might give extra context for interpreting PET quantification values appropriately. These are suggested as future research topics to help standardize PET analytical methods for lung imaging. The ability of ^{18}F-fluoroproline PET quantification of collagenogenesis in pulmonary myofibroblast cells introduced another non-invasive molecular technique in assessment of early lung fibrosis. This review highlights the necessity for lung imaging collaboration that promotes data and protocol exchange. This will allow validation across a broad array of lung diseases, resulting in uniform data collection and processing.

Author Contributions

Qi Cao, PI of the project, oversaw the project and wrote the manuscript.

Youssef Elshafee Ali, Hongxia Chen, Liya Pi, Jingqing Liang, and Li Yang provided data collection.

Hongxia Chen, Minjie Chen, and Xicui Sun provided data analysis and created the figures.

Li Yang, Liya Pi, Bin Ren, and Zhekang Ying are collaborators who worked with clinical and basic medical studies and revised the manuscript.

Funding

Institutional Review Board Statement

Not application.

Informed Consent Statement

Not application.

Data Availability Statement

Not application.

Acknowledgements

References

1. Antin Ozerkis D, Rubinowitz A, Evans J, Homer RJ, Matthay RA (2012) Interstitial lung disease in the connective tissue diseases. Clin Chest Med 33(1): 123-149.

2. B Bradley, H M Branley, J J Egan, M S Greaves, D M Hansell, et al. (2008) Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society. Thorax 63 Suppl (5): 1-58.

3. Brown KK (2006) Chronic cough due to chronic interstitial pulmonary diseases: ACCP evidence-based clinical practice guidelines. Chest 129(1 Suppl): 180S-185S.

4. Wallis A, Spinks K (2015) The diagnosis and management of interstitial lung diseases. BMJ 350: h2072.

5. Ferguson EC, Berkowitz EA (2012) Lung CT: Part 2, The interstitial pneumonias--clinical, histologic, and CT manifestations. AJR Am J Roentgenol 199(4): W464-476.

6. Wittram C, Mark EJ, McLoud TC (2003) CT-histologic correlation of the ATS/ERS 2002 classification of idiopathic interstitial pneumonias. Radiogr Rev Publ Radiol Soc N Am Inc 23(5): 1057-1071.

7. Kim EA, Lee KS, Johkoh T, Kim TS, Suh GY, et al (2002) Interstitial lung diseases associated with collagen vascular diseases: radiologic and histopathologic findings. Radiogr Rev Publ Radiol Soc N Am Inc Spec No: S151-165.

8. Mueller Mang C, Grosse C, Schmid K, Stiebellehner L, Bankier AA (2007) What every radiologist should know about idiopathic interstitial pneumonias. Radiogr Rev Publ Radiol Soc N Am Inc 27(3): 595-615.

9. Attili AK, Kazerooni EA, Gross BH, Flaherty KR, Myers JL, et al. (2008) Smoking-related interstitial lung disease: radiologic-clinical-pathologic correlation. Radiogr Rev Publ Radiol Soc N Am Inc 28(5): 1383-1396; discussion 1396-1398.

10. Zarkowski P (1988) Bite mark evidence: its worth in the eyes of the expert. J Law Ethics Dent 1(1): 47-57.

11. Raphael Borie, Pierre Le Guen, Mada Ghanem, Camille Taillé, Clairelyne Dupin, et al. (2019) The genetics of interstitial lung diseases. Eur Respir Rev [Internet]. 28(153): 190053.

12. Selman M, Rojas M, Mora AL, Pardo A (2010) Aging and interstitial lung diseases: unraveling an old forgotten player in the pathogenesis of lung fibrosis. Semin Respir Crit Care Med 31(5): 607-617.

13. Meyer KC, Decker CA (2017) Role of pirfenidone in the management of pulmonary fibrosis. Ther Clin Risk Manag 13: 427-437.

14. Jiurong Liang, Yanli Zhang, Ting Xie, Ningshan Liu, Huaiyong Chen, et al. (2016) Hyaluronan and TLR4 promote surfactant- protein-C-positive alveolar progenitor cell renewal and prevent severe pulmonary fibrosis in mice. Nat Med 22(11): 1285-1293.

15. King TE, Schwarz MI, Brown K, Tooze JA, Colby TV, et al. (2001) Idiopathic pulmonary fibrosis: relationship between histopathologic features and mortality. Am J Respir Crit Care Med 164(6): 1025-1032.

16. Fulmer JD, Roberts WC, von Gal ER, Crystal RG (1979) Morphologic-physiologic correlates of the severity of fibrosis and degree of cellularity in idiopathic pulmonary fibrosis. J Clin Invest 63(4): 665-676.

17. Watters LC, King TE, Schwarz MI, Waldron JA, Stanford RE, et al. (1986) A clinical, radiographic, and physiologic scoring system for the longitudinal assessment of patients with idiopathic pulmonary fibrosis. Am Rev Respir Dis 133(1): 97-103.

18. Glaspole IN, Wells AU, du Bois RM (2001) Lung biopsy in diffuse parenchymal lung disease. Monaldi Arch Chest Dis Arch Monaldi Mal Torace 56(3): 225-232.

19. American Thoracic Society, European Respiratory Society (2002) American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med 165(2): 277-304.

20. Miller JD, Urschel JD, Cox G, Olak J, Young JE, et al. (2000) A randomized, controlled trial comparing thoracoscopy and limited thoracotomy for lung biopsy in interstitial lung disease. Ann Thorac Surg 70(5): 1647-1650.

21. Harlow L, Rosas IO, Gochuico BR, Mikuls TR, Dellaripa PF, et al. (2013) Identification of citrullinated hsp90 isoforms as novel autoantigens in rheumatoid arthritis-associated interstitial lung disease. Arthritis Rheum 65(4): 869-879.

22. (2000) American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med 161(2 Pt 1): 646-664.

23. (2018) Diagnosis of Idiopathic Pulmonary Fibrosis. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. Am J Respir Crit Care Med 198(5): e44-e68.

24. B Felson (1979) A new look at pattern recognition of diffuse pulmonary disease. AJR Am J Roentgenol 133(2): 183-189.

25. Pipavath S, Godwin JD (2004) Imaging of interstitial lung disease. Clin Chest Med 25(3): 455-465, v-vi.

26. (2021) Interstitial lung disease. Radiology Reference Article. Radiopaedia.org.

27. Yoshinouchi T, Ohtsuki Y, Fujita J, Yamadori I, Bandoh S, et al. (2005) Nonspecific interstitial pneumonia pattern as pulmonary involvement of rheumatoid arthritis. Rheumatol Int 26(2): 121-125.

28. Hunninghake GW, Fauci AS (1979) Pulmonary involvement in the collagen vascular diseases. Am Rev Respir Dis. 119(3): 471-503.

29. Peljto AL, Zhang Y, Fingerlin TE, Ma S F, Garcia JGN, et al. (2013) Association between the MUC5B promoter polymorphism and survival in patients with idiopathic pulmonary fibrosis. JAMA 309(21): 2232-2239.

30. Juge P A, Lee JS, Ebstein E, Furukawa H, Dobrinskikh E, et al. (2018) MUC5B Promoter Variant and Rheumatoid Arthritis with Interstitial Lung Disease. N Engl J Med 379(23): 2209-2219.

31. B A Rajasekaran, D Shovlin, P Lord, C A Kelly (2001) Interstitial lung disease in patients with rheumatoid arthritis: a comparison with cryptogenic fibrosing alveolitis. Rheumatology (Oxford) 40(9):1022-5.

32. Lee H K, Kim DS, Yoo B, Seo JB, Rho J Y, et al. (2005) Histopathologic pattern and clinical features of rheumatoid arthritis-associated interstitial lung disease. Chest 127(6): 2019- 2027.

33. Ley B, Elicker BM, Hartman TE, Ryerson CJ, Vittinghoff E, et al. (2014) Idiopathic pulmonary fibrosis: CT and risk of death. Radiology 273(2): 570-579.

34. Jan Stolk, HeinPutter, Els M Bakker, Saher B Shaker. David G Parr (2007) Progression parameters for emphysema: A clinical investigation. Respiratory Medicine 101(9): 1924-1930.

35. Hartley PG, Galvin JR, Hunninghake GW, Merchant JA, Yagla SJ, et al. (1994) High-resolution CT-derived measures of lung density are valid indexes of interstitial lung disease. J Appl Physiol Bethesda Md 1985 76(1): 271-277.

36. Sverzellati N, Calabrò E, Chetta A, Concari G, Larici AR, et al. (2007) Visual score and quantitative CT indices in pulmonary fibrosis: Relationship with physiologic impairment. Radiol Med (Torino) 112(8): 1160-1172.

37. Do K H, Lee JS, Colby TV, Kitaichi M, Kim DS (2005) Nonspecific Interstitial Pneumonia Versus Usual Interstitial Pneumonia: Differences in the Density Histogram of High-Resolution CT. J Comput Assist Tomogr 29(4): 544-548.

38. Sverzellati N, Calabrò E, Chetta A, Concari G, Larici AR, et al. (2007) Visual score and quantitative CT indices in pulmonary fibrosis: Relationship with physiologic impairment. Radiol Med (Torino) 112(8): 1160-1172.

39. Sverzellati N, Zompatori M, Luca GD, Chetta A, Bnà C, Ormitti F, et al. (2005) Evaluation of quantitative CT indexes in idiopathic interstitial pneumonitis using a low-dose technique. Eur J Radiol 56(3): 370-375.

40. Rosas IO, Yao J, Avila NA, Chow CK, Gahl WA, et al. (2011) Automated quantification of high-resolution CT scan findings in individuals at risk for pulmonary fibrosis. Chest 140(6): 1590-1597.

41. Delorme S, Keller Reichenbecher MA, Zuna I, Schlegel W, Van Kaick G (1997) Usual interstitial pneumonia: quantitative assessment of high-resolution computed tomography findings by computer-assisted texture-based image analysis. Invest Radiol 32(9): 566-574.

42. Xu Y, van Beek EJ, Hwanjo Y, Guo J, McLennan G, et al. (2006) Computer-aided classification of interstitial lung diseases via MDCT: 3D adaptive multiple feature method (3D AMFM). Acad Radiol 13(8): 969-978.

43. Maldonado F, Moua T, Rajagopalan S, Karwoski RA, Raghunath S, et al. (2014) Automated quantification of radiological patterns predicts survival in idiopathic pulmonary fibrosis. Eur Respir J 43(1): 204-212.

44. Jacob J, Bartholmai BJ, Rajagopalan S, Brun AL, Egashira R, et al. (2016) Evaluation of computer-based computer tomography stratification against outcome models in connective tissue disease-related interstitial lung disease: a patient outcome study. BMC Med 14(1): 190.

45. Srivastava GN, Chokhani A, Verma A, Siddiqui Z (2020) Transthoracic ultrasonography in patients with interstitial lung disease. Lung India 37(5): 400-406.

46. Volpicelli G, Elbarbary M, Blaivas M, Lichtenstein DA, Mathis G, et al. (2012) International evidence-based recommendations for point-of-care lung ultrasound. Intensive Care Med 38(4): 577-591.

47. Sperandeo M, Varriale A, Sperandeo G, Filabozzi P, Piattelli ML, et al. (2009) Transthoracic ultrasound in the evaluation of pulmonary fibrosis: our experience. Ultrasound Med Biol 35(5): 723-729.

48. Song G, Bae SC, Lee YH (2016) Diagnostic accuracy of lung ultrasound for interstitial lung disease in patients with connective tissue diseases: a meta-analysis. Clin Exp Rheumatol 34(1): 11-16.

49. Romei C, Turturici L, Tavanti L, Miedema J, Fiorini S, et al. (2018) The use of chest magnetic resonance imaging in interstitial lung disease: a systematic review. Eur Respir Rev 27(150): 180062.

50. Hatabu H, Gaa J, Tadamura E, Edinburgh KJ, Stock KW, et al. (1999) MR imaging of pulmonary parenchyma with a half-Fourier single-shot turbo spin-echo (HASTE) sequence. Eur J Radiol 29(2): 152-159.

51. Biederer J, Mirsadraee S, Beer M, Molinari F, Hintze C, et al. (2012) MRI of the lung (3/3)-current applications and future perspectives. Insights Imaging 3(4): 373-386.

52. Hochhegger B, Souza VVS de, Marchiori E, Irion KL, Souza AS, et al. (2015) Chest magnetic resonance imaging: a protocol suggestion. Radiol Bras 48(6): 373-380.

53. Lonzetti L, Zanon M, Pacini GS, Altmayer S, Martins de Oliveira D, et al. (2019) Magnetic resonance imaging of interstitial lung diseases: A state-of-the-art review. Respir Med 155: 79-85.

54. Kauczor HU, Hanke A, van Beek EJ (2002) Assessment of lung ventilation by MR imaging: status and future perspectives. Eur Radiol 12(8): 1962-1970.

55. Lee CU, White DB, Sykes AMG (2014) Establishing a Chest MRI Practice and its Clinical Applications: Our Insight and Protocols. J Clin Imaging Sci 4:17.

56. Yi CA, Lee KS, Han J, Chung MP, Chung MJ, et al. (2008) 3-T MRI for Differentiating Inflammation-and Fibrosis-Predominant Lesions of Usual and Nonspecific Interstitial Pneumonia: Comparison Study with Pathologic Correlation. Am J Roentgenol 190(4): 878-885.

57. Ciet P, Tiddens HAWM, Wielopolski PA, Wild JM, Lee EY, et al. (2015) Magnetic resonance imaging in children: common problems and possible solutions for lung and airways imaging. Pediatr Radiol 45(13): 1901-1915.

58. (2021) Research C for DE and. Drug Development Tool (DDT) Qualification Programs. FDA.

59. Lin E, Limper AH, Moua T (2018) Obliterative bronchiolitis associated with rheumatoid arthritis: analysis of a single-center case series. BMC Pulm Med 18(1): 105.

60. Peelen DM, Zwezerijnen BGJC, Nossent EJ, Meijboom LJ, Hoekstra OS, et al. (2020) The quantitative assessment of interstitial lung disease with positron emission tomography scanning in systemic sclerosis patients. Rheumatology 59(6): 1407-1415.

61. Win T, Thomas BA, Lambrou T, Hutton BF, Screaton NJ, Porter JC, et al. (2014) Areas of normal pulmonary parenchyma on HRCT exhibit increased FDG PET signal in IPF patients. Eur J Nucl Med Mol Imaging 41(2): 337-342.

62. Groves AM, Win T, Screaton NJ, Berovic M, Endozo R, Booth H, et al. (2009) Idiopathic pulmonary fibrosis, and diffuse parenchymal lung disease: implications from initial experience with ^{18}F-FDG PET/CT. J Nucl Med Off Publ Soc Nucl Med 50(4): 538-545.

63. Groves AM, Win T, Screaton NJ, Berovic M, Endozo R, Booth H, et al. (2009) Idiopathic pulmonary fibrosis, and diffuse parenchymal lung disease: implications from initial experience with ^{18}F-FDG PET/CT. J Nucl Med Off Publ Soc Nucl Med 50(4): 538-545.

64. Win T, Lambrou T, Hutton BF, Kayani I, Screaton NJ, Porter JC, et al. (2012) 18F-Fluorodeoxyglucose positron emission tomography pulmonary imaging in idiopathic pulmonary fibrosis is reproducible: implications for future clinical trials. Eur J Nucl Med Mol Imaging 39(3): 521-528.

65. Lambrou T, Groves AM, Erlandsson K, Screaton N, Endozo R, Win T, et al. (2011) The importance of correction for tissue fraction effects in lung PET: preliminary findings. Eur J Nucl Med Mol Imaging 38(12): 2238-2246.

66. Bahtouee M, Saberifard J, Javadi H, Nabipour I, Raeisi A, et al. (2015) 99mTc-MIBI Lung Scintigraphy in the Assessment of Pulmonary Involvement in Interstitial Lung Disease and Its Comparison with Pulmonary Function Tests and High-Resolution Computed Tomography: A Preliminary Study. Medicine (Baltimore) 94(47): e2082.

67. Ramsay Stuart C, Yeates Michael G, Burke William MJ, Bryant David H, Morgan Graeme W, et al. (1992) Quantitative pulmonary gallium scanning in interstitial lung disease. Eur J Nucl Med 19(2): 80-85.

68. Nagai S, Nagao T, Kitaichi M, Izumi T (1998) Clinical courses of asymptomatic cases with idiopathic pulmonary fibrosis and a histology of usual interstitial pneumonia. Eur Respir J 11: 131s.

69. Ley B, Collard HR, King TE (2011) Clinical Course and Prediction of Survival in Idiopathic Pulmonary Fibrosis. Am J Respir Crit Care Med 183(4): 431-440.

70. Jin GY, Lynch D, Chawla A, Garg K, Tammemagi MC, et al. (2013) Interstitial Lung Abnormalities in a CT Lung Cancer Screening Population: Prevalence and Progression Rate. Radiology 268(2): 563-571.

71. Rosas IO, Ren P, Avila NA, Chow CK, Franks TJ, et al. (2007) Early Interstitial Lung Disease in Familial Pulmonary Fibrosis. Am J Respir Crit Care Med 176(7): 698-705.

72. Lavalaye J, Grutters JC, van de Garde EM, van Buul MM, van den Bosch JM, et al. (2009) Imaging of fibrogenesis in patients with idiopathic pulmonary fibrosis with cis-4- [(18)F]-Fluoro-L-proline PET. Mol Imaging Biol 11(2): 123-127.

73. Cao Q, Lu X, Azad B, Pomper M, Smith M, et al. (2020) *cis*-4-[18F] fluoro-L-proline Molecular Imaging Experimental Liver Fibrosis. Frontiers in Molecular Bioscience 7: 90-107.

74. Sun X, Duan S, Cao A, Villagomez B, Lin R, et al. (2021) RRY Inhibits Amyloid-β_{1-42} Peptide Aggregation and Neurotoxicity. J Alzheimer's disease Report 5(1): 479-495.

75. Li S, Sun X, Chen H, Liang J, Chen M, et al. (2022) Deletion of Interleukin 1 Receptor-Associated Kinase 1 Improves TLR4/STAT1/PPARγ-Cd36 Signaling-M3. Ediated Alcoholic Fatty Liver. Advanced Res Gastroenterol Hepatol 18(2): 1-9.

76. Wan Y, Chen H, Li Z, Liang J, Sun X, et al. (2022) Alcohol and Advanced Glycation End Product Synergistically Stimulate TNF-α and TGF-β1 Production by Cytochrome P450 2E1 Expressing Macrophages. Am J Biomed Sci Res 15 (1): 54-65.