frontiers | Research Topics

Immune-mediated lung injury

Edited by
Theodoros Karampitsakos, Argyrios Tzouvelekis and Paolo Spagnolo

Published in
Frontiers in Medicine

ISSN 1664-8714
ISBN 978-2-8325-3962-0
DOI 10.3389/978-2-8325-3962-0

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Immune-mediated lung injury

Topic editors

Theodoros Karampitsakos — University of South Florida, United States

Argyrios Tzouvelekis — Alexander Fleming Biomedical Sciences Research Center, Greece

Paolo Spagnolo — University of Padua, Italy

Citation

Karampitsakos, T., Tzouvelekis, A., Spagnolo, P., eds. (2023). *Immune-mediated lung injury*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-3962-0

Table of contents

frontiers | Frontiers in Medicine

TYPE Editorial
PUBLISHED 03 November 2023
DOI 10.3389/fmed.2023.1292074

OPEN ACCESS

EDITED AND REVIEWED BY
Ilias C. Papanikolaou,
General Hospital of Corfu, Greece

*CORRESPONDENCE
Argyris Tzouvelekis
✉ atzouvelekis@upatras.gr

RECEIVED 10 September 2023
ACCEPTED 13 October 2023
PUBLISHED 03 November 2023

CITATION
Karampitsakos T, Spagnolo P and Tzouvelekis A (2023) Editorial: Immune-mediated lung injury. *Front. Med.* 10:1292074. doi: 10.3389/fmed.2023.1292074

Editorial: Immune-mediated lung injury

Theodoros Karampitsakos[1], Paolo Spagnolo[2] and Argyris Tzouvelekis[3]*

[1]Division of Pulmonary, Critical Care and Sleep Medicine, Ubben Center for Pulmonary Fibrosis Research, Department of Internal Medicine, Morsani College of Medicine, University of South Florida, Tampa, FL, United States, [2]Respiratory Disease Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova, Padova, Italy, [3]Department of Respiratory Medicine, University Hospital of Patras, Patras, Greece

KEYWORDS

post-COVID, long-COVID, post-COVID-19-interstitial lung disease, idiopathic pulmonary fibrosis, immunity, monocytes

Editorial on the Research Topic
Immune-mediated lung injury

The dawn of a new immune-mediated lung disease: post-COVID-19 interstitial lung disease

The role of immunity in acute and chronic lung injury has been now established (Sweis et al.) (1, 2). With regards to interstitial lung diseases (ILDs), a possible association with immunity was first reported almost 50 years ago. In particular, Crystal et al. reported that "The fibrotic process is probably irreversible, however the inflammatory and immune processes causing it may be amenable to therapy if diagnosed early" (3). Despite further reports linking immune deregulation and chronic lung injury, the role of immunity had been severely underscored in the past mainly due to the disappointing results of immunosuppressive and immunomodulatory agents such as corticosteroids in patients with idiopathic pulmonary fibrosis (IPF) (4). Most recently, the interest has been revived. Clinical and translational observations fueled mechanistic discoveries on the role of immunity in lung injury. For example, cellular deconvolution of the 52-gene signature, a highly reproducible biomarker in IPF, showed that monocytes are the cellular source of the upregulated genes. This paved the way for clinical studies showing the prognostic potential of monocyte count in ILDs as well as mechanistic studies investigating the role of monocytes/myeloid derived suppressor cells in pulmonary fibrosis (5–9).

Given the increased interest for the association of immunity with lung injury, this Research Topic included articles presenting immune insights in the context of IPF as well as articles highlighting the impact of ILD in patients with connective tissue diseases such as scleroderma and myositis (Kirgou et al.; Liossis and Bounia; Liossis and Staveri; Karampitsakos et al.). Importantly, this Research Topic highlighted the dawn of a new immune-mediated lung disease, named post-COVID-19-ILD (Bernardinello et al.; Karampitsakos et al.). In particular, data from the Greek registry of patients with post-COVID-19-ILD showed that Forced Vital Capacity% predicted and Diffusing capacity for carbon monoxide% predicted were below 80% in 25.8 and 30.6% of patients in the 3-month follow up, respectively (Karampitsakos et al.). Of note, 5.6% of patients presented with "fibrotic-like" changes and persistent functional impairment at the 6-month follow-up leading thus to implementation of antifibrotics. Similarly, an Italian study demonstrated that 6.9% of the cohort had not recovered in terms of lung disease in the 1-year follow-up (Bernardinello et al.). Patients that did not recover in the 1-year follow-up were older, more

frequently current smokers and had worse PaO_2/FiO_2 on admission at the time point of hospitalization compared to patients that recovered (Bernardinello et al.). Given that both studies showed that a (not negligible) minority of patients with COVID-19 exhibit persistent lung disease even 1 year following acute infection, questions about the long-term trajectory of these patients arise. Taking into consideration that COVID-19 and fibrotic-ILDs have (1) common radiographic features and (2) common innate and adaptive immune responses [the aforementioned gene-signature that predicted outcomes in IPF, predicted outcomes in COVID-19, as well (10)], the following remain to be addressed:

1) Are "fibrotic-like" changes in patients with post-COVID-19 reversible? Does radiologic fibrosis necessarily mean histologic fibrosis? Will the treatment of "immature fibrosis" prevent irreversible disease or radiographic findings will resolve/not progress irrespective of treatment? Ongoing studies will hopefully shed light to these questions.
2) Are genes that predict mortality in IPF and COVID-19, still abundantly expressed in post-COVID-19-ILD? Extensive investigation of genes that predict mortality in IPF suggested that monocytes/myeloid derived suppressor cells persist during the disease course, while T cells might exhibit exhaustion (5–11). Similarly in COVID-19, myeloid cells have been shown to be highly activated (12), with dysfunctional HLA-$DR^{lo}CD163^{hi}$ and HLA-$DR^{lo}S100A^{hi}$ $CD14^{+}$ monocytes being present in patients with severe disease (13). Moreover, T cell subpopulations of patients with SARS-CoV-2 infection had exhaustion features (14–16).
   However, further data are needed to understand if the aforementioned phenomenon persists in post-COVID-19-ILD. If not, a less abundant expression of genes that are directly related with monocytes, might mean a gradual "immune recovery" in post-COVID-19-ILD and probably a favorable long-term course. Contrary to IPF, persistence of monocytes and impaired T cell response might not be the case in post-COVID-19-ILD, as the epithelial injury happened only in the acute phase of infection and is not repetitive (17, 18). Studies implementing single-cell RNA-sequencing to compare post-COVID-19-ILD and IPF could hopefully address this unmet need and help clinicians "predict" the long-term outcomes of patients with post-COVID-19-ILD.
   Addressing the two aforementioned questions, will substantially contribute to the management of this new entity with unknown long-term consequences. Despite that the acute phase of the pandemic is thankfully over, clinicians should not forget that a minority of COVID-19 survivors have persistent lung disease. Timely and appropriate management of this new entity might positively impact patients' health-related quality of life on a long-term basis.

Author contributions

TK: Conceptualization, Investigation, Writing—original draft. PS: Conceptualization, Investigation, Writing—original draft. AT: Conceptualization, Investigation, Writing—original draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Publisher's note

References

1. Kumar V. Pulmonary innate immune response determines the outcome of inflammation during pneumonia and sepsis-associated acute lung injury. *Front Immunol.* (2020) 11:1722. doi: 10.3389/fimmu.2020.01722

2. Karampitsakos T, Papaioannou O, Tsiri P, Katsaras M, Katsimpris A, Kalogeropoulos AP, et al. Tocilizumab versus baricitinib in hospitalized patients with severe COVID-19: an open label, randomized controlled trial. *Clin Microbiol Infect.* (2023) 29:372–8. doi: 10.1016/j.cmi.2022.10.015

3. Crystal RG, Fulmer JD, Roberts WC, Moss ML, Line BR, Reynolds HY. Idiopathic pulmonary fibrosis. Clinical, histologic, radiographic, physiologic, scintigraphic, cytologic, and biochemical aspects. *Ann Intern Med.* (1976) 85:769–88. doi: 10.7326/0003-4819-85-6-769

4. Idiopathic Pulmonary Fibrosis Clinical Research N, Raghu G, Anstrom KJ, King TE, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. *The New England J Med.* (2012) 366:1968–77. doi: 10.1056/NEJMoa1113354

5. Scott MKD, Quinn K, Li Q, Carroll R, Warsinske H, Vallania F, et al. Increased monocyte count as a cellular biomarker for poor outcomes in fibrotic diseases: a retrospective, multicentre cohort study. *Lancet Respir Med.* (2019) 7:497–508. doi: 10.1016/S2213-2600(18)30508-3

6. Karampitsakos T, Torrisi S, Antoniou K, Manali E, Korbila I, Papaioannou O, et al. Increased monocyte count and red cell distribution width as prognostic biomarkers in patients with idiopathic pulmonary fibrosis. *Respir Res.* (2021) 22:140. doi: 10.1186/s12931-021-01725-9

7. Kreuter M, Lee JS, Tzouvelekis A, Oldham JM, Molyneaux PL, Weycker D, et al. Monocyte count as a prognostic biomarker in patients with idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* (2021) 204:74–81. doi: 10.1164/rccm.202003-0669OC

8. Herazo-Maya JD, Sun J, Molyneaux PL Li Q, Villalba JA, Tzouvelekis A, et al. Validation of a 52-gene risk profile for outcome prediction in patients with idiopathic pulmonary fibrosis: an international, multicentre, cohort study. *Lancet Respir Med.* (2017) 5:857–68. doi: 10.1016/S2213-2600(17)30349-1

9. Karampitsakos T, Juan-Guardela BM, Tzouvelekis A, Herazo-Maya JD. Precision medicine advances in idiopathic pulmonary fibrosis. *EBioMedicine.* (2023) 95:104766. doi: 10.1016/j.ebiom.2023.104766

10. Juan Guardela BM, Sun J, Zhang T, Xu B, Balnis J, Huang Y, et al. 50-gene risk profiles in peripheral blood predict COVID-19 outcomes: a retrospective, multicenter cohort study. *EBioMedicine.* (2021) 69:103439. doi: 10.1016/j.ebiom.2021.103439

11. Desai SR, Hansell DM, Walker A, MacDonald SL, Chabat F, Wells AU. Quantification of emphysema: a composite physiologic index derived from CT estimation of disease extent. *Eur Radiol.* (2007) 17:911–8. doi: 10.1007/s00330-006-0369-0

12. Melms JC, Biermann J, Huang H, Wang Y, Nair A, Tagore S, et al. A molecular single-cell lung atlas of lethal COVID-19. *Nature.* (2021) 595:114–9. doi: 10.1038/s41586-021-03569-1

13. Schulte-Schrepping J, Reusch N, Paclik D, Baßler K, Schlickeiser S, Zhang B, et al. Severe COVID-19 is marked by a dysregulated myeloid cell compartment. *Cell.* (2020) 182:1419–40. doi: 10.1016/j.cell.2020.08.001

14. Kusnadi A, Ramírez-Suástegui C, Fajardo V, Chee SJ, Meckiff BJ, Simon H, et al. Severely ill COVID-19 patients display impaired exhaustion features in SARS-CoV-2-reactive CD8(+) T cells. *Sci Immunol.* (2021) 6:782. doi: 10.1126/sciimmunol.abe4782

15. Davis HE, McCorkell L, Vogel JM, Topol EJ. Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol.* (2023) 21:133–46. doi: 10.1038/s41579-022-00846-2

16. Alahdal M, Elkord E. Exhaustion and over-activation of immune cells in COVID-19: challenges and therapeutic opportunities. *Clin Immunol.* (2022) 245:109177. doi: 10.1016/j.clim.2022.109177

17. Mehta P, Estrada BS, Denneny EK, Foster K, Turner CT, Mayer A, et al. Single-cell analysis of bronchoalveolar cells in inflammatory and fibrotic post-COVID lung disease. *medRxiv.* (2023) 2023:23287759. doi: 10.1101/2023.03.28.23287759

18. Tourki B, Vera I, Perrot C, Farsaei F, Arsenault A, Juan-Guardela B, et al. Peripheral Blood Gene Expression Changes Predict Mortality and Treatment Response in Acute COVID-19 and Associate with Lung Fibrosis. In: *c32 Interrogation of Genetic Variation, Gene Expression, and Other Biological Layers With Omics Techniques to Understand Respiratory Traits*. Washington, DC: American Thoracic Society (2022), p. A3929-A.

frontiers in Medicine

ORIGINAL RESEARCH
published: 22 March 2022
doi: 10.3389/fmed.2022.846480

A Bibliometric Analysis of Pulmonary Alveolar Proteinosis From 2001 to 2021

Shixu Liu[1,2†], Xiangning Cui[1†], Kun Xia[1], Dandan Wang[1,2], Jing Han[3], Xiaoyan Yao[1], Xiaohong Liu[1,2], Lingjie Bian[4], Jinzhi Zhang[1,2] and Guangxi Li[1]*

[1] Guanganmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [2] Graduate School of China Academy of Chinese Medical Sciences, Beijing, China, [3] Affilated Hospital of Weifang Medical University, Weifang, China, [4] Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China

Background: Pulmonary alveolar proteinosis (PAP) is a rare syndrome first described by Rosen et al. in 1958. Despite our considerably evolved understanding of PAP over the past decades, no bibliometric studies have been reported on this field. We aimed to analyze and visualize the research hotspots and current trends of the PAP research field using a bibliometric analysis to help understand the future development of basic and clinical research.

Methods: The literature regarding PAP was culled from the Web of Science Core Collection (WoSCC) database. Data were extracted from the relevant articles and visually analyzed using CiteSpace and VOSviewer software.

Results: Nine hundred and nine qualifying articles were included in the analysis. Publications regarding PAP increased over time. These articles mainly come from 407 institutions of 57 countries. The leading countries were the USA and Japan. University of Cincinnati (USA) and Niigata University (Japan) featured the highest number of publications among all institutions. Bruce C Trapnell exerts a significant publication impact and has made the most outstanding contributions in the field of PAP. *American Journal of Physiology-Lung Cellular and Molecular Physiology* was the journal with the most publications, and *American Journal of Respiratory and Critical Care Medicine* was the most commonly cited journal. All the top 5 co-cited journals belong to Q1. Keyword citation bursts revealed that inflammation, deficiency, tissue resident macrophage, classification, autoimmune pulmonary alveolar proteinosis, sarcoidosis, gm csf, high resolution ct, and fetal monocyte were the emerging research hotspots.

Conclusion: Research on PAP is prosperous. International cooperation is also expected to deepen and strengthen in the future. Our results indicated that the etiology and pathogenesis of PAP, current and emerging therapies, especially the novel pathogenesis-based options will remain research hotspots in the future.

Keywords: pulmonary alveolar proteinosis (PAP), bibliometric analysis, CiteSpace, VOSviewer, autoimmune pulmonary alveolar proteinosis (aPAP), alveolar macrophage, granulocyte-macrophage colony-stimulating factor (GM-CSF)

OPEN ACCESS

Edited by:
Argyrios Tzouvelekis, Alexander Fleming Biomedical Sciences Research Center, Greece

Reviewed by:
Raphael Borie, Assistance Publique Hopitaux De Paris, France
Ilaria Campo, San Matteo Hospital Foundation (IRCCS), Italy

***Correspondence:**
Guangxi Li
lgx0410@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *31 December 2021*
Accepted: *28 February 2022*
Published: *22 March 2022*

Citation:
Liu S, Cui X, Xia K, Wang D, Han J, Yao X, Liu X, Bian L, Zhang J and Li G (2022) A Bibliometric Analysis of Pulmonary Alveolar Proteinosis From 2001 to 2021. Front. Med. 9:846480. doi: 10.3389/fmed.2022.846480

INTRODUCTION

Pulmonary alveolar proteinosis (PAP) is a rare syndrome that was first described in 1958 by Rosen et al. (1). Recently, the prevalence of PAP has been estimated to be 6.87 per million in the general population, without gender predilection (2); the evidence backing global variation in the epidemiology of PAP is insubstantial (3). PAP is characterized by altered surfactant homeostasis and resultant accumulation of lipoproteinaceous material in pulmonary alveoli and alveolar macrophages (AMs) (4, 5). Pathologically, PAP is a heterogeneous group of diseases that result from either poor surfactant clearance or abnormal surfactant production due to AMs dysfunction (3). Granulocyte-macrophage colony-stimulating factor (GM-CSF) plays a pivotal role in the terminal differentiation of AMs, which is crucial for AMs regulating innate immunity and surfactant catabolism (6).

The typical physiological consequence of PAP is impaired gas exchange, resulting in progressive dyspnoea, hypoxemia, or even respiratory failure and death (5). There is currently no cure for PAP, but it can be treated. By tradition, the standard gold therapy of PAP has been whole-lung lavage (WLL), where large quantities of saline are instilled into the lungs to clear the abnormal surfactant. Other therapeutic strategies have been investigated by targeting AMs with GM-CSF augmentation or reducing the levels of GM-CSF autoantibodies with CD20 antibody rituximab and experimental plasmapheresis. New treatment modalities, such as statins, pioglitazone, lung transplantation, and pulmonary macrophage transplantation, are promising approaches requiring further research. Notwithstanding significant progress in our understanding of PAP over the past decades, comprehensive reports that can help scholars obtain an intuitive overview and disclose trends in the PAP research field are still absent.

Bibliometric analysis is a novel scientific method used to analyze large amounts of heterogeneous literature (7). Combining visualizing processing tools like CiteSpace (8) and VOSviewer (9) helps comprehend the knowledge structure and explore developmental trends. Bibliometric analysis can not only evaluate the contributions of various authors, institutions, countries/regions, and journals, but also can predict the research hotspots and trends of a specific research field, laying the foundation for the development of future study (10, 11). However, there is still a lack of bibliometric analysis in PAP research. The present study aimed to explore the hotspots and developmental trends of PAP by analyzing historic achievements from 2001 to 2021 to provide new visions for future researchers, especially for those who have an interest but are new to this field.

MATERIALS AND METHODS

Data Collection

A literature search was conducted using the Web of Science Core Collection database on December 11, 2021. Editions selected "Science Citation Index Expanded (SCI-EXPANDED)." The search strategy was set to TS = ("pulmonary alveolar proteinosis") AND Language = English from 2001 to 2021. A total of 1,504 articles were retrieved, 595 irrelevant articles, including meeting abstracts, editorial materials, corrections, letters, retractions, and proceedings papers, were excluded. A total of 909 papers were exported in the form of full records and cited references and saved in download_txt format within 1 day (**Figure 1**).

Data Analysis

Records retrieved from Web of Science Core Collection were imported to Microsoft Excel 2019, using CiteSpace and VOSviewer to analyze and visualize retrieval results. In the atlas, nodes represent countries/regions, institutions, authors, co-cited literature, journals, and keywords; links between nodes usually mean cooperation or co-citation relationships; colors of the nodes and links alter over time.

CiteSpace version 5.8.R3 (Drexel University, Philadelphia, PA, USA) was used to draw the maps of country/region and institutional cooperation, literature, and journal co-citation. CiteSpace is a tool for progressive knowledge domain visualization developed by Chen (12). It is particularly beneficial to visualize and analyze trends and patterns in scientific literature. The primary objective of knowledge domain visualization is to uncover critical points in the development of the domain. CiteSpace provides a visual aid that portrays research hotspots and evolution processes intuitively and predicts the developmental trends of the research field (8, 13).

VOSviewer version 1.6.17 (Leiden University, Leiden, Netherlands) was used to analyze and visualize the author's cooperation and keyword co-occurrence. VOSviewer is software for constructing and viewing bibliometric networks. Unlike the conventional bibliometric tools, VOSviewer concentrates on the graphic representation of bibliometric networks. Its most prominent feature is displaying large bibliometrics in an easy-to-explain way (9).

RESULTS

The Trend of Publication Outputs

The number of publications and times cited in each period reflects the research trends in this field. As shown in **Figure 2**, the number of studies concerning PAP manifested an overall upward trend. Nine hundred and nine included scientific literature (including 735 Articles and 174 Review Articles) with the times cited of 28,550, the average citations of 31.41 per item, and an h-index of 81. 2015–2017 were the most productive period with a total of 175 articles. Despite reducing publications since 2018, Results showed that research on PAP remains at a relatively high level.

Contribution of Countries/Regions and Institutions

The maps of intercountry/regional cooperation (**Figure 3**, N = 57, E = 131) and inter-institutional cooperation (**Figure 4**, N = 473, E = 789) were generated using CiteSpace. Researchers from 407 institutions in 57 countries/regions contributed to publications on PAP between 2001 and 2021. As shown in these figures, each node represents a country or institution; the node's size is proportional to the publications. The lines between the nodes stand for cooperation between countries or institutions;

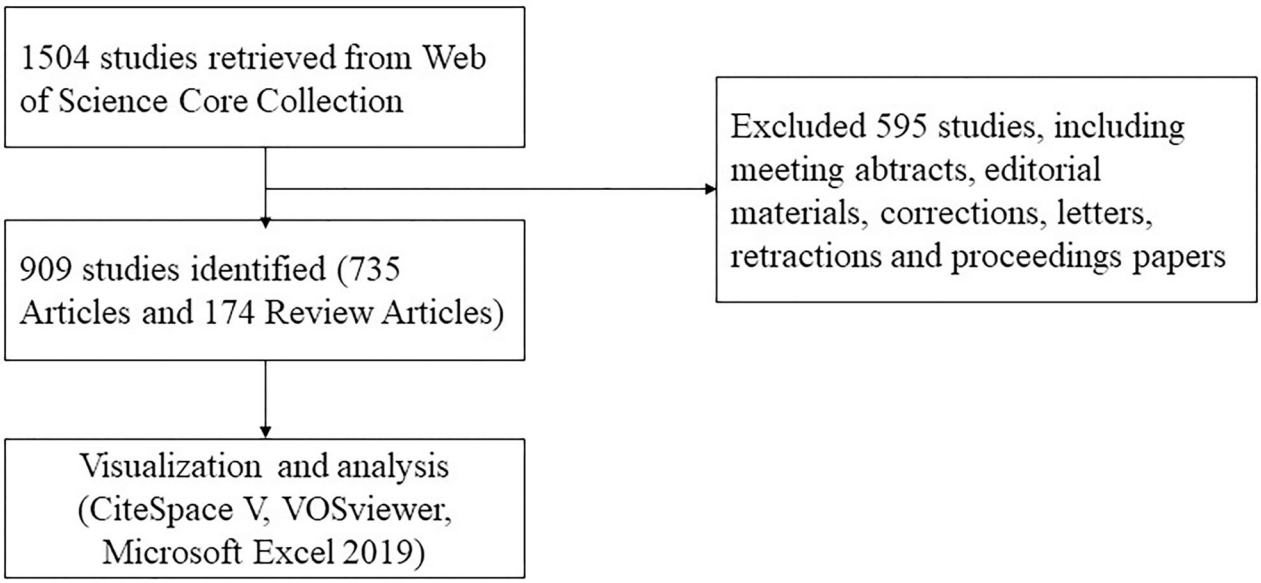

FIGURE 1 | Flowchart of literature selection.

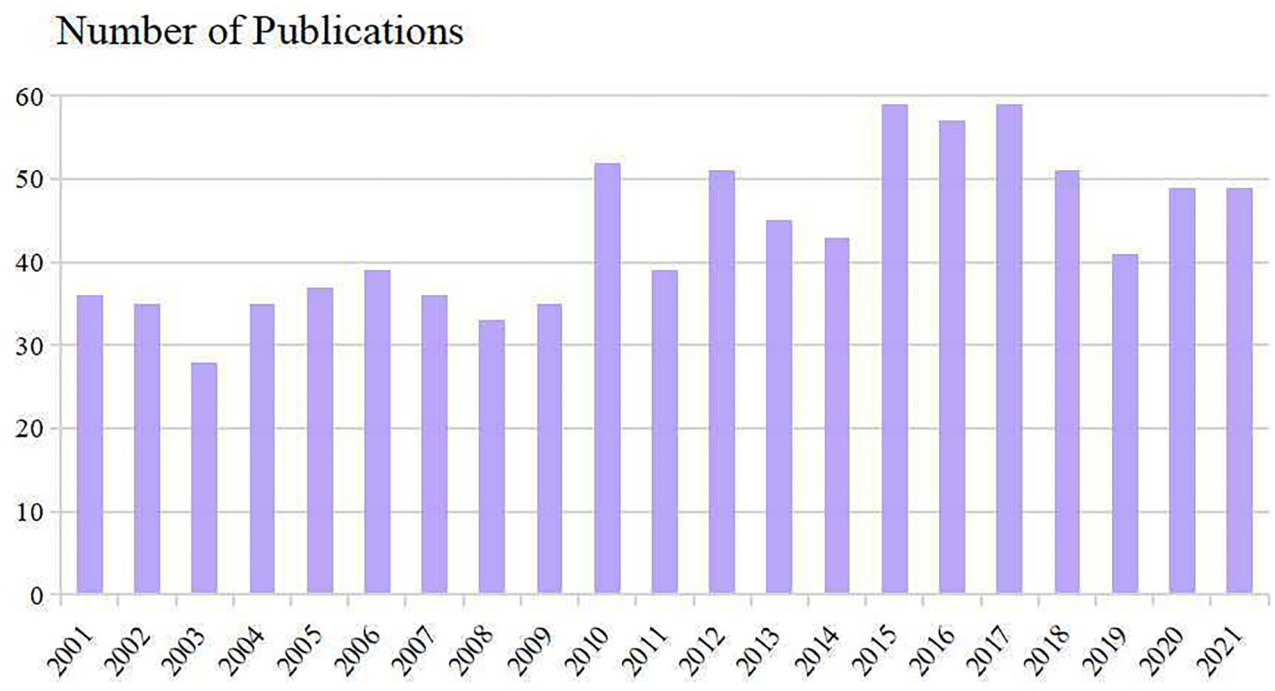

FIGURE 2 | Trends of PAP publications over the past 20 years.

the wider the lines, the closer the cooperation. **Tables 1**, **2** list the top 5 countries and institutions in this field. The largest contributor was the United States ($n = 382$, 42.0%), followed by Japan ($n = 160$, 17.6%), Germany ($n = 116$, 12.8%), China ($n = 74$, 8.1%), and Italy ($n = 56$, 6.2%). The publications from the two highest-ranked countries were nearly two-thirds of the total. University of Cincinnati (Cincinnati, Ohio, USA) was the most authoritative institution in the PAP research field, followed by Niigata University (Niigata, Niigata, Japan) and Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio, USA).

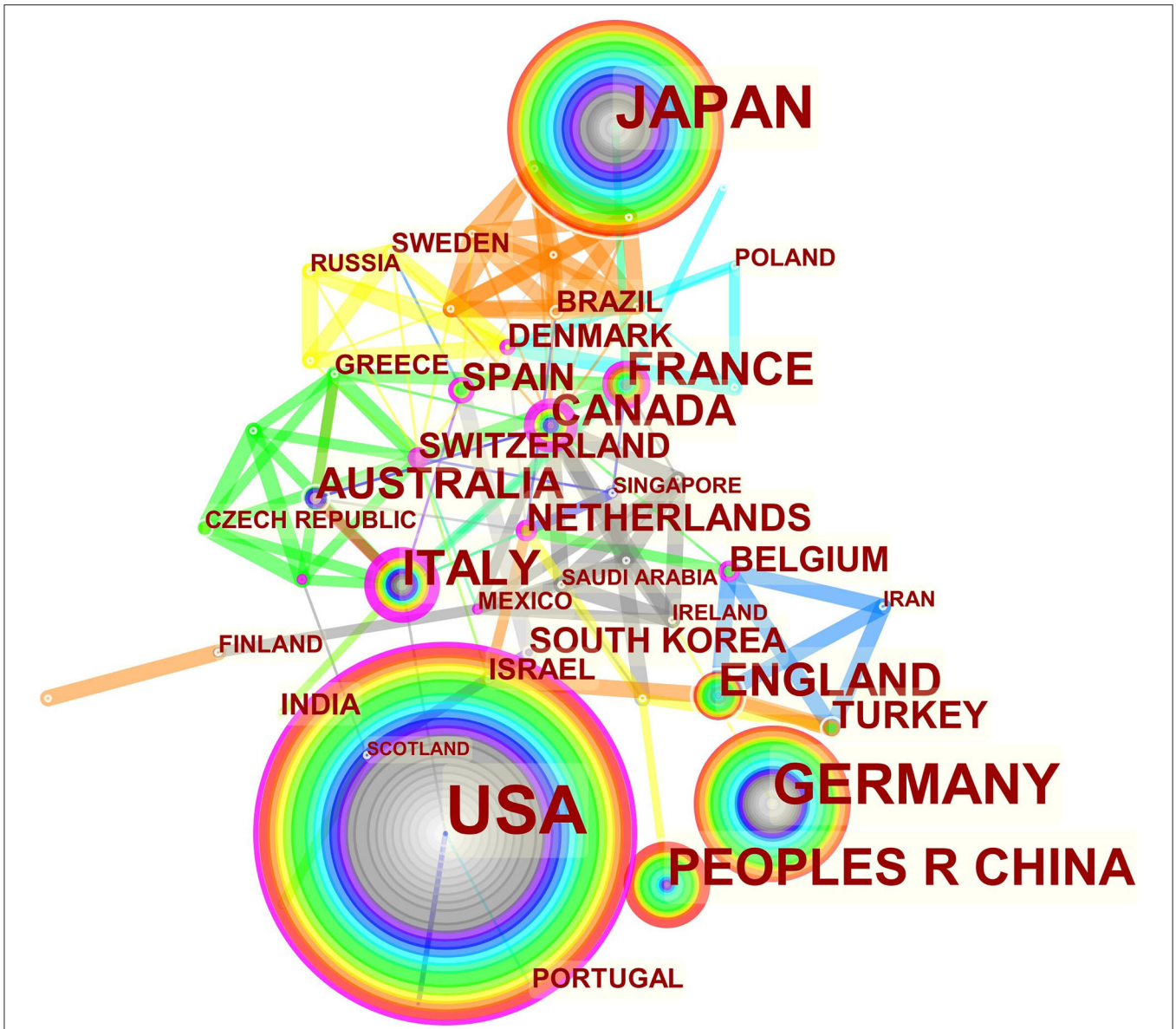

FIGURE 3 | Distribution of publications from different countries/regions.

Countries such as Switzerland, Italy, and Canada, demonstrated a high degree of centrality, as indicated by the thickness of the purple rings in **Figure 3**. It is a measure associated with the transformative potential of a scientific contribution. Such nodes tend to bridge different stages of the development of a scientific field (14). There was active cooperation among countries and institutions, including Switzerland, Australia, Italy, Niigata University, Tohoku University, Hannover Medical School, and the University of Toronto. However, most countries/regions and institutions were dispersed and lacked intensive cooperation.

Authors and Co-cited Authors

Table 3 lists the top 5 most active authors and co-cited authors. BC Trapnell had the highest number of published papers (40, 4.4%). Among the top 5 authors, K Nakata (0.05) and Y Inoue (0.05) have high centralities, which implicates that these two authors significantly impact each other's work and studies from other groups.

Co-cited authors are two or more authors cited simultaneously, and these authors form a co-citation relationship. Among all the co-cited authors, four had a citation frequency of more than 300 times. JF Seymour (503) was the most frequently cited author, followed by BC Trapnell (405).

Figure 5 generated by VOSviewer presented the collaborative relationships and found 11 cooperation teams. Each node symbolizes an author, with larger nodes indicating more publications. Wider lines represent the closer connection between authors. The connection network of different colors

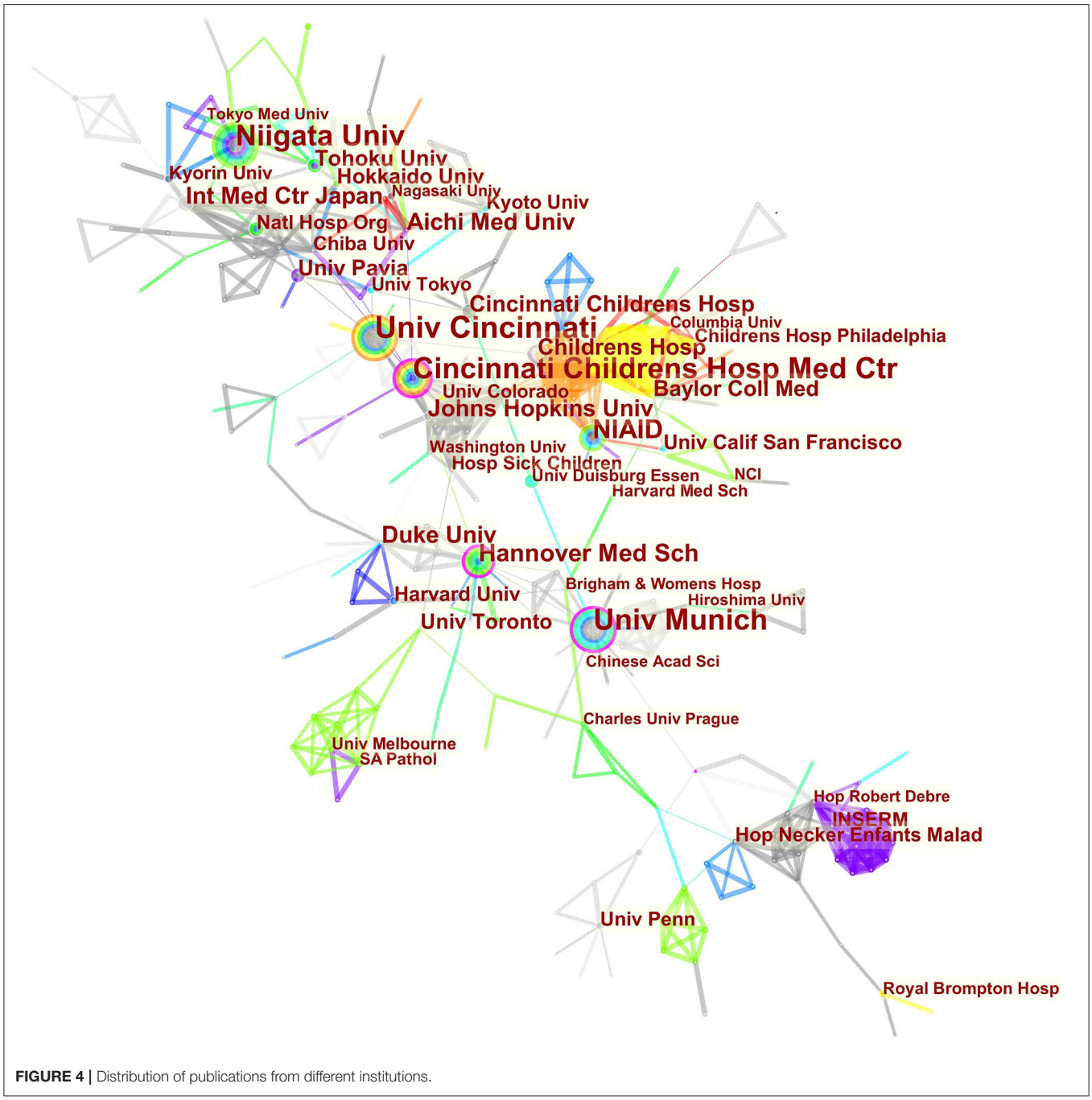

FIGURE 4 | Distribution of publications from different institutions.

manifests the cooperation cluster among authors in PAP research. As shown in **Figure 5**, there was an obvious connection network between different authors, for example, BC Trapnell, T Suzuki, K Uchida, and K Nakata, R Tazawa, T Ichiwata.

Journals and Co-cited Journals

There were 398 academic journals related to PAP. The journal *American Journal of Physiology-Lung Cellular and Molecular Physiology* had the highest number of outputs (25, 2.8%). The *American Journal of Respiratory and Critical Care Medicine* ranked second (24, 2.6%) with the highest impact factor (IF = 16.671) among the top 10 academic journals. It can be seen in **Table 4** that 80% of journals belong to Q1 and Q2 according to the journal citation reports (JCR) in 2020.

The influence of academic journals relies on the number of times they are co-cited, which shows whether the journal has notable influence in a domain. Among the top 10 co-cited journals, five journals have been cited more than 1,000 times. As shown in **Table 4**, the *American Journal of Respiratory and Critical Care Medicine* had the highest number of citations

TABLE 1 | The top 5 countries for publications and centrality in PAP research.

No.	Country	Count (%)	Country	Centrality
1	USA	382 (42.0%)	Switzerland	0.33
2	Japan	160 (17.6%)	Italy	0.31
3	Germany	116 (12.8%)	Canada	0.31
4	China	74 (8.1%)	Netherlands	0.2
5	Italy	56 (6.2%)	France	0.17

TABLE 2 | The top 5 institutions for publications and centrality in PAP research.

No.	Institution	Count (%)	Institution	Centrality
1	Univ Cincinnati	36 (4.0%)	Cincinnati Childrens Hosp Med Ctr	0.26
2	Niigata Univ	33 (3.6%)	Hannover Med Sch	0.22
3	Cincinnati Childrens Hosp Med Ctr	32 (3.5%)	Univ Munich	0.21
4	Univ Munich	30 (3.3%)	Hop Enfants Armand Trousseau	0.15
5	NIAID	19 (2.1%)	Childrens Hosp Pittsburgh	0.13

(2,565), followed by *The New England Journal of Medicine (NEJM)* (1,525), *Chest* (1,519), *Blood* (1,374), and *European Respiratory Journal* (1008). All the co-cited journals were distributed in the Q1 and Q2 region, and the *NEJM* had the highest impact factor (IF = 91.245).

Co-cited References and References Burst

Co-citation means that two or more articles are cited together by at least one later publication. It is a measurement to quantify the degree of relationship between articles. Among the 24,740 cited references retrieved, **Table 5** demonstrates the 10 most often cited references, of which Bruce C Trapnell's article published in *NEJM* in 2003 ranks the first. **Figure 6** reveals that the first burst of co-cited reference began in 2001. The majority of them have been cited frequently during 2001–2021, which suggests that the research related to PAP may continue to be flourishing in the future.

The Analysis of Hotspots and Frontiers

Keywords sum up research topics and essences. High-frequency keywords are usually the dominant research direction in this field. Keyword co-occurrence analysis helps us determine research hotspots and predict research trends in a specific field (12). According to **Table 6,** excluding pulmonary alveolar proteinosis (476), The top 10 keywords with the highest frequency in 2001–2021 are colony-stimulating factor (258), disease (122), gm-csf (119), lung (95), diagnosis (67), expression (64), surfactant (23), deficient mice (24), therapy (25). Among these keywords, GM-CSF (including colony-stimulating factor and gm-csf) appeared over 400 times, indicating that it was the topical issue in the study of PAP.

Clustered keywords reflect structures of knowledge in related study fields. We used VOSviewer software for this procedure. Nodes and labels form a unit, and units of different colors constitute different clusters. As shown in **Figure 7**, there are red, green, blue, and yellow clusters, representing four research directions. The main keywords of the red cluster are gm-csf, autoantibodies, diagnosis, therapy, efficacy, patient. The green cluster includes alveolar macrophages, cells, deficient mice, differentiation, expression, inflammation. Blue cluster mainly includes children, mutations, gene, deficiency, interstitial lung disease, pulmonary surfactant, respiratory distress syndrome, and of the yellow cluster are bronchoalveolar lavage, whole-lung lavage, high-resolution ct, inhalation, fibrosis.

Strong citation bursts can disclose hot words at the frontier of research. **Table 7** cataloged the top 20 keywords with the strongest citation bursts. The top five hotspots were: pulmonary surfactant (11.69), respiratory distress syndrome (9.53), congenital alveolar proteinosis (6.38), surfactant protein b (5.95), deficient mice (5.23). Subsequently, keywords such as "inflammation," "deficiency," "tissue resident macrophage," "classification," "autoimmune pulmonary alveolar proteinosis," "sarcoidosis," "gm csf," "high-resolution ct," and "fetal monocyte" appeared frequently in the last 5 years (**Table 8**). It indicates that the early stage of PAP research was focused on surfactant metabolism and the etiology and pathogenesis of different classifications of PAP. Recently, the efficacy of emerging pathogenesis-based therapies (such as PMT) has addressed our attention. Autoimmune PAP will remain research hotspots in the coming years.

DISCUSSION

General Information

Research on PAP can be roughly divided into three time periods during 2001–2021. The early-stage (2001–2009) has been increasing steadily. From 2010 to 2016, publication outputs began to increase rapidly, with three times more published in 2016 than in 2001. However, decreased publications were seen in the past 5 years but remained at a high level. This reminds us that although significant advances in our understanding of PAP over the past several decades, many important questions remain unanswered and research on PAP has still attracted great attention from scholars.

Using visual analysis of the contribution of countries and institutions, we can see that the USA, Japan, and Germany are the leading countries where PAP research is occurring. All the top 5 institutions were in these three countries. Centrality is an indicator to measure the importance of nodes in network analysis. It mainly quantifies the node's value acts as a bridge in the entire network structure. Generally, centrality values >0.1 are regarded as relatively essential nodes. As shown in **Table 1**, Switzerland has the highest centrality (0.55), implying it plays a pivotal role as a bridge in the worldwide international cooperation network.

TABLE 3 | The top 10 authors and co-cited authors in PAP research.

No.	Author	Count (%)	Centrality	Co-cited author	Citation	Centrality
1	BC Trapnell	40 (4.4%)	0.03	JF Seymour	503	0
2	K Nakata	39 (4.3%)	0.05	BC Trapnell	405	0
3	T Suzuki	21 (2.3%)	0.04	LM Nogee	377	0
4	M Griese	19 (2.1%)	0	T Kitamura	352	0
5	Y Inoue	18 (2.0%)	0.05	T Suzuki	299	0

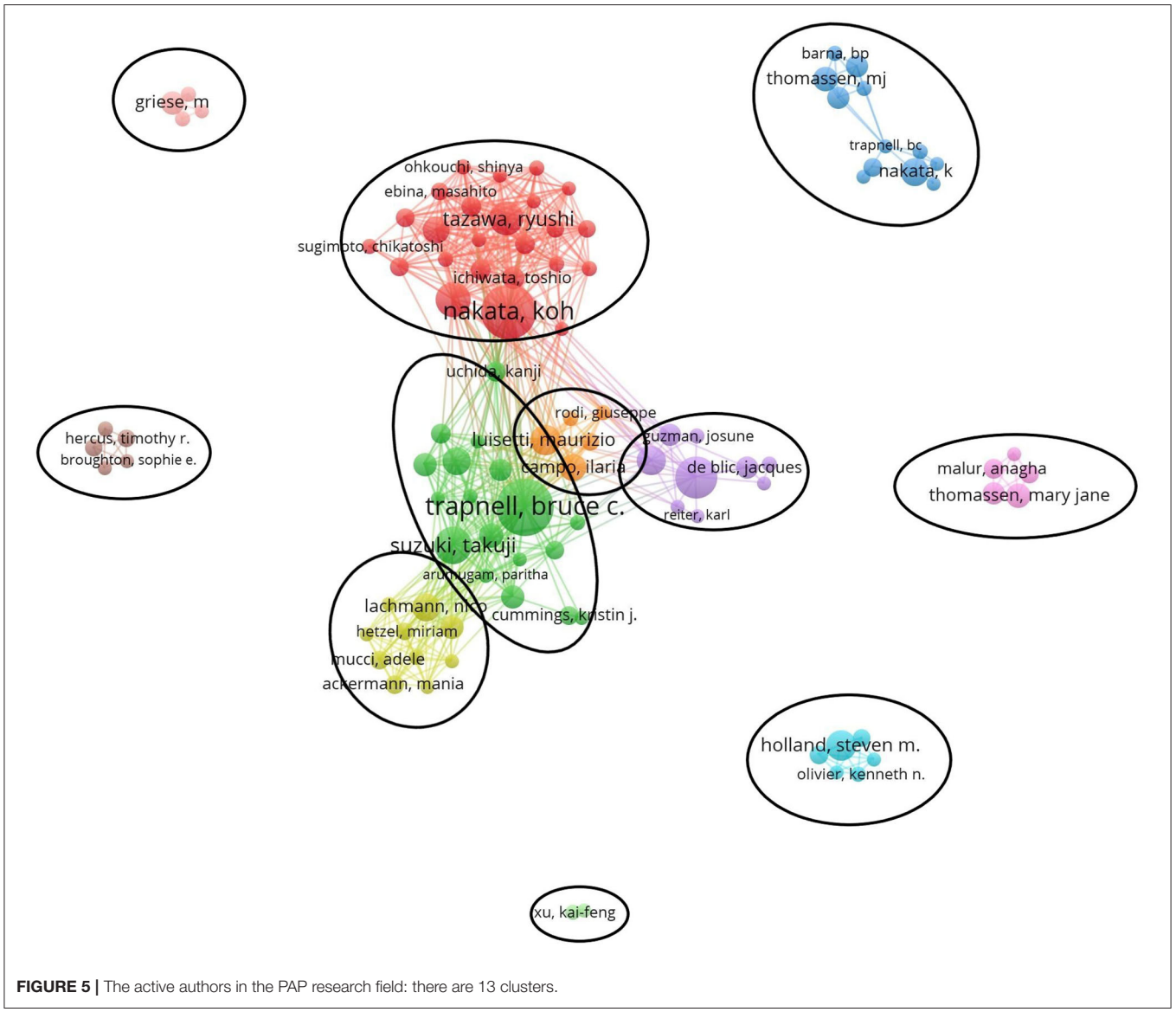

FIGURE 5 | The active authors in the PAP research field: there are 13 clusters.

Nevertheless, from **Figures 3**, **4**, the distribution of countries and institutions is scattered. The breadth and intensity of leading countries' collaboration were not ideal. For instance, there is a lack of academic cooperation between China and the USA. As for institutions, most collaborating institutions are limited to domestic ones, and there is relatively less international cooperation and exchange of findings. Considering this disease is very rare, this situation greatly hinders the development of the research field. Therefore, it is strongly recommended that institutions worldwide remove academic barriers and enhance cooperation to boost the development of PAP research.

TABLE 4 | The top 10 journals and co-cited journals related to PAP.

No.	Journal	Count (%)	IF (2020)	JCR	Co-cited Journal	Citation	IF (2020)	JCR
1	Am J Physiol Lung Cell Mol Physiol	25(2.8%)	5.464	Q2	Am J resp Crit Care	2,565	21.4	Q1
2	Am J resp Crit Care	24(2.6%)	21.4	Q1	New Engl J Med	1,525	91.245	Q1
3	Chest	24(2.6%)	9.410	Q1	Chest	1,519	9.410	Q1
4	Respirology	21(2.3%)	6.424	Q2	Blood	1,374	22.113	Q1
5	Orphanet J Rare Dis	17(1.9%)	4.123	Q2	Eur Respir J	1,008	16.671	Q1
6	Eur Respir J	16(1.8%)	16.671	Q1	J Immunol	997	5.422	Q2
7	Intern Med	14(1.5%)	1.271	Q4	J Biol Chem	976	5.157	Q2
8	BMC Pulm Med	13(1.4%)	3.317	Q3	Am J Physiol Lung Cell Mol Physiol	969	5.422	Q2
9	J Immunol	13(1.4%)	5.422	Q2	J Exp Med	957	14.307	Q1
10	Respir Res	13(1.4%)	5.631	Q2	P Natl Acad Sci USA	873	11.205	Q1

TABLE 5 | The top 10 co-cited references related to PAP.

No.	References	Author	Year	Journal	Citation	References
1	Pulmonary alveolar proteinosis	BC Trapnell	2003	New Engl J Med	277	(15)
2	Pulmonary alveolar proteinosis: progress in the first 44 years	JF Seymour	2002	Am J Resp Crit Care	251	(16)
3	Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor	T Kitamura	1999	J Exp Med	246	(17)
4	Pulmonary alveolar proteinosis	SH Rosen	1958	New Engl J Med	201	(1)
5	Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis	G Dranoff	1994	Science	167	(18)
6	Characteristics of a large cohort of patients with autoimmune pulmonary alveolar proteinosis in Japan	Y Inoue	2008	Am J Resp Crit Care	139	(19)
7	Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology	E Stanley	1994	P Natl Acad Sci Usa	135	(20)
8	GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU. 1	Y Shibata	2001	Immunity	111	(6)
9	High-affinity autoantibodies specifically eliminate granulocyte-macrophage colony-stimulating factor activity in the lungs of patients with idiopathic pulmonary alveolar proteinosis	K Uchida	2004	Blood	108	(21)
10	Familial pulmonary alveolar proteinosis caused by mutations in *CSF2RA*	T Suzuki	2008	J Exp Med	101	(22)

From the perspective of authors and co-cited authors, Koh Nakata (129, 8.6%) contributed the most, followed by Bruce C Trapnell (114, 7.6%), Ryushi Tazawa (78, 5.2%), Brenna Carey (69, 4.6%), and Takuji Suzuki (65, 4.3%). It is noteworthy that Bruce C Trapnell (0.19) exerts a significant publication impact and has made the most outstanding contributions in the field of PAP during the last 20 years. Dr. Trapnell is Professor of Medicine and Pediatrics at the University of Cincinnati and an attending physician at Cincinnati Children's Hospital Medical Centers. He focused on the pathogenesis and therapy of rare lung diseases, including PAP. In 2007, Dr. Trapnell and Kanji Uchida et al. identified that GM-CSF autoantibodies caused autoimmune PAP (aPAP) and initiated clinical laboratory tests for its diagnosis (26). Since 2010, Dr. Trapnell and Takuji Suzuki et al. found

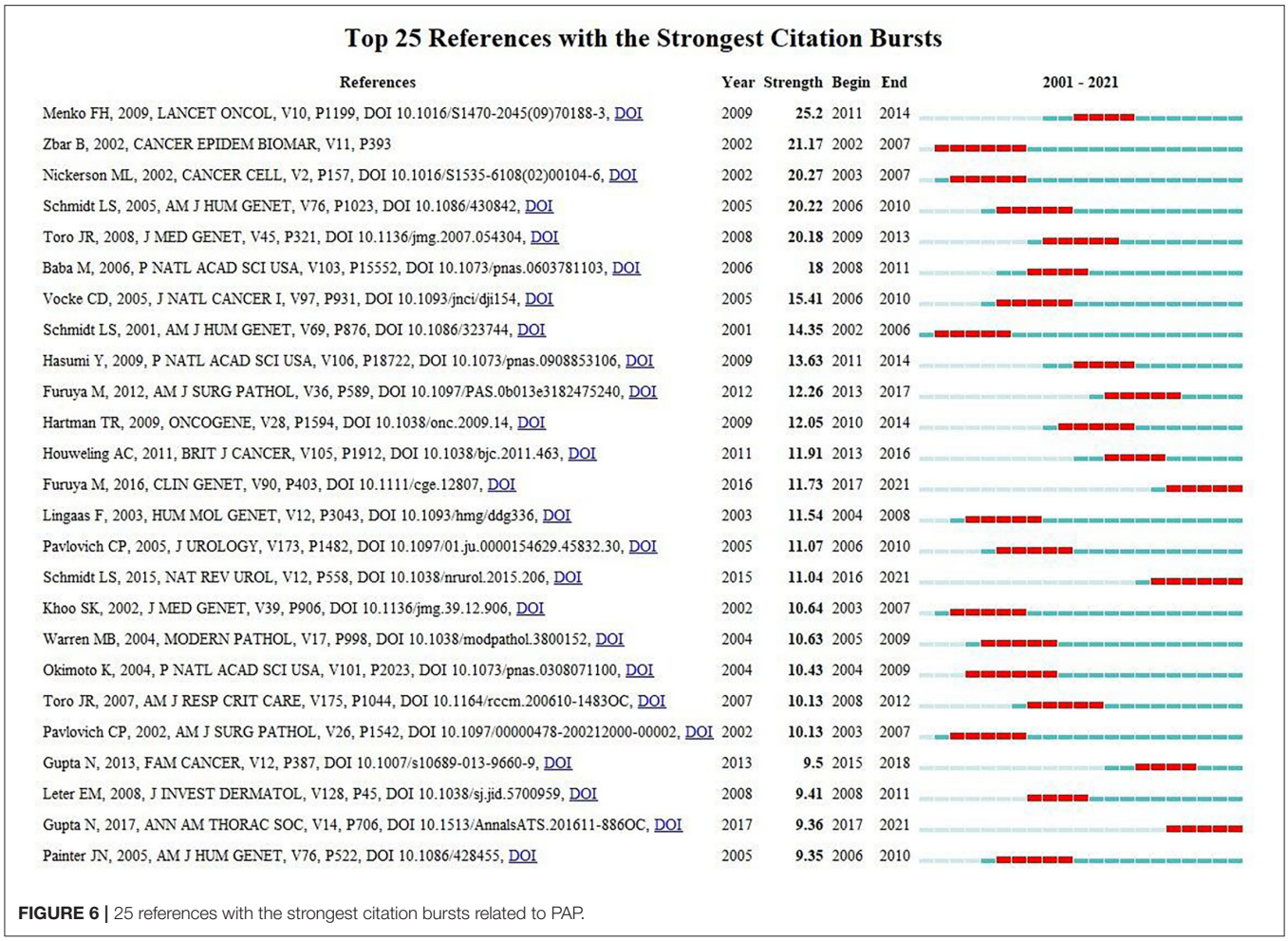

FIGURE 6 | 25 references with the strongest citation bursts related to PAP.

TABLE 6 | Top 20 keywords related to PAP.

Rank	Keywords	Count	Rank	Keywords	Count
1	pulmonary alveolar proteinosis	476	11	pulmonary surfactant	51
2	colony-stimulating factor	258	12	autoantibodies	50
3	disease	122	13	whole-lung lavage	50
4	gm-csf	119	14	macrophages	49
5	lung	95	15	mice	49
6	diagnosis	67	16	bronchoalveolar lavage	45
7	expression	64	17	mutations	43
8	surfactant	58	18	children	39
9	deficient mice	53	19	gene	39
10	therapy	52	20	inflammation	39

hereditary PAP (hPAP) as a new genetic disease due to mutations in CSF2RA and CSF2RB (22, 27, 28). In 2014, his research group developed pulmonary macrophage transplantation (PMT), an innovative type of cell transplantation, and is currently translating it as the first specific therapy in patients with hereditary PAP (29). In 2018, Dr. Trapnell and Cormac McCarthy found that the GM-CSF signaling abnormalities lead to AMs dysfunction, including its ability to process and clear out cholesterol. They creatively use cholesterol-busting statins as a novel pathogenesis-based pharmacotherapy of PAP (30). Among the top 5 co-cited authors, Takayuki Kitamura (357) and John F. Seymour (290) established that idiopathic PAP, aka autoimmune

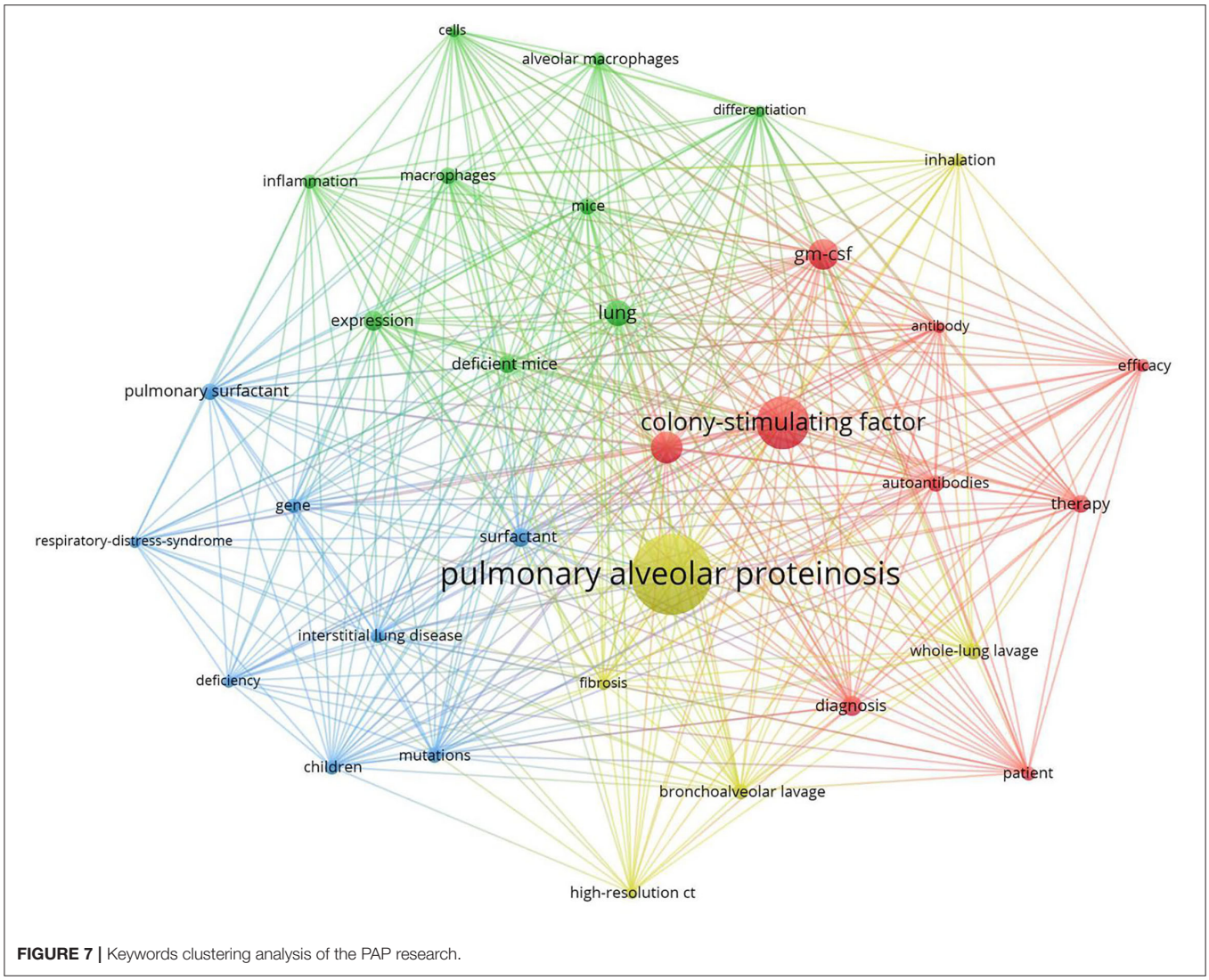

FIGURE 7 | Keywords clustering analysis of the PAP research.

PAP, neutralizes GM-CSF in the early research period (17). Therefore, based on this finding, a novel serological diagnosis is proposed (31).

According to the journals and co-cited journals in **Table 4**, the *American Journal of Respiratory and Critical Care Medicine* had the highest number of publications and citations. Almost all the top 5 co-cited journals belong to Q1. It can be seen that the research regarding etiology, pathogenesis, current and emerging therapies, and management is a hot topic presently and also a future development trend. The analysis of the literature sources is helpful to find the core journals in the research field. It can be seen that the cited articles are all from high-impact journals, indicating that a study on PAP is considered of high value by academics worldwide.

As shown in **Table 5**, the most frequently cited article was published by Bruce C Trapnell in *NEJM* in 2003 (15), which reports that GM-CSF autoantibodies are markedly elevated in autoimmune PAP but not in patients with secondary PAP, congenital PAP, other lung diseases, or healthy people. This finding considerably changed our concepts of the pathogenesis and treatment of PAP.

The Hotspots and Frontiers

Keyword co-occurrence analysis focused on understanding the distribution and development of research hotspots in a particular field. As shown in **Table 6**, pulmonary alveolar proteinosis (522), colony-stimulating factor (285), gm-csf (121), surfactant (32), whole lung lavage (25), macrophages (33), and mutations (33) are keywords with high occurrence frequency. Cluster analysis was performed based on keywords. Eventually, four colors clusters were generated. Based on these two analyses, the research hotspots and development frontiers in the pulmonary alveolar proteinosis research field are as follows:

Pathogenesis and Classification of PAP

Pulmonary alveolar proteinosis (PAP) results from abnormalities of pulmonary surfactant homeostasis, usually owing to AM dysfunction. Attenuated AM maturation is generally caused by

TABLE 7 | Top 20 keywords with the strongest citation bursts.

Keywords	Strength	Begin	End	2001–2021
pulmonary surfactant	11.69	2001	2021	
respiratory distress syndrome	9.53	2001	2021	
congenital alveolar proteinosis	6.38	2001	2021	
surfactant protein b	5.95	2001	2021	
deficient mice	5.23	2001	2021	
icell	5.19	2005	2021	
bronchoalveolar lavage fluid	5.15	2004	2021	
lung surfactant	5.08	2001	2021	
newborn	4.55	2005	2021	
alveolar macrophage	4.23	2004	2021	
innate immunity	4.04	2006	2021	
respiratory failure	3.7	2008	2021	
common beta chain	3.49	2008	2021	
lung disease	3.43	2004	2021	
gm csf therapy	3.43	2004	2021	
hermansky pudlak syndrome	3.43	2004	2021	
gene expression	3.33	2005	2021	
inhalation	3.31	2011	2021	
spc	3.3	2001	2021	
macrophage differentiation	3.18	2008	2021	

TABLE 8 | The strongest citation bursts keywords after 2016.

Keywords	Strength	Begin	End	2016–2021
inflammation	3.83	2016	2021	
deficiency	4.41	2017	2021	
tissue resident macrophage	4.06	2017	2021	
classification	3.51	2017	2021	
autoimmune pulmonary alveolar proteinosis	3.51	2017	2021	
sarcoidosis	3.45	2017	2021	
gm csf	4.06	2018	2021	
high resolution ct	3.77	2018	2021	
fetal monocyte	3.5	2018	2021	

insufficient granulocyte-macrophage colony-stimulating factor (GM-CSF) signaling, which is essential for the development of AMs metabolic and immune functions (34). Autoimmune PAP, formerly known as idiopathic PAP, is the best-studied PAP-causing disease. It occurs when elevated levels of GM-CSF autoantibodies lead to the shortage of bioavailable GM-CSF. Congenital PAP (cPAP) or hereditary PAP, occurs when genetic defects of GM-CSF receptor α or β chains (*CSF2RA, CSF2RB*) lead to impaired AMs differentiation (27). Finally, secondary PAP (sPAP) results from AMs dysfunction due to hematopoietic disorders, immune dysregulation, environmental exposures, and pharmaceutical agents (16). In some rare cases, the etiology of PAP remains uncertain and the patient is diagnosed with unclassified PAP. Autoimmune PAP comprises the biggest share (90–95%) of adult patients, whereas secondary PAP accounts for 5–10% of adult cases (19).

Role of GM-CSF in PAP

As a 23 kDa glycoprotein cytokine produced by type II alveolar epithelial cells, GM-CSF is named for its capacity for stimulating the formation of neutrophil and macrophage colonies (35). It plays a pivotal role in the terminal differentiation of AMs. GM-CSF signaling via transcription factor PU.1 (36) and peroxisome proliferator-activated receptor-γ (PPARγ) (37, 38) is essential for the functions of AMs, including cholesterol export, surfactant clearance, immunity, and others (39). It means that GM-CSF signaling links with cholesterol homeostasis in the lungs.

The pathogenesis of PAP remained obscure until the fortuitous discovery that GM-CSF knock-out mice developed pulmonary surfactants accumulation, remarkably identical to phenotype to human PAP (18, 20). Moreover, knock-out mice deficient in the GM-CSF receptor β-chain (*Csf2rb*$^{-/-}$ mice) (40, 41) or GM-CSF receptor α-chain (*Csf2ra*$^{-/-}$ mice) (42, 43) also developed a lung phenotype similar to PAP caused by *CSF2RA* or *CSF2RB* mutations observed in children. They also had reduced expression of PPARγ and PU.1, which resulted in cholesterol accumulation within AMs, leading to a frothy appearance of macrophages and a decline in the uptake and clearance of surfactant (6, 39). GM-CSF augmentation was also found to correct the alveolar proteinosis in GM-CSF-deficient mice. These and further animal observations established the critical part played by GM-CSF in the proper functioning of human AMs (37). Additionally, it indicates that GM-CSF controls cholesterol efflux in a constitutive, reversible, and dose-dependent style (39).

Neutralizing GM-CSF autoantibodies are observed at high levels in autoimmune PAP patients (17, 34) but not in those with congenital or secondary PAP, other respiratory diseases, or healthy people (21, 44). Furthermore, injection of GM-CSF autoantibodies derived from patients with autoimmune PAP into healthy non-human primates reproduced the characteristics of PAP (45, 46), thus proving the hypothesis that autoimmune PAP results from an autoantibodies-mediated disorder of GM-CSF signaling.

Treatment of PAP

Therapeutic whole-lung lavage

Since its initial description in 1964, WLL has been the forefront therapy for PAP (but not congenital PAP) (47). In brief, it is a single or sequential bilateral lavage accompanied by isolation of the two lungs using a double-lumen endotracheal tube under general anesthesia (48). Normal saline is instilled into the lung, then the milky and opaque effluent is drained repeatedly until the effluent becomes clear (49). Segmental lavage has also been done through bronchoscopy (bronchoalveolar lavage) in some medical centers. Therapeutic efficacy derives from removing the excessive surfactant by physically "washing" the alveoli with saline (50). Although WLL is generally regarded as a safe procedure that could improve symptoms, radiographic abnormalities, and oxygenation in patients, it is not without complications, including hypoxia, pneumothorax, hydrothorax, infection, and acute respiratory distress syndrome (3). Moreover, the procedure has not yet been standardized remains highly operator-dependent. In conclusion, WLL, notwithstanding being an invasive procedure, remains the cornerstone of therapy for moderate to severe PAP.

Emerging Pathogenesis-Based Therapies

GM-CSF therapy

The discovery that insufficient GM-CSF bioavailability is the pathogenesis of autoimmune PAP aroused interest in the therapeutic use of GM-CSF. In 1996, a patient received recombinant human GM-CSF (rhGM-CSF) by subcutaneous administration for the first time, resulting in a significant improvement in symptoms and oxygenation (51). Since then, several other cohort studies using subcutaneous delivery have reported similar findings with objective improvements in ∼50% of cases and accumulated plenty of available efficacy data (33, 52).

Previously, aerosolized GM-CSF has been the most promising therapy in autoimmune PAP. In a prospective trial conducted in Japan (25), 35 Japanese patients with autoimmune PAP inhaled GM-CSF over a total period of 24 weeks, using a regimen of initial high-dose followed by a maintenance low-dose. 62% of patients demonstrated an improvement in both subjective (dyspnoea) and objective parameters (6-minute walk test), whereas serum GM-CSF autoantibody levels remained static. After an extended follow-up of 30 months, 66% of patients require no further additional treatments. Most importantly, unlike the subcutaneous administration, no significant side-effects have been observed in any of the trials of inhaled GM-CSF. In a recent randomized, controlled trial, inhaled recombinant human GM-CSF (sargramostim) was associated with a modest salutary effect on the laboratory outcome of arterial oxygen tension, and no clinical benefits were noted (24). In 2020, a double-blind, placebo-controlled, three-group trial assigned patients with autoimmune PAP to receive the recombinant GM-CSF molgramostim (300 μg once daily by inhalation), either continuously or intermittently (every other week), or matching placebo. For multiple end points, improvement was greater with continuous molgramostim than with intermittent molgramostim or placebo (53). Overall, clinical trials show that inhaled GM-CSF in patients with autoimmune PAP is safe and effective. However, the optimal dose, timing, or duration of administration should be further defined for response rate improvement.

Therapy Targeting GM-CSF Autoantibodies

Since the finding of the pathogenesis of GM-CSF autoantibodies, different therapeutic strategies targeting a lower level of the autoantibodies have been adopted in autoimmune PAP. By analogy with other autoimmune diseases, corticosteroids seem reasonable to treat autoimmune PAP. However, results from PAP patients treated with corticosteroids show more harm than good (54). Other therapies include rituximab (a monoclonal anti-CD20 antibody) to remove B lymphocyte and autoantibodies depletion using plasmapheresis. Plasma exchange has been used with marginal success, and the results have not been consistent enough for a recommendation (55, 56). As for rituximab, one open-label trial demonstrated improvement in A-aDO_2 gradient in seven out of the nine patients completing the study (23); A retrospective study showed that no patient had marked improvement after 6-month treatment (32). Further prospective studies are demanded before the utility of those conceptually feasible therapies.

Pulmonary Macrophage Transplantation

Hereditary PAP deficient in GM-CSF receptor, thus, other therapeutic options are needed. Animal studies showed that bone marrow transplantation (BMT) can re-establish surfactant homeostasis and correct hereditary PAP. Although BMT had minimal success in hereditary PAP children, it is restricted by the morbidity and mortality of myeloablation, and secondary PAP can itself be a complication of BMT (57). Pulmonary macrophage

transplantation (PMT) is a novel cell transplantation method that has demonstrated therapeutic efficacy in animal studies (29, 42, 43). These reports support the translation of PMT as a specific therapy for children with hereditary PAP.

Targeting Pulmonary Cholesterol Homeostasis

GM-CSF signaling interruption reduced cholesterol clearance from AMs, which is the leading pathogenesis of PAP. This led to the consideration of therapy targeting cholesterol homeostasis as an alternative option for PAP. PPARγ agonist therapy escalated cholesterol clearance and reduced PAP disease severity of knock-out mice (39). This discovery has translated to a clinical trial of pioglitazone (a PPARγ agonist). In addition, statin is associated with decreased cholesterol accumulation of AMs and PAP disease remission. As for patients with autoimmune PAP, statin therapy ameliorates PAP significantly (30), supporting the possibility of statins as innovative pathogenesis-based therapies.

CONCLUSIONS

This study summarizes the research status of PAP in the past 20 years. Publications related to PAP are increasing over time. Different countries/regions and institutions need to deepen and strengthen cooperation. The majority of the articles regarding PAP are published in and cited from influential international journals, suggesting that PAP has attributed much attention. Several cardinal questions remain unanswered, such as the etiology of autoimmune PAP and the pathogenesis of secondary PAP. Those critical issues need to be put on the front burner. This study provides assistance for scholars to find core literature and partners in PAP, contributes direction for journals publication, and guidelines identifying research hotspots in this field.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

SL and GL conceived and designed the study. SL, KX, and JH collected the data. DW and XY re-examined the data. JZ and XL analyzed the data. SL wrote the first draft of the manuscript. LB and KX wrote sections of the manuscript. XC reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (Grant No. 82074262).

ACKNOWLEDGMENTS

The authors thank Guang'anmen Hospital, China Academy of Chinese Medical Sciences, for their support of this work and the reviewers for allowing us to improve the manuscript.

REFERENCES

1. Rosen SH, Castleman B, Liebow AA. Pulmonary alveolar proteinosis. *N Engl J Med.* (1958) 258:1123–42. doi: 10.1056/NEJM195806052582301
2. McCarthy C, Avetisyan R, Carey BC, Chalk C, Trapnell BC. Prevalence and healthcare burden of pulmonary alveolar proteinosis. *Orphanet J Rare Dis.* (2018) 13:1–5. doi: 10.1186/s13023-018-0846-y
3. Trapnell BC, Nakata K, Bonella F, Campo I, Griese M, Hamilton J, et al. Pulmonary alveolar proteinosis. *Nat Rev Dis Primers.* (2019) 5:1–17. doi: 10.1038/s41572-019-0066-3
4. Iftikhar H, Nair GB, Kumar A. Update on diagnosis and treatment of adult pulmonary alveolar proteinosis. *Ther Clin Risk Manag.* (2021) 17:701–10. doi: 10.2147/TCRM.S193884
5. Kumar A, Abdelmalak B, Inoue Y, Culver DA. Pulmonary alveolar proteinosis in adults: pathophysiology and clinical approach. *Lancet Respir Med.* (2018) 6:554–65. doi: 10.1016/S2213-2600(18)30043-2
6. Shibata Y, Berclaz P-Y, Chroneos ZC, Yoshida M, Whitsett JA, Trapnell BC. GM-CSF regulates alveolar macrophage differentiation and innate immunity in the lung through PU. 1. *Immunity*. (2001) 15:557–67. doi: 10.1016/S1074-7613(01)00218-7
7. Gu D, Li J, Li X, Liang C. Visualizing the knowledge structure and evolution of big data research in healthcare informatics. *Int J Med Informatics.* (2017) 98:22–32. doi: 10.1016/j.ijmedinf.2016.11.006
8. Chen C. CiteSpace II: detecting and visualizing emerging trends and transient patterns in scientific literature. *J Am Soc Information Sci Technol.* (2006) 57:359–77. doi: 10.1002/asi.20317
9. Van Eck NJ, Waltman L. Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics.* (2010) 84:523–38. doi: 10.1007/s11192-009-0146-3
10. Ma D, Yang B, Guan B, Song L, Liu Q, Fan Y, et al. A bibliometric analysis of pyroptosis from 2001 to 2021. *Front Immunol.* (2021) 12:731933. doi: 10.3389/fimmu.2021.731933
11. Cooper ID. Bibliometrics basics. *J Med Libr Assoc.* (2015) 103:217. doi: 10.3163/1536-5050.103.4.013
12. Chen C. Searching for intellectual turning points: progressive knowledge domain visualization. *Proc Natl Acad Sci USA.* (2004) 101(Suppl. 1):5303–10. doi: 10.1073/pnas.0307513100
13. Synnestvedt MB, Chen C, Holmes JH, editors. CiteSpace II: visualization and knowledge discovery in bibliographic databases. In: *AMIA Annual Symposium Proceedings*. Washington, DC: American Medical Informatics Association (2005).
14. Chen C, Hu Z, Liu S, Tseng H. Emerging trends in regenerative medicine: a scientometric analysis in CiteSpace. *Expert Opinion Biol Ther.* (2012) 12:593–608. doi: 10.1517/14712598.2012.674507
15. Trapnell BC, Whitsett JA, Nakata K. Pulmonary alveolar proteinosis. *N Engl J Med.* (2003) 349:2527–39. doi: 10.1056/NEJMra023226
16. Seymour JF, Presneill JJ. Pulmonary alveolar proteinosis: progress in the first 44 years. *Am J Respir Crit Care Med.* (2002) 166:215–35. doi: 10.1164/rccm.2109105
17. Kitamura T, Tanaka N, Watanabe J, Kanegasaki S, Yamada Y, Nakata K. Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. *J Exp Med.* (1999) 190:875–80. doi: 10.1084/jem.190.6.875
18. Dranoff G, Crawford AD, Sadelain M, Ream B, Rashid A, Bronson RT, et al. Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. *Science.* (1994) 264:713–6. doi: 10.1126/science.8171324
19. Inoue Y, Trapnell BC, Tazawa R, Arai T, Takada T, Hizawa N, et al. Characteristics of a large cohort of patients with autoimmune pulmonary

alveolar proteinosis in Japan. *Am J Respir Crit Care Med.* (2008) 177:752–62. doi: 10.1164/rccm.200708-1271OC
20. Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, Gall J, et al. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci USA.* (1994) 91:5592–6. doi: 10.1073/pnas.91.12.5592
21. Uchida K, Nakata K, Trapnell BC, Terakawa T, Hamano E, Mikami A, et al. High-affinity autoantibodies specifically eliminate granulocyte-macrophage colony-stimulating factor activity in the lungs of patients with idiopathic pulmonary alveolar proteinosis. *Blood.* (2004) 103:1089–98. doi: 10.1182/blood-2003-05-1565
22. Suzuki T, Sakagami T, Rubin BK, Nogee LM, Wood RE, Zimmerman SL, et al. Familial pulmonary alveolar proteinosis caused by mutations in CSF2RA. *J Exp Med.* (2008) 205:2703–10. doi: 10.1084/jem.20080990
23. Kavuru MS, Malur A, Marshall I, Barna BP, Meziane M, Huizar I, et al. An open-label trial of rituximab therapy in pulmonary alveolar proteinosis. *Euro Respir J.* (2011) 38:1361–7. doi: 10.1183/09031936.00197710
24. Tazawa R, Ueda T, Abe M, Tatsumi K, Eda R, Kondoh S, et al. Inhaled GM-CSF for pulmonary alveolar proteinosis. *N Engl J Med.* (2019) 381:923–32. doi: 10.1056/NEJMoa1816216
25. Tazawa R, Trapnell BC, Inoue Y, Arai T, Takada T, Nasuhara Y, et al. Inhaled granulocyte/macrophage-colony stimulating factor as therapy for pulmonary alveolar proteinosis. *Am J Respir Crit Care Med.* (2010) 181:1345–54. doi: 10.1164/rccm.200906-0978OC
26. Uchida K, Beck DC, Yamamoto T, Berclaz PY, Abe S, Staudt MK, et al. GM-CSF autoantibodies and neutrophil dysfunction in pulmonary alveolar proteinosis. *N Engl J Med.* (2007) 356:567–79. doi: 10.1056/NEJMoa062505
27. Suzuki T, Sakagami T, Young LR, Carey BC, Wood RE, Luisetti M, et al. Hereditary pulmonary alveolar proteinosis: pathogenesis, presentation, diagnosis, and therapy. *Am J Respir Crit Care Med.* (2010) 182:1292–304. doi: 10.1164/rccm.201002-0271OC
28. Mucci A, Kunkiel J, Suzuki T, Brennig S, Glage S, Kühnel MP, et al. Murine iPSC-derived macrophages as a tool for disease modeling of hereditary pulmonary alveolar proteinosis due to Csf2rb deficiency. *Stem Cell Rep.* (2016) 7:292–305. doi: 10.1016/j.stemcr.2016.06.011
29. Suzuki T, Arumugam P, Sakagami T, Lachmann N, Chalk C, Sallese A, et al. Pulmonary macrophage transplantation therapy. *Nature.* (2014) 514:450–4. doi: 10.1038/nature13807
30. McCarthy C, Lee E, Bridges JP, Sallese A, Suzuki T, Woods JC, et al. Statin as a novel pharmacotherapy of pulmonary alveolar proteinosis. *Nat Commun.* (2018) 9:1–9. doi: 10.1038/s41467-018-05491-z
31. Kitamura T, Uchida K, Tanaka N, Tsuchiya T, Watanabe J, Yamada Y, et al. Serological diagnosis of idiopathic pulmonary alveolar proteinosis. *Am J Respir Crit Care Med.* (2000) 162:658–62. doi: 10.1164/ajrccm.162.2.9910032
32. Soyez B, Borie R, Menard C, Cadranel J, Chavez L, Cottin V, et al. Rituximab for auto-immune alveolar proteinosis, a real life cohort study. *Respir Res.* (2018) 19:1–7. doi: 10.1186/s12931-018-0780-5
33. Venkateshiah SB, Yan TD, Bonfield TL, Thomassen MJ, Meziane M, Czich C, et al. An open-label trial of granulocyte macrophage colony stimulating factor therapy for moderate symptomatic pulmonary alveolar proteinosis. *Chest.* (2006) 130:227–37. doi: 10.1378/chest.130.1.227
34. Tanaka N, Watanabe J, Kitamura T, Yamada Y, Kanegasaki S, Nakata K. Lungs of patients with idiopathic pulmonary alveolar proteinosis express a factor which neutralizes granulocyte-macrophage colony stimulating factor. *FEBS Lett.* (1999) 442:246–50. doi: 10.1016/S0014-5793(98)01668-8
35. Gearing DP, King J, Gough NM, Nicola N. Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor. *EMBO J.* (1989) 8:3667–76. doi: 10.1002/j.1460-2075.1989.tb08541.x
36. Schneider C, Nobs SP, Kurrer M, Rehrauer H, Thiele C, Kopf M. Induction of the nuclear receptor PPAR-γ by the cytokine GM-CSF is critical for the differentiation of fetal monocytes into alveolar macrophages. *Nat Immunol.* (2014) 15:1026–37. doi: 10.1038/ni.3005
37. Bonfield TL, Raychaudhuri B, Malur A, Abraham S, Trapnell BC, Kavuru MS, et al. PU. 1 regulation of human alveolar macrophage differentiation requires granulocyte-macrophage colony-stimulating factor. *Am J Physiol Lung Cell Mol Physiol.* (2003) 285:L1132–6. doi: 10.1152/ajplung.00216.2003
38. Moore KJ, Rosen ED, Fitzgerald ML, Randow F, Andersson LP, Altshuler D, et al. The role of PPAR-γ in macrophage differentiation and cholesterol uptake. *Nat Med.* (2001) 7:41–7. doi: 10.1038/83328
39. Sallese A, Suzuki T, McCarthy C, Bridges J, Filuta A, Arumugam P, et al. Targeting cholesterol homeostasis in lung diseases. *Sci Rep.* (2017) 7:1–14. doi: 10.1038/s41598-017-10879-w
40. Nishinakamura R, Nakayama N, Hirabayashi Y, Inoue T, Aud D, Mcneil T, et al. Mice deficient for the IL-3/GM-CSF/IL-5 βc receptor exhibit lung pathology and impaired immune response, while βIL3 receptor-deficient mice are normal. *Immunity.* (1995) 2:211–22. doi: 10.1016/1074-7613(95)90046-2
41. Robb L, Drinkwater CC, Metcalf D, Li R, Köntgen F, Nicola NA, et al. Hematopoietic and lung abnormalities in mice with a null mutation of the common beta subunit of the receptors for granulocyte-macrophage colony-stimulating factor and interleukins 3 and 5. *Proc Natl Acad Sci USA.* (1995) 92:9565–9. doi: 10.1073/pnas.92.21.9565
42. Shima K, Suzuki T, Arumugam P, Lachmann N, Moritz T, Ma Y, et al. Pulmonary macrophage transplantation therapy in Csf2ra gene-ablated mice: a novel model of hereditary pulmonary alveolar proteinosis in children. In: *B108 Cystic Fibrosis, Primary Ciliary Dyskinesia, and ILD*. Washington, DC: American Thoracic Society (2017). p. A4857-A.
43. Suzuki T, Shima K, Arumugam P, Ma Y, Black D, Chalk C, et al. Development and validation of Csf2ra gene-deficient mice as a clinically relevant model of children with hereditary pulmonary alveolar proteinosis. In: *B108 Cystic Fibrosis, Primary Ciliary Dyskinesia, and ILD*. Washington, DC: American Thoracic Society (2017). p. A4837-A.
44. Bendtzen K, Svenson M, Hansen MB. GM-CSF autoantibodies in pulmonary alveolar proteinosis. *N Engl J Med.* (2005) 353:2111–20. doi: 10.1056/NEJMc070650
45. Sakagami T, Beck D, Uchida K, Suzuki T, Carey BC, Nakata K, et al. Patient-derived granulocyte/macrophage colony-stimulating factor autoantibodies reproduce pulmonary alveolar proteinosis in nonhuman primates. *Am J Respir Crit Care Med.* (2010) 182:49–61. doi: 10.1164/rccm.201001-0008OC
46. Sakagami T, Uchida K, Suzuki T, Carey BC, Wood RE, Wert SE, et al. Human GM-CSF autoantibodies and reproduction of pulmonary alveolar proteinosis. *N Engl J Med.* (2009) 361:2679–81. doi: 10.1056/NEJMc0904077
47. Ramirez-R J, Kieffer RF Jr, Ball WC Jr. Bronchopulmonary lavage in man. *Ann Internal Med.* (1965) 63:819–28. doi: 10.7326/0003-4819-63-5-819
48. Abdelmalak BB, Khanna AK, Culver DA, Popovich MJ. Therapeutic whole-lung lavage for pulmonary alveolar proteinosis: a procedural update. *J Bronchol Interventional Pulmonol.* (2015) 22:251–8. doi: 10.1097/LBR.0000000000000180
49. Michaud G, Reddy C, Ernst A. Whole-lung lavage for pulmonary alveolar proteinosis. *Chest.* (2009) 136:1678–81. doi: 10.1378/chest.09-2295
50. Campo I, Luisetti M, Griese M, Trapnell BC, Bonella F, Grutters J, et al. Whole lung lavage therapy for pulmonary alveolar proteinosis: a global survey of current practices and procedures. *Orphanet J Rare Dis.* (2016) 11:1–10. doi: 10.1186/s13023-016-0497-9
51. Seymour JF, Dunn AR, Vincent JM, Presneill JJ, Pain MC. Efficacy of granulocyte-macrophage colony-stimulating factor in acquired alveolar proteinosis. *N Engl J Med.* (1996) 335:1924–5. doi: 10.1056/NEJM199612193352513
52. Seymour JF, Presneill JJ, Schoch OD, Downie GH, Moore PE, Doyle IR, et al. Therapeutic efficacy of granulocyte-macrophage colony-stimulating factor in patients with idiopathic acquired alveolar proteinosis. *Am J Respir Crit Care Med.* (2001) 163:524–31. doi: 10.1164/ajrccm.163.2.2003146
53. Trapnell BC, Inoue Y, Bonella F, Morgan C, Jouneau S, Bendstrup E, et al. Inhaled molgramostim therapy in autoimmune pulmonary alveolar proteinosis. *N Engl J Med.* (2020) 383:1635–44. doi: 10.1056/NEJMoa1913590
54. Akasaka K, Tanaka T, Kitamura N, Ohkouchi S, Tazawa R, Takada T, et al. Outcome of corticosteroid administration in autoimmune pulmonary alveolar proteinosis: a retrospective cohort study. *BMC Pulmonary Med.* (2015) 15:1–9. doi: 10.1186/s12890-015-0085-0
55. Jezequel A, Kerjouan M, Lederlin M, Laine-Caroff C, Camus C, Delaval P, et al. Plasmapheresis failure in the treatment of auto-immune pulmonary alveolar proteinosis. *Revue Maladies Respir.* (2017) 34:240–3. doi: 10.1016/j.rmr.2016.06.002

56. Luisetti M, Rodi G, Perotti C, Campo I, Mariani F, Pozzi E, et al. Plasmapheresis for treatment of pulmonary alveolar proteinosis. *Euro Respir J.* (2009) 33:1220–2. doi: 10.1183/09031936.00097508
57. Pidala J, Khalil F, Fernandez H. Pulmonary alveolar proteinosis following allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant.* (2011) 46:1480–3. doi: 10.1038/bmt.2010.321

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

REVIEW
published: 30 June 2022
doi: 10.3389/fmed.2022.937561

Treating Autoimmune-Related Interstitial Lung Disease With B Cell Depletion

Stamatis-Nick C. Liossis[1,2]* *and Constantina A. Bounia*[1]

[1] Division of Rheumatology, Patras University Hospital, Patras, Greece, [2] Department of Internal Medicine, University of Patras Medical School, Patras, Greece

Autoimmune rheumatic diseases may affect vital organs with lung involvement being severe and difficult to treat manifestation. Systemic sclerosis (SSc) commonly affects the lung in the form of interstitial lung disease (ILD). ILD may be also seen in patients with rheumatoid arthritis (RA), Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), inflammatory myositis (IM), antisynthetase syndrome (AS), and the ANCA-associated vasculitides (AAV). Rituximab (RTX) is an anti-CD20 B lymphocyte depleting mAb, often administered in the treatment of autoimmune rheumatic diseases. Although RTX is an off-label treatment for CTD–ILD, there are numerous reports providing data that is effective in improving both pulmonary function tests (PFTs) and chest computed tomography findings consistent with ILD. There are retrospective uncontrolled studies that assess RTX as a treatment of ILD in autoimmune diseases. These studies, apart from one, do not include patients with AAV-ILD. In SSc-ILD, in particular, there are both controlled and uncontrolled studies displaying encouraging results following B cell depletion. In addition, a number of retrospective uncontrolled studies and fewer prospective studies evaluate RTX in connective tissue diseases CTD–ILD. Although RTX is an approved treatment for AAV there are scarce only data focusing on patients with AAV-ILD specifically. The results of a handful of studies comparing treatment of CTD-ILD with RTX to treatment with other agents are in favor of RTX. Results from large, still ongoing controlled trials are awaited to ascertain RTX effects in ILD encountered in autoimmune rheumatic diseases. We review herein the results of the different RTX trials in patients with autoimmune disease–associated with ILD. Despite the heterogeneity of these studies, RTX may be considered an alternative and safe but still off-label treatment for patients with refractory CTD–ILD.

Keywords: interstitial lung disease, connective tissue diseases, B cell depletion, systemic sclerosis, rheumatoid arthritis

OPEN ACCESS

Edited by:
Argyrios Tzouvelekis, Alexander Fleming Biomedical Sciences Research Center, Greece

Reviewed by:
Theodoros Dimitroulas, Aristotle University of Thessaloniki, Greece
Nicol Bernardinello, University of Padua, Italy

***Correspondence:**
Stamatis-Nick C. Liossis
snliossis@med.upatras.gr

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *06 May 2022*
Accepted: *08 June 2022*
Published: *30 June 2022*

Citation:
Liossis SNC and Bounia CA (2022) Treating Autoimmune-Related Interstitial Lung Disease With B Cell Depletion. Front. Med. 9:937561. doi: 10.3389/fmed.2022.937561

INTRODUCTION

Interstitial lung diseases (ILD) are a group of parenchymal lung disorders sharing clinical and radiological phenotypes. CTDs such as systemic sclerosis (SSc), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), inflammatory myositis [polymyositis (PM), dermatomyositis(DM), and antisynthetase syndrome (AS)] may affect lung parenchyma (1). In addition, ANCA—associated vasculitides (AAV) may present with ILD. CTD-ILD is divided

into seven histological types including usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia (NSIP), desquamative interstitial pneumonia (DIP), respiratory bronchiolitis (RB), organizing pneumonia (OP), diffuse alveolar damage (DAD), and lymphoid interstitial pneumonia (LIP). NSIP and UIP are the most common ILD subtypes encountered in patients with connective tissue diseases (CTDs) (2). Although treating lung involvement in CTDs has been extensively studied it is still a challenge to improve the prognosis of these patients (3, 4). Immunosuppressive agents currently represent the standard of care for patients with CTD-ILD. SSc beholds the majority of studies assessing the effects of immunosuppressants and also of studies regarding RTX administration in ILD (5). RTX is a monoclonal antibody against the surface marker CD 20 of B lymphocytes. RTX depletion of B lymphocytes is achieved in three ways (**Figure 1**): (a) anti-CD20 antibody induces internal signaling within the B cell, causing antiproliferative effects or cell death (direct cytotoxicity) (b) the first component of complement (C1) binds to the Fc portion of the anti-CD20 resulting in the activation of the complement cascade and cell lysis through the formation of membrane attack complexes (MAC) of the B cell (complement-dependent cytotoxicity) and (c) the effector cells such as natural killer cells or macrophages bind to the Fc portion of the anti-CD20 molecule through their Fc γ RIIIa receptors (antibody-dependent cell-mediated cytotoxicity-ADCC) (6).

We searched the PUBMED and SCOPUS using the following terms: interstitial lung disease, rheumatic diseases, connective tissue diseases, systemic sclerosis, rheumatoid arthritis, antisynthetase syndrome, Sjogren's syndrome, systemic lupus erythematosus, Anca-associated vasculitis, rituximab, and B-cell depletion. Preference was given in clinical trials, randomized controlled trials and metanalyses over the past 10 years. Reviews and case reports were not included (**Figure 2**). In this review, we discuss the results of studies evaluating RTX as an alternative treatment for ILD in patients with CTDs either collectively in groups of patients with different CTDs or as a separate group with a single CTD. All studies evaluate the changes of pulmonary function tests (PFTs) like forced vital capacity (FVC) and diffusing lung capacity for carbon dioxide (DLCO) and fewer studies examine for alterations of lung parenchyma in chest high resolution computed tomography (HRCT) following treatment of patients with RTX.

RETROSPECTIVE STUDIES IN CTD–ILD

Duarte et al. evaluated 48 patients with CTD–ILD (7); 30 patients with RA (two of them with secondary SS), four with pSS, four with SLE, three with SSc, two with overlap SSc/PM, two with PM, twowith AS, one with DM, and one with an overlap of SLE/pSS. Most patients initiated RTX treatment because of lung involvement and a few were already on RTX treatment for other

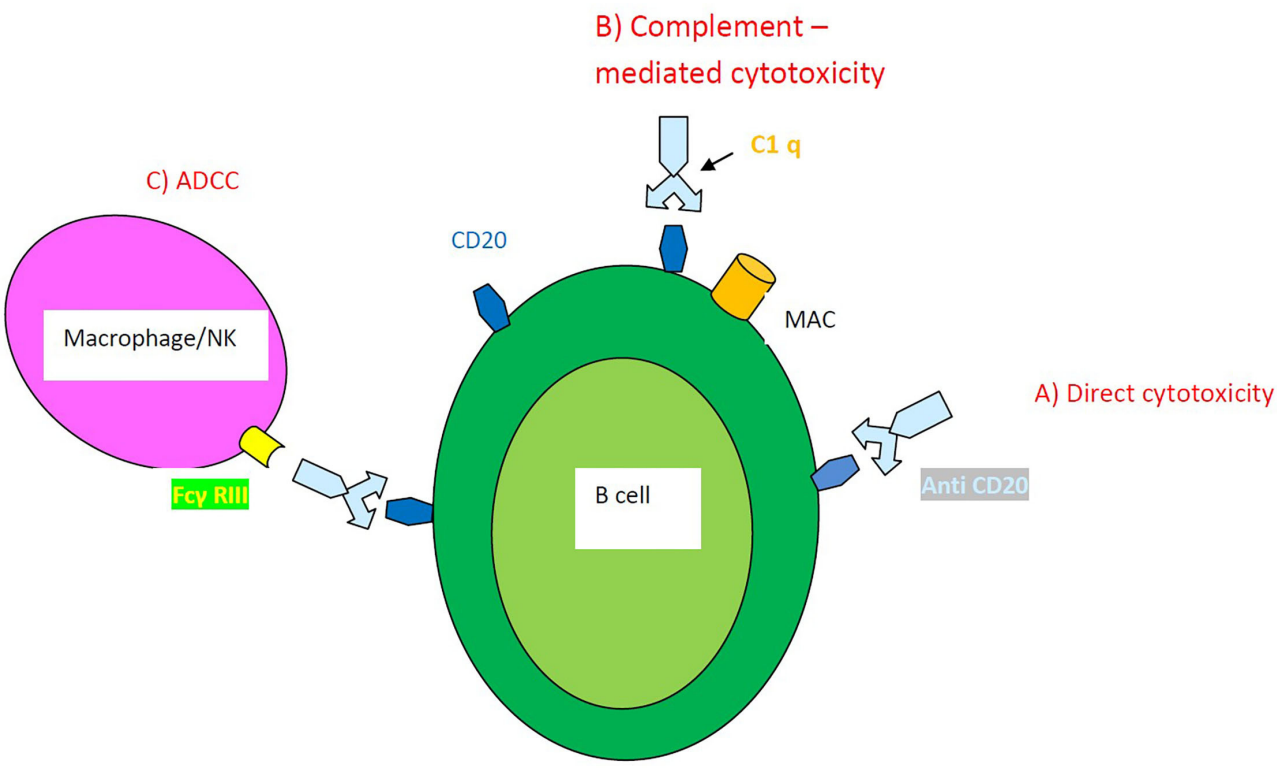

FIGURE 1 | Mechanisms of action of RTX-induced B cell depletion. **(A)** Direct cytotoxicity **(B)** Complement–mediated cytotoxicity **(C)** Antibody-dependent cell-mediated cytotoxicity ADCC.

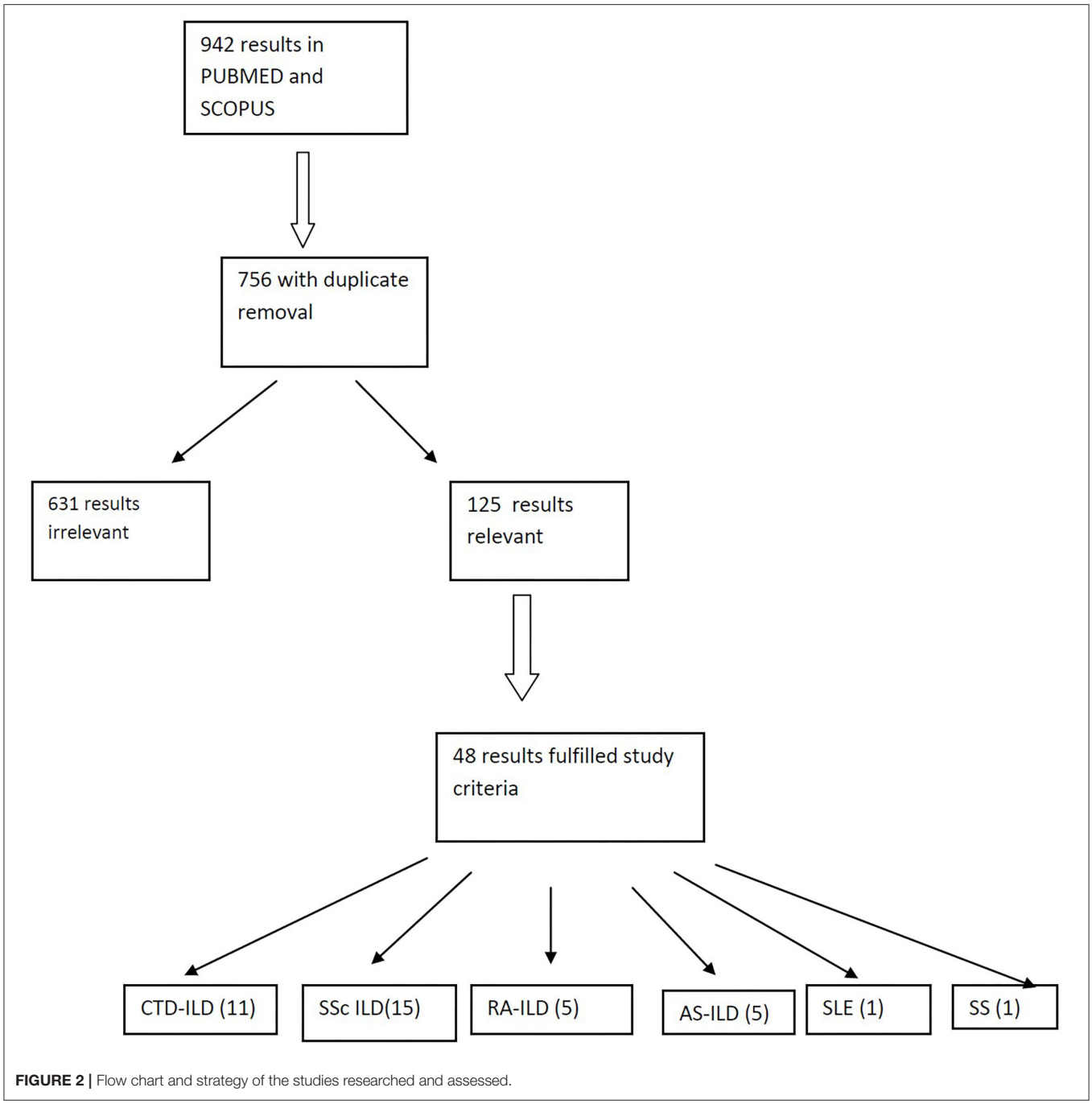

FIGURE 2 | Flow chart and strategy of the studies researched and assessed.

disease manifestations. Thirteen patients had already received other immunosuppressants for ILD such as cyclophosphamide (CYC) ($n = 10$) followed by mycophenolate mofetil (MMF) ($n = 5$ out of 10) or azathioprine (AZA) ($n = 5$ out of 10), and AZA as first choice ($n = 3$). RTX as the initial treatment for ILD was administrated in 35 patients. All patients were evaluated with PFTs and HRCTs performed at 6 and 12 months following RTX administration for a 3-year period of follow-up. At 12 months, DLCO values remained stable (mean: + 5.4%, $p = 0.12$) compared to baseline values and FVC improved (mean + 4.3%, $p = 0.03$) compared to baseline values. When the patients were separately evaluated according to NSIP or UIP pattern in HRCT, the following were observed: in patients with NSIP DLCO values slightly, but not significantly, increased at 12 months after RTX (8.5%, $p = 0.08$), while FVC values increased significantly (4.5%, $p = 0.04$) compared to baseline. However, patients with UIP had no significant change at 12 months after RTX compared to baseline either in DLCO (2.5%, $p = 0.77$) or in FVC (4.2%, $p = 0.16$). Potential changes in HRCT ILD pattern and extent were not addressed.

Lepri et al. retrospectively enrolled 44 patients in their study (23 with SSc, 15 with AS, and 6 with MCTD) (8). All patients were diagnosed with ILD and were treated with RTX. Most patients (15/23 with SSC, 8/15 with AS, and 5/6 patients with MCTD) were also treated with other DMARDs. At 12 months after RTX administration patients with SSc displayed a statistically non-significant increase of FVC from 81.0% at baseline to 89.0%. Patients with AS displayed a stabilization of FVC (53% at baseline vs. 51.4% at 12 months) similar to the group of patients with MCTD (64.5% at baseline vs. 63% at 12 months). DLCO values in the SSc group increased minimally (61% prior RTX to 63% at 12 months) and were considered as stable at 2 years of treatment compared to baseline levels. Similar results were reported for patients with AS a non-significant increase of DLCO from 41.7% at baseline to 52% at 12 months of treatment with RTX and stability of DLCO at 2 years of treatment with RTX compared to baseline levels before RTX administration. Finally, patients with MCTD had no significant changes at 1 year but displayed a trend in the improvement of DLCO at 2 years following treatment with RTX compared to baseline.

Atienza-Mateo et al. enrolled in their retrospective study 26 patients with autoimmune diseases that were treated with RTX due to lung involvement (9). Patients were diagnosed with SSc ($n = 7$), IIM ($n = 6$), ASS ($n = 6$), amyopathic DM ($n = 1$), RA ($n = 5$), interstitial pneumonia with autoimmune features (IPAF) ($n = 3$), pSS ($n = 3$), and myeloperoxidase anti-neutrophil cytoplasmic antibody (MPO-ANCA) positive ($n = 2$). Nineteen out of 26 patients were on immunosuppressive therapy [MMF, hydroxychloroquine (HCQ), methotrexate (MTX), AZA] and 3/26 patients were on antifibrotic therapy at the time of enrollment. RTX was administrated 1,000 mg biweekly ($n = 20$) ("RA scheme"), 500 mg biweekly ($n = 4$), and 375 mg/m^2 weekly for 4 weeks ($n = 2$) ("lymphoma scheme"). The study reported a slight improvement of all PFTs (FVC, FEV1, and DLCO) that was preserved after 6, 12, and 24 months of treatment with RTX compared to baseline levels. In addition, a significant increase in DLCO values was observed at 12 months compared to baseline (mean ± SD: 34.02 ± 14.75% at baseline vs. 38.22 ± 15.86% at 12 months, respectively, $p = 0.025$). Lung involvement was also evaluated with chest HRCT in 23 out of 26 patients. Interstitial lung involvement was unchanged in 15/23 patients (65.2%), worsened in 5/23 patients (21.7%) and improved in 3/23 patients (13.1%). This study finally emphasizes that RTX unlike other previous studies was administered early in ILD diagnosis before any worsening of ILD disease was confirmed.

Sharp et al. studied retrospectively 24 patients with rheumatic diseases that developed ILD refractory to conventional therapy and therefore received RTX (10). The trial included 10 patients with AS, 3 with DM, 3 with SSc, 2 with SS, 2 with SLE and 4 with unclassifiable CTD-ILD. RTX was administered in the "RA scheme" and PFTs and HRCT images were evaluated before and after 6 months of treatment with RTX. FVC improved with a mean change of 4.1%, ($p = 0.01$) at 6 months after RTX compared to baseline, and DLCO did not change significantly. HRCT imaging after treatment with RTX revealed a non-significant mean change of disease extent of −3.75% compared to baseline; it should be noted that HRCT depicted deterioration in 9/22 patients following treatment with RTX.

Robles-Perez et al. evaluated 18 patients with rapidly progressive ILD awaiting in a list for lung transplantation (11); progressive ILD was defined as an FVC < 60% or worsening FVC > 10% in the last 6 months and/or DLCO < 40%. The patients included were 7 with SSc, 5 with RA, 4 with SLE, 1 with SS and 1 with AS. All patients received RTX 1,000 mg biweekly as an add-on therapy on top of immunosuppressive therapy and were evaluated with PFTs and HRCTs at time-points 0, 12 and 24 months after treatment with RTX. FVC increased significantly (+6.3%) at 12 months after RTX compared to baseline ($p = 0.033$) and DLCO values also clearly increased (+12.4%) at 12 months after RTX compared to baseline values $p \leq 0.001$. Ten out of 18 patients were evaluated 2 years following treatment with RTX and they again displayed a significant increase of DLCO compared to baseline levels (+ 15.3%, $p = 0.001$). In contrast, FVC values did not change significantly 2 years after treatment with RTX compared with pre-treatment levels in these 10 patients. Thirteen patients also underwent chest HRCT before and after 1 year of RTX administration. Stabilization or improvement was seen in 10/13 patients (76.9%) when scored by two independent radiologists and deterioration was reported in 3/13 patients.

Keir et al. analyzed 50 patients with ILD 33 of which had CTDs (12). Patients with CTDs included 10 with IIM, 8 with SSc, 9 with UCTD, 2 with MCTD, 2 with RA, 1 with SLE, and 1 with SS. All patients had advanced ILD with a mean FVC of 44.0% and a mean DLCO of 24.5%. In addition, they all had resting state hypoxia (mean PaO2: 8.3 kPa). RTX was administered in the "RA scheme" resulting in a median improvement of FVC of 6.7% ($p < 0.01$) and a stabilization of DLCO with a median change of 0% ($p < 0.01$) at 6 and 12 months of RTX treatment compared to baseline levels. Patients ($n = 50$) were considered as responders according to stabilization ($n = 22$) or improvement (n=14) of PFTs and as non-responders ($n = 14$) due to deterioration of PFTs. Comparison between the two categories depicted a lesser decline of FVC at 6–12 months after RTX compared to baseline in responders (−12.9%) vs. non-responders (−20.0%) after 6 to 12 months of RTX compared to baseline, ($p = 0.05$).

However, Zhu et al. conducted a retrospective controlled trial comparing the effects of combined RTX plus MMF treatment in patients with CTD-ILD ($n = 15$) (RTX group) vs. MMF treatment alone ($n = 68$) in patients with CTD-ILD (control group) (13). The evaluation of PFTs between the two groups reveals the following: the absolute change in FVC (% predicted, post-treatment—baseline) was −3.0 in the RTX group thus showing a significant decrease. On the other hand, the control group had a significant increase in the absolute change of FVC of + 2.0, $p = 0.03$. Moreover, the absolute change of DLCO was significantly decreased at −3.0 in the RTX group, whereas it improved significantly at + 4.5 in the control group, $p = 0.046$. The mixed model analysis performed however in the two groups did not display a significant difference in both PFTs over time. HRCT scores showed no significant differences between the RTX group (17.5 ± 4.6) compared to the control group (12.6 ± 4.4). It should be noted that the steroid dosages decreased in the RTX group, whereas it did not in the control group ($p = 0.017$). The mortality rate after treatment was calculated at 3/15 (20.0%) in the RTX group vs. 7/68 (10.3%) in the control group. Results of

TABLE 1 | Trials of RTX use in CTD-ILD.

References	No of patients	Disease	RTX scheme	Follow up	Outcomes	Safety of RTX
Duarte et al. (7)	30	CTDs	RA scheme	3 y	Improved FVC Stable DLCO	13/30 pts discontinued RTX 4/13 due to infection
Lepri et al. (8)	44	CTDs	RA scheme	1y/2y	Stable FVC and DLCO	12/44 pts serious AE
Atienza-Mateo et al. (9)	26	CTDs	RA scheme ($n = 20$) Lymphoma scheme ($n = 2$)	2 y	Stable PFTs at 6 m, 1, 2 y ↑DLCO at 1 y stable HRCT	6/26 discontinued RTX due to AE 3/26 death
Sharp et al. (10)	24	CTDs	RA scheme	6 m	Improved FVC stable DLCO 13/22 stable HRCT	No serious AE
Robles-Peres et al. (11)	18	CTDs	RA scheme	1y/2y	Increased PFTs at 1 y 10/18 stable HRCT at 1 y	No serious AE
Zhu et al. (13)	15 RTX vs. 68 non-RTX	CTDs			Significant DLCO change and steroid reduction vs. control Similar FVC, HRCT score vs. control	No serious AE
Keir et al. (12)	33	CTDs	RA scheme	6 m/1 y	Improved FVC, stable DLCO	–
Mena-Vazquez et al. (14)	37	CTDs	RA scheme	1 y	Stable PFTs HRCT stable or improved	29/37 lung infection, 7/27 deaths

No, Number; m, month; y, year; AE, adverse events; CTDs, connective tissue diseases; RTX, rituximab; PFTs, pulmonary function tests; FVC, forced vital capacity; DLCO, diffusing lung capacity for carbon monoxide; HRCT, high resolution computed tomography; RA, rheumatoid arthritis. ↑, Increased.

this trial do not favor the addition of RTX on top of standard of care treatment. The authors point-out that patients in the RTX group had longer disease duration and lower DLCO percentages at baseline.

A PROSPECTIVE TRIAL OF B CELL DEPLETION IN PATIENTS WITH CTD–ILD

Mena-Vazquez et al. evaluated 37 patients with CTD-ILD that received RTX as add-on therapy upon the deterioration of either clinical symptoms or PFTs compared to the time of diagnosis (14). Nineteen patients with RA (51.4%), 14 with SS (37.8%), and 4 with IM (10.8%) received RTX in the "RA scheme" and were evaluated before and 12 months after treatment. The above parameters improved ($n = 6$) or stabilized ($n = 7$) in 62.2% of patients and worsened ($n = 7$) or died ($n = 7$) in 37.8% of patients.

In the whole group of patients on RTX or in any disease-specific subgroup mean PFT and DLCO values did not decrease significantly during the first 12 months of treatment with RTX compared with baseline. HRCT revealed radiological progression in 14/37 patients (37.8%) while in 16/37 patients (43.2%) revealed stabilization and in 7/37 (18.9%) revealed improvement, but differences were not significant.

Table 1 summarizes the studies of RTX use in CTD-ILD.

ONGOING CLINICAL TRIALS OF RTX IN PATIENTS WITH CTD-ILD

An ongoing, multicentre, prospective, randomized, double-blind, controlled trial (RECITAL) compares RTX vs. CYC in the treatment of patients with ILD in CTD such as SSc, IIM (including AAS), or MCTD (15). RTX will be administered in the "RA scheme" and CYC will be administered IV at 600 mg/m^2 body surface area monthly. A total of 116 patients is expected to be randomized 1:1 to each of the two treatment arms in this 48-wk trial. The primary end-point is the change in FVC at 24 weeks and secondary endpoints include treatment safety, changes in FVC at 48 weeks as well as survival, change in oxygen requirements, and total 48-wk corticosteroid exposure.

Another multicentre, prospective, randomized, double-blind, placebo controlled, superiority trial evaluates the efficacy and safety of RTX plus MMF in patients with ILDs (EvER-ILD) (16). A broad range of patients with non-responding, resistant to previous therapy ILD was recruited to receive different treatment schemes; one group ($n = 61$) will receive RTX ("RA scheme") plus MMF, while the other group ($n = 61$) will receive one placebo infusion plus MMF for 6 months. Pulmonary function, with the changes of FVC as the primary endpoint, will be evaluated at 6 months.

A META-ANALYSIS OF PATIENTS WITH CTD-ILD

In their meta-analysis, Xing et al. selected 6 retrospective studies in which 242 patients with CTD-ILD had been included (17). Five out of 6 studies included patients with SSc-ILD and 1 included patients with RA-ILD. All patients were analyzed for potential changes in the PFTs after being treated with RTX. The FVC was evaluated in 210 patients from all 6 studies. Analysis disclosed that the mean difference of FVC between RTX and conventional therapy was 5.4 (95% CI 3.73 to 7.08; $p < 0.00001$) in favor of RTX. However, DLCO was evaluated in 111 only patients from four studies (due to the high heterogeneity of results). The mean difference

between post-treatment RTX and conventional therapy was-−2.22 (95% CI −6.83–2.40) in favor of conventional treatment though it was not statistically significant. This meta-analysis suffers from high heterogeneity between the studies included; it mainly refers to SSc-ILD and not ILD due to other CTDs. In addition, the very small number of studies included represents another limitation.

SYSTEMIC SCLEROSIS SSC

Prospective Controlled Studies in Patients With SSc-ILD

A controlled study by Daoussis et al. evaluated 8 patients with SSc-ILD who received RTX as an add-on treatment vs. 6 patients with severe SSc-ILD who continued their standard treatment (18). Patients in the RTX group demonstrated a significant increase of FVC compared to baseline values (mean ± S.D.: 68.13% ± 19.69 vs. 75.63% ± 19.73, at baseline vs. 1 year, respectively, $p = 0.0018$). DLCO increased significantly as well (mean ± S.D.: 52.25 ± 20.71 vs. 62 ± 23.21, at baseline vs. 1 year, respectively, $p = 0.017$). No significant changes in PFTs of the control group were observed. Chest HRCT scores, based on a semi-quantitative program, disclosed no worsening at 24 weeks in the RTX group, but patients in the control group displayed a slight worsening in HRCT involvement scores at 24 weeks.

Jordan et al. studied 25 patients with SSc-ILD initiating RTX vs. 25 patients with SSc-ILD that were not treated with RTX from the EUSTAR database (19). Patients in the RTX-group at 6 months displayed a stable FVC (60.6 ± 2.4 vs. 61.3 ± 4.1%; $p = 0.5$) and a significantly increased DLCO (41.1 ± 2.8 vs. 44.8 ± 2.7%; p=0.03) when compared to baseline values. DLCO did not change significantly in a direct comparison between the two groups. A comparison between the RTX group *vs.* the non-RTX group revealed significant changes in percentages of predicted FVC (0.4 ± 4.4 vs. −7.7 ± 3.6; $p = 0.02$) and in the absolute FVC values changes (0.8 ± 2.2 vs. −4.8 ± 1.7; $p = 0.01$) as well.

A multicentre controlled trial in Greece enrolled 51 patients with SSc-ILD receiving RTX vs. 33 patients with SSc-ILD receiving standard treatment, for a period of 4 years (20). FVC in the RTX group during the first 2 years significantly increased (mean ± SD of FVC: 80.6 ± 21.21 vs. 86.90 ± 20.56 compared to baseline $p = 0.041$, but the patients receiving standard treatment displayed no significant changes in FVC. In addition, patients treated with RTX for 7 years ($n = 5$) had numerically, but not significantly higher FVC compared to baseline (mean ± SD of FVC: 91.60 ± 14.81 vs. 86.90 ± 20.56, $p = 0.158$). However, the patients ($n = 9$) on standard treatment had significant FVC deterioration at the 7th year of follow-up ($p < 0.01$). Moreover, a direct *comparison between the two groups revealed a significant benefit for the RTX group vs.* the non-RTX group ($p = 0.013$). Patients in the RTX group preserved their DLCO values for the 7-yr period, but patients in the non-RTX group displayed a significant reduction of their DLCO values ($p = 0.004$).

Boonstra et al. studied 16 patients with early SSc for a 2-yr follow-up period; one group was treated with RTX ($n = 8$) with the "RA scheme" and the control group ($n = 8$) with placebo (21). Previous immunosuppressive therapy was allowed for all patients during the 2 yrs of follow-up. FVC and the extent of lung involvement slightly but non-significantly improved with RTX after 2 years; FVC (placebo: −1.4 vs. RTX: +4, $p = 0.65$), and DLCO% (placebo: −2.2, RTX: −6.0, $p = 0.77$). Analysis of HRCT lesions according to criteria set by Goh disclosed a mean change in the percentage of affected lung tissue between baseline and 12 months of −1.6% for the RTX group and +2.8% for the placebo groups ($p = 0.28$).

The EUSTAR study evaluated 146 out of 254 patients with SSc-ILD who received RTX plus standard treatment vs. standard treatment alone, for a 2-year period (22). More specifically, Elhai et al. studied 254 patients from the EUSTAR database with SSc-ILD treated with RTX from a pool of 9,575 patients with SSc-ILD receiving standard treatment for a period of 2 years. During the 2 years of evaluation in both groups FVC, as well as DLCO values, remained practically stable. RTX-treated vs. standard-treated patients did not have significantly different rates of decrease in FVC > 10% [6.5 vs. 6.6 events per 100 person-years; OR: 1.03 (0.55–1.94); $p = 0.93$] and in DLCO as well. However, the patients in the RTX group discontinued or reduced the daily dosages of steroids earlier [OR: 2.34 (1.56–3.53), $p < 0.0001$]. Even though the number of patients was large enough, the trial results may have been confounded by patient heterogeneity. There were differences in the extent of lung involvement of enrolled patients, in the chronicity of the disease, and even in the RTX administration protocol among the various participating centers to such an extent that may preclude the extraction of safe conclusions. In secondary analyses of PFTs patients treated with RTX plus MMF ($n = 37$) showed better outcomes as compared with patients receiving RTX alone [delta FVC in RTX plus MMF: 5.22 (0.83–9.62); $p = 0.019$ vs. delta FVC: 3 (0.66–5.35); $p = 0.012$ in patients receiving rituximab alone].

In a recent, open-label, prospective, randomized, controlled trial the authors compared head-to-head RTX vs. monthly cyclophosphamide (CYC) treatment (23). Sixty patients with early, diffuse SSc with ILD and anti-Scl70(+), were enrolled to receive CYC or RTX as first-line treatment. Patients in the CYC treatment arm received 500 mg/m^2 CYC IV pulses every 4 weeks for 24 weeks. Patients in the RTX group received RTX as in the "RA scheme." The RTX group revealed an improvement of FVC% at the end of 6 months when compared to baseline values (RTX group: 61.3–67.5%, $p = 0.002$) while the CYC group did not (CYC: 59.3–58.1%, $p = 0.496$). RTX turned out to be safer than CYC regarding cases of malignancy, gangrene, and ovarian failure, but it displayed higher rates of (minor) infusion reactions. Based on these data RTX may be considered a first-line therapy instead of CYC especially if we take into account the safety demonstrated too in this trial. However, one should take into account that the current standard-of-care treatment for SSc-ILD is MMF and not CYC. The controlled trials of RTX effect on SSc-ILD are depicted in **Table 2**.

TABLE 2 | Controlled trials of RTX in SSc-ILD.

References	No of patients	Disease	RTX scheme	Follow up	Outcomes	Safety of RTX
Daoussis et al. (18)	8 RTX vs. 6 Control	SSc	Lymphoma scheme	1 y	Improved PFTs and stable HRCT score in favor of RTX	3 serious AE/2 lung infections, 1 infusion reaction
Jordan et al. (19)	25 RTX vs. 25 control	SSc	RA scheme	6 m	Increased FVC, stable DLCO in RTX group	11/53 infections No other serious AE
Daoussis et al. (20)	33 RTX vs. 18 control	SSc	Lymphoma scheme	7 y	Improved FVC, stable DLCO vs. control	3 serious infections, 2 infusion reactions, 5 deaths
Boonstra et al. (21)	8 RTX vs. 8 con	SSc	RA scheme	2 y	Stable PFTs and HRCT similar to control group	7 serious AE in RTX(infusion reaction) vs. 4 serious AE in control (weight loss)
Eustar et al. (22)	254 RTX vs. 9,575 control	SSc	RA scheme ($n = 203$) lymphoma scheme ($n = 4$) else ($n = 27$)	2 y	Stable PFTs similar to control/steroid reduction vs. control	36/254 serious AE, 24/254 discontinue, 6 deaths unrelated
Theibaut et al. (24)	23 RTX vs. 26 non-RTX	SSc	RA scheme	2 y	Improved PFTs	Infusion reactions, mild infections/2 deaths
Sircar et al. (23)	30 RTX vs. 60 CYC	SSc	RA scheme	6 m	Improved FVC on RTX vs. control	RTX AE 9/30 vs. CYC AE 21/30

Prospective Uncontrolled Studies in Patients With SSc-ILD

Lafyatis et al. studied 15 patients with early SSc treated with RTX only ("RA scheme") without concomitant disease modifying anti-rheumatic (DMARD) therapy (25); they did not find a clear beneficial effect on skin fibrosis and pulmonary function at 6 and 12 months of follow-up. Patients with severe lung disease were excluded from this trial; therefore this might explain why the average FVC and DLCO values did not change significantly at 6 months. In detail, baseline FVC changed from 89.2 to 92.7% after RTX treatment and DLCO changed from 79.7% at baseline to 77.9% after RTX administration. In agreement with the above, little or no progression of pulmonary disease was depicted on HRCT. Because patients included in this study had a near-normal respiratory function, no safe conclusions can be drawn regarding the effects of B cell depletion treatment.

Daoussis et al. presented a beneficial effect of RTX ("lymphoma scheme") when the follow-up of our initial cohort of 8 patients that were previously reported at 1 year was extended to 2 years of treatment (26). FVC values displayed a significant improvement at 2 years (mean ± SEM: 77.13 ± 7.13 vs. 68.13 ± 6.96, respectively, $p < 0.0001$) as did the DLCO values (mean ± SEM: 63.13 ± 7.65 vs. 52.25 ± 7.32, respectively, $p < 0.001$). Moreover, semiquantitatively ground glass lesions were depicted in HRCT of 5/8 patients. The results are considered encouraging, even though the numbers of patients enrolled were small, RTX was administered concurrently with other immunosuppressing drugs (MMF in the majority) and there is not a control group in this study.

Another open-label study by Smith et al. in patients with early diffuse SSc who received RTX for a 2 year period, displayed a statistically significant overall decrease of FVC percentages, with a mean FVC of 92.8% at baseline vs. 84.7% at 24 months ($p = 0.047$) (27). However, no clinical worsening was DLCO values remained stable over the 2-years.

Bosello et al. administered RTX ("RA scheme") in 20 patients with early (<3 years) and extensive SSc-ILD (28). During a follow-up period of 2 years, the evaluation of PFTs every 6 months and of chest HRCT every 12 months indicated no significant changes in FVC, DLCO, and chest HRCT lesions over time. However, there was marked heterogeneity regarding the duration of follow-up and the number of RTX cycles administered. When analyzed separately, the patients with the restrictive disease ($n = 6$) in PFTs (representing perhaps the most interesting patient subset) had an increase in their FVC but insignificant changes in their DLCO and HRCT scores during the first year of RTX treatment. In contrast, patients without restrictive disease in PFTs ($n = 8$) displayed no increases in the above parameters throughout the study.

Fraticelli et al. studied 15 patients with SSc-ILD receiving RTX for 6 months followed by MMF administration escalated to 2 gr for 6 months (29). After 6 months of RTX, there was a significant increase in FVC ($p = 0.0197$) and FEV1 ($p = 0.0278$) compared to baseline. The HRCT pattern was analyzed with two different methods. The first was a semiquantitative standardized method, but the results did not show significant improvement. On the contrary, the second, which is an automatic software-based assessment revealed a significant improvement ($p = 0.0331$) which is consistent with a significant reduction in the quantity of pulmonary fibrosis. At 12 months of combined therapy with RTX initially and MMF afterward, a significant increase in FVC percentages were observed ($p = 0.0093$) and of FEV1 values ($p = 0.0061$) as well, compared to baseline. DLCO values remained stable ($p = 0.3375$). Despite the small number of patients and the absence of a control group, the authors felt optimistic about the sequential treatment of RTX followed by MMF in SSc-ILD. The uncontrolled studies of RTX administration in SSc-ILD are shown in **Table 3**.

TABLE 3 | Uncontrolled trials of RTX in SSc-ILD.

References	No of patients	Disease	RTX scheme	Follow up	Outcomes	Safety of RTX
Lafyatis et al. (25)	15	SSc	RA scheme	6 m	Stable PFTs and HRCT score	No serious AE
Daoussis et al. (26)	8	SSc	Lymphoma scheme	2 y	Improved PFTs and HRCT score	3 serious infections, 2 infusion reactions
Smith et al. (27)	8	SSc	RA scheme	2 y	Increased FVC, stable DLCO	5 serious AE/1 death
Bosello et al. (28)	20	SSc	RA scheme	2 y	Stable PFTs and HRCT score	4 serious AE
Fraticelli et al. (29)	15	SSc	RA scheme	6 m	Improved FVC, stable DLCO, Imroved HRCT	No serious AE
Vilela et al. (30)	10	SSc	RA scheme	6 m	Stable PFTs	–
Sari et al. (31)	15	SSc	Ra scheme	Variable	Stable PFTs	No serious AE

Retrospective Trials in Patients With SSc-ILD

A placebo-controlled trial by Thiebaut et al. studied retrospectively 13 patients with SSc-ILD who were treated with RTX vs. 26 patients with SSc-ILD that were not for a follow up period of 2 years (24). Both FVC and DLCO values did not change significantly after 1 year. FVC showed 72% at baseline and 85% at month 12 ($p = 0.6$) and DLCO values [40% at baseline vs. 49% at month 12 ($p = 0.9$)]. However, after 2 years of follow-up, 7/13 patients treated with RTX improved their FVC by a gain of 12 points, whereas 14 patients not treated with RT? worsened their FVC by −1.5. DLCO values also improved in the RTX-treated group with a gain of 4, while it worsened with a loss of −4.5 in the non-RTX-treated group. A direct comparison between the 2 groups (RTX group vs. non-RTX) revealed a significant improvement of PFTs in RTX-treated patients regarding both FVC percentages ($p = 0.003$) and DLCO percentages ($p = 0.03$). The authors also further analyzed a total of 42 patients (35 from the literature and 7 from their own series). They reported an increase of FVC from 71% at baseline to 84% at 12 months ($p = 0.0006$) and in DLCO from 58% at baseline to 64% at 12 months ($p = 0.02$) in the RTX group.

Vilela et al. reported 10 patients with diffuse SSc treated with RTX ("RA scheme") that were evaluated after 6 months (30). No significant changes were seen in PFTs in all patients regardless of the patients had early (<4 yrs) or late progressive disease. Sari et al. claimed a beneficial effect of RTX therapy in 14 patients with SSc and chronic extensive restrictive lung disease resistant to previous therapy for 15 months of follow up (31). However, heterogeneity in treatment cycles and in time-points of evaluation limit the value of any potential conclusions of this report.

A META-ANALYSIS OF PATIENTS WITH SSc-ILD

A recent systematic review and meta-analysis by Goswami et al. evaluated the efficacy of RTX treatment on pulmonary function parameters of patients with SSc-ILD (32). Twenty studies were included in this meta-analysis among which there were two randomized controlled trials, six prospective studies, five retrospective studies, and seven conference abstracts. RTX was reportedly associated with increases of FVC by 4.49% (95% CI 0.25, 8.73) and similarly with increases of DLCO by 3.47% (95% CI 0.99, 5.96) at 6 months. Furthermore, FVC values were increased by 7.03% (95% CI 4.37, 9.7) and percentages of DLCO by 4.08% (95% CI 1.51, 6.65) after 1 year of treatment with RTX. Two studies comparing RTX treatment to other immunosuppressive treatments showed improvements in FVC in the RTX group that was 1.03% (95% CI 0.11, 1.94) greater than the improvement of FVC values of patients in the immunosuppressive group at 6 months but not at 12 months. Changes in DLCO values were similar in both groups at both time points. Patients on RTX had lower rates of infection as well. The authors acknowledge another limitation of this meta-analysis such as the different disease traits, RTX administration schemes, and evaluation of PFTs at the standard time points of 6 and 12 months.

RETROSPECTIVE TRIALS IN PATIENTS WITH RA-ILD

Fui et al. studied 28 patients with RA 21 of which had ILD (33). Fourteen of 28 were treated with RTX due to worsening ILD. PFTs and HRCTs were evaluated before and 6 and 12 months later. FVC before RTX administration was 87.11 ± 6.13% and FVC after 12 months of RTX treatment was 93.01 ± 23.01%, p = NS. DLCO percentages before RTX treatment were 53.76 ± 5.37% and DLCO 12 months after RTX administration was 54.9 ± 15.2%, p = NS. Therefore, we conclude that patients receiving RTX had stabilization of their PFTs. Patients were also separately analyzed as UIP HRCT patterns and NSIP HRCT patterns. For the UIP-ILD pattern, there was a loss of 301 ml of FVC at 1 year, while for the NSIP-ILD the loss was limited to 51 ml compared to baseline volumes, non-significant reductions. However, a direct comparison of volume losses between the UIP and NSIP–ILD volume losses disclosed that reductions seen in the UIP-ILD subgroup were significant. The study included also 14/28 patients with RA that did not receive RTX and were evaluated as well at baseline and 12 months. However, a direct comparison between the RTX and the non-RTX group revealed no significant changes. The patients with RA–UIP pattern had a bad prognosis, despite the observation that PFTs of these patients did not worsen after

RTX administration. It is of interest that in this retrospective study another subpopulation not receiving RTX was evaluated and was used as a control subpopulation.

Md Yusof et al. evaluated retrospectively 700 patients with RA with 56/700 diagnosed with ILD (34). Some patients were treated with RTX due to worsening ILD ($n = 10$) or due to unresponsiveness to previous treatment for RA ($n = 6$). PFTs were evaluated in all 56 patients with RA-ILD. A numerical improvement was observed in FVC after 6–12 months of RTX administration compared to baseline from −2.4% pre—RTX treatment to +1.2% post-RTX treatment, with a median difference of +4.2%, $p = 0.025$. DLCO values had an increase from−4.4% pre-RTX to −1.3% post-RTX with a median difference of +3.7%, $p = 0.045$. Fourteen patients with worsening ILD were evaluated with HRCT before and after treatment with RTX. Improvement of HRCT images was seen in one patient (7%), stabilization in six patients (42%), and worsening in seven patients (50%) after RTX administration. A direct comparison between the patients with responsive NSIP-ILD ($n = 33$) and the patients with progressive non-responsive NSIP-ILD revealed that DLCO values declined more in the non-responsive group (−3.8% responsive NSIP-ILD vs. −17.5% non-responsive NSIP-ILD, $p = 0.037$).

In their retrospective study Narvaez et al. analyzed 31 patients with RA that developed serious ILD refractory to the previous DMARD therapy (35). Some patients switched to RTX from other biologic agents (n=12) or had already failed to respond to previous ILD treatment (n=3). HRCTs were performed after RTX treatment, only in those patients that had worsening dyspnea or deterioration of PFTs. RTX treatment prevented the further reduction of FVC at 1 year compared to baseline (delta: percentage change from the start of therapy): Δ% pFVC +8.06% (95% CI: −10.9–−5.2; $p < 0.001$). Moreover, DLCO did not further decline after 1 year compared to baseline Δ% pDLCO: +12.7% (95% CI: −16.3 to −9.1; $p < 0.001$. When the patients were divided into a UIP ($n = 13$) or a non-UIP- pattern ($n = 18$) and analyzed separately, PFTs in both groups improved at 1 year compared to baseline levels. A quantitative ILD score (QILD) score was used in HRCTs to define the extent of lung disease. Eighteen/31 patients had a QILD of 18.6 ± 12% before treatment with RTX. Six of the 18 patients (33%) worsened their QILD score (mean −5% ± 0.7), 2/18 (11%) improved their score (mean +6.5% ± 1.5), and 10/18 (56%) had the same QILD score after 21 months of RTX administration in comparison to baseline QILD score. In addition, 25 patients with RA evaluated at 2 years of treatment with RTX displayed a significant improvement of both FVC and DLCO compared to pretreatment levels.

Prospective Trials in Patients With RA-ILD

In their study, Matteson et al. evaluated 10 patients with RA-ILD treated with RTX (7/10) for a maximum of 48 weeks (36). By week 48, FVC values declined by at least 10% in 1/7 patients, remained stable in 4/7, and increased by at least 10% in 2/7 patients. DLCO worsened by at least 15% in 1/7 patients, was stable in 4/7 patients, and increased by >15% of baseline in 2/7 patients. HRCT scores improved in 1 patient, worsened in another patient and stabilized in the rest of 5/7 patients assessed at week 48 after RTX administration.

Vadillo et al. enrolled 68 patients from two different medical centers diagnosed with RA ILD (37). Functional impairment was defined as a reduction of FVC < 5%. By the time ILD was diagnosed treatment of patients changed with preferential use of RTX, abatacept, and azathioprine and reduced use of MTX, LEF, and anti-TNFα mAb. Thirty-one out of 68 patients with RA were treated with RTX with a mean follow-up of 20.6 months and maximum exposure of 8.8 years. Multivariate analysis revealed that PFTs were preserved in patients with RA treated with RTX compared to patients on other treatment regimes. The same analysis disclosed that RTX had less risk of deterioration (Hazard Ratio and 95% confidence interval) [HR 0.51 (95% CI 0.31, 0.85), $p = 0.01$] vs. patients with RA on other treatments. The doses of RTX administered, the repetition of cycles as well as the number of cycles of RTX differed in each patient. Despite such confounding factors, the results of this trial could be considered as promising.

RETROSPECTIVE STUDIES IN PATIENTS WITH AS

Marie et al. studied seven patients with AS that manifested with severe ILD that had previously failed treatments with immunosuppressive agents (38). After having received RTX treatment for 1 year, the patients exhibited improvement of PFTs.

FVC percentages increased from a pre-treatment mean of 66% to a post-treatment of 74 %, $p = 0.04$ and an increased DLCO from 39% (pre-treatment) to 59% (post treatment), $p = 0.001$. HRCT scoring disclosed an improvement of ILD abnormalities at 1 year after RTX treatment in 5/7 patients and stabilization of ILD abnormalities in 2/7 patients. The dosages of steroids were tapered by almost 50% of the pretreatment dosages after treatment with RTX. This small uncontrolled and retrospective study favors RTX use in refractory cases of AS—associated ILD.

Twenty-five patients with AS autoAb and ILD treated with RTX were retrospectively evaluated in the study of Doyle et al. (39). RTX was administered because of recurrent or progressive ILD. Twenty-one out of 25 patients were treated with RTX as a second-line medication due to unresponsiveness to previous DMARDs and 4 out of 25 were treated with RTX as a treatment of the first choice. Prednisone and other DMARDs were allowed. PFTs and HRCT scores were evaluated before and 1 year after treatment with RTX. FVC ($n = 19$) was stable or improved by 79% at 1 year after treatment with RTX compared to baseline. In addition, HRCT average scores ($n = 8$) were stable or improved in 88% of subjects. FVC was also statistically increased for seven patients with AS 3 years after RTX treatment compared to pretreatment levels (+21%, $p = 0.016$). DLCO percentages on the contrary were reduced 1 year after treatment with RTX compared to baseline, from 42 ± 17 to 36 ± 16. Nevertheless, 2 years after RTX administration, DLCO percentages increased compared to baseline from 36 ± 16 to 53 ± 26. The authors report that the dosages of glucocorticoids were stable or reduced in 88% of patients. Moreover, it is of importance that the patients

who received more than one cycle of RTX ($n = 17$) compared to the patients that received only one cycle of RTX ($n = 8$) had an increase of FVC of 9.3% ($p = 0.0077$). Patients having NSIP patterns in HRCT had a better response to RTX treatment. In addition, patients with mild disease (based on good FVC and DLCO parameters) responded better to treatment with RTX.

Andersson et al. studied retrospectively 24 patients with AS with severe ILD involvement and were administrated RTX (40). During the follow-up period (median: 52 months after RTX treatment), FVC values were increased from 58% at baseline to 72% at post-treatment ($p < 0.018$). DLCO values similarly increased from 41% at baseline to 48% post-treatment, ($p < 0.025$). The extend of ILD-affected lung parenchyma on HRCT was reduced by a median of 33% compared to pre-treatment HRCT scores. The follow-up period after RTX treatment is more than 4 years, which is long enough. Patients treated with RTX that were followed up for <12 months had a significant improvement in PFTs and HRCT extent of ILD after RTX treatment.

Langlois et al. compared CYC ($n = 32$) followed by standard immunosuppressive agents vs. RTX ($n = 28$) treatment administered every 6 months in patients with ASS-related ILD (41). CYC was administered (750 mg/m^2/month) for 6 months and then was switched to other immunosuppressive drugs (AZA, MMF, MTX). PFTs showed a significantly increased median FVC from 53% at baseline to 62% at 6 months after CYC treatment, $p = 0.01$. DLCO percentages also increased from 31.5% at baseline to 35% post treatment, $p = 0.01$. Additionally, eight patients had HRCT scores improved, 14 stabilized, and eight worsened their HRCT scores at 18 months of follow-up after treatment with CYC. RTX was given in the "RA scheme" every 6 months. FVC increased significantly at 6 months after therapy with RTX compared to baseline from 64 to 74%, $p = 0.002$ and DLCO increased slightly, from 45% at baseline to 48% at 6 months after RTX, $p = 0.1$. FVC values were statistically and significantly higher at 1 and 2 years after RTX treatment compared to pre-RTX values. DLCO percentages did not display significant increases at 1 and 2 years of treatment with RTX compared to baseline. Eleven patients improved their HRCT scores, 12 stabilized and 1 worsened their HRCT scores after RTX administration during a mean follow-up of 17.5 months. Upon comparison of the 2 groups treated with CYC vs. treated with RTX, it was observed that the group that initiated CYC had already lower PFT values compared to the group that initiated RTX. More specifically, FVC was 53% in the CYC group vs. 64% in the RTX group, ($p = 0.04$) and DLCO was 32% in the CYC group vs. 45% in the RTX group ($p = 0.01$), respectively. In addition, it was recorded that RTX was administered in patients with the more refractory disease compared to the cases in that CYC was given. The number of previous immunosuppressive treatments contributed to this conclusion: 2.32 ± 1.45 in the RTX group vs. 1.35 ± 1.39 in the CYC group, ($p = 0.004$), respectively.

RTX and CYC demonstrated similar pulmonary progression-free survival (PFS) at 6 months after treatment (92% RTX vs. 85% CYC, respectively), but RTX was superior to CYC at 2 years of treatment (HR 0.263, 95% CI 0.094–0.732, $p = 0.011$). RTX treatment was superior in the maintenance phase compared with immunosuppressants continued after CYC. No significant differences were reported in the dosage of the steroid and in the adverse events of the two treatments as well. Despite the retrospective nature of this study and the dissimilarity of the patients comprising the 2 treatment groups this study is acknowledged as the first comparison study of RTX vs. other immunosuppressive treatment in AS-ILD.

The majority of the studies of RTX conducted in groups of patients with different CTDs–ILD or in patients with one underlying disease–ILD such as SSc, RA, AS, has noted that B cell depletion treatment was safe and well-tolerated. A few gastrointestinal complaints have been reported and to a lesser extent infusion reactions. Our major concern is focused on infections, especially respiratory tract infections (42).

STUDIES IN PATIENTS WITH SS

A few case reports and even fewer retrospective studies have evaluated RTX in ILD in patients with SS (43, 44). Chen et al. studied retrospectively 10 patients with primary SS (pSS) with moderate to severe ILD that had previously been treated with DMARDs (45). PFTs at 6 months after RTX treatment revealed an improvement of DLCO from 49.3 ± 12.6% at baseline to 56.9 ± 11.4% at 6 months after RTX, ($p = 0.011$) but a non-significant change of FVC from 74.7 ± 16.2% at baseline to 76.4 ± 16.1 % at 6 months, ($p = 0.484$). Mean HRCT scores decreased but not significantly after rituximab in 7/10 patients (from 8.7 ± 4.1 to 7.6 ± 4.6, $p = 0.419$). A significant improvement in HRCT imaging was reported only in 1 pSS patient at 6 months. In addition, all 10 pSS patients with ILD had a restrictive pattern with low DLCO (<75%) and 2/10 had a mixed type of obstructive and restrictive patterns (decreased FVC, <60%). According to this observation, RTX may be beneficial for patients with pSS during active phases of ILD.

SLE

ILD, although uncommon in SLE, is highly associated with increased mortality. ILD in SLE presents as NSIP, OP, LIP, follicular bronchitis, and UIP. No clinical trials and head-to-head studies exist assessing the treatment of SLE-related ILD, let alone with RTX treatment. Only case reports and case series have been reported regarding the effects of RTX treatment on all manifestations of patients with SLE (46).

Acute lupus pneumonitis is also a serious manifestation of SLE as well as pulmonary hemorrhage. Immunosuppressive agents including RTX have been used. RTX has also been employed with promising results in shrinking lung syndrome in patients with SLE as a few case reports have reported (47, 48).

One retrospective study included 11 patients with refractory SLE to the previous use of steroids and immunosuppressive agents (49). The patients were given RTX as an add-on therapy and were evaluated at 6 months after RTX administration. Seven out of 11 patients were simultaneously treated with CYC and 6 out of 11 were treated with IV steroids. Three patients

TABLE 4 | Trials of RTX use in RA/AS/SS/SLE-ILD.

References	No of patients	Disease	RTX scheme	Follow up	Outcomes	Safety of RTX
Fui et al. (33)	14 RTX vs. 14 non-RTX	RA	RA scheme	12 m	Stable PFTs and HRCT score vs. reduced PFTs in control	No serious AE
Md Yusof et al. (34)	56	RA	RA scheme	12 m	Increased PFTs/HRCT score	33/56 serious AEs/infections 12/56 deaths
Narvaez et al. (35)	31	RA	RA scheme	12 m	Improved PFTs improved/stable HRCT	Few serious AE 10/31 2/31 deaths
Mattesson et al. (36)	10	RA	RA scheme	12 m	Stable PFTs/stable HRCT	3/7 AEs 2/7 death
Vadillo et al. (37)	31 RTX/37 non-RTX	RA	RA scheme	6 m	Stable PFTs vs. non-stable PFTs in control group	A few AEs
Marie et al. (38)	7	AS	RA scheme	12 m	Improved PFTs/HRCT score/steroid reduction	No serious AE
Doyle et al. (39)	21	AS	RA scheme	12 m	Improved PFTs and HRCT/steroid reduction	No serious AE
Andersson et al. (40)	24	AS	RA scheme	52 m	Improved PFTs and HRCT	6/34 serious infection, 7/34 death
Langlois et al. (41)	28 RTX vs. 32 CYC	AS	RA scheme	2 y	Improved PFTs and HRCT score in both groups	Similar AE
Allenbach et al. (43)	10	AS	RA scheme	1 y	Improve PFTs/stable HRCTscore	No serious AE
Chen et al. (45)	10	SS	RA scheme	6 m	Improved FVC, stable DLCO,Stable HRCTscore	No serious AE
Reynolds et al. (49)	11	SLE	RA scheme ($n = 9$) Lymphoma scheme ($n = 2$)	6 m	Stable PFTs	No serious AE

had severe lung disease with significant morbidity. One patient responded well to RTX with improvement in FVC and total lung capacity. Two patients preserved a non-deteriorating respiratory function, and 1 patient had an objective improvement in lung function as he remained stable after RTX treatment. FVC did not change significantly in the whole group of 11 patients, $p = 0.11$. One might assume that RTX halted the deterioration and progression of lung disease in these few patients, whereas previous immunosuppressants had previously failed in halting ILD progression. **Table 4** collects all studies mentioned above regarding RTX in RA-, AS-, SS-, and SLE-related-ILD.

STUDIES IN PATIENTS WITH AAV-ILD

ILD has been related to ANCA-associated vasculitis (AAV) over the past three decades. ILD can be present in 23% of patients with granulomatosis with polyangiitis (GPA) and up to 45% of patients with microscopic polyangiitis (MPA). ILD may precede or manifest concurrently with other manifestations of AAV (50). Radiologic patterns in HRCT include UIP (up to 78% of cases), NSIP (ranging from 13 to 64% of cases), desquamative interstitial pneumonia-like pattern, OP, and combined pulmonary fibrosis and emphysema (CPFE). Although there are treatment guidelines for the initial therapy of patients with AAV, limited data exist for the treatment of patients with AAV (51). RTX is a licensed and commonly used immunosuppressive treatment in patients with AAV-ILD both for induction of, and for maintenance of disease remission.

The study of Maillet et al. evaluated retrospectively 62 patients with AAV-ILD vs. 124 patients with AAV without ILD who were gathered from five prospective trials and were considered as a control group (52). Patients with AAV–ILD received more frequent immunosuppressive treatment for induction and maintenance of disease remission than the control group did. The comparison concerns the two groups of AAV patients, those with ILD and those without ILD. RTX was administrated for induction of remission in 6/62 patients with AAV-ILD and as maintenance therapy in 24/62 patients with AAV-ILD. RTX (27%) and CYC (41%) were most commonly prescribed for re-inducing remission. The prognosis of relapsing disease was good since 95% of patients achieved re-remission. Therefore, this study cannot account for RTX effects in patients with AAV-ILD. RTX was administered as a maintenance treatment more frequently in AAV-ILD than in AAV-non-ILD (40 vs. 4%). Clearly, a large study evaluating RTX-treatment effects strictly in lung involvement in AAV is lacking, despite the proven efficacy of RTX to control AAV systemic manifestations.

DISCUSSION

The progressive and life-threatening nature of CTD-ILD represents a major challenge. Thus, it is important to identify and apply an aggressive and effective treatment yet with an acceptable safety profile, that will at least slow down disease progression. MMF has been acknowledged as a first-line treatment agent in SSc-ILD. RTX has been employed in a good number of case series and studies both

controlled and uncontrolled regarding CTD-ILD in two different treatment approaches. The results of these studies are encouraging given the heterogeneity of patients studied and additional potentially confounding factors that affect the evaluation of PFTs and HRCT scoring methods after RTX administration.

Based on data discussed herein, patients with CTD-ILD, either early- or long-standing disease, mild or severe disease, and resistant disease to previous therapy may represent successful candidates for B cell depletion treatment. In addition, despite a semi-quantitative approach, imaging studies may also depict improvements following RTX treatment. B cell depletion administered in repeated cycles may be a better approach in contrast to a single infusion. It is not entirely clear if it is preferred to employ RTX as a monotherapy or as a combination treatment along with other immunosuppressive/anti-fibrotic medications.

Nevertheless, all existing data point to an additional benefit of RTX treatment, i.e., the steroid-sparing effect. Finally, the use of RTX as a first-line agent and not as an agent used in unresponsive cases represents another unanswered question and an additional challenge in the successful treatment of patients with CTD-ILD.

Published trials suffer from a marked multifactorial heterogeneity: differences in the groups of patients included regarding the CTD disease type, the disease duration, previous and current treatments, the extent, the pattern and chronicity of lung disease, the lack of clearly defined cut-off values for PFT parameters such as the FVC and DLCO, as well as a non-digital scoring system for HRCT lesions, all limit our clear understanding of the benefits expected from B cell depletion treatment in patients with CTD -ILD. Well-designed, large enough studies are needed to overcome such limitations. Despite all the above limitations and downsides, one should keep in mind that the vast majority of studies published so far support a significant improvement of CTD-ILD in patients treated with a B cell depleting therapeutic approach.

AUTHOR CONTRIBUTIONS

S-NL conceived, wrote, and edited manuscript. CB wrote and edited manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

1. De Zorzi E, Spagnolo P, Cocconcelli E, Balestro E, Iaccarino L, Gatto M, et al. Thoracic involvement in systemic autoimmune rheumatic diseases: pathogenesis and management. *Clin Rev Allergy Immunol.* (2022). doi: 10.1007/s12016-022-08926-0
2. Shao T, Shi X, Yang S, Zhang W, Li X, Shu J, et al. Interstitial lung disease in connective tissue disease: a common lesion with heterogeneous mechanisms and treatment considerations. *Front Immunol.* (2021) 12:684699. doi: 10.3389/fimmu.2021.684699
3. Maher TM, Wuyts W. Management of fibrosing interstitial lung diseases. *Adv Ther.* (2019) 36:1518–31. doi: 10.1007/s12325-019-00992-9
4. Karampitsakos T, Vraka A, Bouros D, Liossis SN, Tzouvelekis A. Biologic treatments in interstitial lung diseases. *Front Med.* (2019) 6:41. doi: 10.3389/fmed.2019.00041
5. Distler O, Volkmann ER, Hoffmann-Vold AM, Maher TM. Current and future perspectives on management of systemic sclerosis-associated interstitial lung disease. *Expert Rev Clin Immunol.* (2019) 15:1009–17. doi: 10.1080/1744666X.2020.1668269
6. Maloney DG. Anti-CD20 antibody therapy for B-cell lymphomas. *N Engl J Med.* (2012) 366:2008–16. doi: 10.1056/NEJMct1114348
7. Duarte AC, Cordeiro A, Fernandes BM, Bernardes M, Martins P, Cordeiro I, et al. Rituximab in connective tissue disease-associated interstitial lung disease. *Clin Rheumatol.* (2019) 38:2001–9. doi: 10.1007/s10067-019-04557-7
8. Lepri G, Avouac J, Airò P, Anguita Santos F, Bellando-Randone S, Blagojevic J, et al. Effects of rituximab in connective tissue disorders related interstitial lung disease. *Clin Exp Rheumatol.* (2016) 34 (Suppl. 100):181–5.
9. Atienza-Mateo B, Remuzgo-Martínez S, Prieto-Peña D, Mora Cuesta VM, Iturbe-Fernández D, Llorca J, et al. Rituximab in the treatment of interstitial lung disease associated with autoimmune diseases: experience from a single referral center and literature review. *J Clin Med.* (2020) 9:3070. doi: 10.3390/jcm9103070
10. Sharp C, McCabe M, Dodds N, Edey A, Mayers L, Adamali H, et al. Rituximab in autoimmune connective tissue disease-associated interstitial lung disease. *Rheumatology.* (2016) 55:1318–24. doi: 10.1093/rheumatology/kew195
11. Robles-Perez A, Dorca J, Castellví I, Nolla JM, Molina-Molina M, Narváez J. Rituximab effect in severe progressive connective tissue disease-related lung disease: preliminary data. *Rheumatol Int.* (2020) 40:719–26. doi: 10.1007/s00296-020-04545-0
12. Keir GJ, Maher TM, Hansell DM, Denton CP, Ong VH, Singh S, et al. Severe interstitial lung disease in connective tissue disease: rituximab as rescue therapy. *Eur Respir J.* (2012) 40:641–8. doi: 10.1183/09031936.00163911
13. Zhu L, Chung MP, Gagne L, Guo HH, Guenther Z, Li S, et al. Rituximab versus mycophenolate in the treatment of recalcitrant connective tissue disease-associated interstitial lung disease. *ACR Open Rheumatol.* (2021) 3:3–7. doi: 10.1002/acr2.11210
14. Mena-Vázquez N, Redondo-Rodríguez R, Rojas-Gimenez M, Romero-Barco CM, Manrique-Arija S, Ortega-Castro R, et al. Efficacy and Safety of rituximab in autoimmune disease-associated interstitial lung disease: a prospective cohort study. *J Clin Med.* (2022) 11:927. doi: 10.3390/jcm11040927
15. Saunders P, Tsipouri V, Keir GJ, Ashby D, Flather MD, Parfrey H, et al. Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease (RECITAL): study protocol for a randomised controlled trial. *Trials.* (2017) 18:275. doi: 10.1186/s13063-017-2016-2
16. Bejan-Angoulvant T, Naccache JM, Caille A, Borie R, Nunes H, Ferreira M, et al. Evaluation of efficacy and safety of rituximab in combination with mycophenolate mofetil in patients with nonspecific interstitial pneumonia non-responding to a first-line immunosuppressive treatment (EVER-ILD): a double-blind placebo-controlled randomized trial. *Respir Med Res.* (2020) 78:100770. doi: 10.1016/j.resmer.2020.100770
17. Xing NS, Fan GZ, Yan F, Liu YP, Zhang R. Safety and efficacy of rituximab in connective tissue disease-associated interstitial lung disease: a systematic review and meta-analysis. *Int Immunopharmacol.* (2021) 95:107524. doi: 10.1016/j.intimp.2021.107524
18. Daoussis D, Liossis SN, Tsamandas AC, Kalogeropoulou C, Kazantzi A, Sirinian C, et al. Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. *Rheumatology.* (2010) 49:271–80. doi: 10.1093/rheumatology/kep093
19. Jordan S, Distler JH, Maurer B, Huscher D, van Laar JM, Allanore Y, et al. Effects and safety of rituximab in systemic sclerosis: an analysis from the European scleroderma trial and research (EUSTAR) group. *Ann Rheum Dis.* (2015) 74:1188–94. doi: 10.1136/annrheumdis-2013-204522
20. Daoussis D, Melissaropoulos K, Sakellaropoulos G, Antonopoulos I, Markatseli TE, Simopoulou T, et al. A multicenter, open-label, comparative study of B-cell depletion therapy with rituximab for systemic sclerosis-associated interstitial lung disease. *Semin Arthritis Rheum.* (2017) 46:625–31. doi: 10.1016/j.semarthrit.2016.10.003

21. Boonstra M, Meijs J, Dorjée AL, Marsan NA, Schouffoer A, Ninaber MK, et al. Rituximab in early systemic sclerosis. *RMD Open.* (2017) 3:e000384. doi: 10.1136/rmdopen-2016-000384
22. Elhai M, Boubaya M, Distler O, Smith V, Matucci-Cerinic M, Alegre Sancho JJ, et al. Outcomes of patients with systemic sclerosis treated with rituximab in contemporary practice: a prospective cohort study. *Ann Rheum Dis.* (2019) 78:979–87. doi: 10.1136/annrheumdis-2018-214816
23. Sircar G, Goswami RP, Sircar D, Ghosh A, Ghosh P. Intravenous cyclophosphamide vs rituximab for the treatment of early diffuse scleroderma lung disease: open label, randomized, controlled trial. *Rheumatology.* (2018) 57:2106–13. doi: 10.1093/rheumatology/key213
24. Thiebaut M, Launay D, Rivière S, Mahévas T, Bellakhal S, Hachulla E, et al. Efficacy and safety of rituximab in systemic sclerosis: french retrospective study and literature review. *Autoimmun Rev.* (2018) 17:582–7. doi: 10.1016/j.autrev.2017.12.010
25. Lafyatis R, Kissin E, York M, Farina G, Viger K, Fritzler MJ, et al. B cell depletion with rituximab in patients with diffuse cutaneous systemic sclerosis. *Arthritis Rheum.* (2009) 60:578–83. doi: 10.1002/art.24249
26. Daoussis D, Liossis SN, Tsamandas AC, Kalogeropoulou C, Paliogianni F, Sirinian C, et al. Effect of long-term treatment with rituximab on pulmonary function and skin fibrosis in patients with diffuse systemic sclerosis. *Clin Exp Rheumatol.* (2012) 30 (2 Suppl. 71):S17–22.
27. Smith V, Piette Y, van Praet JT, Decuman S, Deschepper E, Elewaut D, et al. Two-year results of an open pilot study of a 2-treatment course with rituximab in patients with early systemic sclerosis with diffuse skin involvement. *J Rheumatol.* (2013) 40:52–7. doi: 10.3899/jrheum.120778
28. Bosello SL, De Luca G, Rucco M, Berardi G, Falcione M, Danza FM, et al. Long-term efficacy of B cell depletion therapy on lung and skin involvement in diffuse systemic sclerosis. *Semin Arthritis Rheum.* (2015) 44:428–36. doi: 10.1016/j.semarthrit.2014.09.002
29. Fraticelli P, Fischetti C, Salaffi F, Carotti M, Mattioli M, Pomponio G, et al. Combination therapy with rituximab and mycophenolate mofetil in systemic sclerosis. A single-centre case series study. *Clin Exp Rheumatol.* (2018) 36 (Suppl. 113):142–5.
30. Vilela VS, Maretti GB, Gama LM, Costa CH, Rufino RL, Levy RA. Rituximab for the therapy of systemic sclerosis: a series of 10 cases in a single center. *Rev Bras Reumatol Engl Ed.* (2016) 56:458–63. doi: 10.1016/j.rbre.2016.06.003
31. Sari A, Guven D, Armagan B, Erden A, Kalyoncu U, Karadag O, et al. Rituximab experience in patients with long-standing systemic sclerosis-associated interstitial lung disease: a series of 14 patients. *J Clin Rheumatol.* (2017) 23:411–5. doi: 10.1097/RHU.0000000000000584
32. Goswami RP, Ray A, Chatterjee M, Mukherjee A, Sircar G, Ghosh P. Rituximab in the treatment of systemic sclerosis-related interstitial lung disease: a systematic review and meta-analysis. *Rheumatology.* (2021) 60:557–67. doi: 10.1093/rheumatology/keaa550
33. Fui A, Bergantini L, Selvi E, Mazzei MA, Bennett D, Pieroni MG, et al. Rituximab therapy in interstitial lung disease associated with rheumatoid arthritis. *Intern Med J.* (2020) 50:330–6. doi: 10.1111/imj.14306
34. Md Yusof MY, Kabia A, Darby M, Lettieri G, Beirne P, Vital EM, et al. Effect of rituximab on the progression of rheumatoid arthritis-related interstitial lung disease: 10 years' experience at a single centre. *Rheumatology.* (2017) 56:1348–57. doi: 10.1093/rheumatology/kex072
35. Narváez J, Robles-Pérez A, Molina-Molina M, Vicens-Zygmunt V, Luburich P, Yañez MA, et al. Real-world clinical effectiveness of rituximab rescue therapy in patients with progressive rheumatoid arthritis-related interstitial lung disease. *Semin Arthritis Rheum.* (2020) 50:902–10. doi: 10.1016/j.semarthrit.2020.08.008
36. Matteson EL, Bongartz T, Ryu JH, Crowson CS, Hartman TE, Dellaripa PF. Open-Label, pilot study of the safety and clinical effects of rituximab in patients with rheumatoid arthritis-associated interstitial pneumonia. *Open J Rheumatol Autoimmune Dis.* (2012) 2:53–8. doi: 10.4236/ojra.2012.23011
37. Vadillo C, Nieto MA, Romero-Bueno F, Leon L, Sanchez-Pernaute O, Rodriguez-Nieto MJ, et al. Efficacy of rituximab in slowing down progression of rheumatoid arthritis-related interstitial lung disease: data from the NEREA registry. *Rheumatology.* (2020) 59:2099–108. doi: 10.1093/rheumatology/kez673
38. Marie I, Dominique S, Janvresse A, Levesque H, Menard JF. Rituximab therapy for refractory interstitial lung disease related to antisynthetase syndrome. *Respir Med.* (2012) 106:581–7. doi: 10.1016/j.rmed.2012.01.001
39. Doyle TJ, Dhillon N, Madan R, Cabral F, Fletcher EA, Koontz DC, et al. Rituximab in the treatment of interstitial lung disease associated with antisynthetase syndrome: a multicenter retrospective case review. *J Rheumatol.* (2018) 45:841–50. doi: 10.3899/jrheum.170541
40. Andersson H, Sem M, Lund MB, Aaløkken TM, Günther A, Walle-Hansen R, et al. Long-term experience with rituximab in anti-synthetase syndrome-related interstitial lung disease. *Rheumatology.* (2015) 54:1420–8. doi: 10.1093/rheumatology/kev004
41. Langlois V, Gillibert A, Uzunhan Y, Chabi ML, Hachulla E, Landon-Cardinal O, et al. Rituximab and cyclophosphamide in antisynthetase syndrome-related interstitial lung disease: an observational retrospective study. *J Rheumatol.* (2020) 47:1678–86. doi: 10.3899/jrheum.190505
42. Bounia CA, Liossis SC. B cell depletion treatment in resistant systemic sclerosis interstitial lung disease. *Mediterr J Rheumatol.* (2022) 33:1–6. doi: 10.31138/mjr.33.1.1
43. Allenbach Y, Guiguet M, Rigolet A, Marie I, Hachulla E, Drouot L, et al. Efficacy of rituximab in refractory inflammatory myopathies associated with anti- synthetase auto-antibodies: an open-label, phase II trial. *PLoS ONE.* (2015) 10:e0133702. doi: 10.1371/journal.pone.0133702
44. Klinowski G, Gozzi F, Trentacosti F, Andrisani D, Sebastiani M, Clini EM. Rituximab for the treatment of acute onset interstitial lung disease in primary Sjogren's syndrome. *Pulmonology.* (2021) 27:575–8. doi: 10.1016/j.pulmoe.2021.06.004
45. Chen MH, Chen CK, Chou HP, Chen MH, Tsai CY, Chang DM. Rituximab therapy in primary Sjögren's syndrome with interstitial lung disease: a retrospective cohort study. *Clin Exp Rheumatol.* (2016) 34:1077–84.
46. Amarnani R, Yeoh SA, Denneny EK, Wincup C. Lupus and the lungs: the assessment and management of pulmonary manifestations of systemic lupus erythematosus. *Front Med.* (2021) 7:610257. doi: 10.3389/fmed.2020.610257
47. Benham H, Garske L, Vecchio P, Eckert BW. Successful treatment of shrinking lung syndrome with rituximab in a patient with systemic lupus erythematosus. *J Clin Rheumatol.* (2010) 16:68–70. doi: 10.1097/RHU.0b013e3181d0757f
48. Langenskiöld E, Bonetti A, Fitting JW, Heinzer R, Dudler J, Spertini F, et al. Shrinking lung syndrome successfully treated with rituximab and cyclophosphamide. *Respiration.* (2012) 84:144–9. doi: 10.1159/000334947
49. Reynolds JA, Toescu V, Yee CS, Prabu A, Situnayake D, Gordon C. Effects of rituximab on resistant SLE disease including lung involvement. *Lupus.* (2009) 18:67–73. doi: 10.1177/0961203308094653
50. Kadura S, Raghu G. Antineutrophil cytoplasmic antibody-associated interstitial lung disease: a review. *Eur Respir Rev.* (2021) 30:210123. doi: 10.1183/16000617.0123-2021
51. Sebastiani M, Manfredi A, Vacchi C, Cassone G, Faverio P, Cavazza A, et al. Epidemiology and management of interstitial lung disease in ANCA-associated vasculitis. *Clin Exp Rheumatol.* (2020) 38 (Suppl. 124):221–31.
52. Maillet T, Goletto T, Beltramo G, Dupuy H, Jouneau S, Borie R, et al. Usual interstitial pneumonia in ANCA-associated vasculitis: a poor prognostic factor. *J Autoimmun.* (2020) 106:102338. doi: 10.1016/j.jaut.2019.102338

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Medicine

REVIEW
published: 04 July 2022
doi: 10.3389/fmed.2022.936182

The Role of B Cells in Scleroderma Lung Disease Pathogenesis

Stamatis-Nick C. Liossis[1,2*] *and Chrysanthi Staveri*[1]

[1] *Division of Rheumatology, Department of Internal Medicine, Patras University Hospital, Patras, Greece,* [2] *Division of Rheumatology, Department of Internal Medicine, University of Patras Medical School, Patras, Greece*

Systemic sclerosis (SSc) is a chronic, autoimmune, multisystem disease characterized by tissue fibrosis that, apart from the skin, may affect the lungs among other organs. B cells have been found in tissue lymphocytic infiltrates; in the lungs are encountered in lymphoid aggregates. The abnormal and hyperreactive B cell in SSc may initiate and perpetuate the fibrotic process via incompletely understood mechanisms. Studies in animal models of SSc have demonstrated that B cell dysregulation is an early event in disease pathogenesis. Functional disturbances of BCR signaling such as decreased inhibitory CD22 signal transduction or augmented CD19-mediated signaling result in prolonged B cell activation. Antagonism of BAFF, a cytokine known for his central role in B cell survival and maturation, not only suppresses the production of fibrogenic cytokines such as IL-6 and IL-10, but also amplifies antifibrogenic cytokine secretion such as IFN-γ and it finally contributes to skin fibrosis attenuation. B cells subsets in SSc patients display several abnormalities. Naïve B cells are increased, in contrast to switched memory B cells that are not only decreased but also activated. Disturbances in the expression of molecules that are involved in B cell tuning have also been described. Interestingly, a distinct B cell population characterized by anergy and exhaustion has been found to be increased in patients with SSc-ILD. Another B cell subset, the $CD30^{+}$GM-Beff, is capable to differentiate monocytes to dendritic cells and is increased in SSc patients with ILD. Of note, patients with SSc-ILD exhibit increased expression of the inhibitory receptor FcγRIIB on naïve and double negative B cells aiming perhaps to counterbalance the abnormal B cell activation. Studies of B cell targeted treatments have demonstrated promising clinical efficacy. Therefore, B cell eliminating therapies could be integrated into the therapeutic armamentarium of patients suffering from SSc-ILD aiming to at least stabilize the fibrotic lung process.

Keywords: B cell, scleroderma (systemic sclerosis), interstial lung disease, pathogenesis, human

OPEN ACCESS

Edited by:
Argyrios Tzouvelekis, Alexander Fleming Biomedical Sciences Research Center, Greece

Reviewed by:
Theodoros Dimitroulas, Aristotle University of Thessaloniki, Greece
Charalampos Papagoras, Democritus University of Thrace, Greece

***Correspondence:**
Stamatis-Nick C. Liossis
snliossis@med.upatras.gr

Specialty section:
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

Received: *04 May 2022*
Accepted: *14 June 2022*
Published: *04 July 2022*

Citation:
Liossis SNC and Staveri C (2022) The Role of B Cells in Scleroderma Lung Disease Pathogenesis. Front. Med. 9:936182. doi: 10.3389/fmed.2022.936182

INTRODUCTION

Systemic sclerosis (SSc) is a chronic, autoimmune, multisystem disease characterized by tissue fibrosis affecting the skin, the lungs, and other organs. In patients with SSc, B cells have been found in tissue lymphocytic infiltrates and in the lungs are encountered in lymphoid aggregates (1). Additionally, B cells produce specific SSc-associated autoantibodies (autoAb) and may also present antigens, produce cytokines and regulate T cell function. Based on these, it has been proposed that hyperreactive B cells in SSc may not only initiate but also perpetuate the fibrotic process via incompletely understood mechanisms.

B Cells May Have a Direct Pathogenic Effect

Recombination activating gene 2 (Rag2) is implicated in the V(D)J recombination in T- and B-cell maturation. Interleukin-2 receptor gamma chain gene (IL2Rγ) is involved in natural killer (NK) cell activity. Therefore, in double knockout $Rag2^{-/-}IL2R\gamma^{-/-}$ mice the survival of transferred human immune cells was enhanced due to the absence of native B cells, T cells and NK cells, as well as Tregs which may interplay with the human immune response in mice. Peripheral blood mononuclear cells (PBMC) derived from patients with SSc and from patients with granulomatosis with polyangiitis (GPA) were transferred to the immunocompromised $Rag^{-/-}$/IL-$2R\gamma^{-/-}$mice (2). Two weeks after the infusion of SSc patient-derived PBMC the recipient mice developed autoAb such as antinuclear antibodies (ANA), as well as cellular tissue infiltrates consisting mainly of B cells in various organs including the lungs. In contrast, passive transfer of PBMC from patients with GPA and from healthy donors did not result in autoAb production or cellular tissue infiltrates. Of note, transfer of PBMC from SSc patients previously treated with RTX failed to induce the pathological findings of human SSc. The study illustrated a potentially direct pathogenic effect of immune cells in SSc in the early stages of the disease progress, assigning a particularly important role to B cells.

B Cell Implication Is an Early Event in Disease Pathogenesis

A study illustrated that the potential role of B cell may begin during the early stages of the disease course (3). Next-generation RNA sequencing of skin biopsies from patients with early SSc (median disease duration: 1.3 years) yielded high proportions of M2 and M1 macrophages, $CD8^+$ T cell, $CD4^+$ T cell and B cell signatures (96, 94, 65, 60, and 69%, respectively). Furthermore, analysis illustrated that T and B cell signatures were significantly associated with shorter disease duration even after adjustment for immunosuppression treatment, underscoring thus the potential role of B cells in early disease.

Cytokine Profile in Th1/Type 1 Effector B Cells Defines the Autoimmune Disease

Type I effector B cells are functionally polarized effectors that produce a Th1-like cytokine profile. A study analyzing 179 patients with 4 different systemic autoimmune diseases including SSc, demonstrated that B cells prosper Th1 differentiation and naïve T cell proliferation resulting in the induction of type I effector B cells that tend to differentiate into plasmablasts (4). This T cell/B cell crosstalk has been associated with the development of a proinflammatory cytokine microenvironment consisting of IL-2, IL-6, CXCL10, and IFN-γ production. This study provides evidence that a shared proinflammatory cytokine profile could define distinct patients of each autoimmune disease. In the proinflammatory group patients with Sjogren's syndrome (SS) and patients with SSc had increased levels of IL-13, patients with SLE had significantly increased levels of IFN-γ and significantly decreased levels of IL-13 and patients with RA had significantly increased levels of IL-8. Scleroderma patients in the proinflammatory group had significantly more often pulmonary, renal and vascular involvement compared to other, "non-inflammatory" patients with SSc (66.5 vs. 13.3%, $p = 0.0011$).

A) ALTERED PHENOTYPE OF THE SCLERODERMA B CELL

Deranged expression of molecules implicated in B cell regulation is evident in SSc patients. B cells from SSc patients exhibit an activated phenotype (5). Surface expression of the co-stimulatory molecule CD86 was increased in SSc patients, especially in naïve and transitional B cell subpopulations, but no differences were noticed in the expression of MHC II molecules, involved in antigen presentation. The amounts of IL-6 produced by B cells were similar between SSc patients and healthy controls, but IL-10 secretion by B cells was significantly reduced in SSc patients. Most B cells that secrete IL-10 were identified in the transitional B cell subpopulation but the percentage of B cells that secrete IL-10 was reduced in all B cell subsets. Furthermore, a subset of B cells ($CD25^{high}CD27^{high}CD86^{high}CD1d^{high}$) that normally express high levels of IL-10 (as well as TGF-β), was decreased in SSc patients. Disturbances in the expression of regulatory receptors on the surface of B cell subsets of patients with SSc are summarized in **Table 1**.

Altered CD19-Dependent Signal Transduction

The tight skin mouse (TSK/+) is a model for human SSc. A tandem duplication in the FBN1 gene in TSK/+ mice is thought to be responsible for the development of skin fibrosis and the production of SSc-specific autoAb. The expression of CD19 on the surface of B cells in the periphery, spleen, bone marrow and peritoneum of TSK/+ mice was similar to wild-type mice (6). However, tyrosine phosphorylation of CD19 was increased by 45% in splenic B cells of TSK/+ compared to wild-type mice. Tyrosine phosphorylation of Vav, a kinase, that mediates downstream CD19 signaling, was also increased in B cells of TSK/+ mice compared to wild-type mice. Lyn, a kinase that is also involved in CD19 tyrosine phosphorylation was

TABLE 1 | Aberrations in the expression of stimulatory (stim) and inhibitory (inh) receptors on the surface membrane of B cell subsets from patients with SSc.

	Naïve B cells	Memory B cells	Transitional B cells
CD19 stim	↑	↑	↑
CD21 stim	–	–	–
CD40 stim	↑	↑	↑
CD35 inh	↓	↓	↓
CD22 inh	–	–	–
FcγRIIB inh	↑	–	↑
Siglec 10 inh	–	–	–

↑, Increased; ↓, Decreased; –, no difference between SSc patients and healthy donors.

amplified by 22% in B cells of TSK/+ mice compared to wild-type mice. Aiming to further examine the role of CD19 signaling in the development of autoimmunity, TSK/+ mice with CD19 deficiency were generated. CD19 loss resulted in reduced activity of Lyn kinase in TSK/+ B cells. This genetic model exhibited an activated CD19-initiated signaling pathway following CD19 ligation and enhanced proliferation of TSK/+ B cells in response to anti-IgM Ab. However, not all signaling pathways of TSK/+ B cells were overreactive because responses to LPS and to anti-CD38 mAb were normal. Therefore, TSK/+ B cells display increased responses specifically via CD19 and the BCR and CD19 deficiency normalizes TSK/+ B cell hyperresponsiveness. Moreover, CD19 deficiency prevented autoAb production and resulted in a decrease of peripheral B cell numbers without significantly affecting B cell development. Phenotypically, CD19 loss was associated with a significantly thinner hypodermal tissue compared to CD19 sufficient mice. Of equal importance, CD19 loss inhibited IL-6 production by TSK/+ B cells. IL-6 induces the production of collagen and glycosaminoglycans by dermal fibroblasts, regulates B cell differentiation and proliferation and it also reinforces autoAb production. IL-4 may also play a role in cutaneous hyperplasia of TSK/+ mice. It has been illustrated that TSK/+ B cells display amplified activation by IL-4 that was reduced by CD19 loss. Collectively, these data suggest that amplified CD19 signal transduction results in chronic activation of B cells leading to autoimmunity and predisposing to fibrosis.

The Role of CD19 in Lung Fibrosis

A study examined the role of CD19 in the development of lung fibrosis evaluating CD19-deficient mice and human CD19 (hCD19)-overexpressing transgenic mice (7). Bleomycin introduction resulted in consolidation, infiltrates of inflammatory cells, increased collagen deposition and reduced lung function in wild type mice but to a lesser extent in CD19 deficient mice. However, the development of such abnormalities was significantly enhanced in hCD19-transgenic mice. The hydroxyproline content of the lung was decreased in CD19 deficient mice, whereas it was significantly increased in hCD19-transgenic mice.

The B220 isoform of CD45 is a pan B cell marker found in mice. B220 B cell numbers in bronchoalveolar lavage (BAL) fluid were significantly decreased in CD19 deficient mice but were significantly increased in hCD19-transgenic mice illustrating a direct correlation with CD19 expression. Therefore, loss of CD19 prevented B cell accumulation in the lungs following bleomycin treatment. In addition, CD19 deficiency decreased the B220+ B cell count/total cell count, but CD19 overexpression significantly increased the ratio. Of note, the ratio of B220 B cells/total cells in BAL fluid in control (adhesion molecule-deficient) mice was similar to the ratio found in wild-type mice. Therefore, these experiments clearly depict that CD19 expression magnitude was associated with B cell migration into sites of lung inflammation. Additionally, it was shown that migration of B cells into the inflamed lungs was associated with up-regulation of CXCR3, a chemokine induced by CD19 signaling. Bleomycin treatment of mice induced IL-6 secretion; IL-6 was directly correlated with levels of CD19 expression on the surface membrane of B cells. In hCD19 overexpressing mice IL-6 levels were upregulated but in CD19-deficient animals IL-6 levels were decreased. hCD19 overexpressing animals displayed decreased IL-10 levels in contrast to CD19 deficient mice that had increased levels of IL-10. Of note, IFNγ levels were not detectable in CD19 deficient mice, wild-type mice and hCD19-transgenic mice, with or without bleomycin introduction. Finally, it was illustrated that immunoglobulin levels that reflect B cell responses, were strongly correlated with CD19 expression levels after bleomycin treatment. However, although CD19 clearly plays a role in experimental SSc models, a trial of inebilizumab, a mAb targeting CD19 did not seem to improve lung function in patients with SSc-ILD (8).

Disrupted CD22 Regulation Affects CD19 Activation Status

CD19 overexpression was associated with IgG anti-topo I autoAb production; even higher levels of expression of endogenous CD19 resulted in even higher IgG and IgM anti-topo I autoAb levels (9). Human CD19-trnsgenic (TG)-1 mice and CD19-TG-4 mice were previously backcrossed more than 4 generations onto the TSK/+ mice. Breedings of CD19TG+/– TSK/+ mice resulted in CD19TG1 and CD19TG-4 TSK/+ mice. IgG anti-topo I production was x7.9-fold in CD19TG-4+/–TSK/+ mice and x20-fold in CD19TG-1+/+TSK compared to TSK/+ mice. Similarly, IgM anti-topo I production was x2.7-fold in CD19TG-4+/–/TSK+ mice and x12-fold in CD19TG-1+/+/TSK+ compared to TSK/+. Therefore, overexpression of CD19 in 2 different TSK genetic variants regulates anti-topo I autoAb production levels. Regarding skin fibrosis, it was depicted that CD19 deficiency may attenuate skin fibrosis, but, contrastingly, CD19 overexpression did not amplify skin fibrosis. Evidence raised the possibility of intristic B cell signaling abnormalities in TSK/+ mice. Circulating B cells from TSK/+ mice express lower surface IgM levels, but higher MHC class II levels compared to wild type B cells. Moreover, TSK/+ mice display a hyperreactive B cell phenotype. It has been demonstrated that TSK/+ B cells exhibit a rapid and sustained increase of the intracellular calcium concentration $[Ca^{2+}]$ following BCR ligation compared to wild-type B cells. To explain this augmented BCR-initiated signaling, levels and activities of signal transducing tyrosine kinases and phosphatases were sought, but no abnormalities were reported. Nevertheless, CD22 phosphorylation was reportedly decreased in TSK/+ B cells, while BCR ligation resulted in a modest only, CD22 phosphorylation, underscoring that there is a dysregulated CD19/CD22 loop in Tsk/+ B cells. Therefore, disturbances of B cell signaling events, and particularly of those related to CD22 pathway may participate in the development of autoimmunity in TSK/+ mice.

CD22 and CD72

CD22 and CD72 are B cell-surface molecules that inhibit BCR-mediated signaling. Deficiency of both CD22 plus CD72 in murine knockout models significantly decreased dermal thickness compared to wild type mice ($p < 0.05$) (10). Additionally, α-ASMA (+) myofibroblasts were significantly decreased in CD22 and CD72 deficient mice compared to wild

type mice. Thus, deficiency of CD22 or CD72 or both attenuated skin fibrosis induced by bleomycin administration.

Lung fibrosis in mutant and wild type mice was also examined. Lung fibrosis scores were significantly better in mutant mice (CD22-deficient, CD72-deficient and CD22 and CD72 double-deficient mice) compared to wild type mice (mice without CD22 or CD72 deficiency). Thus, deficiency of CD22 or CD72 or both decreased bleomycin-induced lung fibrosis. $CD22^{-/-}$, $CD72^{-/-}$ and $CD22^{-/-}CD72^{-/-}$ mice transfused with B cells from wild-type mice demonstrated skin and lung fibrosis comparable to that encountered in wild type mice. Moreover, leukocytes, including T cells and macrophages, profibrotic cytokines such as IL-6, TNF-α, IL-1β, IL-13, TGF-β, and CTGF and chemokines such as ICAM-1 and CXCL2 were all reduced in mutant nice compared to wild type mice. Furthermore, an isolated deficiency of CD22 or of CD72 was sufficient to attenuate lung and skin fibrosis, because CD72 blockade in $CD22^{-/-}$ animals did not offer any additional benefit.

Role of FcγRIIB

A study examined the role of FcγRIIB, a B cell surface receptor for the Fc region of IgG that down-regulates BCR signaling, in the development of a murine bleomycin-induced model of SSc (11). B220+B cells were significantly increased in the skin of FcγRIIB deficient mice compared to the skin of wild type mice. Additionally, FcγRIIB deficient mice demonstrated higher levels of TNF-α and IL-1β production and increased expression of ICAM-1, CCL3, and CXCL2 in the skin compared to wild type animals. Furthermore, an adoptive transfer of splenic $CD19^{+}$B cells and $CD19^{-}$ non-B cells from FcγRIIB deficient mice to wild type mice resulted in lung and skin fibrosis attenuation. Therefore, experimental data suggest that FcγRIIB may play a role in the SSc-related fibrotic process.

Increased Expression of FcγRIIB on the Surface of Specific B Cell Subsets

Increased $CD27^{-}IgD^{+}$ naïve, reduced $CD27^{+}IgD^{+}$ pre-switched memory and increased $CD27^{-}IgD^{-}$ memory B cells were found in SSc patients compared to controls. Flow cytometry of blood samples from 76 SSc patients and 59 healthy controls demonstrated an increased expression of the inhibitory receptor FcγRIIB levels on naïve ($CD19^{+}IgD^{+}CD27^{+}$) and double-negative (DN) $CD19^{+}IgD^{-}CD27^{-}$ memory B cells in SSc patients compared to controls (12). FcγRIIB expression on naïve B cells was increased in patients with ILD treated with glucocorticoids and in patients with a history of intravenous cyclophosphamide (IV CYC) treatment. FcγRIIB expression on switched memory B cells was increased in patients with ILD even without any treatment. There was no significant correlation of FcγRIIB expression levels with skin thickness. Therefore, FcγRIIB expression on naïve and DN memory B cells was increased in SSc patients with ILD specifically. TLC, FVC and DLCO were decreased in patients with high FcγRIIB expression ($p < 0.005$, $p < 0.01$, $p < 0.05$). Apart from ILD, cardiac involvement was similarly over-represented in patients with high FcγRIIB expression on DN memory B cells.

The levels of FcγRIIB expression on DN memory B cells may correlate with disease severity, since the European Scleroderma Group-Activity Index (EScG-AI) was significantly increased in patients with higher levels of FcγRIIB compared to those with normal FcγRIIB levels on the surface of DN memory B cells. FcγRIIB expression levels on pre-switched memory, DN memory and switched memory B cell counts after IV CYC treatment decreased compared to pre-treatment levels, in parallel with ILD improvement. Thus, FcγRIIB expression levels on the surface of certain B cell subsets might serve as a marker of ILD and of disease activity.

B) ALTERED B CELL SUBSETS IN SSc

B cells represent a quite heterogeneous population that includes diverse subsets characterized by their membrane phenotype and/or their cytokine secretion profile. A study of 31 patients with SSc and 53 healthy controls demonstrated altered frequencies of B cell subsets in the peripheral blood of SSc patients. More specifically, there was an increased percentage of $CD19^{+}$ B cells in SSc patients, but the percentage of transitional B cells was increased compared to healthy controls (5). Another study showed that in patients with SSc, marginal zone, memory and switched memory B cells were reduced compared to healthy donors (13). In contrast, mature naïve, naïve and $CD21^{lo}CD38^{lo}$B cells were significantly expanded compared to healthy donors. $CD21^{lo}CD38^{lo}$B cells are characterized by a very low expression of CD21 and low levels of CD38. There was an increased expression of activation markers, such as CD80, CD95, and HLA-DR on the surface membrane of B cells from some B cell subpopulations. More specifically: (i) increased expression of CD80 was reported on marginal zone, mature naïve and switched memory B cells, (ii) increased expression of CD95 on marginal zone, mature naïve and the total B cell population and finally (iii) increased HLA DR expression on the surface of marginal zone B cells. Of note, expression of the apoptosis regulator Bcl-2 was decreased in B cells of SSc patients, particularly in marginal zone B cells. Patients with SSc-ILD have decreased numbers of B cells that secrete IL-10 (B10 cells) when stimulated via a short activation protocol (with the oligodeoxynucleotide CpG for 5 h), but not when activated via a long stimulation protocol (with CpG and CD40L for 48 h), suggesting thereby that B10 cells and not their precursors may be also implicated in the ILD pathogenesis.

Increased Activated Switched Memory B Cells

Memory B cells depending on their IgD expression levels are further divided into switched ($CD19^{+}CD27^{+}IgD^{-}$) and non-switched ($CD19^{+}CD27^{+}IgD^{+}$) memory B cell subpopulations. The percentages of unswitched ($CD19^{+}IgD^{lo}CD27^{+}CD38^{+}$), resting switched ($CD19^{+}IgD^{-}CD27^{+}CD38^{+}CD95^{-}$) and activated switched memory ($CD19^{+}IgD^{-}CD27^{+}CD38^{-}CD95^{+}$) B cells have been reported to be significantly decreased in SSc patients compared to healthy donors (14). The percentage of activated switched memory B cells was found to be increased in dcSSc patients, in

anti-topo I (+) patients and in those with lung involvement ($p = 0.003$) suggesting a correlation of this subset with the severity of SSc disease.

Reduced Non-switched Memory B Cells

Naïve B cell counts were increased in SSc patients compared to controls (15). However, the proportion of memory B cells was reduced and more specifically that of non-switched memory B cells was reduced. Patients with diffuse SSc exhibited higher levels of DN memory that lack IgD and CD27 and switched memory B cells compared to patients with limited SSc ($p = 0.031$ and $p = 0.025$, respectively). Additionally, $CD95^+$ DN memory and $CD95^+CD27^+$ memory B cells were increased in patients with diffuse SSc compared to patients with limited SSc ($p = 0.045$ and $p = 0.038$, respectively). Perhaps the imbalance in B cell repertoire is involved in SSc pathogenesis.

Defective B Cell Tolerance Checkpoints

Newly emigrant/transitional B cells ($CD19^+CD21^{lo}CD10^+IgM^{high}CD27^-$) producing polyreactive antibodies were significantly expanded in SSc patients suggesting a defect in central B cell tolerance (16). Mature naïve B cells ($CD19^+CD21^+CD10^-IgM^+CD27^-$) producing Hep-2-reactive antibodies were also increased in SSc patients compared to healthy donors suggesting an additional defective peripheral B cell tolerance checkpoint. Therefore, incomplete removal of autoreactive B cells may result in the production of self-antigen specific B cells that may implicated in the tissue fibrotic process in SSc.

Defective Regulation of Transitional IL-6^+ B Cells

SSc patients have more transitional ($CD24^{high}CD38^{high}$) B cells compared to healthy subjects (17). Perhaps more importantly, patients with severe lung disease had more peripheral IL-6^+ B cells compared to patients with mild disease, and patients with diffuse SSc had more IL-6^+ B cells compared to patients with limited SSc. However, the differences of previous comparisons were not statistically significant. IL-6 producing transitional B cells may reflect a defect of the checkpoint mechanisms that regulate autoreactive T1-T2 maturation. Stimulation of PBMC enriched in B cells via TLR-9 alone or via TLR-9 along with CD40 resulted in the production of less IL-10 compared to healthy donors. Additionally, transitional B cells were resistant to apoptosis following BCR signaling. Levels of IgM anti-Scl-70 positive transitional B cells were increased in patients with anti-Scl-70 autoantibodies compared to those they did not have anti-Scl-70 autoantibodies. It seems that abnormal regulation of transitional IL-6^+ B cells in patients with SSc may contribute in the disease pathogenesis.

C) DISTINCT B CELL SUBSETS ASSOCIATED WITH SSc-ILD

Decreased B Regulatory Cells

The frequency of IL-10 producing Breg cells was reduced in the peripheral blood of patients with SSc compared to healthy controls (18). The absolute number of Breg cells was significantly lower in SSc patients compared to disease-control and healthy individuals. For example, frequency of Breg cells in dermatomyositis patients was similar to that encountered in healthy controls. Furthermore, frequencies and absolute numbers of $CD24^{hi}CD27^+$ that are characterized by a high expression of CD24 and a moderate expression of CD27 were decreased in SSc patients compared to controls.

SSc patients with decreased Breg cells had more frequently ILD. It has been proposed that decreased Breg cells might accelerate autoimmunity in SSc. Breg cell percentages were correlated negatively with the titer of anti-topo I and anti-centromere autoAb in SSc patients. Treatment significantly increased circulating Breg and $CD24^{hi}CD27^+$ B cells in patients with dcSSc compared to pre-treatment levels. It has been also reported that Breg cells of patients receiving immunosuppressive therapy were numerically, but not significantly increased compared to patients that did not. Thus, Breg cell levels may participate in the development of SSc.

Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)-Producing Effector B Cells (GM-Beffs)

GM-CSF is a proinflammatory cytokine involved in the differentiation of monocytes to myofibroblasts and macrophages and promotes fibrosis in SSc. GM-CSF production is mainly a property of $CD19^+CD27^+$ memory B cells. GM-CSF as well as IL-6 mRNA were up-regulated when B cells were stimulated with IL-4 (a Th2 cytokine), but not upon B cell stimulation with IFN-γ or IL-17 (19). IL4R was induced on the surface of stimulated B cells and consequently IL-4 might have autocrine effects on B cells. Tofacitinib introduction suppressed the expression of GM-CSF mRNA and protein in memory B cells induced by IL-4, but not by TGF-β. The number of GM-Beffs was clearly increased in patients with SSc compared to healthy controls. The number of GM-Beffs in diffuse SSc, limited SSc patients, with and without concomitant ILD were 164.2 ± 257.0, 54.0 ± 47.0, 150.6 ± 245.4, and 55.8 ± 44.3 cells/100 μl, respectively, $p < 0.05$. Thus, GM Beffs may be involved synergistically with IL-4 in SSc pathogenesis via the induction of dendritic cells.

TIM-1 Defines a Specific B Cell Subset in SSc-ILD

TIM-1 (T cell Ig and mucin domain protein 1), a Breg marker was reportedly increased on transitional ($CD19^+$ $CD24^{hi}CD38^{hi}$) compared to naïve ($CD19^+$ $CD24^{med}CD38^{med}$) and memory ($CD19^+$ $CD24^{hi}CD38^{med}$) B cells in patients with SSc (20). TIM-1 was expressed in all B cell subsets except plasmablasts ($CD19^+$ $CD24^-CD38^{hi}$). Reduced frequency of B cells expressing TIM-1 was only reported in transitional B cells in SSc patients. Investigators found a direct correlation between TIM-1 expression and CD19 on the surface of transitional B cells, as well as a significant increase in TIM-1 expression upon activation of BCR and TLR9 in all B cell subsets. Importantly, TIM-1 expression levels on transitional B cells were higher in

patients with dcSSc and worse lung function, expressed as a lower DLCO. TIM-1$^+$ B cells from healthy subjects strongly suppressed the expression of proinflammatory cytokines from co-cultured CD4+ T cells such as IFN-γ, TNF-α, and IL-17 compared to TIM-1$^-$ B cells. On the contrary, TIM-1$^+$ and TIM-1$^-$ B cells did not intensively suppress CD4+ T cells like they did in healthy individuals. It was also proposed that TIM-1 is associated with a subpopulation of Bregs different from transitional B cells. Thus, abnormalities in TIM-1$^+$ B cells may play a role in SSc pathogenesis.

CD21^{low} B Cells

A distinct B cell subset expressing low levels of the complement receptor 2 (CD21^{low}B cells) is reportedly expanded in SSc patients compared to healthy controls (21). These cells are characterized by anergy and exhaustion, but they tend to be resistant to apoptosis when compared to healthy donors resulting in their expansion in the blood of SSc patients. Memory B cells represented the prominent B cell subset in patients with CD21^{low} B cells. DN CD27$^-$IgD$^-$ B cells were also increased in SSc - CD21^{low} patients compared to SSc-CD21$^+$ patients. In contrast, naïve B cells represented the prominent B cell subset in SSc-CD21$^+$ patients. CD21$^+$ and CD21^{low} B cells from patients with SSc illustrated higher CD19 expression compared to similar subsets of healthy controls. DLCO was significantly decreased in SSc-CD21^{low} patients compared to SSc-CD21$^+$ ($p<0.01$). Therefore, expansion of CD21^{low} B cells may define a distinct group of patients with SSc and vascular complications.

Among various clinical manifestations of SSc, only ILD was found to be associated with expansion of CD21^{low} B cells in the periphery (22). Such B cells in addition express lower cytoplasmic amounts of Bruton's tyrosine kinase (BTK) compared to normal B cells; in fact, their anergic state has been assigned to suppression of BTK-mediated signaling.

BTK is believed to be overexpressed in autoimmune diseases. A study examined the effects of ibrutinib, a BTK inhibitor, on B cells of SSc patients (23). High doses of ibrutinib (10 μM) resulted in decreased production of all the detected cytokines including IL-6, TNF-α, IFN-γ, and IL-10 and also in decreased production of anti-Scl-70 autoAb. Interestingly, low-doses of ibrutinib (1 μM) decreased the proinflammatory cytokines IL-6 and TNF-α only, increased the anti-fibrotic cytokine IFN-γ and finally it did not affect the anti-inflammatory cytokine IL-10. Ibrutinib treatment significantly decreased the levels of phosphorylation of the proinflammatory transcription factor NFκB that was previously induced with CpG stimulation. Furthermore, high doses of ibrutinib treatment also significantly reduced IL-6$^+$ B cells among naïve and memory B cells, but the low doses of ibrutinib decreased the IL-6$^+$ cells among naïve only, but not memory B cells, a finding of uncertain significance.

Another study disclosed that the CD21$^{lo/neg}$ B cell subset that express low levels or lack surface CD21 was increased in SSc patients with ILD compared to those without ILD and healthy subjects, but frequencies of CD21$^{lo/neg}$ B cells did not differ between patients with diffuse vs. those with limited disease (24). The DN B cell subset consisted of CD21$^+$ and CD21$^{lo/neg}$ B cells, but only CD21$^{lo/neg}$ B cells were increased in patients with SSc-ILD. Histological examination of an explant from 1 patient with SSc-ILD and from 1 patient with chronic post-obstructive pneumonia disclosed that the patient with SSc-ILD had dense B cell infiltrates throughout all histological sections but the other patient had only 1 focus of B cells. Most B cells in the parenchyma of the lung did not stain for CD21, but the B cells in the germinal center did. Previous reports also suggest that CD21$^{lo/neg}$ cells are localized into sites of inflammation. Further studies are necessary to define the role of CD21$^{low/neg}$ B cells in the pathogenesis of SSc-ILD.

Previously mentioned distinct B cell subsets that are associated with SSc-ILD are shown in **Figure 1**.

D) ROLE OF CYTOKINES ESSENTIAL FOR B CELL SURVIVAL

BAFF

Studies in TSK/+ mice have demonstrated elevated serum BAFF levels, although the expression of the 3 different BAFF-receptors, BAFF-R, TACI and BCMA was not altered (25). In an effort to block the effects of BAFF, 8-wk-old TSK/+ mice treated with BAFF-R-Ig displayed reduced hypodermal thickness by 49% compared to TSK/+ littermates, and also decreased cutaneous collagen content by 51%. Therefore, BAFF blockade via BAFF-R-Ig decreased fibrosis and skin collagen accumulation in TSK/+ mice. It was also shown that BAFF antagonism regulates B cell phenotypes in TSK/+ and wild-type littermates. More specifically, BAFF antagonism reduced total B cells by 75% in the spleen of TSK/+ and wild-type littermates and diminished mature B cells in the spleen and bone marrow; it also decreased transitional type 2 (T2 B cells), marginal zone and follicular B cells in TSK/+ and wild-type littermates. In contrast, BAFF antagonism significantly augmented transitional type 1 (T1 B cells). BAFF antagonism also decreased the levels of anti-topoisomerase I autoAb as well as the levels of IgM, IgG2a, IgG2b, and IgG3 in TSK/+ mice. In addition, it was reported that BAFF antagonism downregulates the previously amplified type 2 cytokines mRNA expression in TSK/+ mice, such as TGF-β, IL-6, and IL-10 and increased the expression of the anti-fibrotic cytokine IFN-γ in the skin of TSK/+ mice.

Median BAFF levels in the sera of patients with SSc were higher compared to healthy controls and significantly increased in patients with dcSSc compared to those with lcSSc (26). Of note, higher BAFF levels were observed also in patients with reduced FVC ($p < 0.05$), but the overall frequency of lung disease in dcSSc patients with increased BAFF levels was similar to those with normal BAFF levels. A 6-year follow-up study of 21 SSc patients illustrated stable BAFF levels in 52%, decreased BAFF levels in 33% and increased, over the time, BAFF levels in 14% compared to baseline levels. Among the 3 different receptors that bind BAFF, BAFF-R is the only one that specifically interacts with BAFF. BAFF-R expression on B cells of SSc patients was found to be increased compared to healthy controls. However, patients with SSc who were treated with belimumab, a mAb blocking soluble BAFF, did not exhibit improvements in FVC and DLCO during treatment (27).

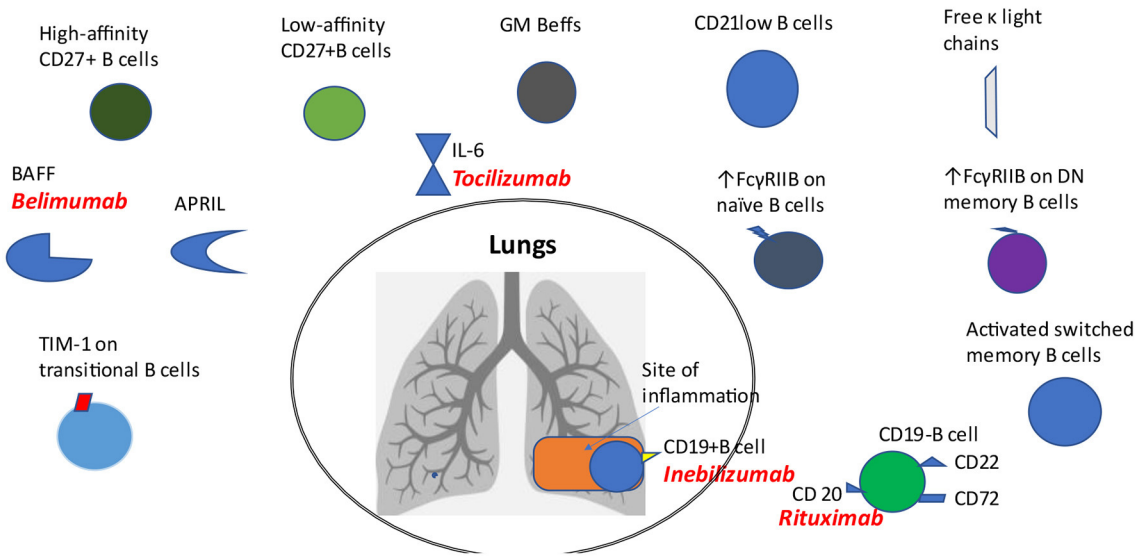

FIGURE 1 | B cell subsets and cytokines in patients with SSc-ILD. Distinct B cell subsets such as $CD21^{lo}$, GM-Beffs, activated switched memory B cells, topo I-reactive B cells with high affinity for topo I, increased expression of FcγRIIB on naïve B cells and that of TIM-1 on transitional B cells, as well as increased levels of free κ light chains, BAFF and APRIL cytokines are associated with ILD in patients with SSc. CD19 modulates the accumulation of B cells in BAL fluid. Loss of CD22 or CD72 decreased lung fibrosis.

A proliferation-inducing ligand (APRIL) is a cytokine demonstrating close homology with BAFF that has an important role in B cell development. Patients with SSc and SLE demonstrated increased serum APRIL concentrations compared to healthy controls (28). SSc patients with increased APRIL concentrations were more likely to develop pulmonary fibrosis than those with normal APRIL levels (65 vs. 37%, $p < 0.05$). Additionally, VC percentages in SSc patients with higher levels of APRIL were significantly reduced compared to those with normal APRIL levels ($p < 0.05$), despite the absence of differences in %DLCO among patients. Elevated levels of APRIL were specifically correlated with lung but not with other organ involvement. Increased APRIL levels were also correlated with hyper-γ-globulinemia, but not with SSc-specific autoAb levels or acute-phase protein levels. Furthermore, serum APRIL levels did not exhibit any association with BAFF levels. Separation of SSc patients in groups according to APRIL and BAFF concentrations illustrated that patients with high BAFF levels had a higher modified Rodnan skin score ($p < 0.05$), while patients with higher APRIL levels had significantly reduced %VC and %DLCO ($p < 0.05$).

E) EVIDENCE FOR THE ROLE OF B CELLS IN THE FIBROTIC PROCESS

B Cells and BAFF Promote Fibrosis

In one study dermal fibroblasts from SSc patients were cocultured with B cells from healthy controls (29). According to the results, B cells induced production of collagen by dermal fibroblasts of both healthy subjects and SSc patients with equal potency to that observed with the addition of TGF-β1 in cell cultures. Coculture of dermal fibroblasts with whole PBMC instead of B cells did not yield any differences in the production of collagen compared to culture of dermal fibroblasts without PBMCs. Coculture of B cells with dermal fibroblasts from SSc patients resulted in the induction of COL1A1, COL1A2, and COL3A1, whereas it increased levels of α-ASMA, TIMP1, and MMP9 expression. BAFF addition also induced the expression of collagen and of profibrotic markers. The use of transwells to prohibit cell-cell contact inhibited collagen production and profibrotic markers, while the addition of BAFF and anti-IgM to stimulate B cells, resulted in a partial inhibition by transwells. The addition of a neutralizing antibody against TGF-β1 inhibited collagen production effectively. Thus, direct cell-cell (B cell—dermal fibroblast) contact and profibrotic cytokines may be implicated in B cell- and BAFF- induced fibrosis, which is eventually mediated by TGF-β1.

B Cells Secrete IL-6 and TGF-β and Activate Fibroblasts

Naïve, Breg, effector memory cell, and plasmablast counts were similar in the periphery of SSc patients and healthy controls (30). The proportion of $IgD^{-}CD27^{+}$ memory B cells was decreased in patients with SSc compared to healthy donors and the $IgD^{-}CD38^{+}$ early Bm5 subpopulation was the most prominently decreased subset. Early Bm5 memory cells express moderately positive levels of CD38 but they do not express IgD. B cells from SSc patients displayed increased expression of the activation

markers CD95, CD69, and CD86 compared to healthy donors. A distinct B cell subset expressing both CD69 and CD95 in patients with SSc was not present in healthy controls. $CD95^+$ B cell count was expanded in early limited SSC compared to those with not-early limited SSc and $CD69^+$ B cell count was increased in early diffuse SSc compared to patients with not-early diffuse SSc. Additionally, the percentages of $CD95^+$ and $CD69^+$ B cells were increased in patients with early disease compared to those without early SSc. B cells that express CD22, CD32, and CD72 were similar between SSc patients and controls. Expression of CD20 on the surface of B cells was increased in patients with SSc compared to healthy controls. Similarly, there was a significant increase in the populations expressing IL-6R or IL-21R in patients with SSc compared to healthy donors but patients without pulmonary disease had a decreased proportion of B cells expressing IL-6R. Of note, the IL-6 receptor inhibitor, tocilizumab, an approved treatment for SSc-ILD, may stabilize lung function in early SSc-associated ILD over 48 weeks of treatment (31). A significant increase of IL-6-producing B cells was found in SSc patients compared to healthy controls. B cells from SSc patients had increased percentages of $TGF\beta^+CD19^+$ B cells compared to healthy controls. B cells from SSc patients secreted increased concentrations of IL-6 and TGFβ and lower amounts of IL-10 upon stimulation, in contrast to a strong increase of IL-10 secretion that was seen in healthy controls after stimulation.

Increased levels of TGFβ were also measured in the supernatants of CpG-stimulated B cells from patients with SSc compared to healthy controls. Supernatants from CpG- or PMA-stimulated SSc B cells enhanced the proliferation of fibroblasts of healthy subjects and SSc patients. Increased expression of vimentin and mRNA for the transcription factor Snail and type I collagen was reported following co-culture of fibroblasts from SSc patients and healthy controls with CpG -stimulated B cell supernatants from patients with SSc. The transcription factor Snail and vimentin are proteins thought to be associated with the trans-differentiation of fibroblasts into myofibroblasts. In agreement with the above, the addition of anti-TGFβ or anti-IL6R mAb in cultures prohibited fibroblast proliferation.

F) AUTOREACTIVITY OF B CELLS, AUTOAB PRODUCTION AND FREE LIGHT CHAINS

Antigen Affinity in B Cell Autoreactivity

In systemic sclerosis specific autoAb are strongly correlated with distinct clinical phenotypes. Anti-topoisomerase I autoAb, also known as anti-Scl-70, are associated with diffuse cutaneous disease and pulmonary fibrosis. A study reported that frequencies of anti-topo I (+) $CD27^+CD19^+$ B cells were significantly expanded in anti-topo I autoAb-positive SSc patients compared to healthy controls and anti-CENP autoAb-positive SSc patients (32). Thus, topo-I-reactive $CD27^+$ B cells are found only in anti-topo I autoAb-positive SSc patients. The majority of topo I-reactive $CD27^+$ B cells produced one type of cytokines (IL-2, IL-6, IL-10, IL-14, IL-16, IL-23, IL-35, and TGF-β1). In contrast, topo I-non-reactive B cells did not express any of these cytokines. Therefore, one may hypothesize that topo I-reactive $CD27^+$ B cells have already been stimulated by self-antigens *in vivo*. Association of the affinity magnitude of topo I reactive $CD27^+$B cells with the cytokine production profile is provided in **Table 2**. The ratio of high-affinity $CD27^+$ B cells to low-affinity $CD27^+$ B cells correlated positively with the mRSS and inversely with the percentage predicted values of FVC and DLCO ($p < 0.0001$). Patients in the high-affinity-dominant group (with a frequency of more than 50% of B cells with high affinity for topo I) demonstrated longer disease duration, higher mRSS, frequent digital scars or ulcers and lower values of %FVC and %DLCO compared to low-affinity-dominant group (with a frequency of more than 50% with low affinity for topo I). Thus, topo I-reactive B cells with high affinity for topo I are associated with severe skin and lung fibrosis. BTK inhibition resulted in increased numbers of cells producing IL-10 and IL-35 and in amplification of IL-10 and IL-35 amount production, whereas the numbers of cells producing IL-6 and IL-23 were reduced. Therefore, BTK inhibition decreased the numbers and the affinity for topo I of topo I-reactive B cells, accompanied by an increased production of inhibitory cytokines, and finally to fibrosis attenuation. One study evaluated 10 patients with SSc treated with RTX; mRSS improved, but anti-topo I autoAb titers did not change. Therefore, one might conclude that it is cytokines and not autoAb production that is critical for the mechanism of BTK inhibitors in SSc pathogenesis. The presence of anti-topo I antibodies in SSc patients' sera could be considered as an epiphenomenon; however, it has been demonstrated that anti-topo I autoAb levels correlate with disease activity and severity. Thus, topo I-reactive B cells may participate in disease pathogenesis, regardless of the presence of anti-topo I autoAb.

Free Light Chains in SSc

Antibody production is accompanied by an increased synthesis of immunoglobulin light chains in the peripheral blood. Increased levels of free κ and λ light chains were found in the sera of SSc patients compared to healthy controls (33). Free κ serum levels were above the cut-off value of 19.4 mg/L in 57 out of 72 patients. The κ/λ ratio and κ + λ sum were reportedly increased in the sera of SSc patients compared to healthy donors. Additionally, free κ light chains were increased in the urine of SSc patients compared to healthy donors. Patients with increased κ + λ sum illustrated higher ESR, C-reactive protein, disease activity index, and disease severity scale scores compared to SSc patients with normal levels of free light chains. According to the results of the study, serum and urine free light chains produced by activated B cells may represent potential biomarkers of disease activity.

Another study examined the possible association of free light chains with organ involvement (34). Free κ light chains were increased in patients with disturbed lung function (FVC<80%): 26.4 ± 7.4 mg/L compared to patients with normal pulmonary function (FVC≥80%): 19.6 ± 7.3 mg/L. There was no difference in free light chains concentrations or κ/λ ratio between patients who had a deterioration of their pulmonary function and patients that had no deterioration. The association between increased levels of free light chains in SSc patients with pulmonary involvement might strongly suggest that B cell overactivation is implicated in disease pathogenesis.

TABLE 2 | Association of the affinity magnitude of anti-topo I reactive CD27$^+$ B cells with cytokine production profile.

Topo I reactive CD27+ B cells	Cytokines
Low affinity	IL-10
	IL-35
High affinity	IL-2
	IL-6
	IL-23
	TGF-β1

TABLE 3 | Effects of B cell depletion treatment on the cytokine profile measured at the skin and spleen of TSK/+ mice.

Skin	TGF-β transcripts	↓
	TNF-α, IL-2, IFN-γ mRNA	↑
	IL-4, IL-6 IL-10 mRNA	↓
Spleen	IL-2, IL-4, IFN-γ mRNA	↑
	IL-6, IL-10, TNF-α mRNA	↑
	TGF-β mRNA	–

↑, *Increased levels;* ↓, *decreased levels;* –, *stable.*

G) INDIRECT EVIDENCE FOR THE ROLES OF B CELLS

B Cell Depletion Treatment in Models

The effects of B cell depletion have been evaluated in murine models of SSc. Low numbers of CD19^{low}B220$^+$B cells were found in the blood, spleen and lymph nodes following B cell depletion therapy (35). These cells were pre-B or immature B cells recently migrating from the bone marrow. B cell depletion treatment decreased dermal and hypodermal thickness in Tsk/+ mice by 31 and 43%, respectively, compared to control mAb treated animals, but it did not affect skin fibrosis in wild-type mice. B cell depletion treatment also decreased content of hydroxyproline by 50% in TSK/+ mice, although this remained significantly higher compared to wild-type littermates. B cell depletion may suppress skin fibrosis at the early stages of the disease process; however, in Tsk/+ mice, it does not affect established sclerosis or lung emphysema. Similarly, autoAb production and hypergammaglobulinemia were decreased when treatment was administered early but not later in the disease process. Tsk/+ and wild-type littermates were treated with anti-CD20 mAb or control mAb at days 3 and 17, with B-cell associated transcripts quantified in the skin from 3-wk-old- mice, at a time-point when blood and spleen CD19$^+$B220$^+$ B cells are eliminated by more than 93%. B cell transcripts were not detected in the skin of Tsk/+ as well as in wild-type littermates. B cell depletion treatment also regulated the cytokine transcript profile in the skin and spleen of the experimental animals **Table 3**.

BAFF Inhibition

A study suggests that BAFF inhibition diminishes fibrosis by affecting the balance between effector and regulatory B cells (Beffs and Bregs, respectively). IL-6- and IL-10-producing Beff counts were found to be increased in inflamed skin of mice treated with bleomycin compared to those treated with phosphate-buffered saline (36). IL-6 deficient B cells induced an attenuation of dermal thickness, collagen expression and lung fibrosis. It is clearly important that IL-6-producing Beffs mediate fibroblast collagen production.

BAFF induced IL-6 production from B cells after stimulation with LPS and/or CD40 and at the same time inhibited IL-10 production from B cells following stimulation with LPS. In addition, BAFF resulted in expansion of IL-6-producing Beffs but at the same time in elimination of IL-10-producing Bregs. Importantly, not only skin but also lung fibrosis of mice treated with the BAFF-blocker BAFFR (BAFF-Receptor)-Fc was significantly diminished compared to those treated with Fc control protein. BAFFR-Fc led to reduced IL-6- producing Beff counts but didn't alter numbers of IL-10-producing Bregs. IL-6-producing Beffs are considered pathogenic, but IL-10-producing Bregs are proposed to have a protective role.

Role of Anti-CD22 AutoAb

A study assessed the effect of autoAb against CD22 present in the sera of SSc patients as well as the sera of TSK/+ mice (37). Titres of anti-CD22 autoAb were higher in the sera of patients with SSc compared to healthy controls (where they were undetectable), and they were higher in patients with diffuse compared to those with limited SSc. Of note, titres of anti-CD22 autoAb were higher in patients with SSc compared to patients with SLE.

Surfactant protein D (SP-D) plays an important role in lung homeostasis due to its immunomodulatory and antiviral properties. It was demonstrated that patients with anti-CD22 autoAb exhibited higher SP-D concentrations compared to those without anti-CD22 autoAb [SP-D: 137 (44–502) ng/dl vs. 73.2 (29.2–311) ng/dl, $p < 0.05$]. Of note, anti-CD22 autoAb were correlated with lower values of vital capacity percentage (%VC) compared to anti-CD22 (–) negative patients, but the difference was not statistically significant (78.2% vs 99.2%). Furthermore, anti-CD22 autoAb reduced CD22 tyrosine phosphorylation by Lyn kinase upon BCR ligation, but this finding was not specific for patients with SSc. Additionally, in the presence of anti-CD22 autoAb the tyrosine phosphorylation of CD19 was augmented following BCR ligation. Thus, one might hypothesize that B cells produce anti-CD22 autoAb which in turn activate B cells in an autocrine manner leading to the perpetuation of the immune response.

Autologous Hematopoietic Stem Cell Transplantation Effects on B Cells

Alterations in B cell subsets that sustained up to 14 months following autologous hematopoietic stem cell transplantation (aHSCT) were reported in 6 SSc patients (38). Regarding lung involvement data were evaluated before and at 10–18 months after aHSCT. The median percentages of DLCO and FVC remained stable during the study ($p = 0.315$ and $p = 0.066$, respectively). On the functional side, another study suggests that aHSCT may restore the previously reduced inhibitory function of Bregs in SSc patients (39). The effects of aHSCT on B cell subsets and cytokine production in patients with SSc are summarized in **Table 4**.

TABLE 4 | Autologous hematopoieitic stem cell transplantation effects on B cell subsets and cytokine production in patients with SSc.

$CD38^{++}/CD10^{+}/IgD^{+}$ transitional	↑
$CD38^{++}/CD27^{++}/IgD^{-}$ plasmablasts	↑
$CD27/IgD^{+}$ naïve	↑
$CD27^{+}/IgD^{+}$ pre-switched memory	↓
$CD27^{+}/IgD^{-}$ post-switched memory	↓
$CD27^{-}/IgD^{-}$ DN	↓
Plasma cell	↓
IL-10 producing Breg cells*	↑
IL-6 and TGF-β producing B cells	↓
Th1 cytokines**	↓
IL-10	↑

↑, *Increased;* ↓, *Decreased.*
**Defined as: $CD19^{+}CD24^{hi}CD38^{hi}$ and $CD19^{+}CD24^{hi}CD27^{+}$ cells.*
***Because $CD19^{+}CD24^{hi}CD38^{hi}$ Bregs recovered their ability to inhibit Th1 cytokine production by $CD4^{+}$ T cells.*

DISCUSSION

A plethora of B cell abnormalities render B cells important players of SSc pathogenesis. The expression of activating and inhibitory molecules tuning B cell functions in B cell subsets has reportedly been disturbed. Studies in murine models of SSc illustrated that CD19 signaling pathway in B cells plays a critical role in the development of skin fibrosis. B cells in Tsk/+ mice upregulated CD23 expression and downregulated surface IgM expression, amplified constitutive CD19 and Vav tyrosine phosphorylation, enhanced the activity of kinase Lyn and also augmented CD19-induced $[Ca^{2+}]$ responses, hyper-γ-globulinemia and response to BCR ligation. Importantly, CD19 deficiency resulted in the reduction of collagen deposition; yet, CD19 overexpression did not amplify skin fibrosis in Tsk/+ mice. It was also proposed that CD19 levels modulate the accumulation of B cells in BAL fluid suggesting thereby a role of CD19 signaling in the development of lung fibrosis.

The inhibitory regulation of CD22 was found to be disturbed in B cells of Tsk/+ mice. The CD19/CD22 signaling loop is strongly correlated with anti-topo I autoAb production. Loss of inhibitory molecules, either CD22 or CD72, decreased skin and lung fibrosis revealing a role for both negative co-receptors of BCR signaling in disease pathogenesis. It has been also proposed that B cells produce anti-CD22 autoAb leading to a vicious circle of B cell activation. Anti-topo I autoAb may not have a direct pathogenic role in the development of fibrosis in SSc. Studies in humans revealed that increased peripheral B cells in patients with SSc exhibit increased expression of activation markers, cytokine receptors and increased levels of IL-6 and TGF-β. Mice with a B cell-specific deficiency in IL-6 exhibited attenuation of their skin and lung fibrosis, whereas those with a B cell-specific deficiency in IL-10 had severe skin and lung fibrosis. BAFF antagonism decreased skin and lung fibrosis in the bleomycin-induced scleroderma model via reduction of Beffs. B cells may increase the expression of FcγRIIB in order to counterbalance their abnormal hyperactivation. Moreover, FcγRIIB expression levels may represent a marker of disease severity, mainly of ILD.

B cell homeostasis also seems to be impaired. $CD21^{lo}$ B cells have been found increased in SSc patients with lung involvement. High counts of GM-Beffs were found to be associated with the diffuse form of SSc with concomitant ILD. The association of activated switched memory B cells with the presence of anti-topoisomerase I autoAb may reflect their potential role in the abnormal autoAb production. Reduced Breg cells were associated with lung disease. Free light chains production by immunoglobulins that reflect B cell overactivation have been found in high concentrations in the sera of patients with SSc and are associated with lung involvement. Disrupted removal of autoreactive naïve B cells may result in the production of self-antigen specific B cells that produce autoAb. Increased BAFF levels were correlated with disease severity, whereas decreased BAFF levels were correlated with attenuation of skin fibrosis. B cells that had been stimulated with BAFF were found to have an amplified capacity to secrete IL-6 and IgG. Profibrotic cytokines and direct cell-cell contact may be participate in TGF-β1-mediated-B cell- and BAFF-induced fibrosis. Increased levels of APRIL in the SSc patients' sera were associated with a greater incidence of lung fibrosis.

Indirect evidence for the role of B cell in SSc pathogenesis result from the efficacy of the anti-CD20 mAb in patients with SSc. A 1-year proof-of-principle study showed that patients treated with rituximab experienced an improvement of lung function (mean FVC ± S.D.: 68.13 ± 19.69 vs. 75.63 ± 19.73, at baseline vs. 1 year, $p = 0.0018$ and mean DLCO ± S.D.: 52.25 ± 20.71 vs. 62 ± 23.21, at baseline vs. 1 year, $p = 0.023$) (40). In contrast, patients that were not treated with rituximab had a deterioration of their pulmonary function. Platelet-derived growth factor (PDGF) participates also in fibrotic process in SSc. A study demonstrated that PDGFRα and PDGFRβ expression as well as phosphorylated PDGFα and PDGXβ expression in the papillary dermis were significantly reduced following rituximab treatment (41). Therefore, rituximab may improve skin fibrosis by altering the pathway mediated by PDGF via unknown mechanisms. Autologous hematopoietic stem cell transplantation causes alterations in B cell subpopulations and seems to restore the previously decreased suppressed function of Breg cells in patients with SSc.

The question on how autoimmunity leads to the clinical phenotypes of SSc, in particular to lung disease, cannot be answered yet. Recently, some light has been shed on B cell abnormalities that are present in patients with SSc and participate in disease pathogenesis through poorly understood mechanisms. However, B cell targeting therapies could be integrated into the therapeutic armamentarium of patients with resistant SSc-ILD aiming to at least stabilize the fibrotic lung process.

AUTHOR CONTRIBUTIONS

S-NL conceived and wrote and edited the manuscript. CS wrote and edited the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

The publication of this article has been financed by the Research Committee of the University of Patras.

REFERENCES

1. Lafyatis R, O'Hara C, Feghali-Bostwick CA Matteson E. B cell infiltration in systemic sclerosis–associated interstitial lung disease. *Arthritis Rheumatism.* (2007) 56:3167–8. doi: 10.1002/art.22847
2. Yue X., Petersen F, Shu Y, Kasper B, Magatsin J, Ahmadi M, et al. Transfer of PBMC from SSc patients induces autoantibodies and systemic inflammation in Rag2-/-/IL2rg-/- mice. *Front Immunol.* (2021) 12:677970. doi: 10.3389/fimmu.2021.677970
3. Skaug B, Khanna D, Swindell WR, Hinchcliff ME, Frech TM, Steen VD, et al. Global skin gene expression analysis of early diffuse cutaneous systemic sclerosis shows a prominent innate and adaptive inflammatory profile. *Ann Rheum Dis.* (2020) 79:379–86. doi: 10.1136/annrheumdis-2019-215894
4. Simon Q, Grasseau A, Boudigou M, Le Pottier L, Bettachioli E, Cornec D, et al. A proinflammatory cytokine network profile in Th1/Type 1 effector B cells delineates a common group of patients in four systemic autoimmune diseases. *Arthritis Rheumatol.* (2021) 73:1550–61. doi: 10.1002/art.41697
5. Soto L, Ferrier A, Aravena O, Fonseca E, Berendsen J, Biere A, et al. Systemic sclerosis patients present alterations in the expression of molecules involved in B-cell regulation. *Front Immunol.* (2015) 6:496. doi: 10.3389/fimmu.2015.00496
6. Saito E, Fujimoto M, Hasegawa M, Komura K, Hamaguchi Y, Kaburagi Y, et al. CD19-dependent B lymphocyte signaling thresholds influence skin fibrosis and autoimmunity in the tight-skin mouse. *J Clin Invest.* (2002) 109:1453–62. doi: 10.1172/JCI0215078
7. Komura K, Yanaba K, Horikawa M, Ogawa F, Fujimoto M, Tedder TF, et al. CD19 regulates the development of bleomycin-induced pulmonary fibrosis in a mouse model. *Arthritis Rheum.* (2008) 58:3574–84. doi: 10.1002/art.23995
8. Schiopu E, Chatterjee S, Hsu V, Flor A, Cimbora D, Patra K, et al. Safety and tolerability of an anti-CD19 monoclonal antibody, MEDI-551, in subjects with systemic sclerosis: a phase I, randomized, placebo-controlled, escalating single-dose study. *Arthritis Res Ther.* (2016) 18:131. doi: 10.1186/s13075-016-1021-2
9. Asano N, Fujimoto M, Yazawa N, Shirasawa S, Hasegawa M, Okochi H, et al. B Lymphocyte signaling established by the CD19/CD22 loop regulates autoimmunity in the tight-skin mouse. *Am J Pathol.* (2004) 165:641–50. doi: 10.1016/S0002-9440(10)63328-7
10. Zhao C, Matsushita T, Ha Nguyen VT, Tennichi M, Fujimoto M, Takehara K, et al. CD22 and CD72 contribute to the development of scleroderma in a murine model. *J Dermatol Sci.* (2020) 97:66–76. doi: 10.1016/j.jdermsci.2019.12.007
11. Sawada K, Hamaguchi Y, Mizumaki K, Oishi K, Maeda S, Ikawa Y, et al. A role for FcγRIIB in the development of murine bleomycin-induced fibrosis. *J Dermatol Sci.* (2021) 104:201–9. doi: 10.1016/j.jdermsci.2021.11.002
12. Numata N, Matsushita T, Takehara K, Hamaguchi Y. Increased expression levels of FcγRIIB on naïve and double-negative memory B cells in patients with systemic sclerosis. *Clin Exp Rheumatol.* (2019) 37(Suppl)119:23–31.
13. Forestier A, Guerrier T, Jouvray M, Giovannelli J, Lefèvre G, Sobanski V, et al. Altered B lymphocyte homeostasis and functions in systemic sclerosis. *Autoimmun Rev.* (2018) 17:244–55. doi: 10.1016/j.autrev.2017.10.015
14. Simon D, Balogh P, Erdo-Bonyár S, Böröcz K, Minier T, Czirják L, et al. Increased frequency of activated switched memory B cells and its association with the presence of pulmonary fibrosis in diffuse cutaneous systemic sclerosis patients. *Front Immunol.* (2021) 12:686483. doi: 10.3389/fimmu.2021.686483
15. Simon D, Balogh P, Bognár A, Kellermayer Z, Engelmann P, Németh P, et al. Reduced non-switched memory B cell subsets cause imbalance in B cell repertoire in systemic sclerosis. *Clin Exp Rheumatol.* (2016) 34(Suppl)100:30–36.
16. Glauzy S, Olson B, May CK, Parisi D, Massad C, Hansen JE, et al. Defective early B cell tolerance checkpoints in patients with systemic sclerosis allow the production of self antigen-specific clones. *Arthritis Rheumatol.* (2022) 74:307–17. doi: 10.1002/art.41927
17. Taher TE, Ong VH, Bystrom J, Hillion S, Simon Q, Denton CP, et al. Association of defective regulation of autoreactive Interleukin-6-producing transitional B Lymphocytes with disease in patients with systemic sclerosis. *Arthritis Rheumatol.* (2018) 70:450–61. doi: 10.1002/art.40390
18. Matsushita T, Hamaguchi Y, Hasegawa M, Takehara K, Fujimoto M. Decreased levels of regulatory B cells in patients with systemic sclerosis: association with autoantibody production and disease activity. *Rheumatology.* (2016) 55:263–7. doi: 10.1093/rheumatology/kev331
19. Higashioka K, Kikushige Y, Ayano M, Kimoto Y, Mitoma H, Kikukawa M, et al. Generation of a novel $CD30^{+}$ B cell subset producing GM-CSF and its possible link to the pathogenesis of systemic sclerosis. *Clin Exp Immunol.* (2020) 201:233–43. doi: 10.1111/cei.13477
20. Aravena O, Ferrier A, Menon M, Mauri C, Aguillón JC, Soto L, Catalán D. TIM-1 defines a human regulatory B cell population that is altered in frequency and function in systemic sclerosis patients. *Arthritis Res Ther.* (2017) 19:8. doi: 10.1186/s13075-016-1213-9
21. Marrapodi R, Pellicano C, Radicchio G, Leodori G, Colantuono S, Iacolare A, et al. $CD21^{low}$ B cells in systemic sclerosis: a possible marker of vascular complications. *Clin Immunol.* (2020) 213:108364. doi: 10.1016/j.clim.2020.108364
22. Vowell KN, Wilfong EM, Crofford LJ, Kendall PL. Autoreactive-prone CD21lo B cells increased in scleroderma patients with interstitial lung disease. *J Immunol.* (2018) 200 (1 Suppl.) 45.48.
23. Einhaus J, Pecher AC, Asteriti E, Schmid H, Secker KA, Duerr-Stoerzer S, et al. Inhibition of effector B cells by ibrutinib in systemic sclerosis. *Arthritis Res Ther.* (2020) 22:66. doi: 10.1186/s13075-020-02153-8
24. Wilfong EM, Vowell KN, Bunn KE, Rizzi E, Annapureddy N, Dudenhofer RB, et al. CD19 + $CD21^{lo/neg}$ cells are increased in systemic sclerosis-associated interstitial lung disease. *Clin Exp Med.* (2021) 22:209–20. doi: 10.1007/s10238-021-00745-5
25. Matsushita T, Fujimoto M, Hasegawa M, Matsushita Y, Komura K, Ogawa F, et al. BAFF antagonist attenuates the development of skin fibrosis in tight-skin mice. *J Invest Dermatol.* (2007) 127:2772–80. doi: 10.1038/sj.jid.5700919
26. Matsushita T, Hasegawa M, Yanaba K, Kodera M, Takehara K, Sato S. Elevated serum BAFF levels in patients with systemic sclerosis: enhanced BAFF signaling in systemic sclerosis B lymphocytes. *Arthritis Rheum.* (2006) 54:192–201. doi: 10.1002/art.21526
27. Gordon JK, Martyanov V, Franks JM, Bernstein EJ, Szymonifka J, Magro C, et al. Belimumab for the treatment of early diffuse systemic sclerosis: results of a randomized, double-blind, placebo-controlled, pilot trial. *Arthritis Rheumatol.* (2018) 70:308–16. doi: 10.1002/art.40358
28. Matsushita T, Fujimoto M, Hasegawa M, Tanaka C, Kumada S, Ogawa F, et al. Elevated serum APRIL levels in patients with systemic sclerosis: distinct profiles of systemic sclerosis categorized by APRIL and BAFF. *J Rheumatol.* (2007) 34:2056–62.
29. François A, Chatelus E, Wachsmann D, Sibilia J, Bahram S, Alsaleh G, et al. B lymphocytes and B-cell activating factor promote collagen and profibrotic markers expression by dermal fibroblasts in systemic sclerosis. *Arthritis Res Ther.* (2013) 15:R168. doi: 10.1186/ar4352
30. Dumoitier N, Chaigne B, Régent A, Lofek S, Mhibik M, Dorfmüller P, et al. Scleroderma peripheral B lymphocytes secrete Interleukin-6 and transforming growth factor β and activate fibroblasts. *Arthritis Rheumatol.* (2017) 69:1078–89. doi: 10.1002/art.40016
31. Khanna D, Lin CJF, Furst DE, Goldin J, Kim G, Kuwana M, et al. Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med.* (2020) 8:963–74. doi: 10.1016/S2213-2600(20)30318-0
32. Fukasawa T, Yoshizaki A, Ebata S, Yoshizaki-Ogawa A, Asano Y, Enomoto A, et al. Single-cell-level protein analysis revealing the roles of autoantigen-reactive B lymphocytes in autoimmune disease and the murine model. *Elife.* (2021) 10:e67209. doi: 10.7554/eLife.67209
33. Gigante A, Pellicano C, Leodori G, Napodano C, Vantaggio L, Gulli F, et al. Serum and urine free light chains measurements in patients with systemic sclerosis: novel biomarkers for disease activity. *Clin Exp Immunol.* (2021) 205:135–41. doi: 10.1111/cei.13611
34. Bosello S, Basile U, De Lorenzis E, Gulli F, Canestrari G, Napodano C, et al. Free light chains of immunoglobulins in patients with systemic sclerosis: correlations with lung involvement and inflammatory milieu. *J Clin Pathol.* (2018) 71:620–5. doi: 10.1136/jclinpath-2017-204656
35. Hasegawa M, Hamaguchi Y, Yanaba K, Bouaziz JD, Uchida J, Fujimoto M, et al. B-lymphocyte depletion reduces skin fibrosis and autoimmunity in the tight-skin mouse model for systemic sclerosis. *Am J Pathol.* (2006) 169:954–66. doi: 10.2353/ajpath.2006.060205

36. Matsushita T, Kobayashi T, Mizumaki K, Kano M, Sawada T, Tennichi M, et al. BAFF inhibition attenuates fibrosis in scleroderma by modulating the regulatory and effector B cell balance. *Sci Adv*. (2018) 4:eaas9944. doi: 10.1126/sciadv.aas9944
37. Odaka M, Hasegawa M, Hamaguchi Y, Ishiura N, Kumada S, Matsushita T, et al. Autoantibody-mediated regulation of B cell responses by functional anti-CD22 autoantibodies in patients with systemic sclerosis. *Clin Exp Immunol*. (2010) 159:176–84. doi: 10.1111/j.1365-2249.2009.04059.x
38. Gernert M, Tony HP, Schwaneck EC, Gadeholt O, Schmalzing M. Autologous hematopoietic stem cell transplantation in systemic sclerosis induces long-lasting changes in B cell homeostasis toward an anti-inflammatory B cell cytokine pattern. *Arthritis Res Ther*. (2019) 21:106. doi: 10.1186/s13075-019-1889-8
39. Lima-Júnior JR, Arruda LCM, Gonçalves MS, Dias JBE, Moraes DA, Covas DT, et al. Autologous haematopoietic stem cell transplantation restores the suppressive capacity of regulatory B cells in systemic sclerosis patients. *Rheumatology*. (2021) 60:5538–48. doi: 10.1093/rheumatology/keab257
40. Daoussis D, Liossis SN, Tsamandas AC, Kalogeropoulou C, Kazantzi A, Sirinian C, et al. Experience with rituximab in scleroderma: results from a 1-year, proof-of-principle study. *Rheumatology (Oxford)*. (2010) 49:271–80. doi: 10.1093/rheumatology/kep093
41. Daoussis D, Tsamandas AC, Liossis SN, Antonopoulos I, Karatza E, Yiannopoulos G, et al. B-cell depletion therapy in patients with diffuse systemic sclerosis associates with a significant decrease in PDGFR expression and activation in spindle-like cells in the skin. *Arthritis Res Ther*. (2012) 14:R145. doi: 10.1186/ar3879

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 08 August 2022
DOI 10.3389/fmed.2022.964722

OPEN ACCESS

EDITED BY
Argyrios Tzouvelekis,
Alexander Fleming Biomedical Sciences Research Center, Greece

REVIEWED BY
Vasilios Tzilas,
Sotiria General Hospital, Greece
Paschalis Ntolios,
Democritus University of Thrace, Greece

*CORRESPONDENCE
Sotirios I. Sinis
sinis1995@windowslive.com

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 08 June 2022
ACCEPTED 18 July 2022
PUBLISHED 08 August 2022

CITATION
Kirgou P, Sinis SI, Dimeas IE, Papanikolaou IC, Tatsis K, Gogali A, Gourgoulianis KI, Bogdanos DP and Daniil Z (2022) Clinical relevance of circulating autoantibodies in idiopathic pulmonary fibrosis; A NAt hard to break. *Front. Med.* 9:964722. doi: 10.3389/fmed.2022.964722

Clinical relevance of circulating autoantibodies in idiopathic pulmonary fibrosis; A NAt hard to break

Paraskevi Kirgou[1], Sotirios I. Sinis[1]*, Ilias E. Dimeas[1], Ilias C. Papanikolaou[2], Konstantinos Tatsis[3], Athena Gogali[3], Konstantinos I. Gourgoulianis[1], Dimitrios P. Bogdanos[4] and Zoe Daniil[1]

[1]Department of Respiratory Medicine, Faculty of Medicine, School of Health Sciences, University of Thessaly, Biopolis, Larissa, Greece, [2]Respiratory Medicine Department, Corfu General Hospital, Corfu, Greece, [3]Respiratory Medicine Department, Faculty of Medicine, University of Ioannina, Ioannina, Greece, [4]Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Biopolis, Larissa, Greece

Patients with idiopathic pulmonary fibrosis are screened for circulating autoantibodies as part of the initial interstitial lung disease workup. Management of seropositive idiopathic pulmonary fibrosis is currently considered no different than that of lone idiopathic pulmonary fibrosis. Emerging data however suggest that the former may possess distinct characteristics in terms of pathophysiology, histopathology, prognosis and amenability to immunomodulation. In that context, the aim of our study was to evaluate the influence of autoantibody status on: (i) the decline of forced vital capacity; (ii) the decline of diffusing capacity of lung for carbon monoxide; and (iii) 3-year survival; in a cohort of 102 idiopathic pulmonary fibrosis patients. In a pilot sub-study, we also sought to evaluate whether changes in antibody status during disease course affect the aforementioned parameters by potentially reflecting activity of the autoimmunity component of the pro-fibrotic mechanism.

KEYWORDS

idiopathic pulmonary fibrosis, autoimmunity, antinuclear antibody, autoantibodies, interstitial lung disease, pulmonary function test (PFTs)

Introduction

Idiopathic pulmonary fibrosis (IPF) is the most common form of progressive idiopathic interstitial pneumonia characterized by a specific type of radiological/histopathological pattern termed Usual Interstitial Pneumonia (UIP) (1). Paramount to the diagnosis of IPF is exclusion of interstitial lung disease in the setting of connective tissue disease (CTD-ILD) as UIP can be found in patients with systemic sclerosis, rheumatoid arthritis and polymyositis/dermatomyositis (1). The 2018 ATS/ERS/JRS/ALAT guidelines recommend routine screening for circulating anti-nuclear antibody (ANA), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibodies (anti-CCP) and anti-myositis panel (1). Autoantibody positivity has been

reported in 23–41% of IPF patients and ∼8–10% of those with an initial diagnosis congruent with IPF will eventually develop overt CTD (2). Another portion of UIP cases may demonstrate clinical, histological and serological aspects of autoimmunity inconsistent with distinct CTD and are categorized as Interstitial Pneumonia with Autoimmune Features (IPAF) (3). In other words, a significant number of seropositive cases under the guise of IPF are likely to present with an autoimmunity phenotype/flare and be therefore rediagnosed as IPAF or CTD-ILD. On the other hand, the significance of circulating autoantibodies in patients with IPF that never fulfill criteria for IPAF or CTD-ILD remains controversial.

In light of the ineffectiveness of current immunomodulatory/immunosuppressive agents in IPF, the pathophysiological implications of autoimmunity have been markedly understated. Recent studies however concerning aberrant function of CD4+ and regulatory T cells and humoral autoreactivity against nuclear and cytoplasmic antigens in IPF have spurred novel interest on the paradigm (4). As a result there have been several publications contemplating the link between circulating autoantibodies and clinically impactful IPF endpoints such as: (i) patient survival, (ii) pulmonary function test parameters at diagnosis, (iii) pulmonary function test (PFT) parameter progression, (iv) acute exacerbation frequency or severity, and (v) response to treatment with antifibrotics or immunosuppression; albeit with conflicting outcomes (5–9). While it cannot be excluded that circulating antibodies are randomly autoreactive, it is entirely possible that they reflect an ongoing autoimmune process that participates in the mechanism culminating in pulmonary fibrosis. Considering the above, it would be prudent to explore whether autoantibody profiling denotes clinical phenotypes that could be more amenable to immunomodulation given the dismal prognosis of IPF and the urgent need for effective pharmacological intervention (10).

In this study we retrospectively assessed the frequency of an extensive panel of circulating autoantibodies in IPF patients belonging to three reference centers in Greece. We sought to perform associations between serological status and demographics, baseline PFT parameters, PFT parameter decline and 3-year survival. In a pilot sub-study the influence of seroconversion and autoantibody clearance on disease trajectory were investigated.

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; anti-dsDNA, anti-double stranded DNA; cANCA, antineutrophil cytoplasmic antibodies, cytoplasmic; pANCA, antineutrophil cytoplasmic antibodies, perinuclear; ANA, antinuclear antibody; anti-RNP, anti-ribonucleoprotein; anti-SM, anti-Smith; anti-Scl70, anti-topoisomerase 1; CTD, connective tissue disease; ILD, interstitial lung disease; DLCO, diffusing capacity for carbon monoxide; anti-ENA, extractable nuclear antigen; FVC, functional vital capacity; IPF, idiopathic pulmonary fibrosis; IPAF, interstitial pneumonia with autoimmune features; RF, rheumatoid factor; PFT, pulmonary function test.

Materials and methods

Study population

The records of 102 patients diagnosed with IPF by an interdisciplinary care team (pulmonologist, rheumatologist, and radiologist) at the Outpatient ILD Clinics of Respiratory Medicine Department of University Hospital of Larissa, Respiratory Medicine Department of University Hospital of Ioannina and Respiratory Medicine Department of Corfu General Hospital between 2017 and 2020 were reviewed retrospectively. The diagnosis was based on the results of high- resolution computed tomography according to the 2018 ATS/ERS/JRS/ALAT guidelines (UIP pattern) (1). Demographics, pulmonary function tests and serum antibody titers were recorded.

Serologic auto-antibodies profile

All patients were tested for serum autoantibodies at diagnosis and at regular visits after 12, 24, and 36 months. The results reported tests for conventional IgG ANA (antinuclear antibody), anti-ENA (extractable nuclear antigen), anti-SSA (Ro), anti- SSB (La), anti- RNP (ribonucleoprotein), anti- SM (Smith), anti- Scl70 (topoisomerase 1), anti-Jo1, antineutrophil cytoplasmic antibodies cytoplasmic and perinuclear (cANCA and pANCA, respectively), anti-dsDNA (double stranded DNA), anti-CCP (cyclic citrullinated peptide) and RF (rheumatoid factor). The titer of all serologic screening antibodies was considered positive if the result was above cut-offs recommended by the manufacturer. ANA testing was performed by conventional indirect immunofluorescence using HEp-2 as antigenic source (Euroimmun, Lübeck, Germany) and a test was considered positive at >1:80. ANCA testing was performed by indirect IFL using human neutrophils (Euroimmun) (>1:20). Positive tests by IFL were cross-checked by an independent diagnostician. All other tests were performed by conventional ELISAs or blot assays, as thoroughly described previously (Euroimmun). All positive tests were re-checked in duplicate (11).

Pulmonary function tests

All patients underwent pulmonary function testing at baseline and at regular follow up every 12 months. Spirometry, diffusing capacity of lung for carbon monoxide (DLCO) and body plethysmography were recorded according to published

guidelines (12). All the parameters were expressed in absolute terms and as a percentage of the predicted value. Gender, age and height were the variables used for calculating the predicted values for each PFT parameter (13).

Statistical analysis

Results are given as mean (±Standard Deviation, SD) in normally distributed parameters and as median (±Standard Error, SE) in non-normally distributed values. Kolmogorov–Smirnov test was used to identify whether a continuous variable was normally or non-normally distributed. Normally distributed continuous indices were compared with Student's *t*-test or one-way Anova; non-normally distributed indices were compared *via* the Mann-Whitney-U and Wilcoxon test or Kruskal-Wallis test. Survival was reviewed by using Kaplan Meier analysis and hazard by implementing Cox Regression. Finally, Chi-square was used when testing categorical data. Data were analyzed using SPSS (IBM SPSS statistics version 25).

Results

The follow up time after diagnosis was 3 years. The study involved 102 participants with a mean age of 71.8 years and a male/female ratio of ∼4:1. The patients were subject to pulmonary function tests and had their blood drawn at diagnosis and at 12, 24,and 36 months post-diagnosis. A positive smoking history was obtained from 76 participants divided in 13 current smokers and 63 ex-smokers. The study population had a mean Forced Vital Capacity (FVC) of 2.67 ± 0.84 L (77.48 ± 19.22%) and a mean DLCO of 3.96 ± 1.53 mmol/min/kPa (48.20 ± 16.4%) at diagnosis (Table 1).

Gender, smoking, age, baseline FVC, FVC percent decline during the first year following diagnosis, average annual percent FVC decline or total percent FVC decline over 3 years, average annual percent DLCO decline or total percent DLCO decline over 3 years were not associated with all-cause mortality. On the other hand, baseline DLCO (HR = 1.66. 95% CI 1.09–2.54 p = 0.018) and DLCO percent decline during the first year following diagnosis were associated with all-cause mortality in IPF patients (HR = 0.94 95% CI 0.89–1, p = 0.049).

It was found that 47 out of 102 (48%) enrolled subjects had detectable circulating autoantibodies at some point during the disease course. ANA was found in 35 patients, RF was found in 14 patients and the antigen-specific antibodies of the study panel were detected in 5 patients (Table 1). No statistical difference was observed in terms of baseline demographics and pulmonary function test values between seropositive and seronegative patients.

The rate of FVC decline was independent of autoreactivity status in this cohort (Table 1). On the other hand, all patients with circulating autoantibodies showed slower deterioration when assessed for changes in DLCO (Table 1). Autoantibody status was not associated with all-cause mortality in IPF patients although a tendency for benefit was observed for seropositive individuals (HR = 0.77. 95% CI 0.23–1.33) (Figure 1).

We speculated that circulating autoantibody temporal kinetics may define distinct disease phenotypes on the basis that innate immunity activity against self antigens may be an important avenue of fibrosis propagation in autoantibody positive IPF. Patients of: (i) Group A (n = 29) were persistently seropositive; (ii) Group B (n = 11) developed autoreactivity after the diagnosis; and (iii) Group C (n = 7) became seronegative as fibrosis progressed. The remaining patients were the 55 seronegative controls comprising Group D. However, no statistically significant differences were observed between groups A, B and C in terms of FVC and DLCO baseline values/trajectory or 3-year survival.

Discussion

Our study contributes to the small but growing body of literature assessing the rate of decline in PFT parameters of IPF patients with and without circulating autoantibodies. This is the first report aiming to determine the implications of autoantibody kinetics in IPF. The results suggest that patients with circulating autoantibodies at some point during the disease course progressed slower in terms of DLCO compared to seronegative controls. There was a tendency for improved survival in seropositive participants but it was not statistically significant. Persistently seropositive patients were no different in terms of study endpoints compared to patients that developed seroconversion or those that became seronegative.

It remains unclear if circulating autoantibodies are more common in IPF patients compared to healthy individuals. In the publication of Lee et al., which is one of the few to address the issue, positive serology was found in 22% of IPF patients (n = 67) similar to that of internal controls (n = 52) (6). However, the lack of statistical significance becomes intriguing when taking into context that; (i) the IPF group had a higher proportion of males (50% males for the control group vs. 75% males for the IPF group); and (ii) healthy females are more likely to harbor circulating ANA.

Analysis and comparison of data regarding circulating autoantibodies in IPF patients is hindered by considerable heterogeneity between studies in critical parameters such as positivity cut-off values, population demographics, autoantibody selection and laboratory methodologies. At a low dilution of ≥1:40, Fischer et al. and Kang et al. reported ANA prevalence of 34 and 31.5% in their cohorts of 285 and 526 patients, respectively (8, 14, 15). We demonstrated that 48% of patients with IPF were seropositive for ANA, RF and/or antigen specific autoantibodies and that 34.4% had ANA ≥ 1:160, a

TABLE 1 Cohort demographic data and evolution of pulmonary function test parameters for seronegative patients and seropositive patients.

	Seronegative patients	Seropositive patients	*p*-value	Cohort
Patient number	55	47	-	102
Age (mean ± SD)	72.40 ± 9.60	71.10 ± 9.43	>0.05	71.80 ± 9.50
Male	83.60%	76.60%	>0.05	80.40%
Smoking status				
Non	11 (20%)	15 (31.9%)	>0.05	26 (25.5%)
Ex	38 (69.1%)	25 (53.2%)	>0.05	13 (61.8%)
Active	6 (10.9%)	7 (14.9%)	>0.05	63 (12.7%)
Autoantibodies				
ANA	-	35 (74.5%)	-	-
RF	-	14 (29.7%)	-	-
Antigen specific	-	5 (10.6%)	-	-
Pulmonary function trajectory				
Baseline absolute FVC (mean ± SD)	2.66 ± 0.9	2.67 ± 0.75	>0.05	2.67 ± 0.84
Baseline FVC % predicted (mean ± SD)	77.66 ± 21.88%	79.02 ± 17.42%	>0.05	77.48 ± 19.22%
Baseline absolute DLCO (mean ± SD)	3.85 ± 1.6	4.09 ± 1.45	>0.05	3.96 ± 1.53
Baseline DLCO % predicted (mean ± SD)	45.92 ± 15.92%	51.01 ± 16.89%	>0.05	48.2 ± 16.4%
Total ΔFVC (mean ± SD)	−2.85 ± 14.1%	−7.73 ± 12.7%	>0.05	−5.26 ± 13.63%
Total DLCO (mean ± SD)	−14.45 ± 14.26%	−7.86 ± 12.42%	0.041	−14.45 ± 2.05%
Mean annual ΔFVC (mean ± SE/SD)	−1.23 ± 2.46%	−1.78 ± 4.01%	>0.05	−1.45 ± 0.61%
Mean annual ΔDLCO (mean ± SD)	−6.92 ± 6.81%	−3.73 ± 7.53%	0.042	−4.82 ± 7.41%
Clinically significant FVC decline	40 (72.7%)	37 (78.7%)	>0.05	77 (75.5%)
Year 1	10 (18.2%)	9 (19.1%)	>0.05	19 (18.7%)
Year 2	9 (16.4%)	10 (21.2%)	>0.05	19 (18.7%)
Year 3	7 (12.7%)	6 (12.8%)	>0.05	13 (12.7%)
Year 1+2	4 (7.3%)	4 (8.5%)	>0.05	8 (7.8%)
Year 1+3	3 (5.5%)	2 (4.3%)	>0.05	5 (4.9%)
Year 2+3	5 (9.1%)	4 (8.5%)	>0.05	9 (8.8%)
Year 1+2+3	2 (3.5%)	2 (4.3%)	>0.05	4 (3.9%)
Clinically significant DLCO decline	34 (61.8%)	28 (59.6%)	>0.05	62 (60.8%)
Year 1	12 (21.7%)	9 (19.2%)	>0.05	21 (20.6%)
Year 2	9 (16.3%)	9 (19.2%)	>0.05	18 (17.7%)
Year 3	4 (7.3%)	7 (14.9%)	>0.05	11 (10.8%)
Year 1+2	3 (5.5%)	1 (2.1%)	>0.05	4 (3.9%)
Year 1+3	3 (5.5%)	1 (2.1%)	>0.05	4 (3.9%)
Year 2+3	3 (5.5%)	0 (0%)	>0.05	3 (2.9)
Year 1+2+3	0 (0%)	1 (2.1%)	>0.05	1 (1%)

Total ΔFVC or ΔDLCO = percent difference between absolute value at diagnosis and at 3 years post-diagnosis, Mean annual ΔFVC or ΔDLCO = mean value of percent decline occurring during year 1, 2, and 3 post diagnosis, Clinically significant FVC decline = annual decline ≥5% at least once during follow-up (max. 3), Clinically significant DLCO decline = annual decline ≥15% at least once during follow-up (max. 3). Patients were further stratified based on which year(s) clinically significant FVC or DLCO decline was observed.
SD, standard deviation; SE, standard error.

level which is more clinically relevant. Two studies involving the same cut-off point showed a large discrepancy in terms of frequency with 41.4% out 58 and 16.8% out 386 individuals being positive, respectively (5, 16). Both research groups screened patients for antigen-specific autoantibodies such as ANCA, anti SSA/SSB, antiScl70 and antiJo-1, a strategy which moderates misclassification of patients as seronegative owing to untested markers of humoral autoimmunity. Nevertheless, no significant correlation between autoantibody presence and survival was found. It must be noted though that Moua et al. screened a portion of the population for antigen-specific autoantibodies which could result in false negative results (16). Even patients with ANA ≥ 1:320 and/or antigen specific autoantibodies showed no alterations in terms of life expectancy (17). The decline of PFT parameters during disease progression (annual absolute and percent predicted FVC or DLCO)

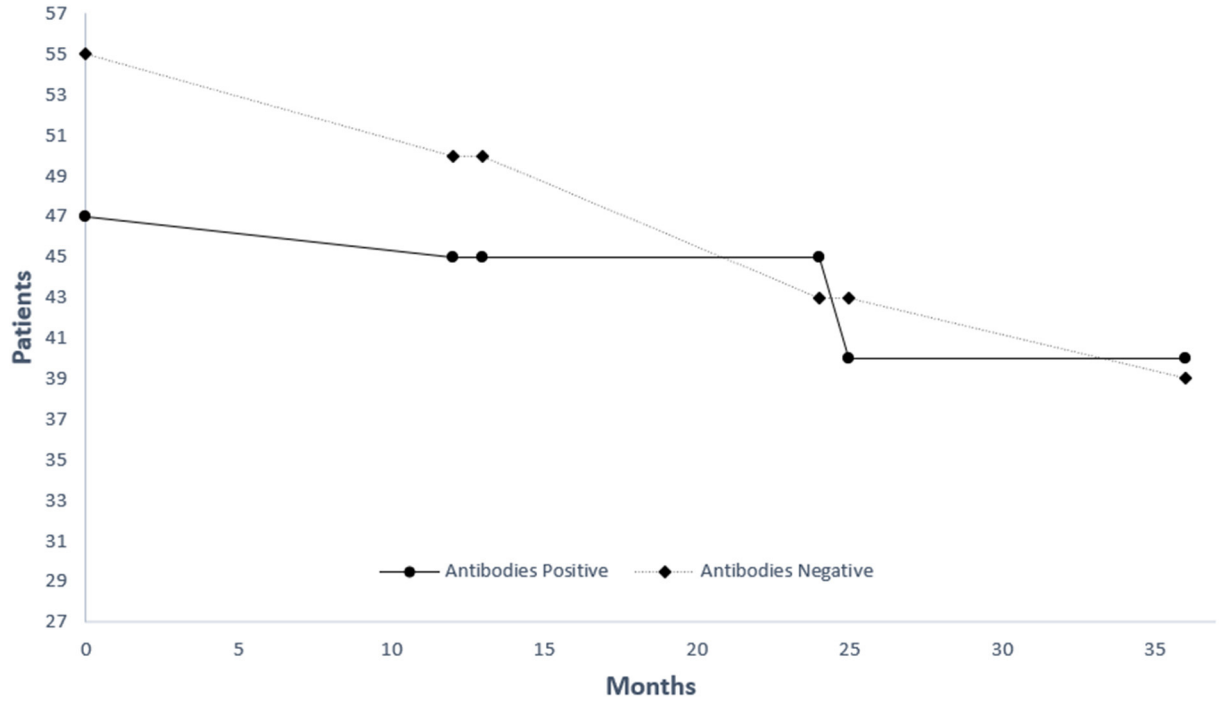

FIGURE 1
Kaplan-Meier estimate of survival for patients with (black line) and without (grey line) antibodies.

remained unaffected in disagreement with our findings (17). However, after adjustment for previously established FVC and DLCO decline cut-offs (>5 and >15%, respectively) associated with survival, the influence of autoantibody positivity on DLCO becomes negligible in our study (Table 1) (18, 19). Taken together, the aforementioned body of evidence support the prevailing notion that CTD-related autoantibodies in IPF patients are probably inconsequential.

We did observe a statistically significant reduction in DLCO decline and Lee et al. reported longer transplant-free survival in seropositive individuals although both outcomes are of ambiguous clinical relevance (6). Extension of the follow-up period in our study could resolve the apparent contradiction, given the prognostic value of DLCO, and produce improvement of survival in line with the latter. It was recently shown by, Ghang et al. that autoantibodies as defined by the IPAF serologic domain and ANCA confer a favorable prognosis (7). Intriguingly, immunomodulation was beneficial to seropositive subjects which is consistent with the fact that circulating autoantibodies in IPF patients have been previously linked to CTD-like findings on lung histology (20). On the other hand, seronegative comparators showed greater 5-year mortality when treated (7). Parallels can be drawn with the study of Tzouvelekis et al. where combined emphysema and fibrosis patients with autoimmune markers showed better prognosis and abundant CD20+ B cell lymphoid follicles compared to those lacking autoreactivity indices (4). In agreement with the above, a recent pilot trial suggested that antibody reduction through plasma exchange, rituximab and intravenous immunoglobulin may quench acute exacerbations of IPF, a condition likely to be characterized by immunity perturbations found in classical autoantibody-mediated disorders (21). Within that context, we suggest that the clinical redundancy of circulating autoantibodies in IPF may need to be revisited.

Serial autoantibody measurements at follow-up visits for patients with an initial diagnosis most congruent with IPF has been proposed to monitor development of CTD given that disease presentation may be limited to pulmonary manifestations and the likelihood of misdiagnosis should be considered. For instance, in a Chinese study 25.1% of patients with ILD became seropositive after the initial diagnostic assessment (22). We sought to unravel additional value beyond CTD exclusion for monitoring levels of circulating autoantibodies in IPF. It is speculated that regular screening could identify patients developing activation of innate immunity against self antigens at some point during disease evolution (23). Given the different types of inflammation between rapid and slow progressors, changes in antibody status could represent a harbinger of the rate of functional decline and even response to immunosuppression (24). Although our exploratory trial did not show any difference in PFT trajectory and survival between groups A, B, and

C, it is worthwhile to further delineate the implications of the dynamic inflammatory process during the course of IPF.

Our study has several limitations. Due to the size of the cohort, stratification based on antibody temporal kinetics produced sub-groups each with a small number of patients, thereby diminishing the strength of statistical analysis. Although we thoroughly screened every participant for an extensive panel of circulating autoantibodies, seropositive patients were not further classified based on autoantibody type considering that a very small minority had humoral autoreactivity markers besides ANA and RF. Therefore, the notion that certain CTD-related circulating autoantibodies may be of greater relevance in the setting of IPF management could not be explored in this study. Unfortunately, no data were recorded in terms of acute exacerbations precluding associations between deviant adaptive immunity responses against self-antigens and frequency or severity of episodes. We performed serial measurements of autoantibodies and regular rheumatologic consultations, however given the follow-up duration of 3 years; it cannot be excluded that some patients will eventually develop features consistent with CTD and were therefore misdiagnosed as IPF.

Large, prospective, multi-center studies involving meticulous serial measurements of standardized ILD-associated autoantibody arrays are required to unfold the dichotomy surrounding the role of circulating autoantibodies in IPF. To this end, the recent discovery of unidentified autoantibody bands by immunoprecipitation in IPF patients provides a new avenue to explore the immunological background of the disease and particularly that of acute exacerbation, when adaptive immunity perturbation may be more prominent (25). Even if detection of CTD-related circulating autoantibodies is unequivocally proven incidental in IPF patients, it is possible that novel autoantibodies with mechanistic, diagnostic, prognostic and therapeutic relevance await discovery.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PK contributed to the conception, design of the study, and the acquisition of data for the work. SS contributed to drafting the work and revised it critically for important intellectual content. ID contributed to the conception, design of the study, and the acquisition and statistical analysis of data for the work. IP, KT, and AG contributed to the acquisition of data for the work and performed critical revision of the manuscript for important intellectual content. KG contributed to the conception, design of the study, and performed critical revision for important intellectual content. DB contributed to acquisition of data for the work and performed critical revision for important intellectual content. ZD contributed to the conception of the study, interpretation of data for the work, and performed critical revision of the manuscript for important intellectual content. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

PK is a recipient of financial support in the context of a doctoral thesis (grant number MIS-5113934). The implementation of the doctoral thesis was co-financed by Greece and the European Union (European Social Fund-ESF) through the Operational Programme—Human Resources Development, Education and Lifelong Learning—in the context of the Act—Enhancing Human Resources Research Potential by undertaking a Doctoral Research—Sub-action 2: IKY Scholarship Programme for Ph.D. candidates in the Greek Universities.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med.* (2018) 198:e44–68. doi: 10.1164/rccm.201807-1255ST

2. Kono M, Nakamura Y, Enomoto N, Hashimoto D, Fujisawa T, Inui N, et al. Usual interstitial pneumonia preceding collagen vascular disease: a retrospective case control study of patients initially diagnosed with idiopathic pulmonary fibrosis. *PLoS ONE.* (2014) 9:e94775. doi: 10.1371/journal.pone.0094775

3. Graney BA, Fischer A. Interstitial pneumonia with autoimmune features. *Ann Am Thorac Soc.* (2019) 16:525–33. doi: 10.1513/AnnalsATS.201808-565CME

4. Tzouvelekis A, Zacharis G, Oikonomou A, Mikroulis D, Margaritopoulos G, Koutsopoulos A, et al. *Increased Incidence of Autoimmune Markers in Patients With Combined Pulmonary Fibrosis and Emphysema*. (2013). Available online at: http://www.biomedcentral.com/1471-2466/13/31 (accessed June 6, 2022).

5. Vij R, Noth I, Strek ME. Autoimmune-featured interstitial lung disease: a distinct entity. *Chest.* (2011) 140:1292. doi: 10.1378/chest.10-2662

6. Lee JS, Kim EJ, Lynch KL, Elicker B, Ryerson CJ, Katsumoto TR, et al. Prevalence and clinical significance of circulating autoantibodies in idiopathic pulmonary fibrosis. *Respir Med.* (2013) 107:249–55. doi: 10.1016/j.rmed.2012.10.018

7. Ghang B, Lee J, Chan Kwon O, Ahn SM, Oh JS, Hong S, et al. Clinical significance of autoantibody positivity in idiopathic pulmonary fibrosis. *Respir Med.* (2019) 155:43–8. doi: 10.1016/j.rmed.2019.07.001

8. Kang BH, Park JK, Roh JH, Song JW, Lee CK, Kim M, et al. Clinical significance of serum autoantibodies in idiopathic interstitial pneumonia. *J Korean Med Sci.* (2013) 28:731. doi: 10.3346/jkms.2013.28.5.731

9. Song MJ, Lee SH, Jung JY, Kang YA, Park MS, Kim YS, et al. Effectiveness of pirfenidone in idiopathic pulmonary fibrosis according to the autoantibody status: a retrospective cohort study. *BMC Pulm Med.* (2021) 21:145. doi: 10.1186/s12890-021-01516-4

10. Karampitsakos T, Vraka A, Bouros D, Liossis SN, Tzouvelekis A. Biologic treatments in interstitial lung diseases. *Front Med.* (2019) 6:41. doi: 10.3389/fmed.2019.00041

11. Liaskos C, Marou E, Simopoulou T, Barmakoudi M, Efthymiou G, Scheper T, et al. Disease-related autoantibody profile in patients with systemic sclerosis. *Autoimmunity*. (2017) 50:414–21. doi: 10.1080/08916934.2017.1357699

12. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J.* (2005) 26:319–38. doi: 10.1183/09031936.05.00034805

13. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. *Eur Respir J.* (2005) 26:948–68. doi: 10.1183/09031936.05.00035205

14. Fischer A, West SG, Swigris JJ, Brown KK, Du Bois RM. Connective tissue disease-associated interstitial lung disease: a call for clarification. *Chest.* (2010) 138:251. doi: 10.1378/chest.10-0194

15. Fischer A, Pfalzgraf FJ, Feghali-Bostwick CA, Wright TM, Curran-Everett D, West SG, et al. Anti-th/to-positivity in a cohort of patients with idiopathic pulmonary fibrosis. *J Rheumatol.* (2006) 33:1600–5.

16. Moua T, Maldonado F, Decker PA, Daniels CE, Ryu JH. Frequency and implication of autoimmune serologies in idiopathic pulmonary fibrosis. *Mayo Clin Proc.* (2014) 89:319–26. doi: 10.1016/j.mayocp.2013.11.018

17. Goobie GC, Ford-Sahibzada CA, Fritzler MJ, Johannson KA, Fell CD. Autoantibody status is not associated with change in lung function or survival in patients with idiopathic pulmonary fibrosis. *Respir Med.* (2019) 153:85–90. doi: 10.1016/j.rmed.2019.05.019

18. Zappala CJ, Latsi PI, Nicholson AG, Colby TV, Cramer D, Renzoni EA, et al. Marginal decline in forced vital capacity is associated with a poor outcome in idiopathic pulmonary fibrosis. *Eur Respir J.* (2010) 35:830–6. doi: 10.1183/09031936.00155108

19. Tzouvelekis A, Karampitsakos T, Ntolios P, Tzilas V, Bouros E, Markozannes E, et al. Longitudinal "real-world" outcomes of pirfenidone in idiopathic pulmonary fibrosis in Greece. *Front Med.* (2017) 4:213. doi: 10.3389/fmed.2017.00213

20. Song JW, Do KH, Kim MY, Jang SJ, Colby TV, Kim DS. Pathologic and radiologic differences between idiopathic and collagen vascular disease-related usual interstitial pneumonia. *Chest.* (2009) 136:23–30. doi: 10.1378/chest.08-2572

21. Kulkarni T, Valentine VG, Fei F, Tran-Nguyen TK, Quesada-Arias LD, Mkorombindo T, et al. Correlates of survival after autoantibody reduction therapy for acute IPF exacerbations. *PLoS ONE.* (2021) 16:0260345. doi: 10.1371/journal.pone.0260345

22. Hu Y, Wang LS, Wei YR, Du SS, Du YK, He X, et al. Clinical characteristics of connective tissue disease-associated interstitial lung disease in 1,044 Chinese patients. *Chest.* (2016) 149:201–8. doi: 10.1378/chest.15-1145

23. Moll SA, Platenburg MGJP, Platteel ACM, Vorselaars ADM, Bonàs MJ, Roodenburg-Benschop C, et al. Prevalence of novel myositis autoantibodies in a large cohort of patients with interstitial lung disease. *J Clin Med.* (2020) 9:1–14. doi: 10.3390/jcm9092944

24. Balestro E, Calabrese F, Turato G, Lunardi F, Bazzan E, Marulli G, et al. Immune inflammation and disease progression in idiopathic pulmonary fibrosis. *PLoS ONE*. (2016) 11:0154516. doi: 10.1371/journal.pone.0154516

25. Cotton CV, Betteridge ZE, Spencer LG, New RP, Lamb J, McHugh NJ, et al. O24 Low level detection of CTD-associated autoantibodies in patients with idiopathic pulmonary fibrosis confirms this as a robust phenotype when diagnosed on clinical grounds alone. *Rheumatology*. (2018) 57:206. doi: 10.1093/rheumatology/key075.206

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 09 January 2023
DOI 10.3389/fmed.2022.1096203

OPEN ACCESS

EDITED BY
Marta Bueno,
University of Pittsburgh, United States

REVIEWED BY
Anna Papazoglou,
University of Pittsburgh, United States
Yongpeng Ge,
China-Japan Friendship Hospital, China
Jacobo Sellares Torres,
Hospital Clinic of Barcelona, Spain

*CORRESPONDENCE
Argyris Tzouvelekis
✉ atzouvelekis@upatras.gr;
✉ argyris.tzouvelekis@gmail.com

†These authors have contributed equally to this work

‡These authors have contributed equally to this work and share last authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 11 November 2022
ACCEPTED 14 December 2022
PUBLISHED 09 January 2023

CITATION
Karampitsakos T, Tzilas V, Papaioannou O, Chrysikos S, Vasarmidi E, Juge P-A, Vizirianaki S, Bibaki E, Reppa A, Sidiropoulos P, Katsaras M, Sotiropoulou V, Tsiri P, Koulousousa E, Theochari E, Tsirikos G, Christopoulos I, Malakounidou E, Zarkadi E, Sampsonas F, Hillas G, Karageorgas T, Daoussis D, Kalogeropoulou C, Dimakou K, Tzanakis N, Borie R, Dieudé P, Antoniou K, Crestani B, Bouros D and Tzouvelekis A (2023) Clinical features and outcomes of patients with myositis associated-interstitial lung disease. *Front. Med.* 9:1096203. doi: 10.3389/fmed.2022.1096203

Clinical features and outcomes of patients with myositis associated-interstitial lung disease

Theodoros Karampitsakos[1†], Vasilios Tzilas[2†], Ourania Papaioannou[1†], Serafeim Chrysikos[2], Eirini Vasarmidi[3,4,5], Pierre-Antoine Juge[4,6], Styliani Vizirianaki[3], Eleni Bibaki[3], Argyro Reppa[7], Prodromos Sidiropoulos[7], Matthaios Katsaras[1], Vasilina Sotiropoulou[1], Panagiota Tsiri[1], Electra Koulousousa[1], Eva Theochari[1], Georgios Tsirikos[1], Ioannis Christopoulos[1], Elli Malakounidou[1], Eirini Zarkadi[1], Fotios Sampsonas[1], Georgios Hillas[2], Theofanis Karageorgas[8], Dimitrios Daoussis[9], Christina Kalogeropoulou[10], Katerina Dimakou[2], Nikolaos Tzanakis[3], Raphael Borie[4,5], Philippe Dieudé[4,6], Katerina Antoniou[3], Bruno Crestani[4,5], Demosthenes Bouros[11‡] and Argyris Tzouvelekis[1*‡]

[1]Department of Respiratory Medicine, University Hospital of Patras, Patras, Greece, [2]5th Department of Pneumonology, General Hospital for Thoracic Diseases Sotiria, Athens, Greece, [3]Laboratory of Molecular and Cellular Pneumonology, Department of Thoracic Medicine, Medical School, University of Crete, Heraklion, Greece, [4]Université de Paris, INSERM UMR 1152, F-75018, Paris, France, [5]Assistance Publique – Hôpitaux de Paris (APHP), Service de Pneumologie A, Centre de Référence Constitutif des Maladies Pulmonaires Rares, Fédération Hospitalo-Universitaire (FHU) APOLLO, Hôpital Bichat, Paris, France, [6]Assistance Publique – Hôpitaux de Paris (APHP), Service de Rheumatologie, Hôpital Bichat-Claude Bernard, F-75018, Paris, France, [7]Department of Rheumatology, Medical School, University of Crete, Heraklion, Greece, [8]Department of Rheumatology, Attikon University Hospital, Athens Medical School, National and Kapodistrian University of Athens, Athens, Greece, [9]Department of Rheumatology, University Hospital of Patras, University of Patras Medical School, Patras, Greece, [10]Department of Radiology, University Hospital of Patras, Patras, Greece, [11]First Academic Department of Pneumonology, Hospital for Thoracic Diseases, "SOTIRIA", Medical School, National and Kapodistrian University of Athens, Athens, Greece

Introduction: Myositis associated interstitial lung disease (ILD) seems to be an under-recognized entity.

Methods: In this multicenter, retrospective study, we recorded between 9/12/2019 and 30/9/2021 consecutive patients who presented in five different ILD centers from two European countries (Greece, France) and received a multidisciplinary diagnosis of myositis associated-ILD. The primary outcome was all-cause mortality over 1 year in specific subgroups of patients. Secondary outcomes included comparison of disease characteristics between patients diagnosed with the amyopathic subtype and patients with evidence of myopathy at diagnosis.

Results: We identified 75 patients with myositis associated-ILD. Median age (95% CI) at the time of diagnosis was 64.0 (61.0–65.0) years. Antinuclear antibody testing was positive in 40% of the cohort (n = 30/75). Myopathy onset occurred first in 40.0% of cases (n = 30), ILD without evidence of myopathy occurred in 29 patients (38.7%), while 16 patients (21.3%) were diagnosed concomitantly with ILD and myopathy. The commonest radiographic pattern was cellular non-specific interstitial pneumonia (NSIP) and was observed in 29 patients (38.7%). The radiographic pattern of organizing pneumonia was significantly more common in patients diagnosed with the amyopathic subtype compared to patients that presented with myopathy [24.1% (n = 7/29) vs. 6.5% (n = 3/46), p = 0.03]. One year survival was 86.7% in the overall population. Kaplan–Meier analysis demonstrated significantly higher all-cause 1-year mortality in patients with the amyopathic subtype compared to patients with evidence of myopathy [HR 4.24 (95% CI: 1.16–15.54), p = 0.03]. Patients diagnosed following hospitalization due to acute respiratory failure experienced increased risk of 1-year all-cause mortality compared to patients diagnosed in outpatient setting [HR 6.70 (95% CI: 1.19–37.81), p = 0.03]. Finally, patients with positive anti-MDA5 presented with higher 1-year all-cause mortality compared to anti-MDA5 negative patients [HR 28.37 (95% CI: 5.13–157.01), p = 0.0001].

Conclusion: Specific ILD radiographic patterns such as NSIP and organizing pneumonia may herald underlying inflammatory myopathies. Hospitalized patients presenting with bilateral organizing pneumonia refractory to antibiotics should be meticulously evaluated for myositis associated-ILD even if there is no overt muscular involvement. Incorporation of ILD radiological patterns in the diagnostic criteria of inflammatory myopathies may lead to timely therapeutic interventions and positively impact patients' survival.

KEYWORDS

myositis, interstitial lung disease, organizing pneumonia, amyopathic, survival

Introduction

Idiopathic inflammatory myopathies (IIMs) or "myositis spectrum disorders" comprise a heterogeneous group of systemic autoimmune diseases characterized by varying degree of skeletal muscle inflammation (1). The most commonly used classification involves three main subtypes of IIMs and specifically dermatomyositis, polymyositis and clinically amyopathic dermatomyositis (CADM) (2). The subgroups of IIM have varied in recent years as knowledge on serotype-phenotype correlations has evolved (3). Clinical features of IIMs vary considerably and may include proximal muscle weakness, skin rash, and extramuscular manifestations such as arthralgia, Raynaud's phenomenon, fever, cardiac arrhythmias, ventricular dysfunction, and interstitial lung disease (ILD). Multiple IIMs phenotypes have been described including overlap syndrome, antisynthetase syndrome, immune mediated necrotizing myopathy and inclusion body myositis. (4–6).

Myositis-associated ILD represents one of the most common extramuscular manifestations occurring in 20–80% of patients with IIMs (7, 8). Importantly, ILD can precede clinically evident muscle or skin disease in a considerable proportion of patients (9–11). Of note, absence of concomitant skin or muscle disease can confound early disease identification and hamper timely interventions leading thus to increased mortality, especially in centers with no expertise in connective tissue disease associated-ILD (9). The need for meticulous evaluation of patients admitted with rapidly progressive respiratory failure seems amenable, given that rapidly progressive-ILD (RP-ILDs) associated with IIMs can be refractory to immunosuppression and result in patients' admission to the intensive care unit (ICU) (9, 12–14). Negative prognostic indicators include anti-MDA5 antibody positivity, absence of myopathy, older age, and skin ulceration (8, 15–17).

Despite the considerable progress on the knowledge of IIMs phenotypes (3, 18–21) and the advent of recent registries from

different parts of the world (1, 22), there is still a pressing need of data for amyopathic cases. This multicenter study aims to present features of patients with myositis associated-ILD from two European countries, compare patients with myopathy and patients with the amyopathic subtype and increase awareness for this under-recognized entity.

Materials and methods

Study design and patient selection

In this multicenter, retrospective study, we recorded between 9/12/2019 and 30/9/2021 consecutive patients who presented in five different ILD centers from two European countries (Greece, France) and received a multidisciplinary diagnosis of myositis associated-ILD. Diagnosis was typically set following multidisciplinary discussion, thus muscle biopsy was not always performed. Patients with a follow-up of at least 1 year were included in the analysis. Data collection and analysis was approved by the Institutional Review Board and the Local Ethics Committee (protocol number 28746/9-12-2019).

Age, smoking history, comorbidities, most commonly encountered antibodies, predominant radiographic pattern, functional indices including Forced Vital Capacity% predicted (FVC% pred) and diffusing capacity of the lung for carbon monoxide% predicted (DLCO% pred), cytologic features of bronchoalveolar lavage (BAL), treatment modalities applied, and survival data. Radiographic patterns were reviewed by a radiologist (CK). Antibody profile was obtained using commercially available myositis panel tests such as EUROLINE test kit. Results were available no later than 7 days in most cases.

Outcome measures

The primary outcome was all-cause mortality over 1 year in specific subgroups of patients: (1) patients diagnosed with the amyopathic subtype compared to patients with evidence of myopathy at diagnosis, (2) patients diagnosed following hospitalization due to acute respiratory failure compared to patients diagnosed in outpatient setting, and (3) patients with positive anti-MDA5 compared to anti-MDA5 negative patients. Secondary outcomes included comparison of disease characteristics such as (1) the frequency of encountered autoantibodies and (2) the most common radiographic patterns, between patients diagnosed with the amyopathic subtype and patients with evidence of myopathy at diagnosis.

Statistical analysis

With regards to baseline data, summary descriptive statistics were generated with categorical data displayed as absolute numbers and relative frequencies. Continuous data were denoted as mean ± standard deviation (SD) or medians with 95% Confidence Interval (95% CI) following Kolmogorov–Smirnov test for normality. The primary outcome was presented with the Kaplan–Meier method and cumulative incidence curves were compared between the pre-specified groups.

Results

Baseline characteristics

We included 75 patients with myositis associated-ILD. Baseline characteristics are summarized in Table 1. Common working diagnoses included antisynthetase syndrome (n = 43, 57.3%) and CADM (n = 29, 38.7%). Median age (95% CI) at the time of diagnosis was 64.0 (61.0–65.0) years. Most patients were female (57.3%, n = 43) and ex-smokers (50.7%, n = 38). Mean FVC% predicted ± SD and DLCO% predicted ± SD at the time of diagnosis were 76.5 ± 22.3 and 59.1 ± 27.7, respectively. Median percentage (95% CI) of lymphocytes in BAL was 15.5 (6.0–22.7) in the overall population. Median percentage (95% CI) of lymphocytes in BAL was 13.0 (6.0–20.0) in patients with evidence of myopathy and 21.0 (3.0–43.8) in patients with CADM. Creatine phosphokinase was elevated in 46 patients with evidence of myopathy and 4 patients with CADM (total number of patients: n = 50, 66.7% of this cohort).

Myopathy onset occurred first in 30 patients (40.0%), ILD without evidence of myopathy occurred in 29 patients (38.7%), while 16 patients (21.3%) were diagnosed concomitantly with ILD and myopathy. Patients with evidence of myopathy (n = 46, 61.3%) had typically proximal muscle weakness, while distal myopathy was observed following disease progression. The percentage of patients diagnosed with IIM at the time point of hospitalization due to acute respiratory failure was significantly higher in CADM compared to the group with evidence of myopathy [31% (n = 9/29) vs. 6.5% (n = 3/46), p = 0.005].

Autoantibody profiles

Antinuclear antibody testing (ANA) (40.0%, n = 30), anti-Jo-1 (26.7%, n = 20), anti-Ro-52 (24.0%, n = 18), anti-MDA5 (18.7%, n = 14), anti-PL-7 (14.7%, n = 11), and anti-PL-12 (14.7%, n = 11) were the most frequently encountered antibodies in the overall population (Table 2). Anti-Jo-1 (27.6%, n = 8), anti-MDA5 (27.6%, n = 8), anti-Ro-52 (24.1%, n = 7), anti-PL-7 (20.7%, n = 6), and anti-OJ (13.8%, n = 4) were the most frequently encountered antibodies in patients diagnosed with CADM. Anti-Ku antibodies were significantly more common in patients with myopathy compared to patients with CADM [13.0%, (n = 6/46) vs. 0.0% (n = 0/29), p = 0.04].

TABLE 1 Baseline characteristics.

Characteristics	(*N*, %)
Total number of patients	75
Age median (%95 CI)	64.0 (61.0–65.0)
Male/Female	32 (42.7%)/43 (57.3%)
Current smokers/Ex-smokers/Never smokers	7 (9.3%)/38 (50.7%)/11 (40.0%)
FVC% predicted ± SD	76.5 ± 22.3
DLCO% predicted ± SD	59.1 ± 27.7
BAL Macrophages% median (%95 CI)	64.0 (59.7–75.7)
BAL Lymphocytes% predicted median (%95 CI)	15.5 (6.0–22.7)
BAL Neutrophils% predicted median (%95 CI)	8.0 (3.0–11.0)
BAL Eosinophils% predicted median (%95 CI)	1.0 (0.0–3.0)
ILD onset first	29 (38.7%)
Concomitant diagnosis of ILD/myopathy	16 (21.3%)
Myopathy onset first	30 (40.0%)
Arterial hypertension	28 (37.3%)
Gastroesophageal reflux disease	14 (18.7%)
Hypothyroidism	11 (14.7%)
Diabetes mellitus	9 (12.0%)
Chronic heart disease	9 (12.0%)
Cancer	8 (10.7%)

BAL, bronchoalveolar lavage; CI, confidence interval; DLCO, diffusing capacity of lung for carbon monoxide; FVC, forced vital capacity; ILD, interstitial lung disease; SD, standard deviation.

TABLE 2 Most frequently encountered autoantibodies.

Auto-antibodies	Overall *N* = 75	Amyopathic at diagnosis *N* = 29	Presence of myopathy at diagnosis *N* = 46
ANA	30 (40.0%)	11 (37.9%)	19 (41.3%)
Anti-Jo-1	20 (26.7%)	8 (27.6%)	12 (26.1%)
Anti-Ro-52	18 (24.0%)	7 (24.1%)	11 (23.9%)
Anti-MDA5	14 (18.7%)	8 (27.6%)	6 (13.0%)
Anti-PL-7	11 (14.7%)	6 (20.7%)	5 (10.9%)
Anti-PL-12	11 (14.7%)	3 (10.3%)	8 (17.4%)
Anti-OJ	9 (12.0%)	4 (13.8%)	5 (10.9%)
Anti-Ku	6 (8.0%)	0 (0.0%)	6 (13.0%)
Anti-Mi-2a	4 (5.3%)	3 (10.3%)	1 (2.2%)
Anti-Mi-2b	4 (5.3%)	3 (10.3%)	1 (2.2%)
Anti-NXP2	3 (4.0%)	2 (6.9%)	1 (2.2%)

ANA, antinuclear antibody; Jo-1, Histidyl-tRNA synthetase; MDA5, melanoma differentiation-associated gene 5; Mi-2a, helicase protein-2a; Mi-2b, helicase protein-2b; NXP2, nuclear matrix protein; OJ, Isoleucyl-tRNA synthetase; PL-7, Threonyl-tRNA synthetase antibodies; PL-12, Alanyl-tRNA synthetase.

TABLE 3 Radiographic patterns identified in the cohort.

Radiographic pattern	Overall *N* = 75	Amyopathic at diagnosis *N* = 29	Presence of myopathy at diagnosis *N* = 46
Cellular NSIP	29 (38.7%)	12 (41.4%)	17 (37.0%)
Overlap NSIP/organizing pneumonia	19 (25.3%)	5 (17.2%)	14 (30.4%)
Fibrotic NSIP	17 (22.7%)	5 (17.2%)	12 (26.1%)
Organizing pneumonia	10 (13.3%)	7 (24.1%)	3 (6.5%)

NSIP, non-specific interstitial pneumonia.

Radiographic patterns

The predominant radiographic pattern was non-specific interstitial pneumonia (NSIP) in 46 patients (61.3%), with fibrotic changes in 17 of them (22.7% of the cohort). Organizing pneumonia was observed in 10 patients (13.3%) and NSIP along with organizing pneumonia in 19 patients (25.3%), (Table 3). The radiographic pattern of organizing pneumonia was significantly more common in patients diagnosed with the amyopathic subtype compared to patients that presented with myopathy [24.1% (n = 7/29) vs. 6.5% (n = 3/46), p = 0.03]. Representative images are shown in Figure 1. NSIP was the most common radiographic pattern in patients with anti-Jo-1 [NSIP: 10/20 (50%), NSIP along with organizing pneumonia: 9/20 (45.0%)], anti-PL-7 [NSIP: 8/11 (72.7%), NSIP along with organizing pneumonia: 2/11 (18.2%)], anti-PL-12 [NSIP: 6/11 (54.5%), NSIP along with organizing pneumonia: 3/11 (27.3%)], anti-Ku [NSIP: 6/6 (100%)], and anti-OJ [NSIP: 7/9 (77.8%)] antibodies. Organizing pneumonia was the most common pattern in patients with positive anti-MDA5 [organizing pneumonia: 5/14 (35.7%), organizing pneumonia along with NSIP: 4/14 (28.6%)].

Treatment modalities

Oral corticosteroids were given to all patients (100%, n = 75), with intravenous pulses of methylprednisolone being implemented in 19 patients (25.3%). Other immunosuppressants including rituximab, mycophenolate mofetil, azathioprine, cyclophosphamide, intravenous immune globulin, and methotrexate were applied in 54.7% (n = 41), 34.7% (n = 26), 20.0% (n = 15), 16.0% (n = 12), 14.7% (n = 11), and 12.0% (n = 9) of patients, respectively. Treatment modalities per myositis antibody are summarized in Table 4.

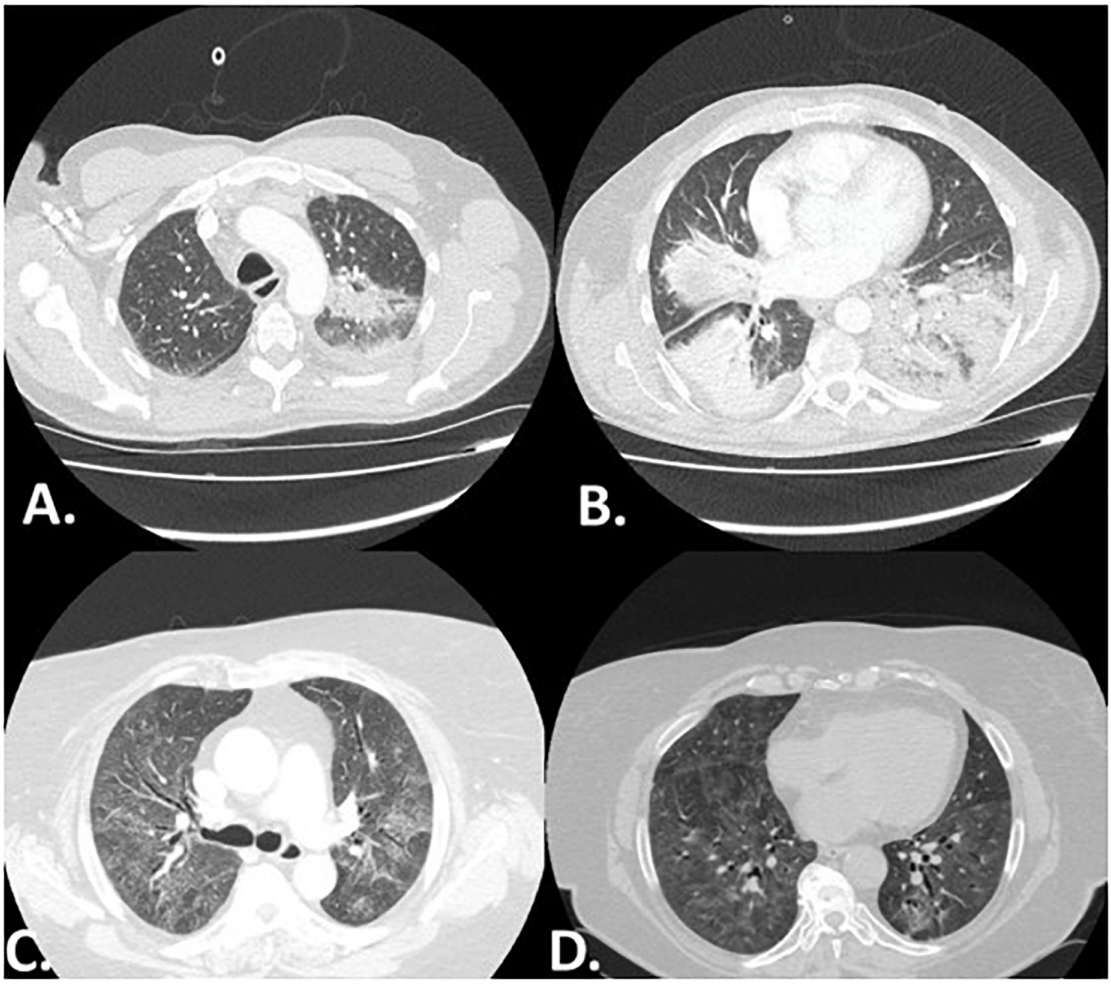

FIGURE 1
Representative high resolution computed tomography (HRCT) images of two patients with myositis associated-ILD. Panel **(A,B)** depict a patient with RP-ILD presenting with the radiographic pattern of organizing pneumonia. Panel **(C,D)** present a case of myositis in which non-specific interstitial pneumonia (NSIP) was observed 10 years following diagnosis.

TABLE 4 Most common treatment regimens per myositis antibody.

Auto-antibodies	Pulses CS	RTX	MMF	Azathioprine	Cyclophosphamide	IVIG	MTX
Anti-Jo-1	6 (30.0%)	12 (60.0%)	5 (25.0%)	4 (20.0%)	2 (10.0%)	3 (15.0%)	4 (20.0%)
Anti-MDA5	7 (50.0%)	4 (28.6%)	5 (35.7%)	2 (14.3%)	6 (42.9%)	5 (35.7%)	1 (7.1%)
Anti-PL-7	4 (36.4%)	4 (36.4%)	4 (36.4%)	1 (9.1%)	1 (9.1%)	2 (18.2%)	0 (0%)
Anti-PL-12	4 (36.4%)	8 (72.3%)	4 (36.4%)	3 (27.3%)	3 (27.3%)	0 (0%)	0 (0%)
Anti-OJ	2 (22.2%)	6 (54.5%)	2 (22.2%)	2 (22.2%)	1 (11.1%)	0 (0%)	0 (0%)

CS, corticosteroids; Jo-1, Histidyl-tRNA synthetase; IVIG, intravenous immune globulin; MDA5, melanoma differentiation-associated gene 5; MMF, mycophenolate mofetil; MTX, methotrexate; OJ, Isoleucyl-tRNA synthetase; PL-7, Threonyl-tRNA synthetase antibodies; PL-12, Alanyl-tRNA synthetase; RTX, rituximab.

Survival

One year survival was 86.7% in the overall population. All-cause 1-year mortality was higher in patients with the amyopathic subtype compared to patients with evidence of myopathy [HR 4.24 (95% CI: 1.16–15.54), $p = 0.03$, Kaplan–Meier analysis], (Figure 2A). Patients diagnosed with RP-ILD and acute respiratory failure experienced increased risk of 1-year all-cause mortality compared to patients diagnosed in outpatient setting [HR 6.70 (95% CI: 1.19–37.81), $p = 0.03$], (Figure 2B).

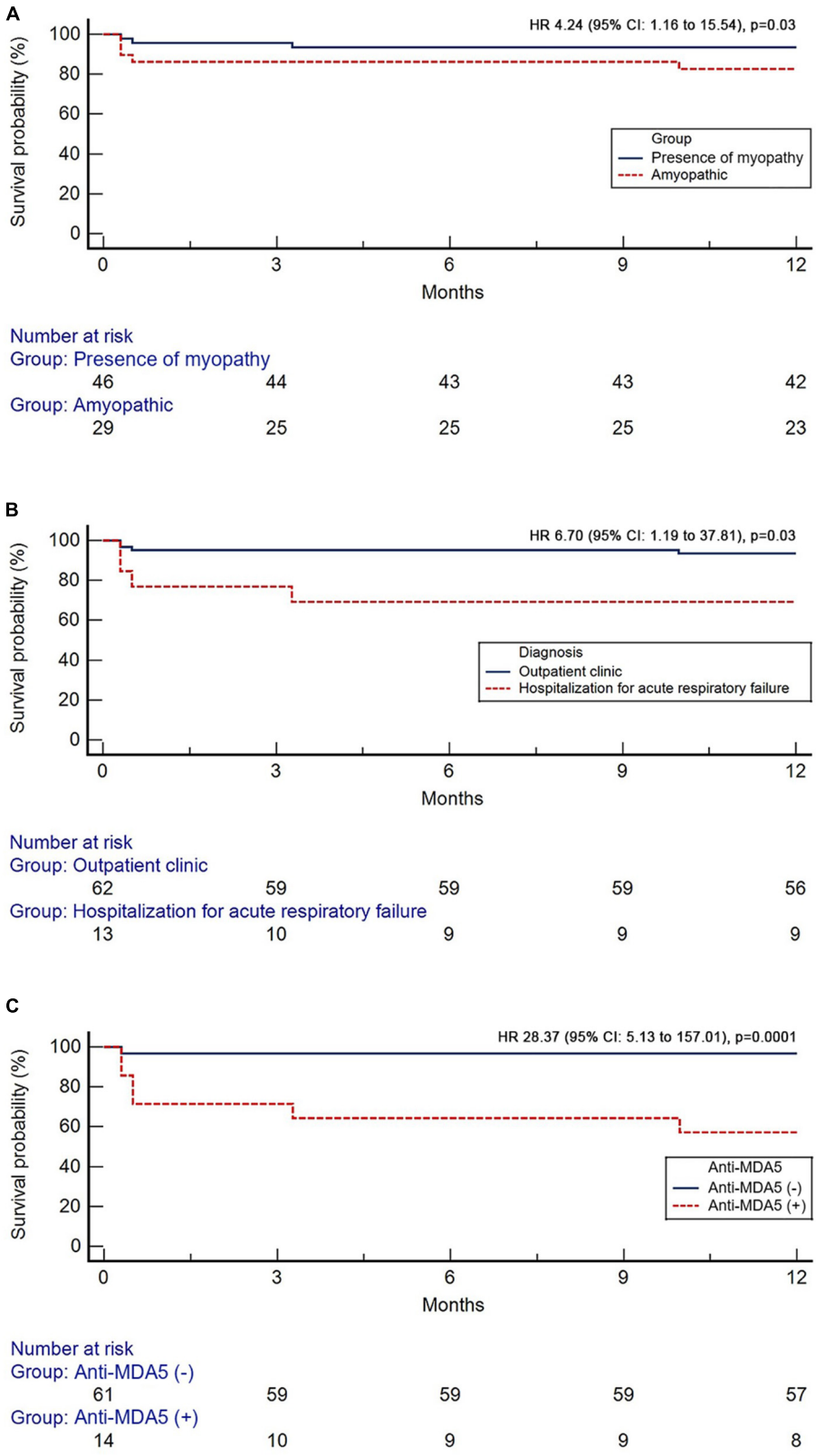

FIGURE 2
Kaplan–Meier curves showing the prognostic impact of the absence of myopathy **(A)**, diagnosis during hospitalization for acute respiratory failure **(B)**, and anti-MDA5 positivity **(C)**.

Finally, patients with positive anti-MDA5 presented with higher 1-year all-cause mortality compared to anti-MDA5 negative patients [HR 28.37 (95% CI: 5.13–157.01), p = 0.0001], (**Figure 2C**).

Discussion

This multicenter study demonstrated that ILD involvement in the setting of amyopathic IIM and diagnosis at the time point of hospitalization due to acute respiratory failure are negative prognostic factors in patients with IIM. This study also validated that presence of anti-MDA5 is associated with increased mortality risk. Specific ILD radiographic patterns such as NSIP and organizing pneumonia may herald underlying inflammatory myopathies and most importantly raise the suspicion of autoimmune-associated ILDs with rapidly progressive clinical course requiring timely intervention with immunosuppressants.

The evidence that patients with the amyopathic subtype have worse prognosis represents an important attribute of that study that should be presented upfront. Indeed, a substantial minority of patients presenting in the clinical setting as antibiotic -refractory organizing pneumonia with rapidly-progressing clinical course may display positive myositis-related autoantibodies without any muscle involvement, as assessed by both clinical signs and laboratory parameters (11, 12, 21, 23). This is an important observation as specific ILD radiological patterns including those of organizing pneumonia/NSIP in patients with rapidly progressing respiratory failure may raise the suspicion of an underlying autoimmune-related pneumonitis that will respond to high-doses of corticosteroids and immunosuppressants while escalation of antibiotics will fail, as it happens in our cohort. Unfortunately, in the majority of these cases the conventional serology profile is negative further hampering the diagnosis. To this end, the myositis panel of autoantibodies may unravel the underlying autoimmunity and guide therapeutic decisions.

We showed that diagnosis at the time point of hospitalization due to acute respiratory failure has a negative prognostic impact. Our report couples with previous evidence showing increased mortality risk for myositis-associated RP-ILD (8, 24–26). Myositis-associated RP-ILD is more common in female and younger patients (27). A high index of suspicion is of paramount importance for these rare but treatable cases. Otherwise, these patients are often admitted to ICU due to Acute Respiratory Distress Syndrome (ARDS). Intriguingly, critically ill patients are frequently not evaluated for myositis-associated pulmonary processes, leading thus to excess mortality due to the absence of appropriate treatment. In the multinational Large Observational Study to Understand the Global Impact of Severe Acute Respiratory Failure -LUNG SAFE- cohort, only 12 out of 234 patients with ARDS of unknown etiology had been screened for autoimmune etiology of RP-ILD (28).

Screening for autoimmune etiology in these patients should not be limited to ANA testing. Antisynthetase antibodies are cytoplasmic and subsequently ANA testing is frequently negative in patients with myositis associated-ILD. Therefore, negative ANA should not preclude further testing with myositis-specific antibodies (9, 29). This has been corroborated by our cohort, in which only 40% of patients were ANA positive. Importantly, classification criteria of the European League Against Rheumatism and American College of Rheumatology had been widely criticized for the exclusion of non-Jo-1 antisynthetase antibodies (9, 30). The aforementioned is further corroborated by our cohort in which anti-Jo-1 were positive only in 26.7% of cases. Patients with myositis presented with substantial variability with regards to myositis-specific positive antibodies.

Myositis-specific positive antibodies might have prognostic significance and unravel specific phenotypes based on this study and previous reports (31). Anti-MDA5 positivity is closely associated with CADM and has a negative prognostic role for patients with myositis (8, 12, 15, 32–36). Features including presence of ILD, mucocutaneous or necrotic ulcerations, Gottron papules, painful, and erythematous papules especially over the palmar surfaces should raise suspicion of anti-MDA5 positivity and fuel meticulous immunologic investigation even in the absence of myopathy (37–39). Early identification of anti-MDA5 can lead to timely therapeutic interventions and may positively impact patients' survival. Several compounds have been used for the management of anti-MDA5 myositis including corticosteroids and steroid sparing agents (40). Most recently, emerging data support the use of tofacitinib in these patients (41, 42). With regards to other autoantibodies, anti-PL7 and anti-PL12 have been characterized as indicators of more severe lung involvement (31). Risk of cancer is elevated in patients with anti- Transcriptional intermediary factor-1γ, Nuclear matrix protein 2 and 3-hydroxy-3-methylglutaryl-CoA reductase antibodies (43). Finally, in our cohort anti-Ku were significantly more common in patients with myopathy.

With regards to radiographic patterns, in consistency with previous studies, most patients presented with NSIP and/or organizing pneumonia (8, 44). Patchy bilateral areas of ground-glass attenuation predominantly localized in the lower lobes along with areas of consolidation, septal thickening and traction bronchiectasis seem to be common radiographic findings in these patients (45). Prominent consolidation refractory to antibiotics is typical of acute onset ILD and responds well to corticosteroids or immunosuppressive compounds if intervention is timely. Presence of ground-glass attenuation combined with reticulation and bronchiectasis is more common in chronic cases potentially underdiagnosed due to lack of extensive immunologic evaluation. Of note, American thoracic society (ATS)/European respiratory society (ERS) statement highlighted that overlap of NSIP and organizing pneumonia or

presence of the fibrosing variant of organizing pneumonia are suspicious for underlying myositis (46).

Our study presents with limitations that should be treated cautiously. First, our sample size is relatively moderate and we could not compare the effect of different treatment regimens in particular subgroups; yet, this sample size seems acceptable for this rare entity. Second, we did not present long term follow-up data; nonetheless, our aim was to increase awareness for timely diagnosis and intervention in this under-recognized entity. Finally, this study has the inherent weakness of a retrospective study.

Collectively, this study showed that ILD is the hallmark of pulmonary involvement in both myopathic and amyopathic forms of inflammatory myopathies and may precede myopathy onset. Respiratory physicians should be alert to organizing pneumonia cases without overt muscular involvement. Hospitalized patients presenting with bilateral organizing pneumonia refractory to antibiotics should be meticulously evaluated for myositis associated-ILD as certain autoimmune profiles such as anti-MDA5 have a negative prognostic role for these patients. The term of amyopathic forms of IIMs in the context of RP-ILD is quite often misjudged and questioned by rheumatologists as autoantibodies presenting in the myositis panel may also independently affect the lung interstitium without muscle involvement. To this end, we believe that the term "autoimmune-induced lung injury or ILD" could be most appropriate in this context. Incorporation of myositis-specific ILD radiological patterns and all myositis-specific antibodies in the diagnostic criteria of inflammatory myopathies may lead to timely and effective therapeutic interventions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Data collection and analysis was approved by the Institutional Review Board and the Local Ethics Committee (protocol number 28746/9-12-2019). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

TdK and AT were involved in study conception, data collection, statistical analysis, and drafting of the initial version of the manuscript. VT and OP were involved in data collection and drafting of the manuscript. SC, EV, P-AJ, SV, EB, AR, PS, MK, VS, PT, EK, ET, GT, IC, EM, EZ, FS, GH, TfK, DD, CK, KD, NT, and RB were involved in data collection and offered important intellectual contribution. RB, PD, KA, BC, and DB supervised the work along with AT. DB and AT supervised the work. All authors offered significant intellectual contribution for the last version of the manuscript and approved the final form.

Conflict of interest

RB has received grants and consulting fees from Sanofi, Roche, Boehringer Ingelheim outside the submitted work. AT has received grants and honoraria from GlaxoSmithKline, AstraZeneca, Chiesi, Roche, and Boehringer Ingelheim outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Lilleker J, Vencovsky J, Wang G, Wedderburn L, Diederichsen L, Schmidt J, et al. The EuroMyositis registry: an international collaborative tool to facilitate myositis research. *Ann Rheum Dis.* (2018) 77:30–9. doi: 10.1136/annrheumdis-2017-211868

2. Lundberg I, Tjärnlund A, Bottai M, Werth V, Pilkington C, Visser M, et al. 2017 European League Against Rheumatism/American College of Rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Ann Rheum Dis.* (2017) 76:1955–64. doi: 10.1136/annrheumdis-2017-211468

3. Lundberg I, Fujimoto M, Vencovsky J, Aggarwal R, Holmqvist M, Christopher-Stine L, et al. Idiopathic inflammatory myopathies. *Nat Rev Dis Primers.* (2021) 7:86. doi: 10.1038/s41572-021-00321-x

4. Dalakas M. Inflammatory muscle diseases. *New Engl J Med.* (2015) 372:1734–47. doi: 10.1056/NEJMra1402225

5. Rider L, Miller F. Deciphering the clinical presentations, pathogenesis, and treatment of the idiopathic inflammatory myopathies. *JAMA*. (2011) 305:183–90. doi: 10.1001/jama.2010.1977

6. Cottin V, Thivolet-Béjui F, Reynaud-Gaubert M, Cadranel J, Delaval P, Ternamian PJ, et al. Interstitial lung disease in amyopathic dermatomyositis, dermatomyositis and polymyositis. *Eur Respir J.* (2003) 22:245–50. doi: 10.1183/09031936.03.00026703

7. Kiely P, Chua F. Interstitial lung disease in inflammatory myopathies: clinical phenotypes and prognosis. *Curr Rheumatol Rep*. (2013) 15:359. doi: 10.1007/s11926-013-0359-6

8. Li Y, Gao X, Li Y, Jia X, Zhang X, Xu Y, et al. Predictors and mortality of rapidly progressive interstitial lung disease in patients with idiopathic inflammatory myopathy: A series of 474 patients. *Front Med (Lausanne).* (2020) 7:363. doi: 10.3389/fmed.2020.00363

9. Jablonski R, Bhorade S, Strek M, Dematte J. Recognition and management of myositis-associated rapidly progressive interstitial lung disease. *Chest.* (2020) 158:252–63. doi: 10.1016/j.chest.2020.01.033

10. Tzilas V, Sfikakis P, Bouros D. Antisynthetase syndrome masquerading as hypersensitivity pneumonitis. *Respiration.* (2021) 100:1105–13. doi: 10.1159/000516508

11. Trallero-Araguás E, Grau-Junyent J, Labirua-Iturburu A, García-Hernández F, Monteagudo-Jiménez M, Fraile-Rodriguez G, et al. Clinical manifestations and long-term outcome of anti-Jo1 antisynthetase patients in a large cohort of Spanish patients from the GEAS-IIM group. *Semin Arthritis Rheum.* (2016) 46:225–31. doi: 10.1016/j.semarthrit.2016.03.011

12. Vuillard C, Pineton de Chambrun M, de Prost N, Guérin C, Schmidt M, Dargent A, et al. Clinical features and outcome of patients with acute respiratory failure revealing anti-synthetase or anti-MDA-5 dermato-pulmonary syndrome: a French multicenter retrospective study. *Ann Intensive Care.* (2018) 8:87. doi: 10.1186/s13613-018-0433-3

13. Morisset J, Johnson C, Rich E, Collard H, Lee J. Management of myositis-related interstitial lung disease. *Chest.* (2016) 150:1118–28. doi: 10.1016/j.chest.2016.04.007

14. Hervier B, Uzunhan Y. Inflammatory myopathy-related interstitial lung disease: From pathophysiology to treatment. *Front Med.* (2019) 6:326. doi: 10.3389/fmed.2019.00326

15. Moghadam-Kia S, Oddis C, Sato S, Kuwana M, Aggarwal R. Anti-Melanoma differentiation-associated gene 5 Is associated with rapidly progressive lung disease and poor survival in US patients with amyopathic and myopathic dermatomyositis. *Arthritis Care Res.* (2016) 68:689–94. doi: 10.1002/acr.22728

16. Sato S, Masui K, Nishina N, Kawaguchi Y, Kawakami A, Tamura M, et al. Initial predictors of poor survival in myositis-associated interstitial lung disease: a multicentre cohort of 497 patients. *Rheumatology (Oxford, England).* (2018) 57:1212–21. doi: 10.1093/rheumatology/key060

17. Fujiki Y, Kotani T, Isoda K, Ishida T, Shoda T, Yoshida S, et al. Evaluation of clinical prognostic factors for interstitial pneumonia in anti-MDA5 antibody-positive dermatomyositis patients. *Mod Rheumatol.* (2018) 28:133–40. doi: 10.1080/14397595.2017.1318468

18. Chen F, Wang J, Zhang P, Zuo Y, Ye L, Wang G, et al. Interstitial lung disease in dermatomyositis without myositis-specific and myositis-associated autoantibodies: Study of a series of 72 patients from a single cohort. *Front Immunol.* (2022) 13:879266. doi: 10.3389/fimmu.2022.879266

19. González-Pérez M, Mejía-Hurtado J, Pérez-Román D, Buendía-Roldán I, Mejía M, Falfán-Valencia R, et al. Evolution of pulmonary function in a cohort of patients with interstitial lung disease and positive for antisynthetase antibodies. *J Rheumatol.* (2020) 47:415–23. doi: 10.3899/jrheum.181141

20. Maloir Q, Laurence S, Christian V, Fanny G, Renaud L, Julien G. Clinical experience in anti-synthetase syndrome: a monocentric retrospective analytical study. *Acta Clin Belg.* (2022) 77:624–30. doi: 10.1080/17843286.2021.1925818

21. Wilfong E, Young-Glazer J, Sohn B, Schroeder G, Annapureddy N, Gillaspie E, et al. Anti-tRNA synthetase syndrome interstitial lung disease: A single center experience. *Respir Med.* (2022) 191:106432. doi: 10.1016/j.rmed.2021.106432

22. Feldon M, Farhadi P, Brunner H, Itert L, Goldberg B, Faiq A, et al. Predictors of reduced health-related quality of life in adult patients with idiopathic inflammatory myopathies. *Arthritis Care Res.* (2017) 69:1743–50. doi: 10.1002/acr.23198

23. Suda T, Fujisawa T, Enomoto N, Nakamura Y, Inui N, Naito T, et al. Interstitial lung diseases associated with amyopathic dermatomyositis. *Eur Respir J.* (2006) 28:1005–12. doi: 10.1183/09031936.06.00038806

24. Won Huh J, Soon Kim D, Keun Lee C, Yoo B, Bum Seo J, Kitaichi M, et al. Two distinct clinical types of interstitial lung disease associated with polymyositis-dermatomyositis. *Respir Med.* (2007) 101:1761–9. doi: 10.1016/j.rmed.2007.02.017

25. Fujisawa T, Hozumi H, Kono M, Enomoto N, Hashimoto D, Nakamura Y, et al. Prognostic factors for myositis-associated interstitial lung disease. *PLoS One.* (2014) 9:e98824. doi: 10.1371/journal.pone.0098824

26. Tillie-Leblond I, Wislez M, Valeyre D, Crestani B, Rabbat A, Israel-Biet D, et al. Interstitial lung disease and anti-Jo-1 antibodies: difference between acute and gradual onset. *Thorax.* (2008) 63:53–9. doi: 10.1136/thx.2006.069237

27. Shi J, Li S, Yang H, Zhang Y, Peng Q, Lu X, et al. Clinical profiles and prognosis of patients with distinct antisynthetase autoantibodies. *J Rheumatol.* (2017) 44:1051–7. doi: 10.3899/jrheum.161480

28. de Prost N, Pham T, Carteaux G, Mekontso Dessap A, Brun-Buisson C, Fan E, et al. Etiologies, diagnostic work-up and outcomes of acute respiratory distress syndrome with no common risk factor: a prospective multicenter study. *Ann Intensive Care.* (2017) 7:69. doi: 10.1186/s13613-017-0281-6

29. Ashton C, Paramalingam S, Stevenson B, Brusch A, Needham M. Idiopathic inflammatory myopathies: a review. *Internal Med J.* (2021) 51:845–52. doi: 10.1111/imj.15358

30. Lundberg I, Tjärnlund A, Bottai M, Werth V, Pilkington C, Visser M, et al. 2017 european league against rheumatism/american college of rheumatology classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups. *Arthritis Rheumatol.* (2017) 69:2271–82. doi: 10.1002/art.40320

31. Pinal-Fernandez I, Casal-Dominguez M, Huapaya J, Albayda J, Paik J, Johnson C, et al. A longitudinal cohort study of the anti-synthetase syndrome: increased severity of interstitial lung disease in black patients and patients with anti-PL7 and anti-PL12 autoantibodies. *Rheumatology (Oxford).* (2017) 56:999–1007. doi: 10.1093/rheumatology/kex021

32. Hozumi H, Enomoto N, Kono M, Fujisawa T, Inui N, Nakamura Y, et al. Prognostic significance of anti-aminoacyl-tRNA synthetase antibodies in polymyositis/dermatomyositis-associated interstitial lung disease: a retrospective case control study. *PLoS One.* (2015) 10:e0120313. doi: 10.1371/journal.pone.0120313

33. Kishaba T, McGill R, Nei Y, Ibuki S, Momose M, Nishiyama K, et al. Clinical characteristics of dermatomyosits/polymyositis associated interstitial lung disease according to the autoantibody. *J Med Invest.* (2018) 65:251–7. doi: 10.2152/jmi.65.251

34. Yoshida N, Okamoto M, Kaieda S, Fujimoto K, Ebata T, Tajiri M, et al. Association of anti-aminoacyl-transfer RNA synthetase antibody and anti-melanoma differentiation-associated gene 5 antibody with the therapeutic response of polymyositis/dermatomyositis-associated interstitial lung disease. *Respir Invest.* (2017) 55:24–32. doi: 10.1016/j.resinv.2016.08.007

35. Tanizawa K, Handa T, Nakashima R, Kubo T, Hosono Y, Aihara K, et al. The prognostic value of HRCT in myositis-associated interstitial lung disease. *Respir Med.* (2013) 107:745–52. doi: 10.1016/j.rmed.2013.01.014

36. Allenbach Y, Uzunhan Y, Toquet S, Leroux G, Gallay L, Marquet A, et al. Different phenotypes in dermatomyositis associated with anti-MDA5 antibody: Study of 121 cases. *Neurology.* (2020) 95:e70–8. doi: 10.1212/wnl.0000000000009727

37. Charrow A, Vleugels R. Cutaneous Ulcerations in Anti-MDA5 Dermatomyositis. *New Engl J Med.* (2019) 381:465. doi: 10.1056/NEJMicm1816147

38. Fiorentino D, Chung L, Zwerner J, Rosen A, Casciola-Rosen L. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. *J Am Acad Dermatol.* (2011) 65:25–34. doi: 10.1016/j.jaad.2010.09.016

39. Tzouvelekis A, Karampitsakos T, Bouros E, Tzilas V, Liossis S, Bouros D. Autoimmune biomarkers, antibodies, and immunologic evaluation of the patient with fibrotic lung disease. *Clin Chest Med.* (2019) 40:679–91. doi: 10.1016/j.ccm.2019.06.002

40. Karampitsakos T, Vraka A, Bouros D, Liossis S, Tzouvelekis A. Biologic treatments in interstitial lung diseases. *Front Med.* (2019) 6:41. doi: 10.3389/fmed.2019.00041

41. Chen Z, Wang X, Ye S. Tofacitinib in amyopathic dermatomyositis-associated interstitial lung disease. *New Engl J Med.* (2019) 381:291–3. doi: 10.1056/NEJMc1900045

42. Kurasawa K, Arai S, Namiki Y, Tanaka A, Takamura Y, Owada T, et al. Tofacitinib for refractory interstitial lung diseases in anti-melanoma differentiation-associated 5 gene antibody-positive dermatomyositis. *Rheumatology (Oxford, England).* (2018) 57:2114–9. doi: 10.1093/rheumatology/key188

43. Benveniste O, Stenzel W, Allenbach Y. Advances in serological diagnostics of inflammatory myopathies. *Curr Opin Neurol.* (2016) 29:662–73. doi: 10.1097/wco.0000000000000376

44. Yura H, Sakamoto N, Satoh M, Ishimoto H, Hanaka T, Ito C, et al. Clinical characteristics of patients with anti-aminoacyl-tRNA synthetase antibody positive idiopathic interstitial pneumonia. *Respir Med.* (2017) 132:189–94. doi: 10.1016/j.rmed.2017.10.020

45. Lega J, Reynaud Q, Belot A, Fabien N, Durieu I, Cottin V. Idiopathic inflammatory myopathies and the lung. *Eur Respir Rev.* (2015) 24:216–38. doi: 10.1183/16000617.00002015

46. Travis W, Costabel U, Hansell D, King T Jr., Lynch D, Nicholson A, et al. An official american thoracic society/european respiratory society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med.* (2013) 188:733–48. doi: 10.1164/rccm.201308-1483ST

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 13 January 2023
DOI 10.3389/fmed.2022.1075465

OPEN ACCESS

EDITED BY
Argyrios Tzouvelekis,
Alexander Fleming Biomedical Sciences Research Center, Greece

REVIEWED BY
Zhengyu He,
Shanghai Jiao Tong University, China
Juan Huang,
Zunyi Medical University, China

*CORRESPONDENCE
Peiqing Liu
✉ liupq@mail.sysu.edu.cn
Jianwen Chen
✉ chenjwen@mail.sysu.edu.cn
Jing Lu
✉ lujing0504@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 20 October 2022
ACCEPTED 29 December 2022
PUBLISHED 13 January 2023

CITATION
Ye Z, Wang P, Feng G, Wang Q, Liu C, Lu J, Chen J and Liu P (2023) Cryptotanshinone attenuates LPS-induced acute lung injury by regulating metabolic reprogramming of macrophage.
Front. Med. 9:1075465.
doi: 10.3389/fmed.2022.1075465

Cryptotanshinone attenuates LPS-induced acute lung injury by regulating metabolic reprogramming of macrophage

Zesen Ye[1†], Panxia Wang[2†], Guodong Feng[1†], Quan Wang[1], Cui Liu[1], Jing Lu[1*], Jianwen Chen[1*] and Peiqing Liu[1*]

[1]Laboratory of Pharmacology and Toxicology, National-Local Joint Engineering Laboratory of Druggability and New Drugs Evaluation, Guangdong Province Engineering Laboratory for Druggability and New Drug Evaluation, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China, [2]School of Pharmaceutical Science, Guangzhou Medical University, Guangzhou, China

Background: Acute lung injury (ALI) is a life-threatening inflammatory disease without effective therapeutic regimen. Macrophage polarization plays a key role in the initiation and resolution of pulmonary inflammation. Therefore, modulating macrophage phenotype is a potentially effective way for acute lung injury. Cryptotanshinone (CTS) is a lipophilic bioactive compound extracted from the root of *Salvia miltiorrhiza* with a variety of pharmacological effects, especially the anti-inflammatory role. In this study, we investigated the therapeutic and immunomodulatory effects of CTS on ALI.

Materials and methods: The rat model of ALI was established by intratracheal instillation of LPS (5 mg/kg) to evaluate the lung protective effect of CTS *in vivo* and to explore the regulation of CTS on the phenotype of lung macrophage polarization. LPS (1 μg/mL) was used to stimulate RAW264.7 macrophages *in vitro* to further explore the effect of CTS on the polarization and metabolic reprogramming of RAW264.7 macrophages and to clarify the potential mechanism of CTS anti-ALI.

Results: CTS significantly improved lung function, reduced pulmonary edema, effectively inhibited pulmonary inflammatory infiltration, and alleviated ALI. Both *in vivo* and *in vitro* results revealed that CTS inhibited the differentiation of macrophage into the M1 phenotype and promoted polarization into M2 phenotype during ALI. Further *in vitro* studies indicated that CTS significantly suppressed LPS-induced metabolic transition from aerobic oxidation to glycolysis in macrophages. Mechanistically, CTS blocked LPS-induced metabolic transformation of macrophages by activating AMPK.

Conclusion: These findings demonstrated that CTS regulates macrophage metabolism by activating AMPK, and then induced M1-type macrophages to transform into M2-type macrophages, thereby alleviating the inflammatory response of ALI, suggesting that CTS might be a potential anti-ALI agent.

KEYWORDS

acute lung injury, Cryptotanshinone, macrophage polarization, metabolic reprogramming, AMPK

1. Introduction

Acute lung injury (ALI) is a life-threatening respiratory disease which can lead to respiratory failure and higher mortality (1). The main pathogenesis of ALI are sharp increase in pulmonary inflammatory responses, diffused alveolar injury and pulmonary edema, which might ultimately lead to acute hypoxemia (2). Currently, the clinical treatment for ALI is limited and specific drugs for ALI are also still lacking (3). Even though mechanical ventilation could partially relieve the pathology of ALI, long-term mechanical ventilation always increases ventilator-related lung injury, higher mortality and heavy financial burden (4, 5). Therefore, it is urgent to explore new strategies to improve ALI.

As the core participants of innate immune response, alveolar macrophages play a pivotal role in the initiation, development and resolution of lung inflammation during acute lung injury (6). In the early exudative stage of ALI, stimulated by Th1-type cytokines such as TNF-α or interferon, macrophages could differentiated into M1 phenotype or proinflammatory macrophages to mediate inflammatory responses via releasing proinflammatory cytokines and chemokines (7). During the repair phase of ALI, activated by Th2-type cytokines, such as IL-4, IL-13 and immune complex, macrophages prefer to polarize into M2 phenotype or anti-inflammatory macrophages (6). Macrophages are key orchestrators in the progress of ALI and modulating the phenotype of macrophage might improve the prognosis of ALI.

Furthermore, accumulating evidence suggests that metabolic reprogramming plays a crucial role in the differentiation of macrophages (8). As indicated by the increasing glucose uptake and lactic acid production, activated M1 phenotypes are highly dependent on aerobic glycolysis to meet energy requirements for rapid proliferation and cytokine production (9). Conversely, M2 phenotypes are mainly dependent on mitochondrial oxidative phosphorylation and fatty acid oxidation for energy supplement (10). The metabolic reprogramming is not only to meet the energy requirements of macrophages in response to vary stimulus, but also a necessary step to drive macrophage polarization (8). Previous studies have shown that overexpression of glucose transporter 1 (GLUT1), a key gene involved in glycolysis, could drive macrophages differentiated into M1 phenotype by promoting glycolysis (11). However, 2-DG (2-deoxy-D-glucose), a well-established inhibitor of glycolysis, inhibits the proinflammatory phenotype of M1 macrophages by blocking glycolysis (12). Additionally, knockout of genes related to fatty acid metabolism or mitochondrial oxidative phosphorylation blocked the activation of M2 phenotype (13, 14). Therefore, intervention of the metabolic pattern of macrophages will control the phenotype of macrophages and might play a pivotal role in ALI.

Cryptotanshinone (CTS) is extracted from *Salvia miltiorrhiza* and belongs to diterpenoid quinones with a variety of pharmacological activities such as anti-inflammatory, anti-cancer, anti-oxidant and anti-fibrosis (15). Previous studies from other's and our laboratory have systematically studied the effects of CTS on arthritis (16), atherosclerosis (17), and Alzheimer's disease (18), all of which indicated the excellent therapeutic effects of CTS. Previously, we have reported that CTS effectively protected lung from pulmonary fibrosis by inhibiting Smad and STAT3 signaling pathways (19). CTS inhibited the occurrence and development of acute colitis and cerebral ischemic stroke by promoting the trans-differentiation of M1 phenotype into the M2 phenotype (20, 21). In addition, CTS has been shown to exert anticancer effects by blocking glycolysis to inhibit tumor cell proliferation and migration (22, 23). Even though CTS could inhibited the progression of protect ALI by inhibiting NF-κB signal pathway (24), it was still unknown whether CTS could alleviate the inflammatory response ALI by altering the metabolic pattern of macrophages.

In this study, we found that CTS effectively improved pulmonary function and relieved LPS-induced pulmonary inflammation in rats with ALI. This study further revealed that CTS inhibited the accumulation of the M1 phenotype (pro-inflammatory) macrophage and increased the M2 phenotype (anti-inflammatory) macrophage in the lung tissue. Additionally, both the *in vivo* and *in vitro* results showed that CTS could regulate metabolic reprogramming of macrophage by activating AMPK.

2. Materials and methods

2.1. Reagents

CTS (purity ≥ 98%) were obtained from the Laboratory of Pharmacology and Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University (Guangzhou, China). LPS was purchased from Sigma-Aldrich (St. Louis, USA). Compound C were purchased from Selleck (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco (NY, USA). Fetal bovine serum (FBS) was obtained from HyClone (Logan, USA). Myeloperoxidase (MPO) and lactic acid assay kits were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The antibodies against CD86 (A1199) and Arg-1 (A4923) were obtained from ABclonal (Wuhan, China). The antibodies against CD206 (ab64693) were purchased from Abcam (Cambridge, MA). The antibody against iNOS (AF0199) was from Affinity Biosciences (OH, United States). The antibodies against β-actin (6600-1-Ig), GLUT1 66290-1-Ig) and PFKFB3 (3763-1-AP) were purchased from Proteintech (Chicago, USA). The antibodies against PKM2 (D78A4), HIF-1α (D1S7W), p-AMPK (40H9) and AMPK (2532) were obtained from Cell Signaling Technology (Danvers, USA). The antibodies for flow cytometry, including phycoerythrin (PE) anti- CD86 (105007) and allophycocyanin (APC) anti-CD206 (141708) were obtained from BioLegend (San Diego, USA).

Abbreviations: ALI, acute lung injury; AMPK, amp-activated protein kinase; BALF, bronchoalveolar lavage fluid; CD68, cluster of differentiation 68; CD86, cluster of differentiation 86; CD206, cluster of differentiation 206; CPT1A, carnitine palmitoyltransferase 1a; CPT2, carnitine palmitoyltransferase 2; CC, compound c; 2-DG, 2-deoxy-D-glucose; ECAR, extracellular acidification rate; EIP, end inspiratory pause; EEP, end expiratory pause; FAO, fatty acid oxidation; GLUT1, glucose transporter 1; LPS, lipopolysaccharide; LKB1, liver kinase b1; MV, minute ventilation volume; MPO, myeloperoxidase; MCAD, medium-chain acyl-coA dehydrogenase; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; Penh, enhanced pause; RT-PCR, real-time polymerase chain reaction; PKM2, pyruvate kinase m2; PFKFB3, 6-phosphofructo-2kinase/fructose-2,6-biphosphatase; RT, relaxation time.

2.2. LPS - Induced acute lung injury rat model

The animal procedures were approved by the Research Ethics Committee of Sun Yat-sen University and conducted following the Guide for the Care and Use of Laboratory (NIH Publication No. 85-23, revised 1996). Sprague-Dawley rats (SD rats, male, SPF grade, 6-8 weeks, weighing 200-230 g) were supplied by the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China) and the certification No. 44008500024762. LPS (5 mg/kg) was dissolved in normal saline (NS) and administrated by intratracheal instillation to SD and the acute lung injury model was established by LPS administration for 24 h. SD rats were randomly divided into several groups (10 rats in each group): the control group, the LPS-induced acute lung injury model group and the CTS treatment groups at three different concentrations. CTS was dissolved in sodium carboxymethylcellulose (CMC-Na, 5%, W/V) at different concentrations (15, 30 and 60 mg/kg/day). Before LPS treatment, the CTS treatment group was pre-administered intragastrically for 5 days, while the control and model groups were given the same volume of solvent solution. The rats in each drug administration group were given drug intervention once at 6 hours, 12 hours and 18 hours after modeling.

2.3. Pulmonary function assessment

Pulmonary function was measured by using a whole-body plethysmograph (Emka Technologies, Paris, France) for rats. The parameters of pulmonary function included enhanced pause (Penh), relaxation time (RT), end inspiratory pause (EIP), end expiratory pause (EEP) and minute ventilation volume (MV). Rats were placed in a plethysmograph chamber and 10 min was used for acclimation before 5 min of assessing respiratory parameters.

2.4. Histopathological assessment and the measurement of lung wet/dry (W/D) weight ratio

At the end of the *in vivo* experiment, all rats were anesthetized and sacrificed. The whole lung of the rat was quickly removed and weighed. The lung weight to body weight ratios were calculated according to the following formula: Lung to Body weight ratio = (Lung weight (g)) / (Body weight (g)) × 100%. Subsequently, left lung tissue was fixed in 4% paraformaldehyde, embedded in paraffin, sectionalized, and stained with hematoxylin and eosin (HE). HE scores were calculated by light microscopic analysis of four parameters including alveolar septal thickness, interstitial edema, infiltration of inflammatory cells, and alveolar congestion/collapse. Each parameter was categorized into four grades: 0 = normal; 1 $\leq$ 25%; 2 = 25–50%; 3 = 50–75%; and 4 $\geq$ 75%, and the mean score of the four parameters was used to represent the overall lung injury (25). Histopathological images were captured and analysis by using light microscope at 400x magnification (EVOS FL Auto Cell Imaging System, USA). The right lung was excised and weighed to assay wet weight, followed by drying at 80 ? for 48 h to obtain dry weight. The lung wet/dry (W/D) weight ratio W/D weight ratio was calculated to indicate pulmonary edema formation.

2.5. Measurement of myeloperoxidase (MPO) activity

MPO activity of lung tissue was measured by a commercial kit according to the manufacturer's instructions (A044, Nanjing Jiancheng Bioengineering Institute, China).

2.6. Bronchoalveolar lavage fluid (BALF) collection

By intratracheal injection of 5 mL sterile saline and then slowly withdrawn, repeated irrigation three times. The collected bronchoalveolar lavage fluid was centrifuged at 300 g for 10 min at 4°C, and the supernatant was extracted for subsequent cytokine and total protein inspection. Cytokine levels in the supernatants of BALF were determined using commercially available TNF-α, IL-1β, IL-6, IL-10 ELISA kits (Wuhan Huamei Biotech Co., Ltd., Wuhan, China) according to the manufacturer's instructions. Total protein concentration in the supernatant BALF was determined using the BCA protein quantification kit (Thermo Fisher Scientific, Waltham, USA).

2.7. Cell culture

RAW264.7 cell line was obtained from ProCell (Wuhan, China) and maintained in 37°C incubators with 5% CO_2. The cultured media was DMEM media with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin. RAW264.7 were pretreated with the indicated concentrations of CTS (2.5, 5, 10 μM) for 2 h before being stimulated with LPS (1 μg/mL) for another 24 h.

2.8. Measurement of glucose uptake and lactic acid in RAW264.7

Glucose uptake of RAW264.7 cells were assayed by using the Glucose Uptake-Glo Assay kit from Promega (Wisconsin, USA). The level of lacticte acid was detected by using a commercial kit (Nanjing Jiancheng Bioengineering Institute, China).

2.9. Immunofluorescence staining

Frozen lung tissue sections were fixed in acetone for 20 min, then permeabilized by 0.3% Triton X-100 (Sigma, St. Louis, USA) for 15 min and blocked by 10% goat serum for 30 min. Subsequently, sections were incubated with anti-CD68 antibody (BIO-RAD, MCA341GA 1:100 dilution) overnight at 4°C, anti-CD86 antibody (ABclonal, A11991, 100 dilution), and anti-CD206 antibody respectively (Abcam, ab64693, 1:100 dilution). Sections were washed with PBS followed by incubation with fluorescent secondary antibodies (Abcam, ab150116, ab150077) in dark for 1 h at room temperature. Finally, the sections were re-stained with 4',6-diamidino-2-phenylindole (DAPI) for 10 min at room temperature. Fluorescent images were captured under a fluorescent microscope (EVOS FL Auto Cell Imaging System, USA).

2.10. Immunohistochemical staining

For immunohistochemistry (IHC) analysis, paraffin-embedded lung tissues were deparaffinized, rehydrated through an alcohol series followed by antigen retrieval with sodium citrate buffer. Tumor sections were blocked with 5% normal goat serum with 0.3% Triton X-100 and 3% H_2O_2 in PBS for 60 min at room temperature and then incubated with anti-iNOS antibody (Affinity Biosciences, AF0199, 1:100 dilution) or anti-Arg-1 antibody (ABclonal, A4923, 1:100 dilution) at 4°C overnight. Then, HRP-conjugated goat anti-rabbit IgG polyclonal antibody (Abcam, ab6721, 1:1000) were used. Alternatively, sections were stained with DAB and restained with hematoxylin, and then photographed using a microscope (EVOS FL Auto Cell Imaging System, USA). The area of the positive area was calculated using Image-Pro Plus 6.0 software (Media Cyber??netics, Silver Spring, USA).

2.11. CCK-8 assay

RAW264.7 cells were grown in 96-well plates at a density of 10000 cells per well and cultured overnight in the incubator. Different concentrations of CTS (2.5, 5, 10 μM) were administrated to cells for 24 h with or without LPS (1 μg/mL) stimulation. Subsequently, 10 μL of CCK-8 solution was added into each well and incubated for another 4 h. The absorbance values of each well were measured at 450 nm using a microplate reader (Bio-Tek, Winooski, USA).

2.12. Flow cytometry analysis

RAW264.7 cell suspension was collected and incubated with anti-CD16/32 (BioLegend, San Diego, USA) at 4 ? for 20 min to block Fc receptor. And then, the cells were washed twice in staining buffer (BioLegend, San Diego, USA) and stained with anti-CD86-PE antibody (BioLegend, San Diego, USA)) or anti-CD206-APC antibody (BioLegend, San Diego, USA) for 30 min. Followed by washing twice with staining buffer (BioLegend, San Diego, USA), resuspended the RAW264.7 cells in 300 μL staining buffer. Flow cytometry data were obtained using a CytoFLEX S flow cytometer (Beckman Coutler, Brea, USA) and analyzed using FlowJo software (Ashland, USA).

2.13. Measurement of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) of RAW264.7 cells

OCR and ECAR were measured by using XF-96 Extracellular flux analyzer (Seahorse Bioscience, North Billerica, MA, USA) to assess mitochondrial oxidative phosphorylation and glycolysis capacity respectively. CTS at different concentrations (2.5, 5, 10 μM) were pre-incubated with RAW264.7 cells for 2 h with co-stimulation of LPS (1 μg/mL) for 24 h. For the ECAR assay, the medium was replaced with XF solution containing 2 mmol/L L-glutamine prior to analysis. Glucose (10 mM), oligomycin (1 μM) and 2-DG (50 mM) were used to determine the glycolysis rate, glycolysis capacity and glycolysis reserve capacity of cells. For the OCR assay, the medium was replaced with XF solution containing glucose (2.5 M), pyruvate (1 mM) and glutamine (1 mM) prior to analysis. Oligomycin (1 μM), FCCP (0.75 μM), rotenone (0.5 μM) and antimycin A (0.5 μM) were used to determine basal respiration, mitochondrial ATP production and maximum respiration.

2.14. Protein extraction and western blot

Total protein was extracted from lung tissues or RAW264.7 cells using RIPA lysis buffers containing protease inhibitors and phosphatase inhibitors. The concentrations of proteins were measured using the BCA protein quantification kit (Thermo Fisher Scientific, Waltham, USA). Equal amount of protein samples was boiled and loaded in 10% SDS-PAGE for separation and transferred to PVDF membrane (Meck Millipore, Burlington, USA). The PVDF membranes were blocked with 5% skim milk at room temperature for 1 hour and then incubated with different primary antibodies at 4°C overnight. The membranes were incubated with the corresponding secondary antibodies for 1 h at room temperature. The proteins were visualized by chemiluminescence using an ECL system (GE Healthcare, Pittsburgh, USA) and the images were captured using an imaging system (Tanon, Shanghai, China).

2.15. Real-time polymerase chain reaction (RT-PCR)

Total RNA was extracted from RAW264.7 cells using Trizol reagent (Invitrogen, Carlsbad, USA) and cDNA was synthesized using the QuantiTect reverse transcription kit (QIAGEN, Valencia, USA) according to the manufacturer's protocol. The relative mRNA expression level was determined using the 2-delta delta Ct analysis method, where GAPDH was used as a home keeper gene. The primer sequences used in this experiment were listed in Table 1.

2.16. Statistical analysis

The results were expressed as mean ± standard error of mean (SEM) from at least three independent experiments and analyzed

TABLE 1 RT-qPCR Primers used in this study.

Primer		Sequence (5′–3′)
GLUT1	Forward Reverse	ACGATCTGAGCTACGGGGT GCCCGTCACCTTCTTGCTG
PFKFB3	Forward Reverse	CAACTCCCCAACCGTGATTGT TGAGGTAGCGAGTCAGCTTCT
PKM2	Forward Reverse	ATTACCAGCGACCCCACAGAA ACGGCATCCTTACACAGCACA
CPT1A	Forward Reverse	TATGGTCAAGGTCTTCTCGGGTCG AGTGCTGTCATGCGTTGGAAGTCTC
CPT2	Forward Reverse	TCGGCCCTTAAGTGCTGTCT TTTAGGGATAGGCAGCCTGGG
MCAD	Forward Reverse	TGACAAAAGCGGGGAGTACC GCACCCCTGTACACCCATAC
GAPDH	Forward Reverse	ACCCTTAAGAGGGATGCTGC CCCAATACGGCCAAATCCGT

by using GraphPad Prism 8.0 software (San Diego, CA, USA). Student's t-test was used to compare differences between two groups. Differences between groups were compared using one-way analysis of variance (ANOVA) followed by *Post hoc* Bonferroni's test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. CTS ameliorated LPS-induced acute lung injury in rats

To determine the effects of CTS on pulmonary function of rats, non-invasive lung function tests were performed in rats. Penh is an indicator of airway resistance to positively reflect the constriction degree of internal bronchi. Compared with control group, a single dose of LPS (5 mg/kg) administration through intratracheal instillation significantly increased the value of enhanced pause (Penh) (**Figure 1A**), shortened the time period of end-inspiratory pause time (EIP) and relaxation time (RT) (**Figures 1B, C**), prolonged end-expiratory pause time (EEP) (**Figure 1D**), and finally decreased minute ventilation volume (MV) (**Figure 1E**). These results indicated that LPS administration successfully induced acute lung injury of rats. In contrast, CTS treatment at 15, 30 and 60 mg/kg effectively improved pulmonary function of rat in a dose-dependent manner (**Figures 1A–E**).

Additionally, pathological changes of lung tissue were analyzed by HE staining. As **Figures 1F, G** shown, the pulmonary structure was destroyed following LPS-stimulation as evidenced by disorganized pulmonary alveoli structure, obvious perivascular edema, widened space and significantly thickened alveolar walls accompanied by a large number of inflammatory cell infiltration, all of which were effectively relieved by CTS. Furthermore, LPS-induced increase in lung coefficient and right lung wet to dry weight ratio (Lung W/D ratio) were also relieved by CTS treatment in a dose dependent manner (**Figures 1H, I**). Total protein concentration in BALF and neutrophil infiltration was used to assess permeability of lung and the severity of pulmonary edema. Compared with the control group, LPS significantly induced the total protein concentration of BALF (**Figure 1J**). while CTS decreased the level of BALF in dose-dependent manner. In addition, LPS induced the neutrophil infiltration as indicated by the increased activity of MPO in lung tissue (**Figure 1K**). Moreover, CTS treatment relieved the protein concentration of BALF and neutrophil infiltration. All these results revealed that CTS alleviated lung pathology and inflammatory cell infiltration in a dose-dependent manner.

3.2. CTS inhibited inflammatory response by regulating macrophage polarization

Macrophages are sentinel cells of the lung innate immune system and can be differentiated into the proinflammatory (M1) phenotype or anti-inflammatory (M2) phenotype macrophages according to different stimulations (26, 27). Excessive activation of M1 phenotype macrophages or deficiency of M2 phenotype macrophages is the key factors causing uncontrolled lung inflammation in acute lung injury

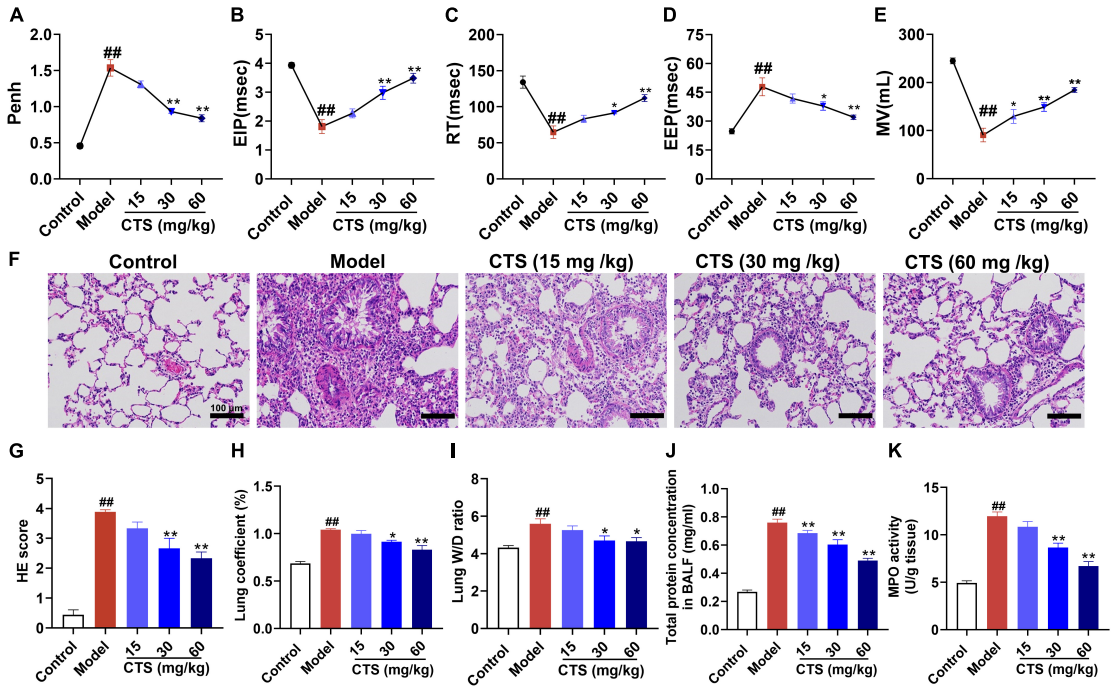

FIGURE 1
Cryptotanshinone (CTS) ameliorates LPS-induced acute lung injury in rats. LPS was used to induce *in vivo* ALI and CTS at different concentrations was administrated to rat. Representative parameters of mice pulmonary function: **(A)** enhanced pause (Penh), **(B)** end-inspiratory pause (EIP), **(C)** relaxation time (RT), **(D)** end-expiratory pause (EEP), **(E)** minute ventilation volume (MV), $n = 6$. **(F)** Representative HE staining results of lung histopathological changes, scale bar: 100 μm, $n = 6$. **(G)** Lung histopathological score, $n = 6$. **(H)** Lung coefficient **(%)** changes in each group, $n = 8$. **(I)** Wet-dry weight ratio of right lung (Lung W/D ratio) in rats, $n = 6$. **(J)** Total protein concentration in BALF, $n = 5$. **(K)** The MPO activity level in the lung tissues was measured, $n = 6$. $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the Model group.

10.3389/fmed.2022.1075465

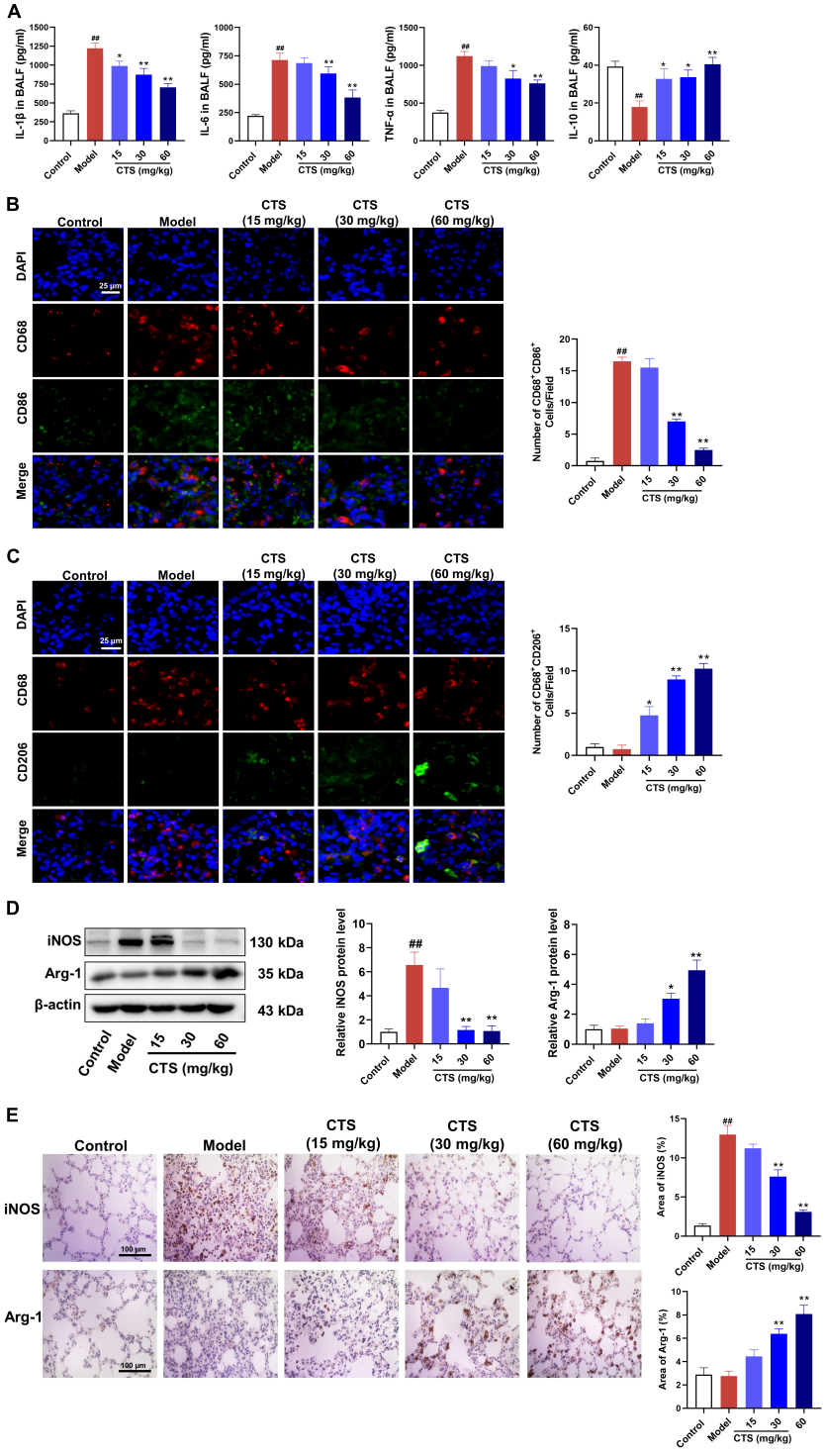

FIGURE 2
Cryptotanshinone (CTS) inhibited M1-type polarization and induces M2-type polarization of macrophages *in vivo*. **(A)** The levels of IL-1β, IL-6, TNF-α, and IL-10 in BALF were determined using ELISA, $n = 6$. **(B,C)** Representative immunofluorescence image of lung tissues. DAPI (blue), CD68 (red), CD86 (green), and CD206 (green), scales: 25 μm, $n = 4$. **(D)** The protein levels of iNOS and Arg-1, $n = 4$. **(E)** Lung sections were immunohistochemically stained by anti-iNOS antibody and anti-Arg-1 antibody, $n = 4$. Data were presented as the mean ± SEM. $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the Model group.

(28). To explore the *in vivo* effects of CTS on inflammatory response and macrophage polarization, we measured the levels of different cytokines in alveolar lavage fluid and the changes of macrophage polarization subtypes in lung tissue. Our ELISA results showed that LPS significantly induced the secretion of pro-inflammatory cytokines (such as IL-1β, IL-6 and TNF-α) and inhibited the secretion of anti-inflammatory factors (IL-10) in alveolar lavage fluid (Figure 2A). However, CTS significantly inhibited the secretion of IL-1β, IL-6 and TNF-α, and increased the IL-10 levels in alveolar lavage fluid (Figure 2A). Cluster of differentiation 68 (CD68) was used

as a pan-macrophage marker, cluster of differentiation 86 (CD86) was used as a specific marker for M1 macrophages, and cluster of differentiation 206 (CD206) was used as a specific marker for M2 macrophages (29). Our immunofluorescence results showed that the number of $CD68^{+}CD86^{+}$ macrophages significantly increased during LPS-induced acute lung injury rats (**Figures 2B, C**). Western blotting and immunohistochemical also showed that the expression of iNOS was also increased following LPS-stimulation (**Figures 2D, E**). All these results indicated that LPS promoted macrophage differentiated into the proinflammatory type. However, we found that CTS could significantly promote the trans-differentiation of M1 macrophages into M2 macrophages, evidence by decreased $CD68^{+}CD86^{+}$ and iNOS level and increased the protein levels of $CD68^{+}CD206^{+}$ and Arg1 level.

3.3. CTS inhibited macrophage polarized to M1 type and induced to M2 type in RAW264.7 cell line

Our *in vivo* results suggested that CTS inhibited LPS-induced inflammatory response of lung tissues by regulating macrophage polarization. Therefore, we further validated the effects of CTS on macrophage polarization by using RAW264.7 cell line. Firstly, CCK-8 results showed that CTS treatment with or without LPS did not alter the cell viability of RAW264.7 cell line (**Figure 3A**). According to our previous studies (30), CTS was used at different concentrations (2.5, 5, 10 μM) to inhibit inflammation. CTS could dose-dependently relieved LPS-induced expression of iNOS and CD86 (**Figure 3B**), and increased expression of Arg-1 and CD206 (**Figure 3C**). Our flow cytometry results furthermore showed that CTS dose-dependently decreased the proportion of $CD86^{+}$ M1 macrophages and increased the proportion of $CD206^{+}$ M2 macrophages (**Figures 3D, E**). Collectively, both *in vitro* and *in vivo* results consistently showed that CTS inhibited the polarization of macrophage and relieved inflammation *in vitro*.

3.4. CTS ameliorated LPS-induced metabolism dysfunction of macrophages

Phenotypic transformation of macrophages are closely related to the metabolism pattern (31). Based on metabolic characteristics of different macrophages phenotypes, M1 phenotype macrophages mainly rely on glycolysis, while M2 phenotype macrophages rely on fatty acid oxidation (FAO) and oxidative phosphorylation (OXPHOS) (32). Therefore, we further detected the effects of CTS on macrophage metabolism. Our results showed that CTS abrogated LPS-induced glucose uptake and lactic acid production in macrophage in a dose-dependent manner (**Figures 4A, B**). Extracellular acidification rate (ECAR) is a key indicator for measuring glycolysis flux and mitochondrial oxygen consumption rate (OCR) is the gold standard for detecting oxidative phosphorylation. Subsequently, we detected ECAR and OCR respectively in macrophage by using XF-96 extracellular flux analyzer. As shown in **Figures 4C, D**, LPS significantly increased glycolysis rate, glycolytic capacity and higher glycolysis reserve capacity, whereas basal respiration, mitochondrial related ATP production and maximum respiration rate were significantly inhibited in macrophage following LPS stimulation (**Figures 4E, F**). Conversely, CTS effectively relieved glycolysis and improved mitochondrial oxidative phosphorylation of macrophage (**Figures 4C–F**). Moreover, we detected the expression of proteins closely related to glycolysis such as pyruvate kinase M2 (PKM2), and 6-phosphofructo-2kinase/fructose-2,6-biphosphatase 3 (PFKFB3) and glucose transporter 1 (GLUT1). As shown in **Figure 4G**, the expression of PKM2, PFKFB3 and GLUT1 were significantly increased by LPS, whereas CTS treatment effectively inhibited the expression of these proteins. These results indicated that CTS might block LPS-induced metabolic dysfunction in macrophage.

3.5. AMPK was involved in the regulation of CTS on RAW264.7 macrophage polarization

As a sensor of intracellular energy metabolism, AMP-activated protein kinase (AMPK) plays an important role in oxidative phosphorylation, cell growth and regulation of immune responses (33). AMPK has been shown to be a metabolic regulator of macrophage polarization (34), which was inhibited in LPS-induced M1 type macrophage and meant increased glycolysis as major metabolism pathway (35). However, activation of AMPK could switch the metabolism pattern from glycolysis to aerobic oxidation and promote the transformation of macrophages from M1 to M2 phenotype (36, 37). According to previous studies (38), CTS is an activator of AMPK pathway. Whether it could relieve LPS induced inflammation and metabolism dysfunction by activating AMPK remains unknown. Therefore, we detected the phosphorylation at ser172 and total protein level of AMPK in macrophage with CTS and LPS co-treatment. As shown in **Figures 5A, B**, both the *in vivo* and *in vitro* results showed that the phosphorylation of AMPK was decreased following LPS stimulation, whereas CTS treatment significantly augmented AMPK ser127 phosphorylation (**Figures 5A, B**). These results indicated that CTS activated AMPK during ALI.

Compound C, a specific pharmacological inhibitor for AMPK, was used to further verify the involvement of AMPK on the protective of CTS. And then the type of macrophage polarization was measured. As **Figure 5C** shown, compound C (5 μM) treatment effectively blocked the phosphorylation of AMPK. Moreover, the expression of iNOS and CD86 were inhibited and the expression of Arg-1 and CD206 were augmented by CTS, which were deprived following compound C stimulation (**Figure 5D**). Flow cytometry results also revealed that compound C inhibited CTS induced M2 type macrophage and promoted polarization of macrophages to M1 type (**Figure 5E**). These results suggest that AMPK was closely involved in the regulation of CTS on macrophage polarization.

3.6. CTS regulates RAW264.7 macrophage metabolism by activating AMPK-HIF-1α

Since changes of macrophage polarization phenotype are closely related to cell metabolism, we further explored whether AMPK is involved in the regulation on metabolism of macrophage. Our results showed that LPS sharply increased rate of glycolysis and deterioration of mitochondrial oxidative phosphorylation of

10.3389/fmed.2022.1075465

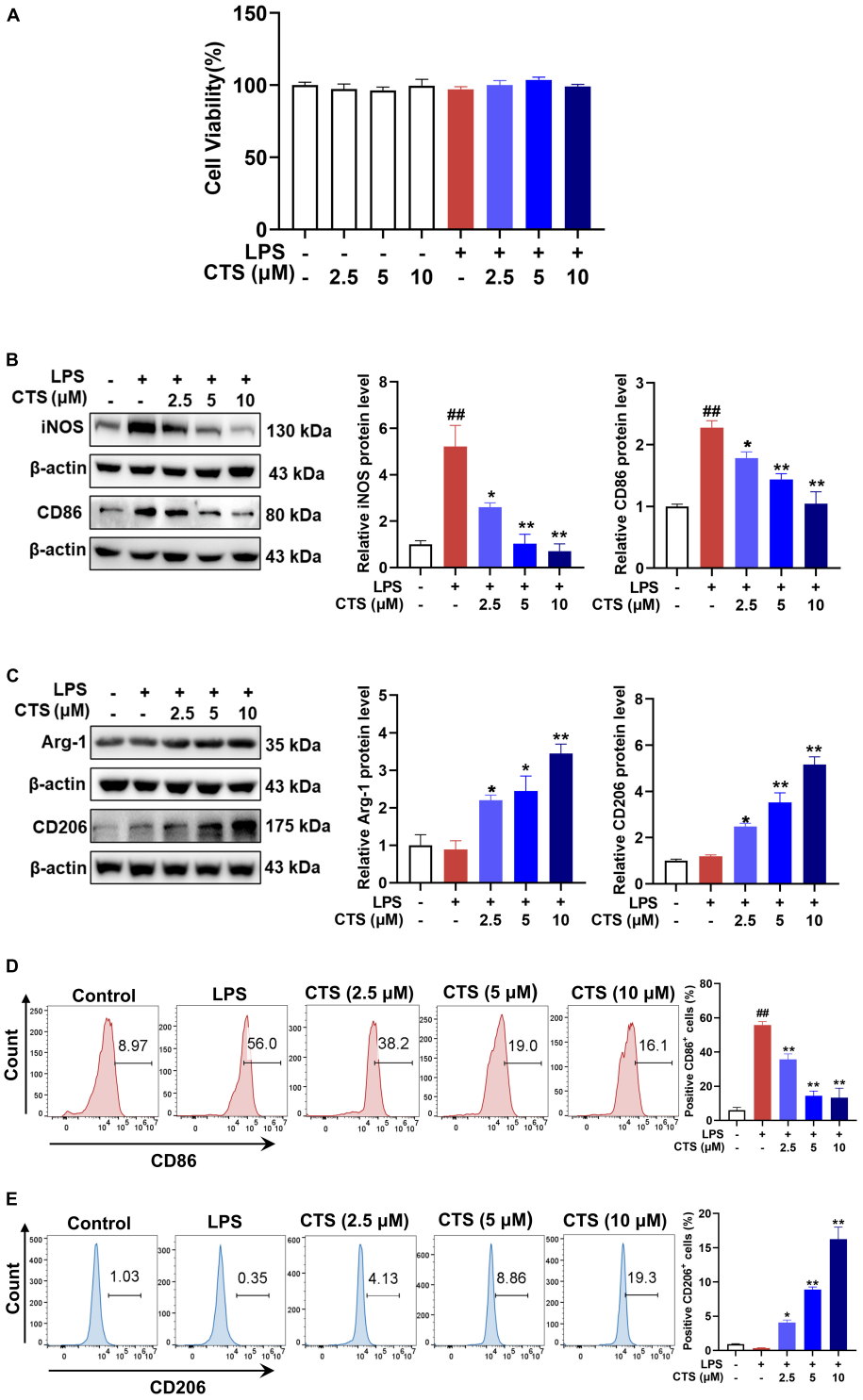

FIGURE 3
Cryptotanshinone (CTS) inhibited M1-type polarization and promoted M2-type polarization of RAW264.7 macrophages. **(A)** CTS alone or co-treatment with or without LPS stimulation and cell viability was measured. RAW264.7 were pretreated with CTS (2.5, 5, and 10 μM) for 2 h and then co-stimulated with LPS (1 μg/mL) for another 24 h. **(B)** The protein expression of iNOS and CD86, $n = 3$. **(C)** The protein expression of Arg-1 and CD206, $n = 3$. **(D)** The proportion of CD86 positive cells was analyzed by flow cytometry, $n = 3$. **(E)** The proportion of CD206 positive cells was analyzed by flow cytometry, $n = 3$. Data were presented as the mean ± SEM. $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the LPS group.

macrophage, while CTS antagonized these results (Figures 6A–D). However, inhibition of AMPK partially blocked the effects of CTS on macrophage metabolism dysfunction. There is ample evidence that AMPK acts as a negative regulator of the "Warburg" effect, by inhibiting HIF-1α-mediated glycolysis (39–41). The LPS-induced metabolic switch from OXPHOS to aerobic glycolysis in macrophages is associated with HIF1α activation (42). This metabolic pattern switch depends on the stabilization of HIF-1α and resulting in in the expression of key glycolytic proteins, such as GLUT1, PKM2, and PFKFB3 (43). Here, our results

10.3389/fmed.2022.1075465

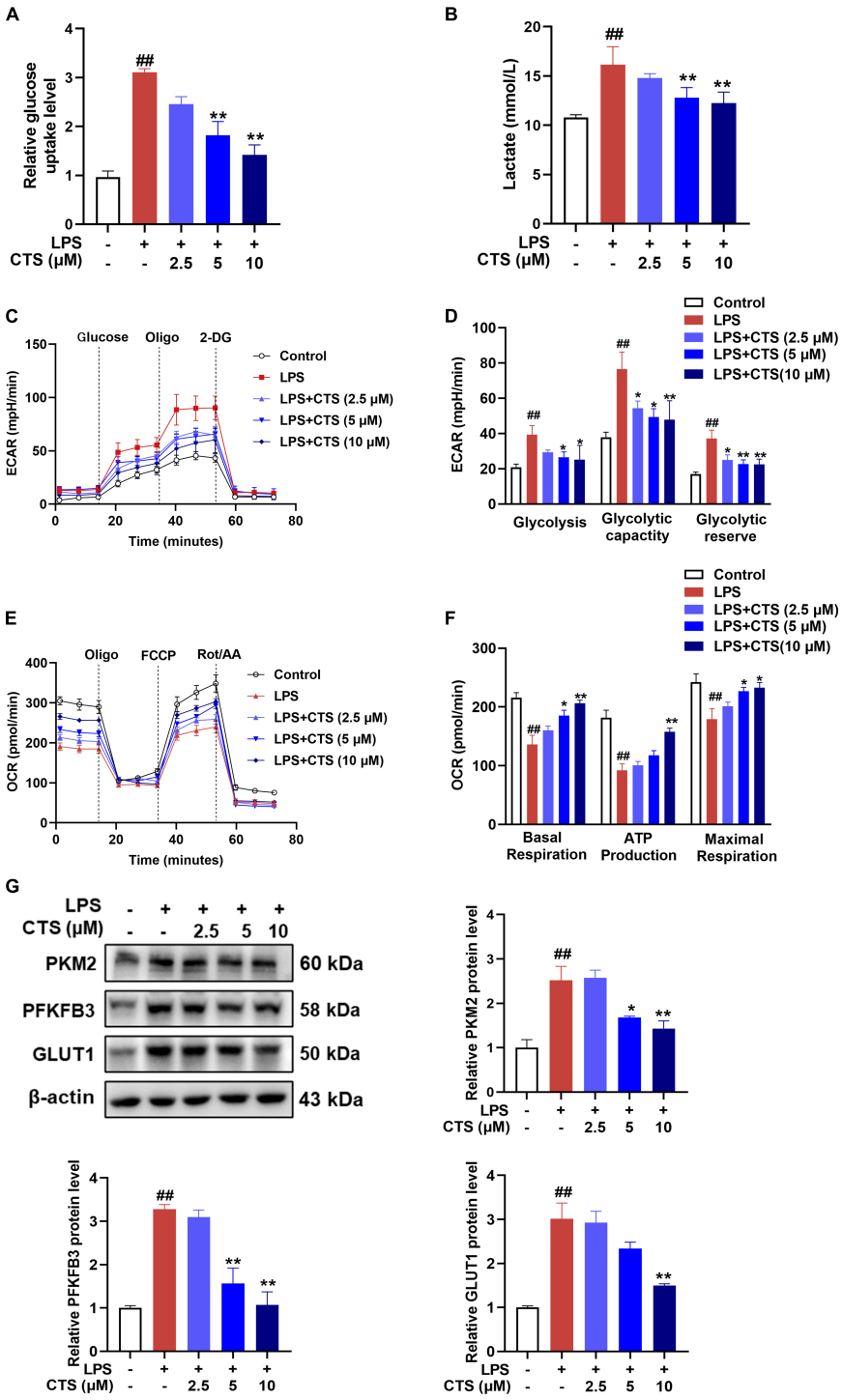

FIGURE 4
Cryptotanshinone (CTS) blocks LPS-induced metabolic reprogramming of RAW264.7 macrophages. RAW264.7 were pretreated with CTS (2.5, 5, and 10 μM) for 2 h and then co-treated with LPS for another 24 h. **(A)** Glucose uptake was detected using assay kit, $n = 4$. **(B)** The level of lactic acid, $n = 4$. **(C,D)** The extracellular acidification rate (ECAR) of macrophages were measured, $n = 4$. **(E,F)** The oxygen consumption rate (OCR) was measured, $n = 4$. **(G)** The protein levels of PKM2, PFKFB3 and GLUT1 were analyzed by using western blotting assay, $n = 3$. Data were presented as mean ± SEM. $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the LPS group.

showed that LPS significantly promoted the expression of HIF-1α, which was ameliorated by CTS treatment (Figure 6E). However, inhibition of AMPK by compound C blocked the effect of CTS on HIF-1α and the mRNA expression of GLUT1 and PFKFB3 (Figures 6E, F). FAO, an important provider of acetyl CoA that fuels the TCA cycle and OXPHOS, is significantly increased in M2 macrophages (44). AMPK is a key regulator of FAO, which promotes the increase of intracellular FAO by increasing the expression of fatty acid metabolism enzymes, such as carnitine palmitoyltransferase 1A (CPT1A), carnitine palmitoyltransferase 2 (CPT2) and medium-chain acyl-coA dehydrogenase (MCAD) (45, 46). Therefore, we speculated that CTS enhanced mitochondrial

10.3389/fmed.2022.1075465

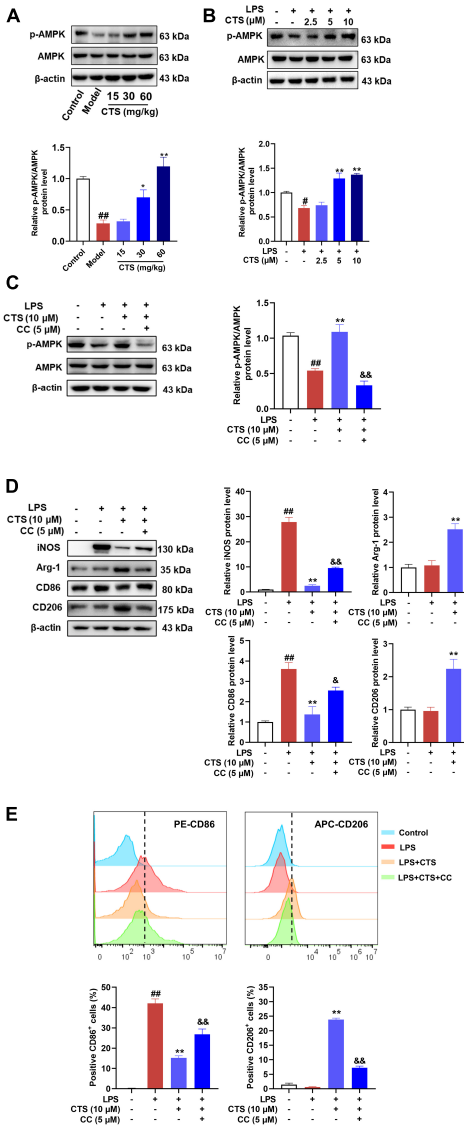

FIGURE 5
Cryptotanshinone (CTS) regulates RAW264.7 macrophage polarization via AMPK. **(A)** The protein levels of p-AMPK/AMPK in LPS-induced acute lung injury were analyzed by western blot, $n = 4$. **(B)** The protein expression of p-AMPK/AMPK in LPS-treated macrophage was analyzed by western blot, $n = 3$. **(C-E)** RAW264.7 pretreated with 5?μM AMPK inhibitor compound C for 2 h were treated with 10 μM CTS for 2?h and then exposed to LPS for another 24 h. **(C)** The protein expression levels of p-AMPK/AMPK were assessed by western blot analysis, $n = 3$. **(D)** The protein expression of iNOS, Arg-1, CD86, and CD206 was detected by Western blot, $n = 3$. **(E)** The proportion of $CD86^+$ cells and $CD206^+$ cells were analyzed by flow cytometry, $n = 3$. Data were presented as the mean ± SEM. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the LPS group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ vs. the LPS + CTS (10 μM) group.

oxidative phosphorylation through activation of AMPK-mediated increases in FAO. The effect of CTS on the expression of fatty acid oxidation genes were determined. Our results showed that the expressions of CPT1A, CPT2 and MCAD were significantly inhibited by LPS while promoted by CTS (Figure 6G). However, Compound C blocked the mRNA expression of CPT1A, CPT-2 and MCAD. These results suggested that AMPK was involved in role of CTS on regulation of macrophage polarization and metabolism reprogramming.

4. Discussion

ALI is a diffuse inflammatory injury of lung parenchyma caused by a variety of non-cardiac internal and external lung pathogenic factors, which is clinically manifested as respiratory failure, hypoxemia and pulmonary edema with high morbidity and mortality (47, 48). In addition, ALI has been considered to be an important factor causing the death of critically ill patients with The Corona Virus Disease 2019 (COVID-19) that is currently circulating worldwide (49, 50). A growing body of evidence indicates that the key pathogenesis of ALI is cytokine storm induced by immune cell. Too much proinflammatory cytokines caused inflammation of the lungs and serious destruction of the alveolar capillary barrier, further resulting in the decrease of lung compliance and a sharp deterioration in pulmonary function (51, 52). Therefore, inhibiting excessive inflammatory response in the lung is a key strategy for the treatment of ALI. However, to date, the treatment strategies for acute lung injury are limited and no drugs targeting inflammatory responses during ALI have been approved. ALI patients can only rely on supportive strategies to save their lives (53). Therefore, it is urgent to develop new and effective drugs to treat patients with acute lung injury.

As the main component of the cell wall of gram-negative bacteria, LPS could activate the *in vivo* innate immune system and induce pulmonary inflammatory responses, and was used to simulate ALI syndrome *in vivo* (54). In this study, intratracheal infusion of LPS was used to induce acute lung injury model of rat and the pulmonary function was rapidly destroyed as indicated by obvious diffuse alveolar injury, excessive infiltration of inflammatory cells and pulmonary edema. CTS is one of the main biologically active ingredient of *salvia miltiorrhiza* with powerful anti-inflammatory activity and higher distribution in the lung tissue (15, 55). Given its anti-inflammatory property and tissue-specific distribution in lung, CTS may be a potential compound for the treatment of acute lung injury. According to our previous results, the dosages of CTS used in this study were 15, 30 and 60 mg/kg/day (19). In this study, our results firmly showed that CTS restored pulmonary function of rats in a dose-dependent manner as indicated by the improved lung compliance and alveolar capillary barrier integrity.

Alveolar macrophages are the most abundant immune cells in the lung tissue and are crucial for maintaining airway homeostasis (56). Different phenotypes of macrophage played different roles during the pathological process of acute lung injury (56). Activation of pro-inflammatory (M1) phenotype macrophage is a key parameter of acute pneumonia, which stimulates cytokine storm by releasing proinflammatory factors such as IL-1β, IL-6 and TNF-α (57). In contrast, activation of the anti-inflammatory (M2) phenotype macrophages ameliorates lung tissue injury by releasing anti-inflammatory mediators Arg-1 and IL-10 to promote inflammation resolution (58). Therefore, modulation of macrophage polarization is a potentially effective treatment for acute lung injury (58).

CTS has been shown to be able to convert M1 to M2 phenotype macrophages to alleviate ulcerative colitis lesions and significantly inhibit neuroinflammation in ischemic stroke (20, 21). This suggests that CTS may be an immunomodulator targeting macrophage polarization to treat inflammatory diseases. Consistently, our results showed that CTS was able to inhibit the activation of M1 phenotype macrophage and promote the activation of M2 phenotype macrophage during acute lung injury. Additionally,

10.3389/fmed.2022.1075465

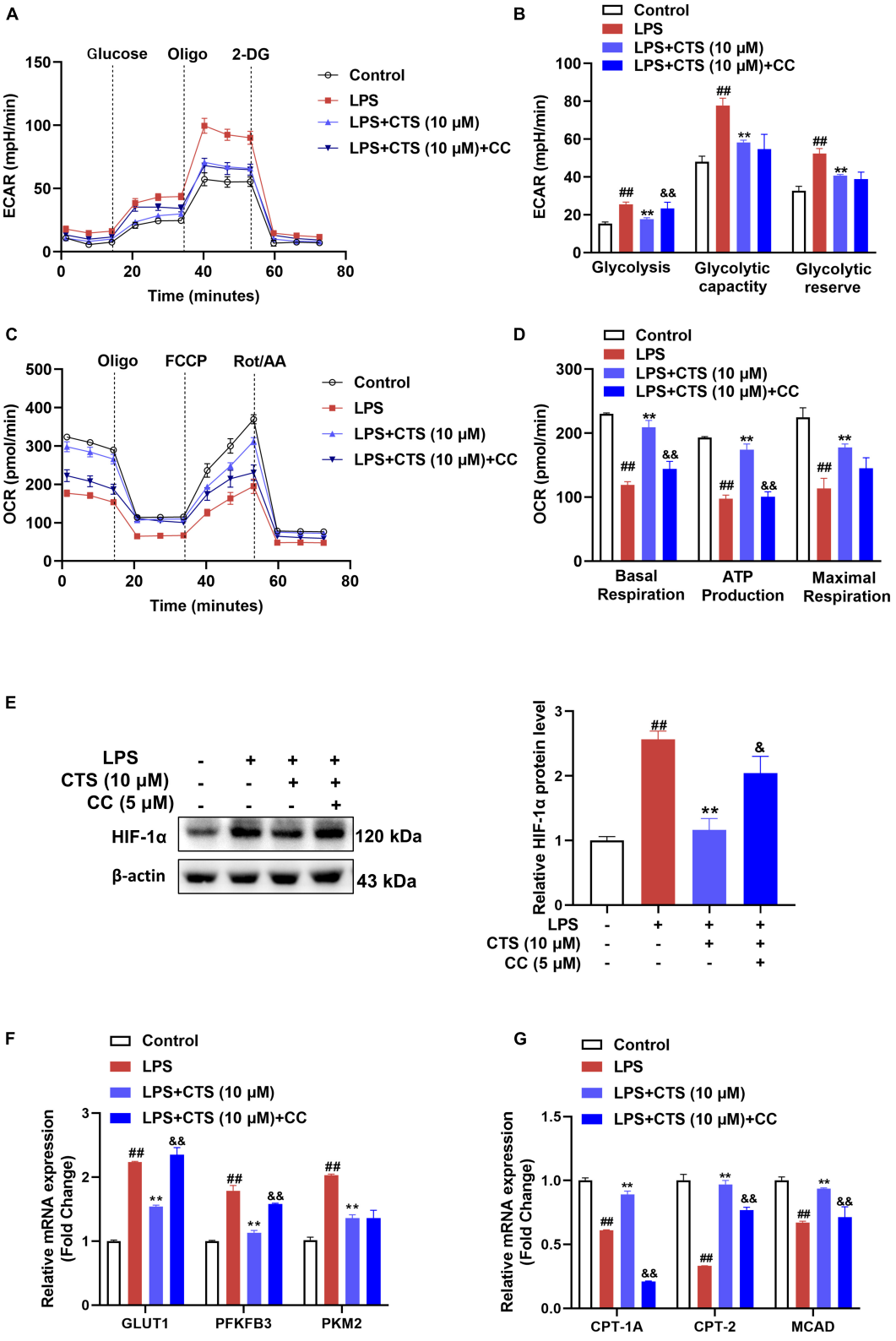

FIGURE 6

Cryptotanshinone (CTS) regulates energy metabolism of LPS-induced RAW264.7 macrophages through AMPK. RAW264.7 pretreated with 5 μM AMPK inhibitor compound C for 2 h were treated with 10 μM CTS for 2 h and then exposed to LPS for another 24 h. **(A)** ECAR of the indicated macrophages were measured with a seahorse analyzer. **(B)** Glycolysis, glycolytic capacity, and glycolytic reserve were calculated and are indicated as ECAR in mpH/min, $n = 4$. **(C)** OCR was measured with the Seahorse analyzer. **(D)** The basal respiration, maximal respiration, and ATP production were calculated and are indicated as OCR in pmoles/min, $n = 4$. **(E)** The protein level of HIF-1α was analyzed by using western blotting assay, $n = 3$. **(F)** The mRNA expressions of glucose transporter type1 (GLUT1), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), pyruvate kinase M2 (PKM2) were detected by using the qPCR assay, $n = 3$. **(G)** The mRNA expressions of carnitine palmitoyltransferase 1A (CPT1A), carnitine palmitoyltransferase 2 (CPT2), medium-chain acyl-coA dehydrogenase (MCAD) were detected by using the qPCR assay, $n = 3$. Data were presented as the mean ± SEM. $^{\#\#}P < 0.01$ vs. the control group; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the LPS group; $^{\&}P < 0.05$, $^{\&\&}P < 0.01$ vs. the LPS + CTS (10 μM) group.

metabolic reprogramming of macrophage plays a key role in the process of macrophage polarization (31). M1 macrophage showed reduced oxidative metabolism and increased glycolysis, while M2 macrophage has a complete tricarboxylic acid cycle and utilizes FAO and OXPHOS for energy supplement (35). The glycolytic inhibitor 2-DG inhibits the activation of M1 type macrophage, whereas viral knockdown of the fatty acid-related gene CPT1A inhibited activation of M2 type macrophage (12, 13, 59). This suggests that blocking or restoring metabolic pathways to modulate macrophage polarization is feasible. Consistent with previous study, we found that CTS inhibited LPS-induced glycolysis and promoted mitochondrial oxidative phosphorylation of macrophage (22, 23). These results firstly revealed that CTS exerted its protective effects by regulating macrophage polarization and metabolism reprogramming.

Previous studies suggested that AMPK acted as a key protein molecule in regulating metabolism reprogramming and polarization of macrophage by repressing the expression of HIF-1α (39, 41). Activation of AMPK could promote transformation of macrophages from the pro-inflammatory type to the anti-inflammatory type (36). Consistent with previous reports, the activation of AMPK was inhibited with LPS stimulation both *in vivo* and *in vitro* (37), which was relieved by CTS in a dose-dependently manner. Previous studies have shown that HIF-1α-mediated glycolysis drove macrophage differentiated into the pro-inflammatory phenotype (60). Furthermore, the M2 phenotype polarization of macrophage was dependent on AMPK-induced increase in FAO (10), including the up-regulation of FAO related enzymes such as CPT1A, CPT2 and MCAD (45, 61). In this study, compound C, an AMPK specific inhibitor, blocked CTS-induced inhibition of HIF-1α and the expression of key glycolysis genes (such as GLUT1 and PFKFB3), subsequently abrogating CTS-induced promotion of key FAO enzyme genes (e.g., CPT1A, CPT2, and MCAD). These findings suggested that the ability of CTS to induce macrophages polarization was mainly attributed to the activation of AMPK to induce FAO and inhibit HIF-1α-mediated glycolysis.

However, the mechanism by which CTS activates AMPK remains unknown. Liver kinase B1 (LKB1) is one of the upstream kinases that regulate AMPK activation and directly phosphorylates Thr172 of the α subunit of AMPK to activate AMPK. Studies have shown that LPS reduced AMPK phosphorylation in macrophages and inhibited LKB1 activation (62). It has been reported that CTS activates AMPK signaling pathway by LKB1 (63). Therefore, it was reasonable to speculate that CTS might promote AMPK phosphorylation through activation of LKB1, but this needs further experimental verification.

In conclusion, our study suggested that CTS promoted the transformation of M1-type macrophages into M2-type macrophages by regulating energy metabolism, thus playing an anti-acute lung injury role. Furthermore, we have shown that AMPK mediated the regulation of CTS on macrophage polarization by affecting energy metabolism patterns of macrophage.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding authors.

Ethics statement

This animal study was reviewed and approved by Sun Yat-sen University Animal Ethics.

Author contributions

ZY, PW, and GF: research design, performed experiments, data collection, and writing—original draft. QW and CL: assisted the research and data collection. JL, PL, and JC: project administration, funding acquisition, and writing—review and editing. All authors have read the author agreement of the magazine and agreed to the published version of the manuscript.

Funding

All research were supported by grants from the National Natural Science Foundation of China (U21A20419 and 82104157), Natural Science Foundation of Guangdong Province (2022A1515012322), the Fundamental Research Funds for the Central Universities, Sun Yat-sen University (22qntd4510), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01Y093), National Engineering and Technology Research Center for New drug Druggability Evaluation (Seed Program of Guangdong Province, 2017B090903004), Guangdong Provincial Key Laboratory of Construction Foundation (2017B030314030), and Guangdong Provincial Key Laboratory of Construction Foundation, No. 2019B030301005, Guangzhou Basic and Applied Basic Research Project (202102020173 and 202206080007), and the Discipline Construction Project of Guangdong Medical University (4SG21233G).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.1075465/full#supplementary-material

References

1. Butt Y, Kurdowska A, Allen T. Acute lung injury: a clinical and molecular review. *Arch Pathol Lab Med.* (2016) 140:345–50. doi: 10.5858/arpa.2015-0519-RA

2. Sweeney R, McAuley D. Acute respiratory distress syndrome. *Lancet.* (2016) 388:2416–30. doi: 10.1016/s0140-6736(16)00578-x

3. Zhu J, Guo M, Cui Y, Meng Y, Ding J, Zeng W, et al. Surface coating of pulmonary siRNA delivery vectors enabling mucus penetration, cell targeting, and intracellular radical scavenging for enhanced acute lung injury therapy. *ACS Appl Mater Interfaces.* (2022) 14:5090–100. doi: 10.1021/acsami.1c23069

4. Nieman G, Gatto L, Andrews P, Satalin J, Camporota L, Daxon B, et al. Prevention and treatment of acute lung injury with time-controlled adaptive ventilation: physiologically informed modification of airway pressure release ventilation. *Ann Intensive Care.* (2020) 10:3. doi: 10.1186/s13613-019-0619-3

5. Xu B, Chen S, Liu M, Gan C, Li J, Guo G. Stem cell derived exosomes-based therapy for acute lung injury and acute respiratory distress syndrome: a novel therapeutic strategy. *Life Sci.* (2020) 254:117766. doi: 10.1016/j.lfs.2020.117766

6. Chen X, Tang J, Shuai W, Meng J, Feng J, Han Z. Macrophage polarization and its role in the pathogenesis of acute lung injury/acute respiratory distress syndrome. *Inflamm Res.* (2020) 69:883–95. doi: 10.1007/s00011-020-01378-2

7. Vichare R, Janjic J. Macrophage-targeted nanomedicines for ARDS/ALI: promise and potential. *Inflammation.* (2022) 45:2124–41. doi: 10.1007/s10753-022-01692-3

8. Galván-Peña S, O'Neill L. Metabolic reprograming in macrophage polarization. *Front Immunol.* (2014) 5:420. doi: 10.3389/fimmu.2014.00420

9. Viola A, Munari F, Sánchez-Rodríguez R, Scolaro T, Castegna A. The metabolic signature of macrophage responses. *Front Immunol.* (2019) 10:1462. doi: 10.3389/fimmu.2019.01462

10. Mills E, O'Neill L. Reprogramming mitochondrial metabolism in macrophages as an anti-inflammatory signal. *Eur J Immunol.* (2016) 46:13–21. doi: 10.1002/eji.201445427

11. Freemerman A, Johnson A, Sacks G, Milner J, Kirk E, Troester M, et al. Metabolic reprogramming of macrophages: glucose transporter 1 (Glut1)-mediated glucose metabolism drives a proinflammatory phenotype. *J Biol Chem.* (2014) 289:7884–96. doi: 10.1074/jbc.M113.522037

12. Wang F, Zhang S, Jeon R, Vuckovic I, Jiang X, Lerman A, et al. Interferon gamma induces reversible metabolic reprogramming of M1 macrophages to sustain cell viability and pro-inflammatory activity. *EBioMedicine.* (2018) 30:303–16. doi: 10.1016/j.ebiom.2018.02.009

13. Vats D, Mukundan L, Odegaard J, Zhang L, Smith K, Morel C, et al. Oxidative metabolism and PGC-1beta attenuate macrophage-mediated inflammation. *Cell Metab.* (2006) 4:13–24. doi: 10.1016/j.cmet.2006.05.011

14. Divakaruni A, Hsieh W, Minarrieta L, Duong T, Kim K, Desousa B, et al. Etomoxir inhibits macrophage polarization by disrupting CoA homeostasis. *Cell Metab.* (2018) 28:490–503.e7. doi: 10.1016/j.cmet.2018.06.001

15. Li H, Gao C, Liu C, Liu L, Zhuang J, Yang J, et al. A review of the biological activity and pharmacology of cryptotanshinone, an important active constituent in danshen. *Biomed Pharmacother.* (2021) 137:111332. doi: 10.1016/j.biopha.2021.111332

16. Wang Y, Zhou C, Gao H, Li C, Li D, Liu P, et al. Therapeutic effect of cryptotanshinone on experimental rheumatoid arthritis through downregulating P300 mediated-STAT3 acetylation. *Biochem Pharmacol.* (2017) 138:119–29. doi: 10.1016/j.bcp.2017.05.006

17. Liu Z, Xu S, Huang X, Wang J, Gao S, Li H, et al. Cryptotanshinone, an orally bioactive herbal compound from Danshen, attenuates atherosclerosis in apolipoprotein E-deficient mice: role of lectin-like oxidized LDL receptor-1 (Lox-1). *Br J Pharmacol.* (2015) 172:5661–75. doi: 10.1111/bph.13068

18. Mei Z, Zhang F, Tao L, Zheng W, Cao Y, Wang Z, et al. Cryptotanshinone, a compound from *Salvia miltiorrhiza* modulates amyloid precursor protein metabolism and attenuates B-amyloid deposition through upregulating A-secretase in vivo and in vitro. *Neurosci Lett.* (2009) 452:90–5. doi: 10.1016/j.neulet.2009.01.013

19. Zhang Y, Lu W, Zhang X, Lu J, Xu S, Chen S, et al. Cryptotanshinone protects against pulmonary fibrosis through inhibiting Smad and STAT3 signaling pathways. *Pharmacol Res.* (2019) 147:104307. doi: 10.1016/j.phrs.2019.104307

20. Mao Y, Qu Y, Wang Q. Cryptotanshinone reduces neurotoxicity induced by cerebral ischemia-reperfusion injury involving modulation of microglial polarization. *Restor Neurol Neurosci.* (2021) 39:209–20. doi: 10.3233/RNN-201070

21. Zhang L, Yu L, Wei Y. Oral administration of cryptotanshinone-encapsulated nanoparticles for the amelioration of ulcerative colitis. *Cell Mol Bioeng.* (2022) 15:129–36. doi: 10.1007/s12195-021-00711-x

22. Yang Y, Cao Y, Chen L, Liu F, Qi Z, Cheng X, et al. Cryptotanshinone suppresses cell proliferation and glucose metabolism via STAT3/SIRT3 signaling pathway in ovarian cancer cells. *Cancer Med.* (2018) 7:4610–8. doi: 10.1002/cam4.1691

23. Zhou J, Su C, Chen H, Du S, Li C, Wu H, et al. Cryptanshinone inhibits the glycolysis and inhibits cell migration through PKM2/β-catenin axis in breast cancer. *Onco Targets Ther.* (2020) 13:8629. doi: 10.2147/OTT.S239134

24. Tang Y, Chen Y, Chu Z, Yan B, Xu L. Protective effect of cryptotanshinone on lipopolysaccharide-induced acute lung injury in mice. *Eur J Pharmacol.* (2014) 723:494–500. doi: 10.1016/j.ejphar.2013.10.019

25. Bi X, Jiang B, Zhou J, Fan X, Yan X, Liang J, et al. CBP bromodomain inhibition rescues mice from lethal sepsis through blocking hmgb1-mediated inflammatory responses. *Front Immunol.* (2020) 11:625542. doi: 10.3389/fimmu.2020.625542

26. Wang Q, Chen S, Li T, Yang Q, Liu J, Tao Y, et al. Critical role of LKB1 in the maintenance of alveolar macrophage self-renewal and immune homeostasis. *Front Immunol.* (2021) 12:629281. doi: 10.3389/fimmu.2021.629281

27. Arora S, Dev K, Agarwal B, Das P, Syed M. Macrophages: their role, activation and polarization in pulmonary diseases. *Immunobiology.* (2018) 223:383–96. doi: 10.1016/j.imbio.2017.11.001

28. Zhang W, Zhang Y, He Y, Wang X, Fang Q. Lipopolysaccharide mediates time-dependent macrophage M1/M2 polarization through the tim-3/galectin-9 signalling pathway. *Exp Cell Res.* (2019) 376:124–32. doi: 10.1016/j.yexcr.2019.02.007

29. Zhu T, Mao J, Cheng Y, Liu H, Lv L, Ge M, et al. Recent progress of polysaccharide-based hydrogel interfaces for wound healing and tissue engineering. *Adv Mater Interfaces.* (2019) 6:1900761.

30. Tang S, Shen X, Huang H, Xu S, Yu Y, Zhou C, et al. Cryptotanshinone suppressed inflammatory cytokines secretion in RAW264. 7 macrophages through Inhibition of the NF-κB and MAPK signaling pathways. *Inflammation.* (2011) 34:111–8. doi: 10.1007/s10753-010-9214-3

31. Liu Y, Xu R, Gu H, Zhang E, Qu J, Cao W, et al. Metabolic reprogramming in macrophage responses. *Biomark Res.* (2021) 9:1.

32. Hu C, Xuan Y, Zhang X, Liu Y, Yang S, Yang K. Immune cell metabolism and metabolic reprogramming. *Mol Biol Rep.* (2022) 49:9783–95. doi: 10.1007/s11033-022-07474-2

33. Herzig S, Shaw R. AMPK: guardian of metabolism and mitochondrial homeostasis. *Nat Rev Mol Cell Biol.* (2018) 19:121–35. doi: 10.1038/nrm.2017.95

34. Day E, Ford R, Steinberg G. AMPK as a therapeutic target for treating metabolic diseases. *Trends Endocrinol Metab.* (2017) 28:545–60. doi: 10.1016/j.tem.2017.05.004

35. Wang S, Liu R, Yu Q, Dong L, Bi Y, Liu G. Metabolic reprogramming of macrophages during infections and cancer. *Cancer Lett.* (2019) 452:14–22. doi: 10.1016/j.canlet.2019.03.015

36. O'Neill L, Hardie D. Metabolism of inflammation limited by AMPK and pseudo-starvation. *Nature.* (2013) 493:346–55. doi: 10.1038/nature11862

37. Steinberg G, Schertzer J. AMPK promotes macrophage fatty acid oxidative metabolism to mitigate inflammation: implications for diabetes and cardiovascular disease. *Immunol Cell Biol.* (2014) 92:340–5. doi: 10.1038/icb.2014.11

38. Chen W, Pan Y, Wang S, Liu Y, Chen G, Zhou L, et al. Cryptotanshinone activates AMPK-TSC2 axis leading to inhibition of mTORC1 signaling in cancer cells. *BMC Cancer.* (2017) 17:34. doi: 10.1186/s12885-016-3038-y

39. Faubert B, Boily G, Izreig S, Griss T, Samborska B, Dong Z, et al. AMPK is a negative regulator of the warburg effect and suppresses tumor growth in vivo. *Cell Metab.* (2013) 17:113–24.

40. Yu Y, Cai W, Zhou J, Lu H, Wang Y, Song Y, et al. Anti-arthritis effect of berberine associated with regulating energy metabolism of macrophages through AMPK/ HIF-1α pathway. *Int Immunopharmacol.* (2020) 87:106830. doi: 10.1016/j.intimp.2020.106830

41. Chen Y, Liu L, Xia L, Wu N, Wang Y, Li H, et al. TRPM7 silencing modulates glucose metabolic reprogramming to inhibit the growth of ovarian cancer by enhancing AMPK activation to promote HIF-1α degradation. *J Exp Clin Cancer Res.* (2022) 41:44. doi: 10.1186/s13046-022-02252-1

42. Wang T, Liu H, Lian G, Zhang S, Wang X, Jiang C. Hif1α-induced glycolysis metabolism is essential to the activation of inflammatory macrophages. *Mediators Inflamm.* (2017) 2017:9029327. doi: 10.1155/2017/9029327

43. Pan X, Zhu Q, Dong X, Li J, Liu H, Ren Z, et al. Macrophage immunometabolism in inflammatory bowel diseases: from pathogenesis to therapy. *Pharmacol Ther.* (2022) 238:108176.

44. Almeida L, Everts B. Fa(C)T checking: how fatty acids shape metabolism and function of macrophages and dendritic cells. *Eur J Immunol.* (2021) 51:1628–40. doi: 10.1002/eji.202048944

45. Yu L, Li Y, Su M, Zhang M, Zhang W, Zhang L, et al. Development of novel alkene oxindole derivatives as orally efficacious AMP-activated protein kinase activators. *ACS Med Chem Lett.* (2013) 4:475–80. doi: 10.1021/ml400028q

46. Bories G, Leitinger N. Macrophage metabolism in atherosclerosis. *FEBS Lett.* (2017) 591:3042–60.

47. Peng L, Yuan M, Shi H, Li J, Song K, Huang J, et al. Protective effect of piceatannol against acute lung injury through protecting the integrity of air-blood barrier and modulating the TLR4/NF-κB signaling pathway activation. *Front Pharmacol.* (2019) 10:1613. doi: 10.3389/fphar.2019.01613

48. Impellizzeri D, Bruschetta G, Esposito E, Cuzzocrea S. Emerging drugs for acute lung injury. *Expert Opin Emerg Drugs.* (2015) 20:75–89.

49. Mehta P, McAuley D, Brown M, Sanchez E, Tattersall R, Manson J. Covid-19: consider cytokine storm syndromes and immunosuppression. *Lancet.* (2020) 395:1033–4.

50. Colunga Biancatelli R, Solopov P, Sharlow E, Lazo J, Marik P, Catravas J. The SARS-CoV-2 spike protein subunit S1 induces covid-19-like acute lung injury in K18-hACE2

transgenic mice and barrier dysfunction in human endothelial cells. *Am J Physiol Lung Cell Mol Physiol.* (2021) 321:L477–84. doi: 10.1152/ajplung.00223.2021

51. Wang C, Xie J, Zhao L, Fei X, Zhang H, Tan Y, et al. Alveolar macrophage dysfunction and cytokine storm in the pathogenesis of two severe covid-19 patients. *EBioMedicine.* (2020) 57:102833.

52. Liu J, Wan M, Lyon C, Hu T. Nanomedicine therapies modulating macrophage dysfunction: a potential strategy to attenuate cytokine storms in severe infections. *Theranostics.* (2020) 10:9591–600. doi: 10.7150/thno.47982

53. Patel V, Biswas Roy S, Mehta H, Joo M, Sadikot R. Alternative and natural therapies for acute lung injury and acute respiratory distress syndrome. *Biomed Res Int.* (2018) 2018:2476824. doi: 10.1155/2018/2476824

54. Zhu J, Feng B, Xu Y, Chen W, Sheng X, Feng X, et al. Mesenchymal stem cells alleviate LPS-induced acute lung injury by inhibiting the proinflammatory function of LY6C(+) CD8(+) T Cells. *Cell Death Dis.* (2020) 11:829. doi: 10.1038/s41419-020-03036-1

55. Pan Y, Bi H, Zhong G, Chen X, Zuo Z, Zhao L, et al. Pharmacokinetic characterization of hydroxylpropyl-B-cyclodextrin-included complex of cryptotanshinone, an investigational cardiovascular drug purified from danshen (*Salvia miltiorrhiza*). *Xenobiotica.* (2008) 38:382–98. doi: 10.1080/00498250701827685

56. Zhou B, Magana L, Hong Z, Huang L, Chakraborty S, Tsukasaki Y, et al. The angiocrine Rspondin3 instructs interstitial macrophage transition via metabolic-epigenetic reprogramming and resolves inflammatory injury. *Nat Immunol.* (2020) 21:1430–43. doi: 10.1038/s41590-020-0764-8

57. Hu Q, Lyon C, Fletcher J, Tang W, Wan M, Hu T. Extracellular vesicle activities regulating macrophage- and tissue-mediated injury and repair responses. *Acta Pharm Sin B.* (2021) 11:1493–512. doi: 10.1016/j.apsb.2020.12.014

58. Li W, Li D, Chen Y, Abudou H, Wang H, Cai J, et al. Classic signaling pathways in alveolar injury and repair involved in sepsis-induced ALI/ARDS: new research progress and prospect. *Dis Markers.* (2022) 2022:6362344. doi: 10.1155/2022/6362344

59. Huang S, Smith A, Everts B, Colonna M, Pearce E, Schilling J, et al. Metabolic reprogramming mediated by the MTORC2-IRF4 signaling axis is essential for macrophage alternative activation. *Immunity.* (2016) 45:817–30. doi: 10.1016/j.immuni.2016.09.016

60. Corcoran S, O'Neill L. Hif1α and metabolic reprogramming in inflammation. *J Clin Invest.* (2016) 126:3699–707. doi: 10.1172/jci84431

61. Kelly B, O'Neill L. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* (2015) 25:771–84. doi: 10.1038/cr.2015.68

62. Dohmen M, Krieg S, Agalaridis G, Zhu X, Shehata S, Pfeiffenberger E, et al. AMPK-dependent activation of the cyclin Y/CDK16 complex controls autophagy. *Nat Commun.* (2020) 11:1032. doi: 10.1038/s41467-020-14812-0

63. Park I, Yang W, Nam S, Hong J, Yang K, Kim J, et al. Cryptotanshinone induces G1 cell cycle arrest and autophagic cell death by activating the AMP-activated protein kinase signal pathway in HEPG2 hepatoma. *Apoptosis.* (2014) 19:615–28. doi: 10.1007/s10495-013-0929-0

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 17 January 2023
DOI 10.3389/fmed.2022.1083264

OPEN ACCESS

EDITED BY
Rifat Hamoudi,
University of Sharjah, United Arab Emirates

REVIEWED BY
Constantina A. Bounia,
General University Hospital of Patras, Greece
Ludovica Capitelli,
University of Naples Federico II, Italy
Athanasios Voulgaris,
Democritus University of Thrace, Greece

*CORRESPONDENCE
Argyris Tzouvelekis
✉ atzouvelekis@upatras.gr;
✉ argyris.tzouvelekis@gmail.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 28 October 2022
ACCEPTED 30 December 2022
PUBLISHED 17 January 2023

CITATION
Karampitsakos T, Sotiropoulou V, Katsaras M, Tsiri P, Georgakopoulou VE, Papanikolaou IC, Bibaki E, Tomos I, Lambiri I, Papaioannou O, Zarkadi E, Antonakis E, Pandi A, Malakounidou E, Sampsonas F, Makrodimitri S, Chrysikos S, Hillas G, Dimakou K, Tzanakis N, Sipsas NV, Antoniou K and Tzouvelekis A (2023) Post-COVID-19 interstitial lung disease: Insights from a machine learning radiographic model.
Front. Med. 9:1083264.
doi: 10.3389/fmed.2022.1083264

Post-COVID-19 interstitial lung disease: Insights from a machine learning radiographic model

Theodoros Karampitsakos[1†], Vasilina Sotiropoulou[1†], Matthaios Katsaras[1], Panagiota Tsiri[1], Vasiliki E. Georgakopoulou[2], Ilias C. Papanikolaou[3], Eleni Bibaki[4], Ioannis Tomos[5], Irini Lambiri[4], Ourania Papaioannou[1], Eirini Zarkadi[1], Emmanouil Antonakis[3], Aggeliki Pandi[3], Elli Malakounidou[1], Fotios Sampsonas[1], Sotiria Makrodimitri[2], Serafeim Chrysikos[5], Georgios Hillas[5], Katerina Dimakou[5], Nikolaos Tzanakis[4], Nikolaos V. Sipsas[2,6], Katerina Antoniou[4] and Argyris Tzouvelekis[1*]

[1]Department of Respiratory Medicine, University General Hospital of Patras, Patras, Greece, [2]Department of Infectious Diseases-COVID-19 Unit, Laiko General Hospital, Athens, Greece, [3]Department of Respiratory Medicine, Corfu General Hospital, Corfu, Greece, [4]Laboratory of Molecular and Cellular Pneumonology, Department of Thoracic Medicine, Medical School, University of Crete, Heraklion, Greece, [5]5th Department of Respiratory Medicine, Hospital for Thoracic Diseases, 'SOTIRIA', Athens, Greece, [6]Medical School, National and Kapodistrian University of Athens, Zografou, Greece

Introduction: Post-acute sequelae of COVID-19 seem to be an emerging global crisis. Machine learning radiographic models have great potential for meticulous evaluation of post-COVID-19 interstitial lung disease (ILD).

Methods: In this multicenter, retrospective study, we included consecutive patients that had been evaluated 3 months following severe acute respiratory syndrome coronavirus 2 infection between 01/02/2021 and 12/5/2022. High-resolution computed tomography was evaluated through Imbio Lung Texture Analysis 2.1.

Results: Two hundred thirty-two (n = 232) patients were analyzed. FVC% predicted was ≥80, between 60 and 79 and <60 in 74.2% (n = 172), 21.1% (n = 49), and 4.7% (n = 11) of the cohort, respectively. DLCO% predicted was ≥80, between 60 and 79 and <60 in 69.4% (n = 161), 15.5% (n = 36), and 15.1% (n = 35), respectively. Extent of ground glass opacities was ≥30% in 4.3% of patients (n = 10), between 5 and 29% in 48.7% of patients (n = 113) and <5% in 47.0% of patients (n = 109). The extent of reticulation was ≥30%, 5–29% and <5% in 1.3% (n = 3), 24.1% (n = 56), and 74.6% (n = 173) of the cohort, respectively. Patients (n = 13, 5.6%) with fibrotic lung disease and persistent functional impairment at the 6-month follow-up received antifibrotics and presented with an absolute change of +10.3 (p = 0.01) and +14.6 (p = 0.01) in FVC% predicted at 3 and 6 months after the initiation of antifibrotic.

Conclusion: Post-COVID-19-ILD represents an emerging entity. A substantial minority of patients presents with fibrotic lung disease and might experience benefit from antifibrotic initiation at the time point that fibrotic-like changes are "immature." Machine learning radiographic models could be of major significance for accurate radiographic evaluation and subsequently for the guidance of therapeutic approaches.

KEYWORDS

post-COVID-19, long COVID, interstitial lung disease, antifibrotics, machine learning

Introduction

The global impact of the ongoing coronavirus disease 2019 (COVID-19) pandemic has been unparalleled. The disease course is variable, manifesting from asymptomatic to fatal forms, and long-term complications could have further devastating effects (1–3). Emerging evidence indicate that a substantial proportion of infected individuals may experience prolonged symptoms lasting for more than 6 months (4–6).

The National Institute for Health and Care Excellence (NICE) and the Centers for Disease Control and prevention (CDC) define long COVID as symptoms and sequalae that persist or develop after the 4-week acute phase of COVID-19 and that cannot be explained by an alternative diagnosis (7, 8). This term includes ongoing symptomatic COVID-19, which encompasses manifestations from 4 to 12 weeks post-infection, and post-COVID-19 syndrome, which refers to symptoms/clinical signs beyond 12 weeks following infection (8). The pathogenic process of this recently reported condition has not been elucidated; yet potential contributing mechanisms include viral toxicity inducing ACE2 downregulation (due to cellular internalization) thus leading to a pro-fibrotic microenvironment as well as immune dysregulation with emergence of autoimmunity phenomena leading to persistent inflammatory damage (9). Post-COVID clinical manifestations are multisystemic and the lasting symptom burden may lead to severe functional limitation and decrement in quality of life (9). Pulmonary sequalae range in a wide clinical, physiologic, and imaging spectrum and have been related to the severity of acute illness (9–11). The most commonly reported pulmonary manifestations are diffusion capacity decline, restrictive pattern with regards to functional impairment and ground glass opacities with or without fibrotic lesions in the context of radiographic signs (12, 13).

Meticulous radiographic evaluation might have a cardinal role for the guidance of therapeutic approaches in this new entity with unknown long-term effects. Toward this direction, we performed patients' radiographic evaluation through a validated machine learning software system, denominated Imbio Lung Texture Analysis (14). Machine learning represents a subgroup of artificial intelligence. In this setting, computers extract patterns from appropriately classified input data and accordingly generate labels for new, unknown data (15). Machine learning and its subset named deep learning have demonstrated great potential in multiple medical imaging classification tasks including prediction of mortality in Idiopathic Pulmonary Fibrosis (16–20).

This multicenter study aimed to present functional and radiographic features of patients with post-COVID-19-interstitial lung disease (ILD), in a quantitative, precise and unbiased fashion using cutting-edge technology, highlighting the importance of screening patients with long-COVID-19 clinical, and functional and radiological impairment.

Materials and methods

Trial design and oversight

In this multicenter, investigator-initiated, retrospective, observational cohort study, we included consecutive patients that had been evaluated 3 months following severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection between 01/02/2021 and 12/05/2022. Trial sites were five referral ILD centers in Greece. Patients with positive polymerase chain reaction test for SARS-CoV-2, treated both in outpatient and inpatient setting were included. Patients with less than 18 years of age were excluded from the analysis.

We recorded PaO_2/FiO_2 during hospitalization, demographics, comorbidities, as well as forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1), diffusing capacity of the lung for carbon monoxide (DLCO) and High-Resolution Computed

TABLE 1 Baseline characteristics of patients enrolled in the study.

Characteristics	(N%)
Total number of patients	232
Age (median% 95 CI)	61.0 (58.0–63.0)
Male/female	160 (68.9%)/72 (31.1%)
Current/ever/never smokers	30 (12.9%)/84 (36.3%)/118 (50.8%)
Hospitalization for COVID-19	178 (76.7%)
Vaccination against COVID-19	6 (2.6%)
Arterial hypertension	88 (37.9%)
Dyslipidemia	78 (33.6%)
Diabetes mellitus	55 (23.7%)
Thyroid disorders	24 (10.3%)
COPD	15 (6.4%)
Depression/anxiety	15 (6.4%)
Gastroesophageal reflux disease	15 (6.4%)
Asthma	13 (5.6%)
History of myocardial infarction	11 (4.7%)
Pre-existing ILD	8 (3.4%)
Cancer	8 (3.4%)
Atrial fibrillation	7 (3.0%)
Congestive heart failure	6 (2.5%)
Chronic kidney disease	5 (2.2%)
OSAS	3 (1.3%)
Pulmonary hypertension	2 (0.8%)

CI, confidence interval; COPD, chronic obstructive pulmonary disease; ILD, interstitial lung disease; OSAS, obstructive sleep apnea syndrome.

TABLE 2 Functional and radiographic findings 3 months following hospitalization.

Parameter	Value
FVC% predicted (±SD)	89.3 (±18.8)
FEV1% predicted (±SD)	90.5 (±19.5)
DLCO% predicted (±SD)	85.8 (±27.3)
Hyperlucent% (±SD)	2.1 (±0.43)
Reticular% (±SD)	3.7 (±0.55)
Ground glass% (±SD)	8.9 (±1.0)
Honeycombing% (±SD)	0.36 (±0.22)

DLCO, diffusing capacity of lung for carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; SD, standard deviation.

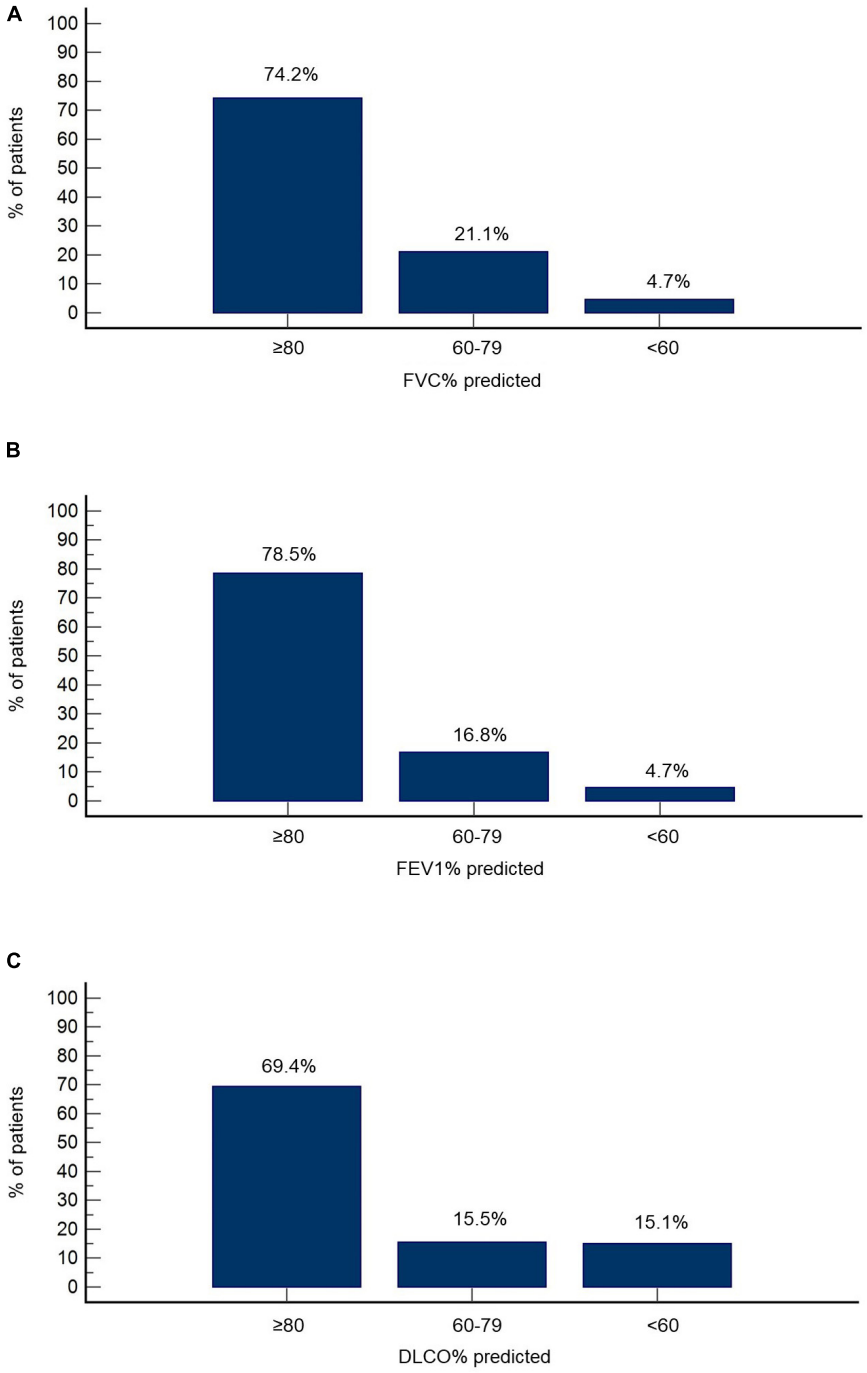

FIGURE 1
Functional features at the 3-month follow-up. Percentage of patients with reduced FVC% predicted **(A)**, FEV1% predicted **(B)**, and DLCO% predicted **(C)** is presented.

Tomography (HRCT) findings 3 months following COVID-19 infection.

High-Resolution Computed Tomography scans were evaluated through the validated machine learning software, named Imbio Lung Texture Analysis version 2.1 (14). Imbio Lung Texture Analysis had the following technical requirements for HRCT analysis: Minimal movement and acceptable position, slice thickness <2.0 mm, revolution time <1 s, pixel spacing <2.0 mm, and slice spacing <2.0 mm. Subsequently, Imbio Lung Texture Analysis version 2.1 provided a report with% of each lobe and % of lung as total that was characterized as: Normal, ground glass, reticular, honeycombing or hyperlucent. A succinct rating scale for % disease extent was applied, providing 1% step evaluation for disease extent.

The trial was conducted in accordance with the International Conference on Harmonization E6 guidelines for Good Clinical Practice, the Declaration of Helsinki and the local regulations. Management was based on a common algorithm. Radiographic findings of the patients were initially meticulously evaluated and split into two groups: (1) Fibrotic-like lesions and (2) inflammatory abnormalities only. Subsequently, patients were further divided based

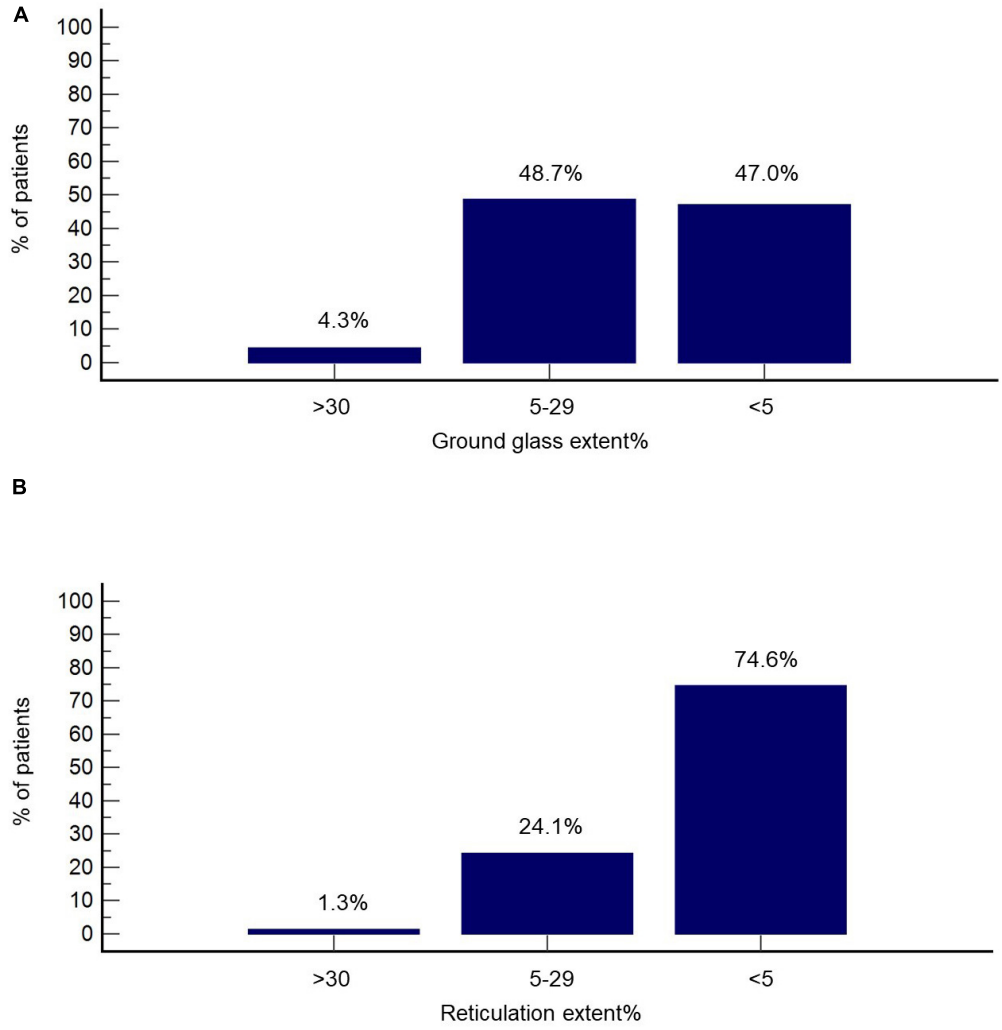

FIGURE 2
Radiographic features at the 3-month follow-up based on Imbio Lung Texture Analysis 2.1. Percentage of patients with% extent ground glass **(A)** and % extent reticulation **(B)** above 30, between 5 and 29, as well as below 5 is presented.

on their functional status. Retrospective data collection and analysis was approved by our institutional review board (protocol number: 16574/29-6-2022).

Outcome measures

Outcome measures included: (1) The frequency of functional impairment as indicated by decline in FVC% predicted, FEV1% predicted or DLCO% predicted, (2) the frequency of specific radiographic findings following Imbio Lung Texture Analysis, and (3) the effectiveness of antifibrotics as indicated by change in FVC% predicted, DLCO% predicted at 3, 6, and 12 months following treatment initiation and changes in HRCT.

Statistical analysis

Continuous data were reported as mean ± standard deviation (SD) or medians with 95% Confidence Interval (95% CI) following Kolmogorov–Smirnov test for normality. Frequency tables and graphs were drawn. ANOVA repeated measures was used to investigate differences in FVC% predicted and DLCO% predicted at different time points. *P*-values < 0.05 were considered statistically significant.

Results

Baseline characteristics

Two hundred thirty-two (n = 232) patients were included in the analysis. Baseline characteristics are summarized in Table 1. Median age (95% CI) was 61.0 (58.0–63.0) years. Most patients were male (n = 160, 68.9%), while the most common comorbid condition was arterial hypertension (n = 88, 37.9%). Six patients (2.6%) had been vaccinated against COVID-19 prior hospitalization. One hundred seventy-eight (n = 178, 76.7%) patients reported history of hospitalization for COVID-19 and 54 (23.3%) patients were treated as outpatients. With regards to hospitalized patients, median value of the worst PaO_2/FiO_2 (95% CI) during hospitalization was 160.0 (130.2–180.0).

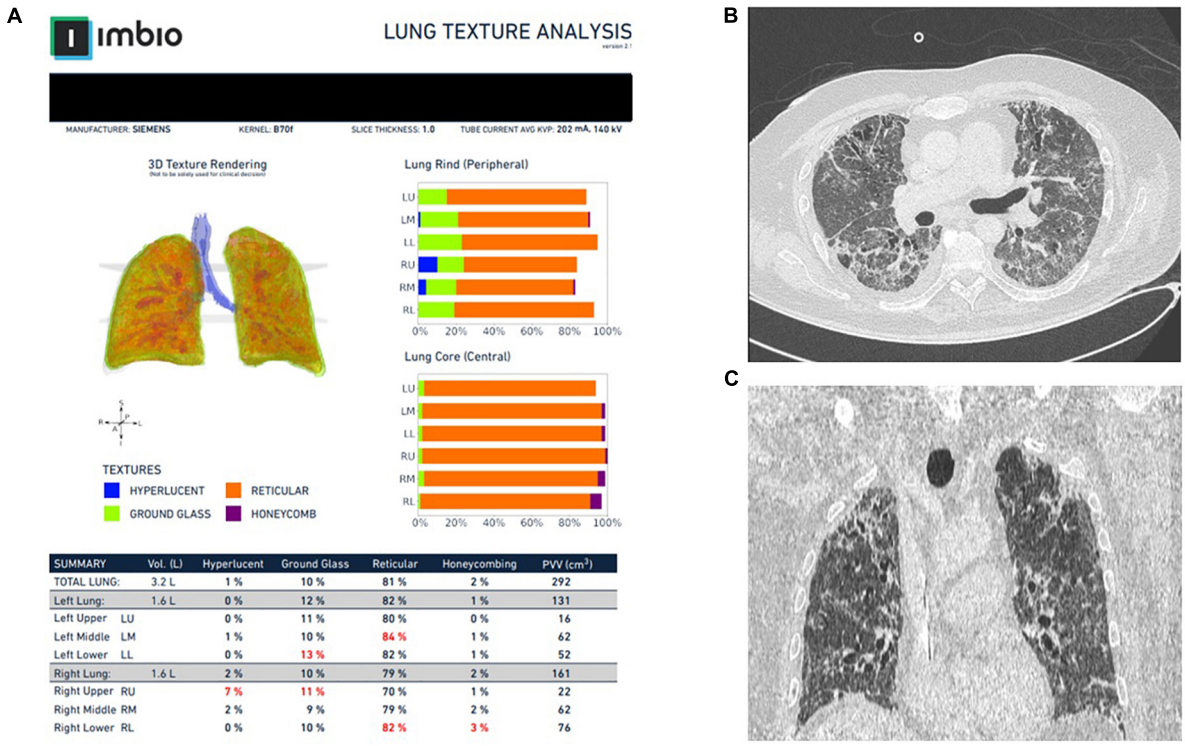

FIGURE 3
Representative images from Imbio Lung Texture Analysis 2.1 of a patient with post-COVID-19-ILD **(A)**. Notice that almost 80% of the lung parenchyma presents with reticular abnormalities that are inconspicuous (at least to that extent) to the bear eye of the operator **(B,C)**.

Three-month follow-up

Functional and radiographic features at the 3-month follow-up are summarized in Table 2. FVC% predicted was ≥80, between 60 and 79 and <60 in 74.2% (n = 172), 21.1% (n = 49), and 4.7% (n = 11) of the cohort, respectively (Figure 1A). FEV1% predicted was ≥80 in 78.5% (n = 182), 60–79 in 16.8% (n = 39) and <60 in 4.7% (n = 11) of patients (Figure 1B). DLCO% predicted was ≥80, between 60 and 79 and <60 in 69.4% (n = 161), 15.5% (n = 36), and 15.1% (n = 35), respectively (Figure 1C).

Imbio Lung Texture Analysis 2.1 demonstrated extent of ground glass opacities ≥30% in 4.3% of patients (n = 10), extent between 5 and 29% in 48.7% of patients (n = 113) and <5% in 47.0% of patients (n = 109) (Figure 2A). Accordingly, the extent of reticulation was ≥30, 5–29, and <5% in 1.3% (n = 3), 24.1% (n = 56), and 74.6% (n = 173) of the cohort, respectively (Figure 2B). Honeycombing was identified in 14 patients (6.0%). Representative images from Imbio Lung Texture Analysis 2.1 are presented in Figure 3.

Post-COVID-19 interstitial lung disease

Management of patients with post-COVID-19 interstitial lung disease was based on a common algorithm as shown in Figure 4A. Patients with reticulation >5% or honeycombing (n = 71, 30.6%) were meticulously evaluated at 6-months. Among them, patients with either of the following: FVC% predicted <70, DLCO% predicted <50 or 6-min walking distance <350 m (n = 16, 6.9%) had been offered a 6-week course of oral prednisolone with gradual tapering at the time point of the 3-month follow-up visit. The rest patients were meticulously evaluated as shown in Figure 4B and to this end none of them presented with disease progression.

At the time point of the 6-month follow-up visit, 13 patients (5.6%) presented with evidence of both "fibrotic-like" abnormalities and persistently decreased functional indices as shown in Figure 4B. Of note, none of these patients was vaccinated against COVID-19 (0/13, 0%). In these patients, antifibrotics were implemented. Antifibrotics were chosen based on patients' comorbidities and preferences following discussion for the potential adverse events of each compound (pirfenidone: 10, nintedanib: 3). A statistically significant improvement was observed with regards to ground glass opacities in the follow-up HRCT 6 months after initiation of antifibrotics [25.0 (95% CI: 6.7–27.9) vs. 5.0 (95% CI: 0.5–8.8), p = 0.04]. A trend for improvement of reticular opacities was also observed [8.0 (95% CI: 2.5–15.0) vs. 2.0 (95% CI: 1.0–8.9), p = 0.13]. Patients presented with an improvement (absolute change) of 10.3 (p = 0.01) and 14.6 (p = 0.01) in FVC% predicted at 3 and 6 months after the initiation of antifibrotic (Figure 5A). An absolute change of +8.6 (p = 0.0003) and +15.1 (p = 0.002) was observed with regards to DLCO% predicted (Figure 6A). Subgroup analysis of patients with 1-year follow-up is presented in Figures 5, 6B.

Discussion

This is the first study in Caucasian population aiming to investigate post-COVID-19-ILD through a machine learning radiographic model. Our report presented in detail the functional and radiographic impact of this entity and validated previous evidence showing that a considerable proportion of patients is experiencing

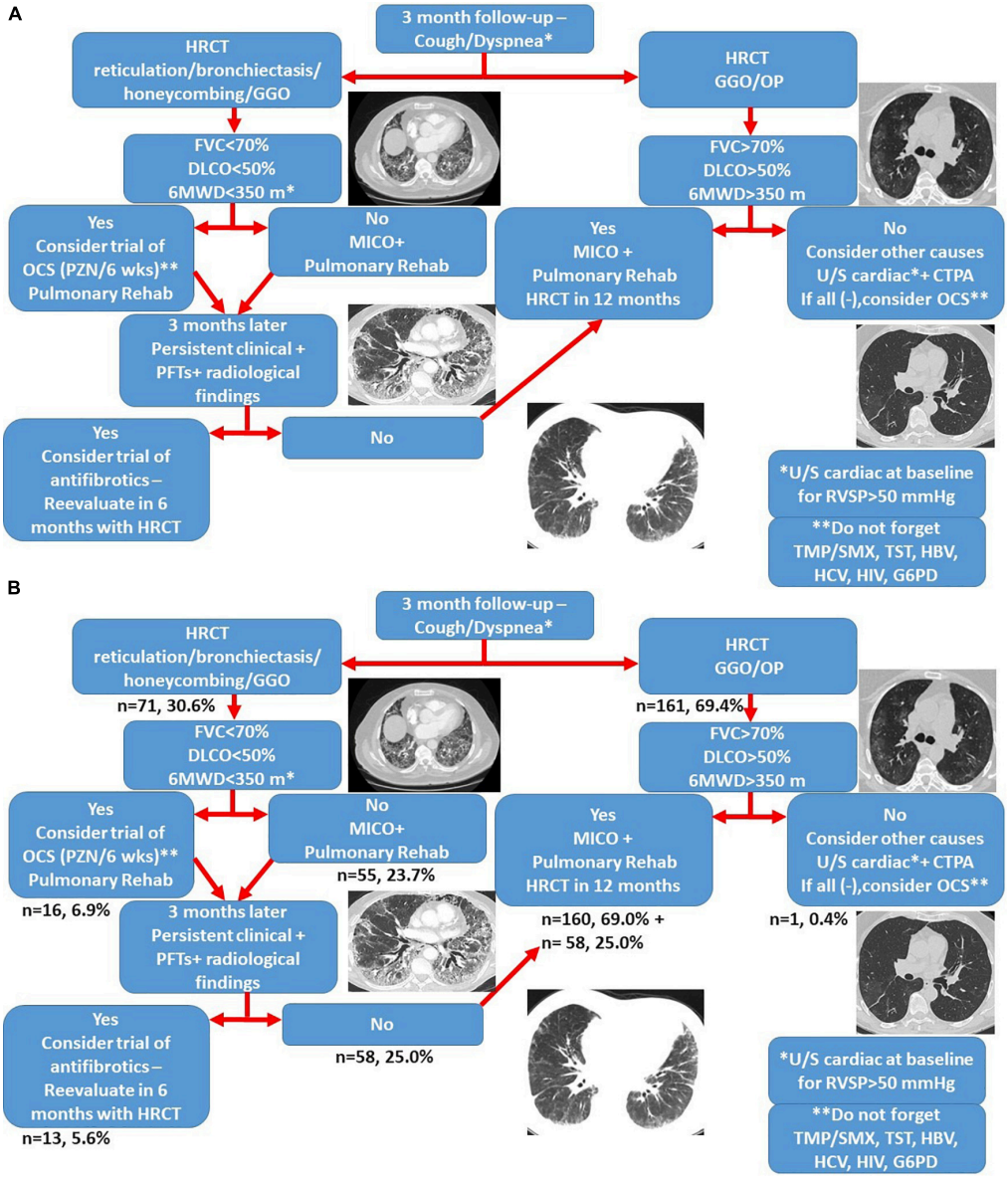

FIGURE 4
Treatment algorithm for patients with post-COVID-19 interstitial lung disease **(A)**. The proportion of patients that belongs to each group is shown in panel **(B)**. CTPA, computed tomography pulmonary angiogram; GGO, ground glass opacities; G6PD, glucose-6-phosphate dehydrogenase; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MICO, masterful inactivity with cat-like observation; OCS, oral corticosteroids; OP, organizing pneumonia; TMP/SMX, trimethoprim-sulfamethoxazole; TST, tuberculin skin test; 6MWD: 6 min walking distance.

functional impairment several months following infection. Fibrotic lung disease was observed in a substantial minority of COVID-19 survivors. These patients might experience benefit from antifibrotic initiation at the time point that fibrotic-like changes are "immature."

Our study exhibited a number of important attributes that should be presented. First, we evaluated disease extent through a machine learning model. Manual interpretation of radiographic abnormalities extent is hindered by variability, especially at centers with lack of expertise (17). Deep learning might be the key to overcome barriers for fast and massive radiological evaluation in an unbiased fashion. Deep learning could be deployed all over the world and provide homogeneous and accurate reporting (21–23). Second, we demonstrated that a short course of antifibrotics could significantly improve lung function. Third, based on the fact that the majority of our cohort was unvaccinated against COVID-19, we provided indirect evidence that vaccination against COVID-19 is not only the best way to contain the pandemic but also the best way to limit post-acute sequelae of COVID-19.

Percentage of patients with functional and radiographic impairment is in line from data of other countries (24–32). In the 3-month follow-up, almost 30% of this cohort presented with DLCO% predicted below 80. A recent, large study in China showed in the 6-month follow-up that DLCO% predicted was below 80 in 21% of patients that were not in need of oxygen supplementation and in 57% of hospitalized patients that presented with WHO ordinal scale of 5 or 6 (32). Reticulation >5% was observed in almost one out of four patients in this study. Other studies reported that fibrotic like changes ranged from 22.5 to 35% in the 6-month follow-up (26, 30). Importantly, we observed fibrotic lung disease and substantial functional impairment in 5.6% of this cohort. Previous studies

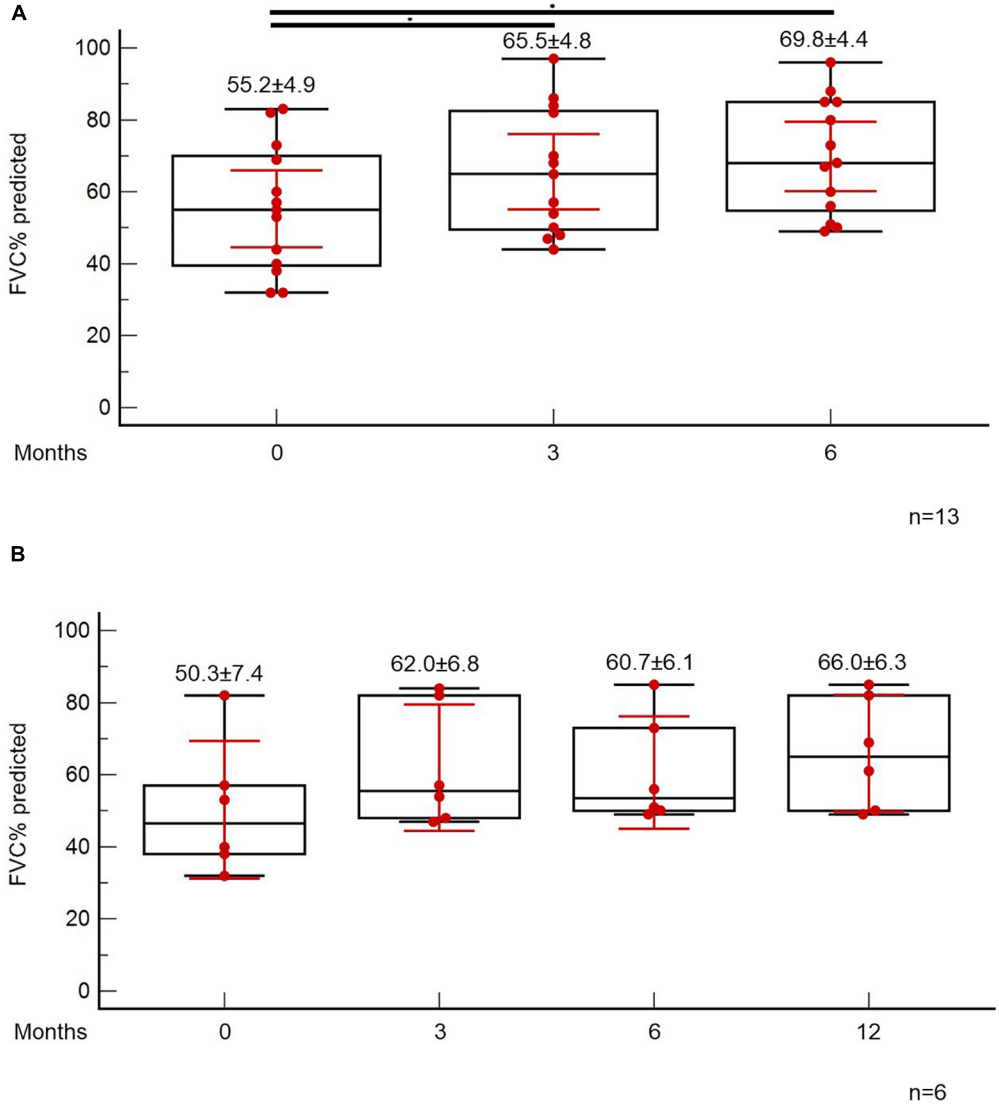

FIGURE 5
Patients with fibrotic-like changes and persistently decreased functional indices received antifibrotics. ANOVA repeated measures was used to investigate differences in FVC% predicted at different time points **(A)**. Subgroup analysis of patients with 1-year follow-up is presented in panel **(B)**.

reported significant functional deficit in almost 5% of COVID-19 survivors, while the prevalence of the so called post-COVID-19 interstitial lung damage was estimated between 6.5 and 8.3% (24, 25). Suggested risk factors for post-COVID-19 fibrotic lung disease have been male gender, increased age, increased body mass index, duration of hospitalization >17 days, disease severity, extent of baseline radiographic lesions, intensity of ventilatory support, persistent viremia and Epstein–Barr reactivation, diabetes mellitus, and presence of auto-antibodies (32–38).

With regards to treatment of post-COVID-19-ILD, a minority of our patients received oral corticosteroids. Percentage of treated patients in this cohort is comparable to a previously published large study reporting that corticosteroids were suggested initially in 35/837 COVID-19 survivors and finally prescribed in 3.6% (n = 30) of patients (24). Importantly, high doses of prednisolone (40 mg tapered) were not superior to lower doses (10 mg tapered) in patients with post-COVID-19-ILD (39). In the context of "fibrotic-like" changes, sequelae consistent with "fibrotic-like" parenchymal lung disease has been observed following COVID-19 infection in a minority of patients and importantly these changes seem to lack of resolution in a considerable proportion of cases (40); yet, estimates must be interpreted cautiously due to substantial heterogeneity, differences in study casemix, and baseline severity among studies (41). Antifibrotics were prescribed in a substantial minority of this cohort. Pirfenidone was administered more often, as a considerable proportion of patients had history of pulmonary embolism following COVID-19 infection or was receiving anticoagulants due to chronic heart disease. While high quality trials aiming to address their role in post-COVID-19 interstitial lung disease are greatly anticipated (42–44), observational studies have suggested that antifibrotics might confer benefit (45, 46) with regards to radiologic improvement and time to recovery in patients with sustained and extensive fibrotic changes. The concept of antifibrotics implementation in a minority of patients at the time point that fibrotic-like changes are "immature" deserves further investigation (47). It is a matter of ongoing debate if all abnormalities

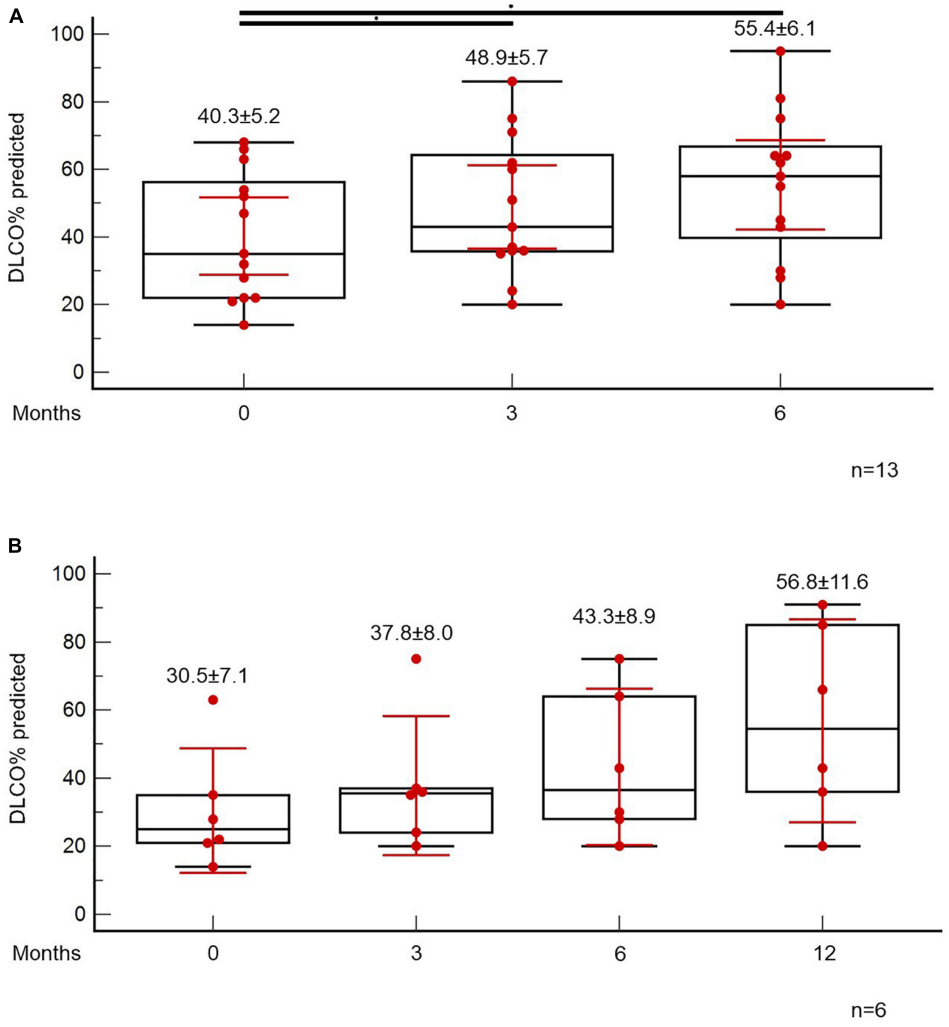

FIGURE 6
Patients with fibrotic-like changes and persistently decreased functional indices received antifibrotics. ANOVA repeated measures was used to investigate differences in DLCO% predicted at different time points **(A)**. Subgroup analysis of patients with 1-year follow-up is presented in panel **(B)**.

characterized as "fibrotic-like" can reliably indicate irreversible disease in a post-Acute Respiratory Distress Syndrome setting (48, 49). Eminent fibrotic-like changes and architectural distortion on HRCT is not necessarily synonymous with irreversible lung scarring, particularly in the context of a post-infectious syndrome (50). Similarly, bronchiectatic-like lesions during the acute phase of a respiratory infection may not necessarily represent irreversible and permanent enlargement of the airways but may largely resolve following resolution of the infection. Even if "fibrotic-like" changes on HRCT represent histologic fibrosis in a subgroup of cases, remodeling and regression of "immature" fibrosis represents an anticipated phenomenon following severe acute lung injury (49–51). In such cases, antifibrotics might be beneficial. Machine learning models might be helpful for the appropriate classification and selection of patients likely to benefit from antifibrotics as they may quantify in an unbiased and accurate fashion the extent and the type of lesions.

Our trial has some limitations. First, our report has the inherent weaknesses of a retrospective study. Second, follow-up period of patients under antifibrotics is relatively short given that post-COVID-19-ILD is a new entity; yet, even in this short period, our study provided evidence that antifibrotics might confer significant benefit if applied early during the post-COVID-19 syndrome when fibrotic-like changes appear to be "immature." Third, a major limitation is that this cohort has not undergone HRCT prior SARS-CoV-2 infection to compare pre and post-infection radiographic abnormalities and identify a subset of patients with pre-existing ILD patterns. Nonetheless, the percentage of patients with functional and radiographic impairment is similar to other published reports and importantly our aim was to highlight the need of early detection and management of interstitial lung disease. Moreover, based on current guidelines, HRCT during infection was not performed in all cases, while PFTs could not be performed at the acute phase due to safety considerations. Thus, we could not compare radiographic and functional status between the acute phase and the 3-month follow-up. Finally, based on the fact we used a common algorithm and treated similarly all cases of same severity, we could not compare patients that receive antifibrotics with other patients of same phenotype that didn't. Besides, this was not a randomized-controlled trial. This was a real-life study aiming to present outcomes following management with a common algorithm and using a highly novel machine learning radiographic model.

Collectively, post-COVID-19-ILD represents an emerging entity. Given that a considerable proportion of infected individuals has functional impairment several months after infection, screening 3 months following infection is encouraged, especially for severe cases that have been hospitalized for prolonged periods. On the other hand, post-COVID-19 HRCT screening may confer several other benefits, including identification of incidental lung cancer lesions, considering that the majority of patients screened for post-COVID-19 present with several risk factors for lung cancer, such as age, smoking status and comorbidities, and including chronic respiratory diseases. Current evidence does not support irrational use of corticosteroids; yet, short-term corticosteroids might confer benefit to specific patients with organizing pneumonia, functional impairment and persistent clinical symptoms without compromising patients' immune status. Finally, a minority of these patients with persistent fibrotic lung lesions and functional disability, despite steroid treatment, could benefit from antifibrotic therapy, if applied early on during disease clinical course. Machine learning radiographic models might have a cardinal role for precise, unbiased and quantitative radiographic evaluation which may guide therapeutic decisions.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the International Conference on Harmonization E6 guidelines for Good Clinical Practice, the Declaration of Helsinki and the local regulation. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

TK and VS: conception and design of the work, acquisition, analysis and interpretation of data, and drafting the work and revising it critically for important intellectual content. MK, PT, VG, IP, EB, IT, IL, OP, EZ, EA, AP, EM, FS, SM, SC, GH, KD, NT, NS, and KA: contributions to the acquisition of data and critical revision for important intellectual content. AT: conception and design of the work, acquisition, analysis and interpretation of data, drafting the work and revising it critically for important intellectual content, formal analysis, and project administration and supervision. All authors final approval for publication of the content, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Funding

The publication fees of this manuscript have been financed by the Research Council of the University of Patras.

Conflict of interest

AT has received grants and honoraria from GlaxoSmithKline, Astra Zeneca, Chiesi, Roche, and Boehringer Ingelheim outside the submitted work.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer CB declared a shared affiliation, with no collaboration, with the authors to the handling editor at the time of the review.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Guan W, Ni Z, Hu Y, Liang W, Ou C, He J, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med.* (2020) 382:1708–20. doi: 10.1056/NEJMoa2002032

2. Karampitsakos, T, Papaioannou O, Tsiri P, Katsaras M, Katsimpris A, Kalogeropoulos A, et al. Tocilizumab versus baricitinib in hospitalized patients with severe COVID-19: an open label, randomized controlled trial. *Clin Microbiol Infect.* (2022). doi: 10.1101/2022.06.13.22276211 [Epub ahead of print].

3. Karampitsakos T, Malakounidou E, Papaioannou O, Dimakopoulou V, Zarkadi E, Katsaras M, et al. Tocilizumab improves 28-day survival in hospitalized patients with severe COVID-19: an open label, prospective study. *Respir Res.* (2021) 22:317. doi: 10.1186/s12931-021-01914-6

4. Vehar S, Boushra M, Ntiamoah P, Biehl M. Post-acute sequelae of SARS-CoV-2 infection: caring for the 'long-haulers'. *Cleve Clin J Med.* (2021) 88:267–72. doi: 10.3949/ccjm.88a.21010

5. Chen C, Haupert S, Zimmermann L, Shi X, Fritsche L, Mukherjee B. Global prevalence of post COVID-19 condition or long COVID: a meta-analysis and systematic review. *J Infect Dis.* (2022) 226:1593–607. doi: 10.1093/infdis/jiac136

6. Brodin P, Casari G, Townsend L, O'Farrelly C, Tancevski I, Löffler-Ragg J, et al. Studying severe long COVID to understand post-infectious disorders beyond COVID-19. *Nat Med.* (2022) 28:879–82. doi: 10.1038/s41591-022-01766-7

7. Datta S, Talwar A, Lee JTA. Proposed framework and timeline of the spectrum of disease due to SARS-CoV-2 infection: illness beyond acute infection and public health implications. *JAMA.* (2020) 324:2251–2. doi: 10.1001/jama.2020.22717

8. Venkatesan P. NICE guideline on long COVID. *Lancet Respir Med.* (2021) 9:129. doi: 10.1016/S2213-2600(21)00031-X

9. Nalbandian A, Sehgal K, Gupta A, Madhavan M, McGroder C, Stevens J, et al. Post-acute COVID-19 syndrome. *Nat Med.* (2021) 27:601–15. doi: 10.1038/s41591-021-01283-z

10. Culebras M, Loor K, Sansano I, Persiva Ó, Clofent D, Polverino E, et al. Histological findings in transbronchial cryobiopsies obtained from patients after COVID-19. *Chest.* (2022) 161:647–50. doi: 10.1016/j.chest.2021.09.016

11. Tourki B, Vera I, Perrot C, Farsaei F, Arsenault A, Juan-Guardela B, et al. Peripheral blood gene expression changes predict mortality and treatment response in acute Covid-19 and associate with lung fibrosis. C32 interrogation of genetic variation, gene expression, and other biological layers with omics techniques to understand respiratory traits. *Am J Respir Crit Care Med.* (2022) 205:A3929. doi: 10.1164/ajrccm-conference.2022.205.1_MeetingAbstracts.A3929

12. Boutou A, Asimakos A, Kortianou E, Vogiatzis I, Tzouvelekis A. Long COVID-19 pulmonary sequelae and management considerations. *J Pers Med.* (2021) 11:838. doi: 10.3390/jpm11090838

13. Boutou A, Georgopoulou A, Pitsiou G, Stanopoulos I, Kontakiotis T, Kioumis I. Changes in the respiratory function of COVID-19 survivors during follow-up: a novel respiratory disorder on the rise? *Int J Clin Pract.* (2021) 75:e14301. doi: 10.1111/ijcp.14301

14. Karampitsakos T, Kalogeropoulou C, Tzilas V, Papaioannou O, Kazantzi A, Koukaki E, et al. Safety and effectiveness of mycophenolate mofetil in interstitial lung diseases: insights from a machine learning radiographic model. *Respiration.* (2022) 101:262–71. doi: 10.1159/000519215

15. Levin D. Deep learning and the evaluation of pulmonary fibrosis. *Lancet Respir Med.* (2018) 6:803–5. doi: 10.1016/S2213-2600(18)30371-0

16. González G, Ash S. Disease staging and prognosis in smokers using deep learning in chest computed tomography. *Am J Respir Crit Care Med.* (2018) 197:193–203. doi: 10.1164/rccm.201705-0860OC

17. Walsh S, Calandriello L, Silva M, Sverzellati N. Deep learning for classifying fibrotic lung disease on high-resolution computed tomography: a case-cohort study. *Lancet Respir Med.* (2018) 6:837–45. doi: 10.1016/S2213-2600(18)30286-8

18. Salisbury M, Lynch D, van Beek E, Kazerooni E, Guo J, Xia M, et al. Idiopathic pulmonary fibrosis: the association between the adaptive multiple features method and fibrosis outcomes. *Am J Respir Crit Care Med.* (2017) 195:921–9. doi: 10.1164/rccm.201607-1385OC

19. Maldonado F, Moua T, Rajagopalan S, Karwoski R, Raghunath S, Decker P, et al. Automated quantification of radiological patterns predicts survival in idiopathic pulmonary fibrosis. *Eur Respir J.* (2014) 43:204–12. doi: 10.1183/09031936.00071812

20. Trusculescu A, Manolescu D, Tudorache E, Oancea C. Deep learning in interstitial lung disease—how long until daily practice. *Eur Radiol.* (2020) 30:6285–92. doi: 10.1007/s00330-020-06986-4

21. Lynch D, Sverzellati N, Travis W, Brown K, Colby T, Galvin J, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner society white paper. *Lancet Respir Med.* (2018) 6:138–53. doi: 10.1016/S2213-2600(17)30433-2

22. Raghu GR-JM, Myers J, Richeldi L, Ryerson C, Lederer D, Behr J, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ATS/ERS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med.* (2018) 198:e44–68. doi: 10.1164/rccm.201807-1255ST

23. Raghu G, Remy-Jardin M, Ryerson C, Myers J, Kreuter M, Vasakova M, et al. Diagnosis of hypersensitivity pneumonitis in adults. An official ATS/JRS/ALAT clinical practice guideline. *Am J Respir Crit Care Med.* (2020) 202:e36–69.

24. Myall K, Mukherjee B, Castanheira A, Lam J, Benedetti G, Mak S, et al. Persistent post-COVID-19 interstitial lung disease. An observational study of corticosteroid treatment. *Ann Am Thorac Soc.* (2021) 18:799–806. doi: 10.1513/AnnalsATS.202008-1002OC

25. Stewart I, Jacob J, George P, Molyneaux P, Porter J, Allen R, et al. Interstitial lung damage following COVID-19 hospitalisation: an interim analysis of the UKILD Post-COVID study. *medRxiv* [Preprint]. (2022). doi: 10.1101/2022.03.10.22272081

26. Tomassetti S, Oggionni T, Barisione E, Bargagli E, Bonifazi M, Confalonieri M, et al. A multidisciplinary multicenter study evaluating risk factors, prevalence and characteristics of post-Covid-19 interstitial lung syndrome (PCOILS). *Eur Respir J.* (2021) 58(Suppl. 65):OA1567. doi: 10.1183/13993003.congress-2021.OA1567

27. Ravaglia C, Doglioni C, Chilosi M, Piciucchi S, Dubini A, Rossi G, et al. Clinical, radiological and pathological findings in patients with persistent lung disease following SARS-CoV-2 infection. *Eur Respir J.* (2022) 60:2102411. doi: 10.1183/13993003.02411-2021

28. Cau R, Faa G, Nardi V, Balestrieri A, Puig J, Suri J, et al. Long-COVID diagnosis: from diagnostic to advanced AI-driven models. *Eur J Radiol.* (2022) 148:110164. doi: 10.1016/j.ejrad.2022.110164

29. Tarraso J, Safont B, Carbonell-Asins J, Fernandez-Fabrellas E, Sancho-Chust J, Naval E, et al. Lung function and radiological findings 1 year after COVID-19: a prospective follow-up. *Respir Res.* (2022) 23:242. doi: 10.1186/s12931-022-02166-8

30. Han X, Fan Y, Alwalid O, Li N, Jia X, Yuan M, et al. Six-month follow-up chest CT findings after severe COVID-19 pneumonia. *Radiology.* (2021) 299:E177–86. doi: 10.1148/radiol.2021203153

31. Caruso D, Guido G, Zerunian M, Polidori T, Lucertini E, Pucciarelli F, et al. Post-acute sequelae of COVID-19 pneumonia: six-month chest CT follow-up. *Radiology.* (2021) 301:E396–405. doi: 10.1148/radiol.2021210834

32. Huang L, Yao Q, Gu X, Wang Q, Ren L, Wang Y, et al. 1-year outcomes in hospital survivors with COVID-19: a longitudinal cohort study. *Lancet.* (2021) 398:747–58. doi: 10.1016/S0140-6736(21)01755-4

33. Wallis T, Heiden E, Horno J, Welham B, Burke H, Freeman A, et al. Risk factors for persistent abnormality on chest radiographs at 12-weeks post hospitalisation with PCR confirmed COVID-19. *Respir Res.* (2021) 22:157. doi: 10.1186/s12931-021-01750-8

34. Ntatsoulis K, Karampitsakos T, Tsitoura E, Stylianaki E, Matralis A, Tzouvelekis A, et al. Commonalities between ARDS. pulmonary fibrosis and COVID-19: the potential of autotaxin as a therapeutic target. *Front Immunol.* (2021) 12:687397. doi: 10.3389/fimmu.2021.687397

35. Lee J, Yim J, Park J. Pulmonary function and chest computed tomography abnormalities 6–12 months after recovery from COVID-19: a systematic review and meta-analysis. *Respir Res.* (2022) 23:233. doi: 10.1186/s12931-022-02163-x

36. Valenzuela C, Waterer G, Raghu G. Interstitial lung disease before and after COVID-19: a double threat? *Eur Respir J.* (2021) 58:2101956. doi: 10.1183/13993003.01956-2021

37. Cocconcelli E, Bernardinello N, Giraudo C, Castelli G, Giorgino A, Leoni D, et al. Characteristics and prognostic factors of pulmonary fibrosis after COVID-19 pneumonia. *Front Med.* (2022) 8:823600. doi: 10.3389/fmed.2021.823600

38. Su Y, Yuan D, Chen D, Ng R, Wang K, Choi J, et al. Multiple early factors anticipate post-acute COVID-19 sequelae. *Cell.* (2022) 185:881–95.e20.

39. Dhooria S, Chaudhary S, Sehgal I, Agarwal R, Arora S, Garg M, et al. High-dose versus low-dose prednisolone in symptomatic patients with post-COVID-19 diffuse parenchymal lung abnormalities: an open-label, randomised trial (the COLDSTER trial). *Eur Respir J.* (2022) 59:2102930. doi: 10.1183/13993003.02930-2021

40. Han X, Fan Y, Alwalid O, Zhang X, Jia X, Zheng Y, et al. Fibrotic interstitial lung abnormalities at 1-year follow-up CT after severe COVID-19. *Radiology.* (2021) 301:E438–40. doi: 10.1148/radiol.2021210972

41. Fabbri L, Moss S, Khan F, Chi W, Xia J, Robinson K, et al. Parenchymal lung abnormalities following hospitalisation for COVID-19 and viral pneumonitis: a systematic review and meta-analysis. *Thorax.* (2022). doi: 10.1101/2021.03.15.21253593 [Epub ahead of print].

42. U.S. National Library of Medicine. *Pirfenidone vs. Nintedanib for Fibrotic Lung Disease After Coronavirus Disease-19 Pneumonia (PINCER)*. Available online at: https://clinicaltrials.gov/ct2/show/NCT04856111

43. U.S. National Library of Medicine. *Nintedanib for the Treatment of SARS-Cov-2 Induced Pulmonary Fibrosis (NINTECOR)*. Available online at: https://clinicaltrials.gov/ct2/show/NCT04541680

44. U.S. National Library of Medicine. *Pirfenidone Compared to Placebo in Post-COVID19 Pulmonary Fibrosis COVID-19 (FIBRO-COVID)*. Available online at: https://clinicaltrials.gov/ct2/show/NCT04607928

45. Singh P, Behera D, Gupta S, Deep A, Priyadarshini S, Padhan P. Nintedanib vs pirfenidone in the management of COVID-19 lung fibrosis: a single-centre study. *J R Coll Physicians Edinb.* (2022) 52:100–4. doi: 10.1177/14782715221103402

46. Umemura Y, Mitsuyama Y, Minami K, Nishida T, Watanabe A, Okada N, et al. Efficacy and safety of nintedanib for pulmonary fibrosis in severe pneumonia induced by COVID-19: an interventional study. *Int J Infect Dis.* (2021) 108:454–60. doi: 10.1016/j.ijid.2021.05.055

47. Hashemian, S, Farhadi T, Varahram M, Velayati A. Nintedanib: a review of the properties, function, and usefulness to minimize COVID-19 induced lung injury. *Expert Rev Anti Infect Ther.* (2022) 1–8. doi: 10.1080/14787210.2023.2153116 [Epub ahead of print].

48. Spagnolo P, Balestro E, Aliberti S, Cocconcelli E, Biondini D, Casa G, et al. Pulmonary fibrosis secondary to COVID-19: a call to arms? *Lancet Respir Med.* (2020) 8:750–2. doi: 10.1016/S2213-2600(20)30222-8

49. Wells A, Devaraj A, Desai S. Interstitial lung disease after COVID-19 infection: a catalog of uncertainties. *Radiology.* (2021) 299:E216–8. doi: 10.1148/radiol.2021204482

50. Wells A, Devaraj A. Residual lung disease at six-month follow-up CT after COVID-19: clinical significance is a key issue. *Radiology.* (2021) 301:E406–8. doi: 10.1148/radiol.2021211284

51. Barini M, Percivale I, Danna P, Longo V, Costantini P, Paladini A, et al. 18 months computed tomography follow-up after Covid-19 interstitial pneumonia. *J Public Health Res.* (2022) 11:2782. doi: 10.4081/jphr.2022.2782

frontiers | Frontiers in Medicine

TYPE Correction
PUBLISHED 13 April 2023
DOI 10.3389/fmed.2023.1194925

OPEN ACCESS

APPROVED BY
Frontiers Editorial Office,
Frontiers Media SA, Switzerland

*CORRESPONDENCE
Argyris Tzouvelekis
atzouvelekis@upatras.gr;
argyris.tzouvelekis@gmail.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 27 March 2023
ACCEPTED 28 March 2023
PUBLISHED 13 April 2023

CITATION
Karampitsakos T, Sotiropoulou V, Katsaras M, Tsiri P, Georgakopoulou VE, Papanikolaou IC, Bibaki E, Tomos I, Lambiri I, Papaioannou O, Zarkadi E, Antonakis E, Pandi A, Malakounidou E, Sampsonas F, Makrodimitri S, Chrysikos S, Hillas G, Dimakou K, Tzanakis N, Sipsas NV, Antoniou K and Tzouvelekis A (2023) Corrigendum: Post-COVID-19 interstitial lung disease: Insights from a machine learning radiographic model. *Front. Med.* 10:1194925. doi: 10.3389/fmed.2023.1194925

Corrigendum: Post-COVID-19 interstitial lung disease: Insights from a machine learning radiographic model

Theodoros Karampitsakos[1†], Vasilina Sotiropoulou[1†], Matthaios Katsaras[1], Panagiota Tsiri[1], Vasiliki E. Georgakopoulou[2], Ilias C. Papanikolaou[3], Eleni Bibaki[4], Ioannis Tomos[5], Irini Lambiri[4], Ourania Papaioannou[1], Eirini Zarkadi[1], Emmanouil Antonakis[3], Aggeliki Pandi[3], Elli Malakounidou[1], Fotios Sampsonas[1], Sotiria Makrodimitri[2], Serafeim Chrysikos[5], Georgios Hillas[5], Katerina Dimakou[5], Nikolaos Tzanakis[4], Nikolaos V. Sipsas[2,6], Katerina Antoniou[4] and Argyris Tzouvelekis[1*]

[1]Department of Respiratory Medicine, University General Hospital of Patras, Patras, Greece, [2]Department of Infectious Diseases-COVID-19 Unit, Laiko General Hospital, Athens, Greece, [3]Department of Respiratory Medicine, Corfu General Hospital, Corfu, Greece, [4]Laboratory of Molecular and Cellular Pneumonology, Department of Thoracic Medicine, Medical School, University of Crete, Heraklion, Greece, [5]5th Department of Respiratory Medicine, Hospital for Thoracic Diseases, 'SOTIRIA', Athens, Greece, [6]Medical School, National and Kapodistrian University of Athens, Zografou, Greece

KEYWORDS

post-COVID-19, long COVID, interstitial lung disease, antifibrotics, machine learning

A corrigendum on
Post-COVID-19 interstitial lung disease: Insights from a machine learning radiographic model

by Karampitsakos, T., Sotiropoulou, V., Katsaras, M., Tsiri, P., Georgakopoulou, V. E., Papanikolaou, I. C., Bibaki, E., Tomos, I., Lambiri, I., Papaioannou, O., Zarkadi, E., Antonakis, E., Pandi, A., Malakounidou, E., Sampsonas, F., Makrodimitri, S., Chrysikos, S., Hillas, G., Dimakou, K., Tzanakis, N., Sipsas, N. V., Antoniou, K., and Tzouvelekis, A. (2023). *Front. Med.* 9:1083264. doi: 10.3389/fmed.2022.1083264

In the published article, the Funding statement was not reported. The correct Funding statement appears below.

Funding

The publication fees of this manuscript have been financed by the Research Council of the University of Patras.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way. The original article has been updated.

Publisher's note

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 02 February 2023
DOI 10.3389/fmed.2023.1084002

OPEN ACCESS

EDITED BY
Jin Woo Song,
Asan Medical Center, Republic of Korea

REVIEWED BY
Nazia Chaudhuri,
Ulster University, United Kingdom
Rodrigo Torres-Castro,
University of Chile, Chile

*CORRESPONDENCE
Elisabetta Balestro
✉ elisabetta.balestro@aopd.veneto.it

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 29 October 2022
ACCEPTED 16 January 2023
PUBLISHED 02 February 2023

CITATION
Bernardinello N, Cocconcelli E, Giraudo C, Daverio M, Castelli G, Petrarulo S, Bovo M, Fichera G, Cavinato S, Cattelan AM, Saetta M, Spagnolo P and Balestro E (2023) Predictors of pulmonary sequelae after COVID-19 pneumonia: A 12-month follow-up study. *Front. Med.* 10:1084002. doi: 10.3389/fmed.2023.1084002

Predictors of pulmonary sequelae after COVID-19 pneumonia: A 12-month follow-up study

Nicol Bernardinello[1†], Elisabetta Cocconcelli[1†], Chiara Giraudo[2], Matteo Daverio[1], Gioele Castelli[1], Simone Petrarulo[1], Matteo Bovo[3], Giulia Fichera[4], Silvia Cavinato[5], Anna Maria Cattelan[5], Marina Saetta[1], Paolo Spagnolo[1] and Elisabetta Balestro[1*]

[1]Respiratory Disease Unit, Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova and Padova City Hospital, Padova, Italy, [2]Department of Medicine, Institute of Radiology, University of Padova and Padova City Hospital, Padova, Italy, [3]School of Medicine and Surgery, University of Padova, Padova, Italy, [4]Pediatric Radiology Unit, University of Padova and Padova City Hospital, Padova, Italy, [5]Division of Infectious and Tropical Diseases, University of Padova and Padova City Hospital, Padova, Italy

Background: Since the beginning of the SARS-CoV-2 pandemic, over 550 million people have been infected worldwide. Despite these large numbers, the long-term pulmonary consequences of COVID-19 remain unclear.

Aims: The aim of this single-center observational cohort study was to identify and characterize pulmonary sequelae of COVID-19 at 12 months from hospitalization and to reveal possible predictors for the persistence of long-term lung consequences.

Methods: Based on the persistence or absence of radiological changes after 12 months from hospitalization, the whole population was categorized into NOT-RECOVERED (NOT-REC) and RECOVERED (REC) groups, respectively. Clinical and pulmonary function data tests and clinical data were also collected and compared in the two groups. In the NOT-REC group, high resolution computed tomography (HRCT) images were semiquantitatively scored analyzing ground-glass opacities (GGO), interstitial thickening (IT), consolidations (CO), linear and curvilinear band opacities, and bronchiectasis for each lung lobe. Logistic regression analyses served to detect the factors associated with 12-month radiological consequences.

Results: Out of the 421 patients followed after hospitalization for SARS-CoV-2 pneumonia, 347 met inclusion and exclusion criteria and were enrolled in the study. The NOT-REC patients (n = 24; 6.9%) were significantly older [67 (62–76) years vs. 63 (53–71) years; p = 0.02], more frequently current smokers [4 (17%) vs. 12 (4%); p = 0.02], and with more severe respiratory failure at the time of hospitalization [PaO_2/FiO_2 at admission: 201 (101–314) vs. 295 (223–343); p = 0.01] compared to REC group (n = 323; 93.1%). On multivariable analysis, being a current smoker resulted in an independent predictor for lung sequelae after 12 months from hospitalization [5.6 OR; 95% CI (1.41–22.12); p = 0.01].

Conclusion: After 12 months from hospital admission, a limited number of patients displayed persistent pulmonary sequelae with minimal extension. Being a current smoker at the time of SARS-CoV-2 infection is an independent predictive factor to lung consequences, regardless of the disease severity.

KEYWORDS

COVID-19, pulmonary fibrosis, 12-month follow-up, CT scan, SARS-CoV-2

Introduction

SARS-CoV-2 has spread quickly around the world since December 2019, infecting hundreds of millions of people. Despite our knowledge about this virus constantly growing, the complete understanding of long-term complications remains unclear (1). The clinical course of COVID-19 could be highly heterogeneous, in some cases with severe respiratory complications, necessitating an intensive care unit hospitalization (ICU) (2). Moreover, a percentage of patients after the acute phase could develop long-term complications of the virus, such as chronic fatigue, dyspnea, brain fog, muscle dizziness, and other neurocognitive conditions, reducing quality of life and daily activity tasks (3, 4). Previous SARS and MERS epidemics demonstrated that symptoms and imaging abnormalities persist over time, hence, it has been suggested to monitor patients after acute SARS-CoV-2 pneumonia (5). In 2020, the British Thoracic Society (BTS) produced a document for post-COVID-19 management, distinguishing severe pneumonia and patients with mild-moderate pneumonia (6). The purpose of this document was to standardize radiological follow-up and then mitigate the pressures on respiratory services after the initial COVID-19 outbreak. Thus, in these last 2 years, more effort was spent to identify specific clinical and biological attributes before and during COVID-19 infection that can be predictive of which symptoms and clinical course patients may develop (7). Other studies tried to investigate, both in severe and non-severe ill patients, the prevalence and the risk factor of pulmonary fibrosis after COVID-19 infection. Several authors reported a percentage of 19% at 4 months (8), while others reported a higher prevalence at 7 and 12-month follow-ups (9–11). However, few data have been published concerning a longer observational period. Thus, the aim of this study was to identify and characterize, among patients hospitalized for SARS-CoV-2 infection, those exhibiting persistent pulmonary sequelae at 12 months of follow-up, and then to investigate which clinical characteristics could predispose to these radiological findings.

Materials and methods

Study population and design

In this single-center observational cohort study, 421 patients were consecutively evaluated at the post-COVID-19 clinic of our hospital after discharge. Eligible patients were previously admitted to the Division of Infectious and Tropical Diseases of the University Hospital of Padova from the end of February 2020 until the end of April 2021. Inclusion criteria were: (i) age $\geq$ 18 years at the moment of hospital admission and (ii) diagnosis of SARS-CoV-2 infection by positive real-time polymerase chain reaction (RT-PCR) on the nasal-pharyngeal swab or on bronchoalveolar lavage (BAL). Exclusion criteria were: (i) pregnancy or breastfeeding status, (ii) having only one or more chest-X-ray (CXR) as a unique radiological investigation, (iii) missing on follow-up visit, or (iv) absence of computed tomography (CT) scan imaging at 12 months. For studying purposes, we completed the recruiting process in April 2021 which allowed the collection of all the data until April 2022, for a global period of 1-year follow-up. During hospitalization, positivity to SARS-CoV-2 was confirmed by a nasal or oropharyngeal swab RT-PCR (12). High-resolution CT (HRCT) was used to evaluate the persistence and characteristics of radiological changes during follow-up visits. Based on the CT changes at 12 months, the whole population was then categorized into two groups: the NOT-RECOVERED group (NOT-REC) when CT still showed lung abnormalities and the RECOVERED group (REC) when CT demonstrated normal lung parenchyma along the follow-up. Symptoms, maximal FiO_2 (FiO_2 max.), gas exchange values (PaO_2/FiO_2), days of hospital stay, and treatment during hospitalization were collected. Comorbidities were categorized as cardiovascular diseases (CVDs), respiratory diseases, metabolic diseases (including diabetes mellitus, obesity, and dyslipidemia), autoimmune diseases, and oncologic diseases (including lung, prostate, pancreatic, breast, and colon cancer). Based on the level of care, we distinguished those requiring low-intensity medical care (LIMC) and high-IMC (HIMC), as previously described (7). Pulmonary function tests were collected during follow-up visits, indeed the study was planned for two follow-up visits at 6 and 12 months from hospital discharge. Results from 6 months follow-up, as well as inclusion and exclusion criteria and study procedures, are summarized in the manuscript by Cocconcelli et al. (13). The study protocol acts by the ethical guidelines of the 1975 Declaration of Helsinki and was reviewed and approved by the Ethics Committee of the University Hospital of Padova (nr.: 46430/03.08.2020).

Radiological evaluation

All the CTs were performed by a 64-slice Siemens Somatom Sensation (Siemens Healthcare, Erlangen, Germany), with a slice $\leq$0.05. Radiological evaluation (REC vs. NOT-REC) was made by two expert radiologists (CG, GF), who were blinded to clinical data and with experience in the evaluation and quantitation of interstitial lung diseases (ILDs) features. After independent evaluation, disagreement between radiologists was resolved by consensus. All the CT images were scored through a composite semi-quantitative scale, as previously described (13). In particular, the extent of ground-glass opacities (GGO), interstitial thickening (IT), and consolidations

(CO) was assessed for each lobe using a scale from 0 to 100 and the result was expressed as the mean value of the five lobes for each radiologic feature. The presence or absence of bronchiectasis and curvilinear or linear band opacities for each of the five lung lobes were also evaluated. The level of interobserver agreement was obtained for each patient and expressed as Cohen's *k* value. For dichotomic parameters (bronchiectasis and band opacities), the patient was considered affected by these abnormalities whenever at least one single lobe was involved.

Statistical analysis

Continuous variables were described as median and interquartile range (IQR; 25–75), while categorical variables were shown as absolute (*n*) and relative values (%). We used the chi-square test and Fisher's exact test for categorical variables, while the Mann–Whitney *U* tests were used for continuous variables. Univariable and multivariable logistic regression analyses were performed to detect the factors associated with radiological consequences (NOT-REC) at 12 months. SPSS Software version 25.0 (IBM Corp., New York, NY, USA) was used for all data analysis. We considered a statistically significant p-value < 0.05.

Results

Baseline characteristics of the entire study population

A total of 421 patients started the follow-up evaluation at our post-COVID-19 clinic and were initially considered the study population. At the end of the study at 12 months, 347 patients met inclusion and exclusion criteria and were enrolled in the study (Figure 1). Analysis of the cohort that completed the 12-month period showed that patients were predominantly men (62%) with a median age of 63 years old (53–72 years) and a body mass index (BMI) of 27 (24–30 kg/m^2), as reported in Table 1. Current smokers were 5%, while non-smokers and former smokers were 63 and 33%, respectively. Patients were predominantly affected by CVDs (50%) and by metabolic diseases (45%). Almost the entire population manifested fever during hospitalization (n = 331; 99%), while cough and dyspnea were present in almost half of the patients (54 and 47%; respectively). Regarding treatment during hospitalization, the most administered therapies were heparins (81%) and corticosteroids (69%). After discharge, corticosteroids were prescribed in 56% of patients, as reported in the Supplementary Table 1.

Baseline characteristics and lung function according to radiological sequelae CT findings at 12 months

At the end of the 1-year follow-up, 24 out of 347 patients (6.9%) presented radiological sequelae at 12 months (NOT-REC). Clinical and demographic characteristics of patients divided into REC (n = 323) and NOT-REC groups (n = 24) are summarized in Table 1. NOT-REC were significantly older [67 (62–76) years vs. 63 (53–71) years; p = 0.02] and more frequently current smokers [4 (17%) vs. 12 (4%); p = 0.02]. Regarding hospital stay and disease severity, the NOT-REC group displayed significantly worsen parameters compared with the REC group: the median of the maximum FiO_2 reached during the hospitalization was higher [75% (32–100) vs. 36% (24–66); p = 0.01], the median duration of hospitalization was longer [17 (10–41) days vs. 10 (6–16) days; p = 0.001], and the median of the PaO_2/FiO_2 ratio at admission was lower [201 (101–314) vs. 295

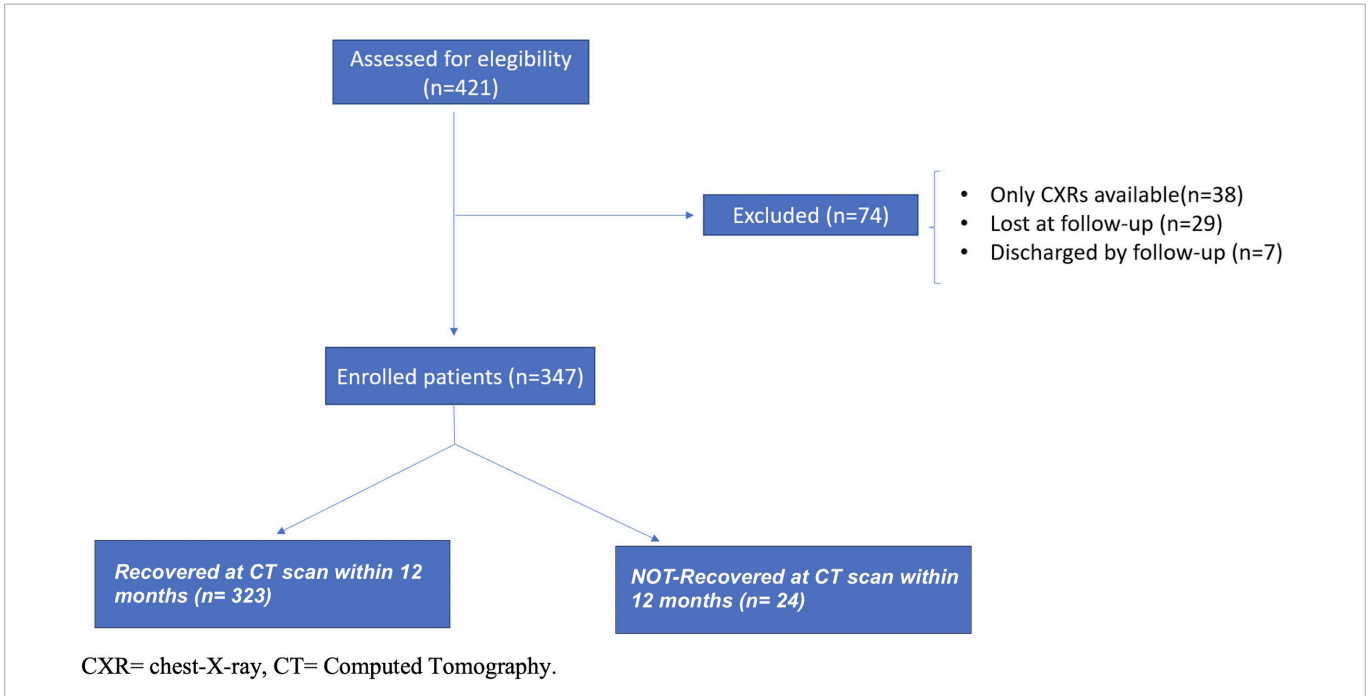

FIGURE 1
Enrollment flow-chart of patients discharged from hospital and included in the study cohort.

TABLE 1 Baseline demographics and clinical characteristics of the overall population evaluated at post-COVID clinic, and of the two subgroups categorized according to the presence or absence of radiological recovery at 12 months.

	Available data	Overall population (n = 347)	REC (n = 323)	NOT-REC (n = 24)	p
Demographic data					
Male–n (%)	347	217 (62)	200 (62)	17 (71)	0.51
Age at admission–years	347	63 (53–72)	63 (53–71)	67 (62–76)	0.02
BMI–kg/m^2	247	27 (24–30)	27 (25–30)	26 (24–30)	0.23
Smoking history					
Pack years	334	0 (0–5)	0 (0–5)	3.1 (0–21)	0.06
Current–n (%)	344	16 (5)	12 (4)	4 (17)	0.02
Non-smoker–n (%)	344	216 (63)	205 (64)	11 (46)	0.08
Former–n (%)	344	114 (33)	105 (33)	9 (37)	0.66
Comorbidities					
Cardiovascular diseases–n (%)	347	174 (50)	161 (50)	13 (54)	0.83
Respiratory diseases–n (%)	347	50 (14)	44 (14)	6 (25)	0.13
Autoimmune diseases–n (%)	347	52 (15)	45 (14)	7 (29)	0.07
Metabolic diseases–n (%)	347	158 (45)	147 (45)	11 (46)	0.99
Oncologic diseases–n (%)	347	57 (16)	52 (16)	6 (21)	0.57
Hospitalization characteristics					
FiO_2 max during hospitalization	337	36 (27–70)	36 (24–66)	75 (32–100)	0.01
Hospitalization–days	347	10 (6–17)	10 (6–16)	17 (10–41)	0.001
High degree of care–n (%)	347	69 (20)	57 (18)	12 (50)	0.0006
PaO_2/FiO_2 at admission	205	295 (218–342)	295 (223–343)	201 (101–314)	0.01
Symptoms during hospitalization					
Fever–n (%)	335	331 (99)	289 (92)	22 (100)	0.39
Asthenia–n (%)	335	119 (35)	112 (36)	7 (32)	0.70
Dyspnea–n (%)	335	158 (47)	140 (45)	18 (75)	0.0008
Anosmia/Ageusia–n (%)	335	104 (31)	96 (31)	8 (33)	0.58
Muscular alterations–n (%)	335	61 (18)	55 (18)	6 (25)	0.25
Headache–n (%)	335	36 (11)	35 (11)	1 (4.2)	0.33
Gastrointestinal–n (%)	335	74 (22)	69 (22)	5 (21)	0.94
Cough–n (%)	335	182 (54)	168 (54)	14 (58)	0.36

Values are expressed as numbers and (%) or median and interquartile range (IQR), as appropriate. BMI: body mass index. To compare demographics between recovered (REC) and not-recovered (NOT-REC), the chi-square test and Fisher's t-test ($n < 5$) for categorical variables and Mann–Whitney t-test for continuous variables were used.

(223–343); $p = 0.01$]. Among all treatments, in the NOT-REC group, other antibiotics (62% vs. 31%; $p = 0.003$) and corticosteroids (87% vs. 67%; $p = 0.04$) were more frequently used during the hospital stay, compared with the REC group (Supplementary Table 1). For all the other drugs, including the administration of corticosteroids after discharge, we did not find any between-group difference ($p = 0.20$). Regarding radiological sequelae, in NOT-REC patients ($n = 24$), the most frequent alteration was IT, which was observed in 21 patients (88%) and with a median extension of 4%. GGO was found in 19 patients (79%) with a median extension of 3.5%, while CO were present in only 2 patients (8%) with an extension of <1% (Figures 2A–C). The linear and curvilinear band opacities were reported in 16 patients (66%) and bronchiectasis in 7 patients (29%), as reported in Table 2. Analyzing pulmonary function tests in the whole population, we observed normal lung volume at first follow-up [Forced Vital Capacity (FVC% pred.): 92% (81–104) and Forced Expiratory Volume in the first second (FEV1% pred.): 95% (84–137)]. Moreover, patients from the NOT-REC group showed similar parameters to patients from the REC group, as reported in Table 3.

Predictors of post-COVID-19 pulmonary sequelae

At the univariable analysis, age ≥ 63 years [2.8 OR; 95% CI (1.09–7.33); $p = 0.03$] and being a current smoker [5.2 OR; 95% CI (1.53–17.53); $p = 0.008$] were identified as risk factors for having persistent radiological sequelae at 12 months follow-up after COVID-19 pneumonia. Among hospital stay characteristics, an hospitalization time ≥ 10 days [OR 2.9; 95% CI (1.14–7.61), $p = 0.03$], the high degree of care [4.7 OR; 95% CI (1.99–10.92); $p = 0.0001$]; FiO_2 max. ≥ 36% [2.6 OR; 95% CI (1.01–6.78); $p = 0.047$] and

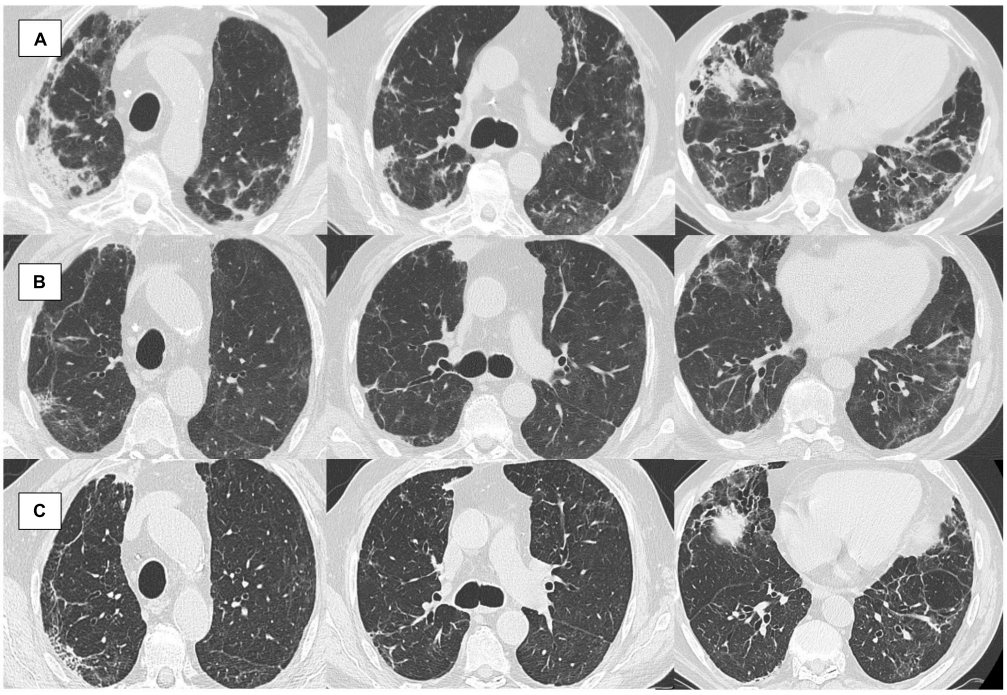

FIGURE 2
(A–C) An 84 years-old male patient followed in our post-COVID-19 clinic. **(A)** Computed tomography (CT) scan at 1 month from hospital admission: diffuse ground-glass opacities (GGO), also with consolidations (CO) in particular in the apical subpleural region and right basal; **(B)** CT at 6 months from hospital admission: ameliorated, GGO persist in the lung bases and the medium lobe; still showing interstitial thickening (IT) with bronchiectasis; **(C)** CT scan at 12 months from hospital admission: sclerosis of the apical subclavian regions, atelectatic thickening in the right upper lobe, diffuse bronchiectasis, and dystelectasis (evaluated through the semi-quantitative grading as follows: 0% of mean GGO extension of the five lobes; 21% of mean IT; 0% CO; 1 bronchiectasis; 1 of linear and curvilinear band opacities).

other antibiotics [3.6 OR; 95% CI (1.54–8.61); p = 0.003], resulted as dependent risk factors for having post-COVID-19 pulmonary changes (Table 4). On the multivariable analysis, adjusted for the previous risk factor, we found that smoking history, particularly being a current smoker, was an independent predictor for lung sequelae after 12 months from COVID-19 pneumonia and hospitalization [5.6 OR; 95% CI (1.41–22.12), p = 0.01].

TABLE 2 Chest high resolution computed tomography (HRCT) scan characteristics of the not-recovered (NOT-REC) group (n = 24) at 12-month.

Ground-glass opacities (GGO)	
GGO [n (%)]	19 (79%)
Extent of GGO	3.5%
Interstitial thickening (IT)	
IT [n (%)]	21 (88%)
Extent of IT	4%
Consolidations (CO)	
CO [n (%)]	2 (8%)
Extent of CO	<1%
Bronchiectasis	
Bronchiectasis [n (%)]	7 (29%)
Curvilinear and liner band opacities	
Curvilinear and liner band opacities [n (%)]	16 (66%)

Values are expressed as numbers and (%).

Discussion

To the best of our knowledge, limited studies have explored the predictive factors of pulmonary sequelae in consecutive patients affected by COVID-19 and with such a long follow-up. In this study, 347 patients were evaluated and we found that 24 (6.9%) subjects still presented radiological changes on CT scans after a 1-year follow-up (NOT-REC group). In line with a previous study (8), these patients were older than those who REC (67 years vs. 63 years; p = 0.02), moreover adults older than 63 years showed nearly three times the risk of developing abnormalities on CT scan at 12 months, as shown by univariable analysis. Furthermore, NOT-REC patients had a worse clinical course, compared to patients who REC, during the hospital stay. In fact, the median maximum FiO_2 required was

TABLE 3 Pulmonary function tests of patients at first follow-up visit according to presence or absence of radiological recovery at 12 months (n = 347).

	Overall population (n = 347)	REC (n = 323)	NOT-REC (n = 24)	p
FVCabs–liters	3.3 (2.8–4.0)	3.3 (2.8–4.0)	3.2 (2.4–4.6)	0.38
FVC pred–%	92 (81–104)	92 (81–104)	97 (70–103)	0.79
FEV1abs–liters	2.8 (2.3–3.3)	2.8 (2.3–3.3)	2.6 (2.0–3.5)	0.37
FEV1 pred–%	95 (84–137)	95 (84–137)	99 (55–121)	0.74

FVC: forced vital capacity, FEV1: flow expiratory volume at first second. Values are expressed as median and interquartile range (IQR). To compare the pulmonary function test between recovered (REC) and NOT-REC, the Mann–Whitney test for continuous variables was used.

two-fold higher and the PaO_2/FiO_2 ratio was lower in NOT-REC patients. Even if a strong correlation resulted between the severity of the acute illness and the persistence of lung changes, none of the indicators received further confirmation as independent predictors in multivariable analysis. This is in line with previous studies, indeed, it has been reported that patients who presented a more severe acute COVID-19 pneumonia, as indicated by ventilatory support, gas exchange index, and duration of hospital stay, are the same who present radiologic involvement during follow-up visits (at 4, 6, or 12 months) (8–11, 13–15). As confirmed by recent evidence, dyspnea was more frequent at hospital admission in the NOT-REC group, this is a further signal of worse clinical presentation during the acute illness (14). Similar results were recently displayed by Faverio et al. (16) who observed a cohort of 287 patients at 12-month follow-up from hospitalization. The authors showed that fibrotic sequelae at HRCT scans were found in a strict minority of patients (3, 1% of the study cohort), while the so-called "mild non-fibrotic radiological abnormalities" were observed in the majority of cases (66% of the entire cohort) with interstitial lung involvement, particularly GGO and reticular abnormalities, as subpleural curvilinear lines, as the main radiologic pattern (16). Besides, as in our cohort, the anatomical extension of these abnormalities was limited, with a mean lobar involvement that ranges between 13 and 17% of each entire single lobe (16). This report, together with our findings, is in contrast with Tarraso et al. (17) who reported in a multicenter prospective study a higher percentage of patients (23%) with fibrotic-like sequelae after 1 year of follow-up. However, it should be mentioned that the authors did not score the extension of lung lesions on CT, thus limiting the comparison with our assessment (17). When analyzing the main radiological characteristics presented in the NOT-REC group, our findings are in line with Huang et al. (18) that found, at 6 months, a more severe involvement in those patients who required a higher degree of care, with the GGO as the most frequent radiological feature, followed by the irregular lines. Overall, in our

TABLE 4 Risk factors associated with the persistence of pulmonary sequela in the overall population (n = 347).

	Univariable analysis	p	Multivariable analysis	p
	OR (95% CI)		OR (95% CI)	
Demographics				
Age ≥ 63 years	2.8 (1.09–7.33)	0.03	2.6 (0.96–7.18)	0.06
BMI ≥ 27 kg/m^2	0.4 (0.15–1.07)	0.07		
Smoking history				
Current smoker–yes	5.2 (1.53–17.53)	0.008	5.6 (1.41–22.12)	0.01
No-smoker–yes	2.0 (0.86–4.58)	0.11		
Comorbidities				
Cardiovascular disease–yes	1.2 (0.52–2.73)	0.68		
Respiratory disease–yes	2.1 (0.79–5.61)	0.13		
Autoimmune disease–yes	2.5 (0.99–6.48)	0.051		
Metabolic disease–yes	1.0 (0.44–2.33)	0.98		
Oncologic disease–yes	1.4 (0.49–3.84)	0.55		
Hospitalization characteristics				
Hospitalization time ≥ 10 days	2.9 (1.14–7.61)	0.03	1.0 (0.25–3.29)	0.89
High degree of care–yes	4.7 (1.99–10.92)	0.0001	2.6 (0.83–8.35)	0.10
FiO_2 max. ≥ 36%	2.6 (1.01–6.78)	0.047	1.1 (0.31–3.86)	0.89
PaO_2/FiO_2 ≥ 295	0.4 (0.14–1.25)	0.12		
Treatment during hospitalization				
Hydroxychloroquine/chloroquine–yes	1.4 (0.61–3.24)	0.42		
Azithromycin–yes	0.7 (0.33–1.75)	0.52		
Ceftriaxone–yes	1.1 (0.44–2.38)	0.95		
Other antibiotics–yes	3.6 (1.54–8.61)	0.003	2.4 (0.84–3.28)	0.10
Lopinavir/ritonavir–yes	1.9 (0.74–4.73)	0.18		
Remdesivir–yes	1.4 (0.58–3.24)	0.47		
Other antiviral–yes	2.7 (0.31–24.6)	0.36		
Tocilizumab–yes	2.9 (0.78–10.9)	0.11		
Corticosteroids–yes	3.4 (0.98–11.6)	0.052		
Heparins–yes	0.88 (0.32–2.46)	0.81		
Corticosteroids during follow-up–yes	1.8 (0.74–4.59)	0.19		

BMI: body mass index.

study, the lung involvement at 12 months was minimal since the median involvement reached 4% for IT and 3.5% for GGO (with the maximum lung involvement of 21 and 22% in one patient, regarding IT and GGO, respectively). Indeed, it remains to be elucidated if the fibrotic lesions are strictly caused by the infection or if the contribution of mechanical ventilation to lung injury should be considered. Interestingly enough, both the univariable and multivariable analyses confirmed that being an active smoker, at the time of infection, represents an independent predictor for long-term pulmonary sequelae with a five times greater risk regardless of the severity of COVID-19 pneumonia. As very recently summarized by Benowitz et al. (19) smokers resulted in a greater risk of developing severe disease following SARS-CoV-2 infection than non-smokers and the main mechanisms underlying this association might include up-regulation of angiotensin-converting enzyme-2 receptors, immune suppression, oxidative stress, inflammation, and vascular injury. As the pandemic has evolved, important research questions have emerged particularly regarding the so-called post-COVID-19 or long COVID and how these long-term sequelae might be affected by tobacco product use. Within this topic, our finding seems to point out, for the first time, the potential association between cigarette exposure and the persistence of fibrotic-like changes in the lung, following SARS-CoV-2 infection. Interestingly, as previously shown in patients with Idiopathic Pulmonary Fibrosis, cigarette smoking exposure has been shown to impair adaptive humoral and cellular responses, and exaggerate proinflammatory and innate immune responses limiting the physiological tissue damage/repair responses after viral infection (20, 21). Moreover, recently, using a murine model in which animals were exposed to cigarette smoking and subsequently infected with H1N1 influenza virus, the authors have found an exaggerated fibroblastic response with the proliferation of lung fibroblasts providing new insights into the role of smoking in the dysregulation of healing and fibroblastic processes after a respiratory viral infection (22). Thus, we can speculate that active smokers, infected with SARS-CoV-2, could have an increased likelihood of developing a lung fibroblastic response and unsuccessful lung repair. When considering hospital treatment strategy we found that the category of antibiotics resulted in a risk factor for the persistence of lung damage long term even though at univariate analysis and we can speculate that the NOT-REC group included severe patients which needed a wider approach to managing acute COVID-19 pneumonia. For the same reason, during hospitalization, the NOT-REC group received, more frequently than the REC-group, corticosteroids which may characterize the management of critically ill patients during the acute phase. On the other hand, the use of corticosteroids after hospitalization, in our cohort, is not a confounder since the percentage of administration in the two groups was similar. Furthermore, lung function tests were normal, in particular lung volume. Our findings seem in contrast with Jutant's (8) study, where they found significant differences in the group with fibrotic lesions both for lung volume and diffusing capacity of the lungs for carbon monoxide compared to those without fibrotic lesions. However, Steinbeis demonstrated that, at 12 months, the degree of pulmonary function impairment still correlates with severity during the acute phase, but it improves over time (23). We cannot ignore some limitations of our study. First, the total lung capacity (TLC) and diffusion of lung carbon monoxide (DLCO) were not routinely assessed. DLCO permits the early detection of interstitial lung involvement, however, is not considered a reliable parameter for monitoring patients with pulmonary fibrosis. Indeed DLCO scores were not used as the primary endpoint in clinical studies of new medications for idiopathic pulmonary fibrosis (IPF) (24–26). Moreover, many studies have reported a reduction in DLCO at 3 or 6 months after SARS-CoV-2 infection (14, 20). Besides, at 12 months, Wu et al. (27) showed how it reverted to normality, so the long-term trend is not still clear and needs further clarification. Lastly, this is not a multicenter study, and it is based on data collected in a single hospital, however, the study cohort we were able to prospectively follow is a very large population and truly reflects all in-hospital disease severity.

Conclusion

After 12 months from hospitalization for COVID-19 pneumonia, fibrotic-like changes on CT are observed in a small percentage of the study population. These radiological sequelae are minimal and do not affect lung function. Finally, being a current smoker, at the time of infection, is an independent predictor of persistent lung changes after 1 year. Further studies are needed to validate these findings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of the University Hospital of Padova, *via* Niccolò Giustiniani n.2, 35128 Padova (nr.: 46430/03.08.2020). The Ethics Committee waived the requirement of written informed consent for participation. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MB, MD, NB, EC, and EB conceived the study. MB and MD coordinated data collection, curation, and analyses. NB analyzed the data. MB, MD, SP, and GC performed data collection. CG and GF scored radiography. NB, MB, and MD wrote the manuscript with revision and supervision from AC, SC, PS, MS, and EB. All authors contributed to the article and approved the submitted version.

Acknowledgments

We thank Elena De Zorzi, Silvia Tonin, and Marta Zuffellato (Department of Cardiac, Thoracic, Vascular Sciences and Public Health, University of Padova and Padova City Hospital, Padova, Italy) for data collection.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary Material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1084002/full#supplementary-material

References

1. Soriano J, Murthy S, Marshall J, Relan P, Diaz J, WHO Clinical Case Definition Working Group on Post-Covid-19 Condition. A clinical case definition of post-COVID-19 condition by a Delphi consensus. *Lancet Infect Dis.* (2022) 22:e102–7. doi: 10.1016/S1473-3099(21)00703-9

2. Kim L, Garg S, O'Halloran A, Whitaker M, Pham H, Anderson E, et al. Risk factors for intensive care unit admission and in-hospital mortality among hospitalized adults identified through the US Coronavirus Disease 2019 (COVID-19)-associated hospitalization surveillance network (COVID-NET). *Clin Infect Dis.* (2021) 72:e206–14. doi: 10.1093/cid/ciaa1012

3. Adeloye D, Elneima O, Daines L, Poinasamy K, Quint J, Walker S, et al. The long-term sequelae of COVID-19: an international consensus on research priorities for patients with pre-existing and new-onset airways disease. *Lancet Respir Med.* (2021) 9:1467–78. doi: 10.1016/S2213-2600(21)00286-1

4. Ceban F, Ling S, Lui L, Lee Y, Gill H, Teopiz K, et al. Fatigue and cognitive impairment in Post-COVID-19 Syndrome: a systematic review and meta-analysis. *Brain Behav Immun.* (2022) 101:93–135. doi: 10.1016/j.bbi.2021.12.020

5. Osuchowski M, Winkler M, Skirecki T, Cajander S, Shankar-Hari M, Lachmann G, et al. The C19 puzzle: deciphering pathophysiology and phenotypes of a new disease entity. *Lancet Respir Med.* (2021) 9:622–42. doi: 10.1016/S2213-2600(21)00218-6

6. British Thoracic Society. *Guidance on Respiratory Follow up of Patients With a Clinico Radiological Diagnosis of COVID-19 Pneumonia*. (2021). Available online at: https://britthoracic.org.uk/about-us/covid-19-information-forthe-respiratory-community/ (accessed May 7, 2021).

7. Cocconcelli E, Biondini D, Giraudo C, Lococo S, Bernardinello N, Fichera G, et al. Clinical features and chest imaging as predictors of intensity of care in patients with COVID-19. *J Clin Med.* (2020) 9:2990. doi: 10.3390/jcm9092990

8. Jutant E, Meyrignac O, Beurnier A, Jaïs X, Pham T, Morin L, et al. Respiratory symptoms and radiological findings in post-acute COVID-19 syndrome. *ERJ Open Res.* (2022) 8:00479–2021.

9. Zhan Y, Zhu Y, Wang S, Jia S, Gao Y, Lu Y, et al. SARS-CoV-2 immunity and functional recovery of COVID-19 patients 1-year after infection. *Sig Transduct Target Ther.* (2021) 6:368.

10. Liu M, Lv F, Huang Y, Xiao K. Follow-up study of the chest CT characteristics of COVID-19 survivors seven months after recovery. *Front Med.* (2021) 8:636298. doi: 10.3389/fmed.2021.636298

11. Watanabe A, So M, Iwagami M, Fukunaga K, Takagi H, Kabata H, et al. One-year follow-up CT findings in COVID-19 patients: a systematic review and meta-analysis. *Respirology.* (2022) 27:605–16. doi: 10.1111/resp.14311

12. WHO. *Clinical Management of Severe Acute Respiratory Infection When Novel Coronavirus (nCoV) Infection is Suspected: Interim Guidance*. (2020). Available online at: https://www.who.int/docs/default-source/coronaviruse/clinical-management-of-novel-cov.pdf (accessed March 13, 2020).

13. Cocconcelli E, Bernardinello N, Giraudo C, Castelli G, Giorgino A, Leoni D, et al. Characteristics and prognostic factors of pulmonary fibrosis after COVID-19 pneumonia. *Front Med (Lausanne).* (2022) 8:823600. doi: 10.3389/fmed.2021.823600

14. Yesudhas D, Srivastava A, Gromiha M. COVID-19 outbreak: history, mechanism, transmission, structural studies, and therapeutics. *Infection.* (2021) 49:199–213. doi: 10.1007/s15010-020-01516-2

15. Mylvaganam R, Bailey J, Sznajder J, Sala M. Recovering from a pandemic: pulmonary fibrosis after SARS-CoV-2 infection. *Eur Respir Rev.* (2021) 30:210194.

16. Faverio P, Luppi F, Rebora P, D'Andrea G, Stainer A, Busnelli S, et al. One-year pulmonary impairment after severe COVID-19: a prospective, multicenter follow-up study. *Respir Res.* (2022) 23:65. doi: 10.1186/s12931-022-01994-y

17. Tarraso J, Safont B, Carbonell-Asins J, Fernandez-Fabrellas E, Sancho-Chust JN, Amat B, et al. COVID-FIBROTIC study team lung function and radiological findings 1 year after COVID-19: a prospective follow-up. *Respir Res.* (2022) 23:242. doi: 10.1186/s12931-022-02166-8

18. Huang C, Huang L, Wang Y, Li X, Ren L, Gu X, et al. 6-month consequences of COVID-19 in patients discharged from hospital: a cohort study. *Lancet.* (2021) 397:220–32.

19. Benowitz N, Goniewicz M, Halpern-Felsher B, Krishnan-Sarin S, Ling P, O'Connor R, et al. Tobacco product use and the risks of SARS-CoV-2 infection and COVID-19: current understanding and recommendations for future research. *Lancet Respir Med.* (2022) 10:900–15. doi: 10.1016/S2213-2600(22)00182-5

20. Baumgartner K, Samet J, Stidley C, Colby T, Waldron J. Cigarette smoking: a risk factor for idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* (1997) 155:242–8.

21. Antoniou K, Hansell D, Rubens M, Marten K, Desai S, Siafakas N, et al. Idiopathic pulmonary fibrosis: outcome in relation to smoking status. *Am J Respir Crit Care Med.* (2008) 177:190–4.

22. Lee S, Sharma L, Kang Y, Kim S, Chandrasekharan S, Losier A, et al. Impact of cigarette smoke exposure on the lung fibroblastic response after influenza pneumonia. *Am J Respir Cell Mol Biol.* (2018) 59:770–81. doi: 10.1165/rcmb.2018-0004OC

23. Steinbeis F, Thibeault C, Doellinger F, Ring R, Mittermaier M, Ruwwe-Glösenkamp C, et al. Severity of respiratory failure and computed chest tomography in acute COVID-19 correlates with pulmonary function and respiratory symptoms after infection with SARS-CoV-2: an observational longitudinal study over 12 months. *Respir Med.* (2022) 191:106709. doi: 10.1016/j.rmed.2021.106709

24. Nathana SD, Meyer KC. IPF clinical trial design and endpoints. *Curr Opin Pulm Med.* (2014) 20:463–71.

25. King T Jr, Bradford W, Castro-Bernardini S, Fagan E, Glaspole I, Glassberg M, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med.* (2014) 370:2083–92.

26. Richeldi L, du Bois R, Raghu G, Azuma A, Brown K, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med.* (2014) 370:2071–82. doi: 10.1056/NEJMoa1402584

27. Wu X, Liu X, Zhou Y, Yu H, Li R, Zhan Q, et al. 3-month, 6-month, 9-month, and 12-month respiratory outcomes in patients following COVID-19-related hospitalization: a prospective study. *Lancet Respir Med.* (2021) 9:747–54. doi: 10.1016/S2213-2600(21)00174-0

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 21 March 2023
DOI 10.3389/fmed.2023.1131070

OPEN ACCESS

EDITED BY
Paolo Spagnolo,
University of Padua,
Italy

REVIEWED BY
Martina Bonifazi,
Marche Polytechnic University,
Italy
Marialuisa Bocchino,
University of Naples Federico II, Italy
Elisabetta Cocconcelli,
University of Padua,
Italy

*CORRESPONDENCE
Jin Woo Song
✉ jwsongasan@gmail.com

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 24 December 2022
ACCEPTED 07 March 2023
PUBLISHED 21 March 2023

CITATION
Oh JH, Kang J and Song JW (2023) Fibrosis score predicts mortality in patients with fibrotic hypersensitivity pneumonitis.
Front. Med. 10:1131070.
doi: 10.3389/fmed.2023.1131070

Fibrosis score predicts mortality in patients with fibrotic hypersensitivity pneumonitis

Ju Hyun Oh[1], Jieun Kang[2] and Jin Woo Song[3]*

[1]Department of Pulmonary and Critical Care Medicine, Sanggye Paik Hospital, Inje University College of Medicine, Seoul, Republic of Korea, [2]Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Ilsan Paik Hospital, Inje University College of Medicine, Goyang-si, Gyeonggi-do, Republic of Korea, [3]Department of Pulmonary and Critical Care Medicine, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea

Background: Variable clinical courses make it challenging to predict mortality resulting from fibrotic hypersensitivity pneumonitis (HP). This study evaluated the usefulness of radiologic parameters for predicting mortality in patients with fibrotic HP.

Methods: Clinical data and high-resolution computed tomography (HRCT) images, which were scored for reticulation, honeycombing, ground glass opacity (GGO), consolidation, and mosaic attenuation (MA) by visual assessment, were retrospectively analyzed in a total of 101 patients with fibrotic HP (all biopsy-proven cases). Fibrosis score was defined as the sum of reticulation and honeycombing scores.

Results: The mean age of the 101 patients was 58.9years, and 60.4% were females. During the follow-up (median: 55.5months; interquartile range: 37.7–89.0months), the 1-, 3-and 5-year mortality rates were 3.9, 16.8, and 32.7%, respectively. The non-survivors were older and had significantly lower lung function and minimum oxygen saturation during the 6-min walk test than the survivors. The non-survivors had higher scores of reticulation, honeycombing, GGO, fibrosis, and MA on HRCT than survivors. In the multivariable Cox analysis, reticulation, GGO, and fibrosis scores were independent prognostic factors for mortality in patients with fibrotic HP, as well as age. Fibrosis score showed great performance for predicting the 5-year mortality (AUC=0.752, $p<0.001$) and higher mortality was recorded for patients with high fibrosis score (≥12.0%) (the mean survival time: 58.3 vs. 146.7months, $p<0.01$) than those without.

Conclusion: Our results suggest that radiologic fibrosis score may be a useful predictor of mortality in patients with fibrotic HP.

KEYWORDS

hypersensitivity pneumonitis, mortality, prognosis, fibrosis, high-resolution computed tomography

Introduction

Hypersensitivity pneumonitis (HP) is a diffuse interstitial lung disease (ILD) caused by an immune response to inhaled antigens in susceptible individuals (1, 2). Historically, HP was categorized into subtypes as acute, subacute, or chronic based on the disease duration. In general, acute HP has favorable outcome by avoiding exposure to the suspected antigen.

However, in the case of subacute or chronic HP, it has been challenging for clinicians to predict the prognosis due to variable disease courses.

Previous studies have shown that several factors are associated with prognosis in chronic HP (3–6). For example, smoking history, unknown inciting antigens, decline in forced vital capacity (FVC) and fibrotic nonspecific interstitial pneumonia pattern or usual interstitial pneumonia (UIP) pattern on histologic examination, have been identified as significant predictors of poor prognosis in patients with chronic HP (5, 7–11). Furthermore, HP patients with radiological fibrosis were reported to have worse survival outcomes than those without (4, 12, 13); Chung et al. showed that of 132 patients with chronic HP, those with reticulation or honeycombing on high-resolution computed tomography (HRCT) had significantly worse survival than those without ($p = 0.016$ or 0.007 in log-rank test, respectively) (4). Hanak et al. also reported that in 69 patients with chronic HP, subjects with fibrosis on HRCT had a higher mortality rate than those without (42% vs. 2%, $p < 0.001$) (14). As such, it has become important to determine the presence of fibrosis in predicting prognosis for patients with HP. Given these points, recent guidelines for diagnosing HP suggest that to better reflect clinical manifestation and courses, HP can be classified as fibrotic or non-fibrotic depending on the radiologic or histopathologic evidence of pulmonary fibrosis (1, 15). However, in patients with fibrotic HP, the prognosis is difficult to predict due to diverse courses, and the role of radiological parameters are not well defined. Therefore, in this study, the role of radiological parameters in predicting mortality was investigated through the quantification of several radiological findings identified in fibrotic HP.

Methods

Study population

From January 2002 to December 2017, 106 patients suspected of fibrotic HP were identified at Asan Medical Center, Seoul, Republic of Korea. Among them, patients with lack of histopathological confirmation ($n = 5$) were excluded, and 101 patients diagnosed with fibrotic HP by histopathologic findings obtained *via* surgical ($n = 99$) or transbronchial lung biopsy ($n = 2$) were included in this study. All diagnoses were made through multidisciplinary discussion. All patients were included in previous studies (12, 16, 17). The diagnosis of HP was reconfirmed by multidisciplinary discussion based on the criteria of the American Thoracic Society (ATS), Japanese Respiratory Society (JRS), and Latin American Thoracic Association (ALAT) (1). This study was approved by the Institutional Review Board of Asan Medical Center (2017–1081), and the requirement for informed consent was waived due to the study's retrospective nature.

Clinical data

Clinical and survival data from all patients were retrospectively collected using medical records and/or the records from the National Health Insurance of Korea. A detailed history of exposure to various environmental factors known to induce HP was obtained from all participants at the time of initial diagnosis *via* questionnaires. FVC by spirometry, total lung capacity (TLC) by plethysmography and diffusing capacity of the lung for carbon monoxide (DLco) were measured according to the ATS/ERS recommendations (18–23). The results were expressed as a percentage of predicted values. The 6-min walk test (6MWT) was performed following the guidelines (24). The ILD-GAP index was calculated based on the type of ILD and clinical variables (gender, age, FVC, and DLco), as suggested previously (25). The time of diagnosis was defined as the date on which the lung biopsy was performed. Baseline clinical data including lung function and 6MWT were obtained at the time of diagnosis.

HRCT images

All HRCT scans were performed at the time of diagnosis. As described in our previous study (17), HRCT images were assessed by two dedicated thoracic radiologists (4 and 17 years of experience, respectively), blinded to the patient's clinical information, and semi-quantitatively scored for reticulation, honeycombing, ground glass opacity (GGO), consolidation, and mosaic attenuation (MA) in six lung zones to the nearest 10^{th} percentile. Fibrosis score was defined as the sum of reticulation and honeycombing scores (26). Level of inter-reader agreement of the HRCT images was evaluated using Cohen's kappa. Inter-reader agreement in assessing HRCT patterns showed moderate strength of agreement ($\kappa = 0.761$; 95% confidence interval [CI], 0.634–0.888) (27). Discrepancies were resolved by a consensus.

HRCT findings were classified as a UIP-like pattern or not; a UIP-like pattern was defined as an HRCT pattern compatible with a UIP or probable UIP pattern according to 2018 Fleischner Society IPF diagnostic guidelines, and some modifications were made to apply to HP (28, 29). Briefly, a UIP-like pattern was defined as reticulation with traction bronchiectasis with/without honeycombing in the absence of features to suggest an alternative diagnosis such as peribronchovascular predominance with subpleural sparing, predominant consolidation, extensive pure GGO or diffuse nodules/cysts; however, presence of MA and distribution of fibrosis were not included in the features of an alternative diagnosis.

Statistical analysis

All values are expressed as mean ± standard deviation for continuous variables or numbers with percentages for categorical variables. Continuous variables were compared using the Student t-test, and categorical variables were compared using the Chi-square test. Correlation analyses were performed using Pearson's correlation coefficients to evaluate the relationship between CT scores and lung function or exercise capacity. We performed Cox proportional hazards analysis to identify risk factors for mortality in patients with HP, and variables with a p-value of <0.1 in the unadjusted analysis were included in the multivariable analysis with backward stepwise elimination. The follow-up time was calculated from the date of the initial HRCT to the date of death or time of censoring (date of vital status ascertainment: 04 February 2020). The Kaplan–Meier survival analysis and log-rank test were used for survival analysis. To compare the performance of each variable for predicting mortality, receiver operating characteristic (ROC) curve analysis for 5-year mortality was performed, and the optimal cut-off

value was estimated by the Youden index method (30). The predicting performance of various combined models was compared by concordance statistics (C-statistics) proposed by Kang et al. (31). Statistical significance was defined as a p-value <0.05. All statistical analyses were performed using the SPSS software (version 21.0; IBM Corporation, Somers, NY, United States), MedCalc Statistical Software (version 12.7.5; MedCalc Software bvba, Ostend, Belgium), or R software (version 4.1.0, The R Foundation for Statistical Computing, Vienna, Austria; URL http://www.R-project.org/).

Results

Baseline characteristics

The mean age of all patients was 58.9 years, 60.4% were females, and 85.1% had a history of exposure to suspected inhalational antigens (Table 1). During the follow-up (median: 55.5 months, IQR: 37.0–89.0 months), 20 (19.8%) patients experienced acute exacerbation and 42 patients (41.6%) died. The 1-, 3-and 5-year mortality rates were 3.9, 16.8, and 32.7%, respectively. Among them, 25 patients showed respiratory-related death. The non-survivors were older, had lower lung function (FVC, DLco, and TLC) and poorer exercise capacity (distance and the lowest oxygen saturation on pulse oximetry [SpO_2] during 6MWT) than the survivors (Table 1).

HRCT parameters

The non-survivors had higher scores of reticulation, honeycombing, fibrosis and MA than the survivors (Table 2). However, there was no significant difference in the frequency of a UIP-like pattern between the non-survivors and survivors.

There were statistically significant negative correlations between fibrosis and reticulation scores and lung function (FVC, DLco, and TLC) and the lowest SpO_2 during 6MWT (online supplementary Figure S1). In addition, honeycombing ($r = -0.207$, $p = 0.038$) and MA ($r = -0.284$, $p = 0.004$) scores were also negatively correlated with exercise capacity (the lowest SpO_2 or distance during 6MWT); however, consolidation score showed no significant correlation with the results of pulmonary function test (PFT) and 6MWT (online Supplementary Table S1).

Prognostic factors for the mortality

In the unadjusted Cox analysis, older age, lower lung function (FVC, DLco, and TLC) and the minimum SpO2 during 6MWT, higher scores of HRCT parameters (reticulation, honeycombing, fibrosis scores, and MA), and the presence of a UIP-like pattern on HRCT were significantly associated with mortality in patients with fibrotic HP (Table 3). In the multivariable analysis, reticulation (HR 1.038; 95% CI 1.004–1.074) and GGO (HR 0.949; 95% CI 0.915–0.984) were independent prognostic factors for mortality in patients with fibrotic HP, as well as age and DLco. Fibrosis score (HR 1.040; 95% CI 1.007–1.075) was also an independent predictor of reduced survival, as well as age (Table 4).

Performance of HRCT parameters

In the ROC analysis, fibrosis scores had a higher area under the curve (AUC) than reticulation and GGO scores for predicting the

TABLE 1 Comparison of baseline characteristics between the non-survivors and survivors among patients with fibrotic HP.

Characteristic	Total	Non-survivors	Survivors	p-value
Patient numbers	101	42	59	
Age, years	58.9 ± 10.8	61.8 ± 11.0	56.8 ± 10.6	0.022
Female sex	61 (60.4)	28 (66.7)	33 (55.9)	0.277
BMI, kg/m^2	25.0 ± 4.1	24.3 ± 4.9	25.5 ± 3.4	0.133
Positive history of exposure*	86 (85.1)	34 (81.0)	52 (88.1)	0.457
Ever-smokers	38 (37.6)	13 (31.0)	25 (42.4)	0.243
Pulmonary function test				
FVC, % predicted	71.2 ± 17.7	66.6 ± 21.3	79.2 ± 14.3	0.047
DLco, % predicted	60.2 ± 16.9	55.4 ± 17.2	63.4 ± 16.1	0.025
TLC, % predicted	73.6 ± 13.4	69.9 ± 14.9	76.1 ± 11.7	0.030
6-min walk test				
Distance, meter	443.5 ± 88.1	407.7 ± 105.3	469.5 ± 62.3	0.001
SpO_2 nadir, %	92.2 ± 4.3	90.8 ± 4.6	93.2 ± 3.8	0.007
Treatment				0.043
No treatment	10 (9.9)	1 (2.4)	9 (15.3)	
Steroid ± cytotoxic agents†	91 (90.1)	41 (97.6)	50 (84.7)	

Data are presented as mean ± standard deviation, or number (%), unless otherwise indicated. HP, hypersensitivity pneumonitis. BMI, body mass index. FVC, forced vital capacity. DLco, diffusing capacity of the lung for carbon monoxide. TLC, total lung capacity. SpO_2 nadir, lowest oxygen saturation during 6-min walk test; *Suspected antigens were animal protein ($n = 8$), chemical agent ($n = 18$), microbes ($n = 50$) plant's proteins ($n = 7$) enzymes ($n=1$), metal ($n=1$) and pharmaceutical agents ($n=1$). †Cytotoxic agents include azathioprine, cyclosporine, or cyclophosphamide.

TABLE 2 Comparison of HRCT scores between the non-survivors and survivors patients with fibrotic HP.

	Total	Non-survivors	Survivors	*p*-value
Patient numbers	101	42	59	
Reticulation, %	14.8 ± 9.8	16.2 ± 9.1	11.6 ± 9.1	<0.001
Honeycombing,%	0.9 ± 2.5	1.7 ± 3.1	0.5 ± 1.9	0.026
GGO, %	10.7 ± 14.9	6.4 ± 10.3	13.7 ± 16.9	0.008
Consolidation, %	0.3 ± 1.8	0.03 ± 0.3	0.5 ± 2.4	0.136
Fibrosis, %	15.7 ± 10.6	20.9 ± 10.1	12.1 ± 9.5	<0.001
MA,%	12.7 ± 10.8	17.3 ± 9.9	9.4 ± 10.3	<0.001
UIP like pattern	56 (55.4)	28 (66.7)	28 (47.5)	0.056

Data are presented as mean ± standard deviation, or number (%), unless otherwise indicated; GGO, ground-glass opacity. HRCT, high-resolution computed tomography. MA, mosaic attenuation. UIP, usual interstitial pneumonia. Fibrosis score was defined as the sum of reticulation and honeycombing score. *P-value was below 0.05.

TABLE 3 Risk factor for mortality in patient with fibrotic HP using unadjusted Cox proportional hazards model.

		Unadjusted analysis	
Parameter	Hazard ratio	95% confidence interval	*p*-value
Age, years	1.059	1.023–1.097	0.001
Female sex	1.241	0.650–2.367	0.513
BMI, kg/m^2	0.948	0.874–1.028	0.199
Positive history of exposure	0.657	0.302–1.429	0.289
Ever-smoker	0.680	0.352–1.316	0.253
Pulmonary function test			
FVC, % predicted	0.973	0.955–0.991	0.003
DLco, % predicted	0.971	0.953–0.989	0.002
TLC, % predicted	0.962	0.938–0.986	0.002
6-min walk test			
Distance, meter	0.994	0.991–0.997	<0.001
SpO_2 nadir, %	0.894	0.837–0.954	0.001
HRCT findings			
Reticulation,%	1.059	1.033–1.086	<0.001
Honeycombing,%	1.159	1.067–1.259	<0.001
GGO,%	0.956	0.923–0.990	0.012
Fibrosis,%	1.065	1.039–1.091	<0.001
MA,%	1.043	1.018–1.068	0.001
Consolidation,%	0.733	0.391–1.377	0.334
UIP-like pattern	2.454	1.259–4.784	0.008

FVC, forced vital capacity. FEV_1, Forced expiratory volume in 1 sec, DLco, diffusing capacity of the lung for carbon monoxide; TLC, total lung capacity; SpO_2 nadir, lowest oxygen saturation during 6-min walk test; GGO, ground glass opacity. MA, mosaic attenuation. UIP, usual interstitial pneumonia.

5-year mortality; however, the difference was not statistically significant (Figure 1A). In addition, the performance of the fibrosis score was comparable to the ILD-GAP index in predicting the 5-year mortality (Figure 1B). The optimal cut-off value of fibrosis score was 12.0% (sensitivity 84.9%, specificity 58.8%) for the 5-year mortality. Patients with high fibrosis score (≥12.0%) showed significantly worse prognosis (the mean survival time: 146.7 months [95% CI 129.5–164.1 months] vs. 58.3 months [95% CI 49.2–67.4 months], $p < 0.01$) than those with low fibrosis score (< 12%) (Figure 2).

To improve the predicting performance of the fibrosis score for the 5-year mortality, various risk prediction models, including clinical variables (age and DLco), were evaluated (online Supplementary Table S2); however, they did not outperform the model using the fibrosis score alone.

Discussion

In this study, the radiologic fibrosis score was useful in predicting mortality in patients with fibrotic HP and showed a predictive performance comparable to that of the ILD-GAP model. Patients with high fibrosis scores (≥12.0%) had poorer survival

TABLE 4 Risk factor for mortality in patient with fibrotic HP using multivariable Cox proportional hazards model.

	HR (95% CI)					
Parameters	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6
Age, years	1.060*(1.020–1.101)	1.059*(1.019–1.100)	1.070*(1.028–1.114)	1.058*(1.019–1.099)	1.053*(1.014–1.094)	1.058*(1.018–1.099)
FVC,% predicted	0.997 (0.974–1.020)	0.987 (0.963–1.010)	0.994 (0.971–1.016)	0.996 (0.974–1.019)	0.994 (0.971–1.017)	0.988 (0.965–1.011)
DLco, % predicted	0.967*(0.936–0.999)	0.966*(0.935–0.998)	0.956*(0.926–0.987)	0.969 (0.938–1.002)	0.961*(0.931–0.992)	0.964*(0.933–0.996)
SpO_2 nadir, %	0.992 (0.912–1.079)	0.987 (0.908–1.074)	0.980 (0.901–1.067)	0.989 (0.909–1.077)	0.997 (0.918–1.083)	0.991 (0.913–1.076)
HRCT pattern						
Reticulation,%	1.038*(1.004–1.074)					
Honeycombing,%		1.092 (0.980–1.218)				
GGO,%			0.949*(0.915–0.984)			
Fibrosis,%				1.040*(1.007–1.075)		
MA,%					1.028 (0.994–1.064)	
UIP-like pattern						1.349 (0.669–2.720)

Variables with $p < 0.1$ in unadjusted Cox analysis were included in multivariable Cox analysis. TLC was excluded from the multivariate analysis due to significant correlation with FVC ($r = 0.873$, $p < 0.001$). Distance of 6-min walk test was excluded because it could be affected by other factor such as leg pain; FVC, forced vital capacity. DLco, diffusing capacity of the lung for carbon monoxide. SpO_2 nadir, lowest oxygen saturation on pulse oximetry during 6-min walk test. GGO, ground glass opacity. MA, mosaic attenuation. UIP, usual interstitial pneumonia. Fibrosis score was defined as the sum of reticulation and honeycombing score. *P-value was below 0.05.

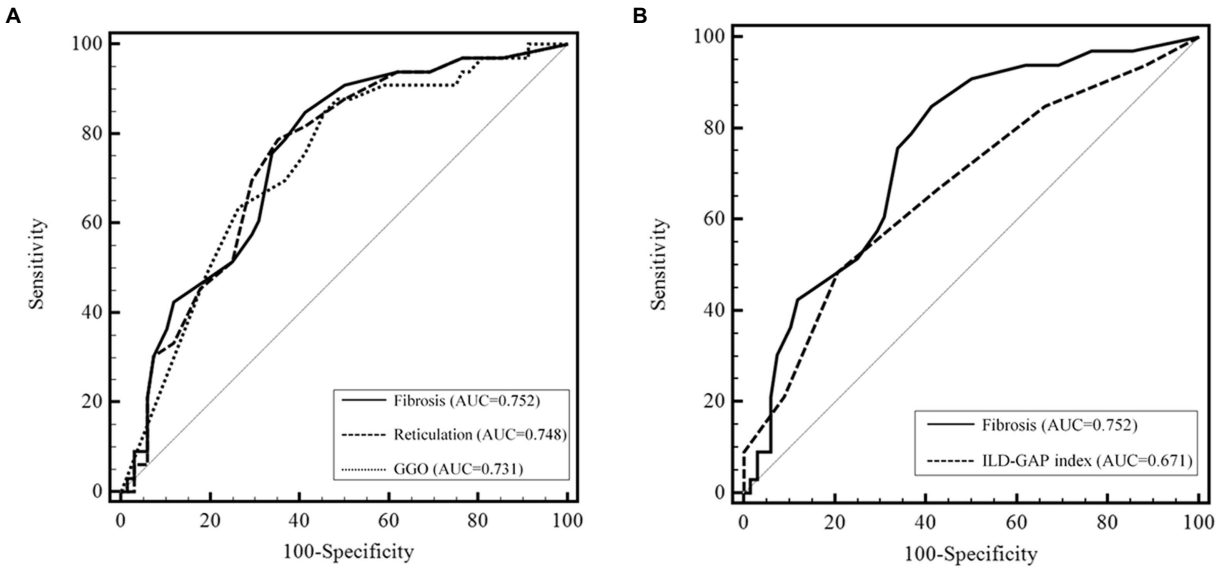

FIGURE 1
Predictive performance of CT scores for the 5year mortality in patients with fibrotic HP. **(A)** Comparison of the predicting performance of the 5year mortality among the CT scores. The area under the ROC curve was higher for the fibrosis score than for reticulation and GGO scores; however, the difference was not statistically significant (fibrosis, AUC=0.752, 95% CI 0.656–0.833, p<0.001; reticulation, AUC=0.748, 95% CI 0.651–0.829, p<0.001; GGO, AUC=0.731, 95% CI 0.634–0.815, p<0.001) (p=0.758 and p=0.697 between reticulation and fibrosis, GGO and fibrosis, respectively). **(B)** Comparison of the predicting performance of the 5year mortality between fibrosis score and ILD-GAP index. CT, computed tomography. AUC, area under the curve. GGO, ground glass opacity.

outcome than those with low fibrosis scores. The radiologic fibrosis score was also an independent predictor for mortality after adjustment for age and physiologic parameters, and even without considering the clinical variables together, fibrosis score itself showed excellent predictive performance.

In this study, a higher fibrosis score on HRCT was associated with an increased mortality risk in patients with fibrotic HP. This result correlates with previous studies (4, 13, 14). Hanak et al. showed that a greater extent of fibrosis on HRCT resulted in higher overall mortality in 26 patients with fibrotic HP (21% vs. 83% in groups with <10% and >40% of fibrosis extent, respectively) (14). In 132 patients of chronic HP, Chung et al. identified increasing reticulation as an independent predictor of reduced survival (HR 1.04; 95% CI 1.02–1.07) after adjustment for the presence of GGO and pulmonary artery/aorta ratio (4). In our study, reticulation was also significantly associated with mortality; however, the

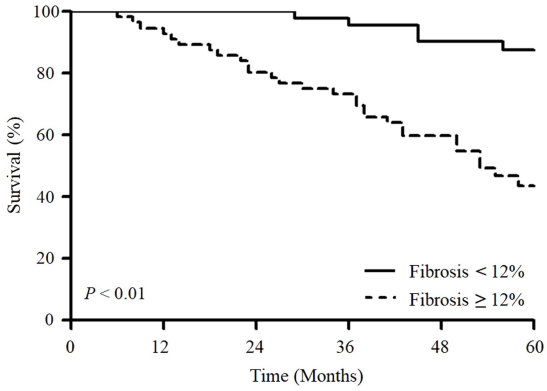

FIGURE 2
Kaplan–Meier survival analysis of overall survival according to fibrosis scores in patients with fibrotic HP. Patients with a fibrosis score above 12% had significantly worse survival than those with a score below 12% (p<0.01).

honeycombing score, one of the components of fibrotic features on HRCT, was not an independent prognostic factor in the multivariable analysis. This discrepancy might be due to the fact that the proportion of patients with radiologic honeycombing is not high, and most of them show low honeycombing scores. Similar to our finding, a previous study found that honeycombing had no statistically significant association with mortality after adjusting for GGO and pulmonary artery/aorta ratio in patients with chronic HP, even though subjects with honeycombing had worse survival than those without (log-rank test, $p = 0.007$) (4).

In our study, MA showed no significant association with mortality in patients with fibrotic HP after adjusting the clinical variables. However, in previous studies, MA was a predictor of better survival outcomes (5, 32). MA is assumed to be a secondary change due to small airway obstruction by obstructive bronchiolitis (33). Chung et al. showed that MA was associated with better survival in 110 patients with chronic HP (HR: 0.26; 95% CI: 0.07–0.97) after adjusting for age, smoking history, and FVC (5). Lima et al. also reported that in 103 patients with subacute/chronic HP, MA was an independent prognostic factor for mortality (HR: 0.05; 95% CI: 0.01–0.39) in the multivariate analysis along with age and oxygen saturation during 6MWT (32). These discordant findings may be due to the different characteristics of each cohort; previous studies included all kinds of HP (non-fibrotic and fibrotic HP) (5, 32), whereas our study included only fibrotic HP. Because MA is a common finding, particularly in non-fibrotic HP, MA may be associated with a relatively better prognosis (5, 14). It is unclear whether MA is associated with the progression or improvement of the fibrotic lesion in patients with fibrotic HP; however, Choe et al., in the study of the serial changes of CT images in patients with chronic HP, reported that the extent of MA remained constant over time, and were finally replaced by fibrosis while in progress (17).

In this study, a UIP-like pattern on HRCT showed no significant association with mortality in patients with fibrotic HP. The result of a previous study supports our findings; Chung et al., in a study of 132 patients with chronic HP, showed that a UIP pattern was not an independent predictor after adjusting for GGO, pulmonary artery/aorta ratio, and GAP index (4). Little is known about the prognostic role of a radiological UIP pattern in patients with fibrotic HP yet. Since UIP classification on chest CT has been predominantly used in IPF, the ability to adequately assess the prognosis of patients with HP may be limited (34–36). According to a previous study identifying the difference in UIP patterns between HP and IPF, HP patients with a UIP pattern had more frequent upper or middle lung dominance or extensive GGO than those with IPF (35). Therefore, in some studies that attempted to classify HRCT patterns in patients with ILD other than IPF, some modifications were applied to account for disease distributions or the presence of MA that was not consistent with a UIP pattern, as in our study (11, 29, 37). However, since there is no consensus on applying the UIP classification to HP, further studies are needed to confirm these results.

In the adjusted model including fibrosis score, only fibrosis score and age were independent risk factors for mortality in our study. Although some previous studies showed that reduced lung function was associated with poor survival in patients with chronic HP (6, 38, 39), these results were from the unadjusted analysis, and radiologic parameters were not considered together. In addition, a previous study, including 92 patients with chronic HP, identified that no individual PFT variables were independent predictors of mortality once HRCT patterns were taken into account for analysis, whereas traction bronchiectasis and honeycombing were associated with mortality (40). These results are comparable with our finding that image parameter was superior to physiologic parameters for predicting mortality in patients with fibrotic HP. These results can be explained by the fact that because HP is a kind of airway involvement disease, lung function abnormalities do not necessarily mean lung parenchymal change (41). Therefore, our results suggest that radiological parameters can be used as useful tools to overcome the limitations of physiologic parameters for predicting mortality in patients with HP.

This study has some limitations. First, it was a retrospective observational study conducted in a single center. Therefore, there might be selection bias leading to the non-generalizability of our findings. However, the baseline characteristics of our cohort were similar to those of other studies (9, 39). Second, the definition of a radiological UIP-like pattern used in this study was inconsistent with guidelines on the classification of IPF diagnostic. However, some modifications were inevitable to consider radiologic features of HP, such as upper or middle lung predominance or MA. Lastly, we did not consider comorbid conditions as confounders in the survival analysis. Because our study analyzed all-cause mortality as the primary outcome, comorbidities commonly found with fibrotic HP, such as pulmonary hypertension or heart failure, may have influenced the survival outcome (42).

In conclusions, our results suggest that a radiologic fibrosis score may be useful in predicting mortality, and high fibrosis score indicates poor prognosis in patients with fibrotic HP. Further prospective multicenter studies involving a larger population are needed to validate our findings.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Board of Asan Medical Center. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

JO, JK, and JWS: study concept and design, data collection, data analysis, interpretation, discussed the results, and reviewed the manuscript. JO and JWS: drafting of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by grants from the Basic Science Research Program (NRF-2022R1A2B5B02001602) and the Bio & Medical Technology Development Program (NRF-2022M3A9E4082647) of the National Research Foundation of Korea (NRF) funded by the Ministry of Science & ICT, Republic of Korea, and also supported by the National Institute of Health research project (2021ER120701) and by Korea Environment Industry & Technology Institute through Core Technology Development Project for Environmental Diseases Prevention and Management Program funded by Korea Ministry of Environment (ARQ202201450001), Republic of Korea.

Acknowledgments

The authors thank Jooae Choe and Eun Jin Chae (Department of Radiology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea) for radiologic analysis and Joon Seon Song (Department of Pathology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea) for the pathology review.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1131070/full#supplementary-material

References

1. Raghu G, Remy-Jardin M, Ryerson CJ, Myers JL, Kreuter M, Vasakova M, et al. Diagnosis of hypersensitivity pneumonitis in adults. An official Ats/Jrs/Alat clinical practice guideline. *Am J Respir Crit Care Med*. (2020) 202:e36–69. Epub 2020/07/25. doi: 10.1164/rccm.202005-2032ST

2. Park SW, Baek AR, Lee HL, Jeong SW, Yang SH, Kim YH, et al. Korean guidelines for diagnosis and Management of Interstitial Lung Diseases: part 1. Introduction. *Tuberc Respir Dis (Seoul)*. (2019) 82:269–76. Epub 2019/06/07. doi: 10.4046/trd.2018.0090

3. Chiba S, Tsuchiya K, Akashi T, Ishizuka M, Okamoto T, Furusawa H, et al. Chronic hypersensitivity pneumonitis with a usual interstitial pneumonia-like pattern: correlation between histopathologic and clinical findings. *Chest*. (2016) 149:1473–81. doi: 10.1016/j.chest.2015.12.030

4. Chung JH, Montner SM, Adegunsoye A, Oldham JM, Husain AN, Vij R, et al. Ct findings associated with survival in chronic hypersensitivity pneumonitis. *Eur Radiol*. (2017) 27:5127–35. Epub 2017/07/09. doi: 10.1007/s00330-017-4936-3

5. Chung JH, Zhan X, Cao M, Koelsch TL, Manjarres DCG, Brown KK, et al. Presence of air trapping and mosaic attenuation on chest computed tomography predicts survival in chronic hypersensitivity pneumonitis. *Ann Am Thorac Soc*. (2017) 14:1533–8. Epub 2017/05/18. doi: 10.1513/AnnalsATS.201701-035OC

6. Wang P, Jones KD, Urisman A, Elicker BM, Urbania T, Johannson KA, et al. Pathologic findings and prognosis in a large prospective cohort of chronic hypersensitivity pneumonitis. *Chest*. (2017) 152:502–9. doi: 10.1016/j.chest.2017.02.011

7. Alberti ML, Malet Ruiz JM, Fernandez ME, Fassola L, Caro F, Roldan IB, et al. Comparative survival analysis between idiopathic pulmonary fibrosis and chronic hypersensitivity pneumonitis. *Pulmonology*. (2020) 26:3–9. Epub 2019/11/19. doi: 10.1016/j.pulmoe.2019.08.007

8. Salisbury ML, Gu T, Murray S, Gross BH, Chughtai A, Sayyouh M, et al. Hypersensitivity pneumonitis: radiologic phenotypes are associated with distinct survival time and pulmonary function trajectory. *Chest*. (2019) 155:699–711. Epub 2018/09/24. doi: 10.1016/j.chest.2018.08.1076

9. Fernandez Perez ER, Swigris JJ, Forssen AV, Tourin O, Solomon JJ, Huie TJ, et al. Identifying an inciting antigen is associated with improved survival in patients with chronic hypersensitivity pneumonitis. *Chest*. (2013) 144:1644–51. Epub 2013/07/06. doi: 10.1378/chest.12-2685

10. Vourlekis JS, Schwarz MI, Cherniack RM, Curran-Everett D, Cool CD, Tuder RM, et al. The effect of pulmonary fibrosis on survival in patients with hypersensitivity pneumonitis. *Am J Med*. (2004) 116:662–8. Epub 2004/05/04. doi: 10.1016/j.amjmed.2003.12.030

11. Sahin H, Brown KK, Curran-Everett D, Hale V, Cool CD, Vourlekis JS, et al. Chronic hypersensitivity pneumonitis: Ct features comparison with pathologic evidence of fibrosis and survival. *Radiology*. (2007) 244:591–8. Epub 2007/07/21. doi: 10.1148/radiol.2442060640

12. Kang J, Kim YJ, Choe J, Chae EJ, Song JW. Acute exacerbation of fibrotic hypersensitivity pneumonitis: incidence and outcomes. *Respir Res*. (2021) 22:152. doi: 10.1186/s12931-021-01748-2

13. Jacob J, Bartholmai BJ, Rajagopalan S, Karwoski R, Mak SM, Mok W, et al. Automated computer-based Ct stratification as a predictor of outcome in hypersensitivity pneumonitis. *Eur Radiol*. (2017) 27:3635–46. Epub 2017/01/29. doi: 10.1007/s00330-016-4697-4

14. Hanak V, Golbin JM, Hartman TE, Ryu JH. High-resolution Ct findings of parenchymal fibrosis correlate with prognosis in hypersensitivity pneumonitis*. *Chest*. (2008) 134:133–8. doi: 10.1378/chest.07-3005

15. Salisbury ML, Myers JL, Belloli EA, Kazerooni EA, Martinez FJ, Flaherty KR. Diagnosis and treatment of fibrotic hypersensitivity pneumonia. Where we stand and where we need to go. *Am J Respir Crit Care Med*. (2017) 196:690–9. doi: 10.1164/rccm.201608-1675PP

16. Kang J, Kim YJ, Choe J, Chae EJ, Song JS, Song JW. Prognostic implication of diagnostic confidence level in patients with fibrotic hypersensitivity pneumonitis. *Respiration*. (2021) 100:940–8. Epub 2021/09/14. doi: 10.1159/000517921

17. Choe J, Chae EJ, Kim YJ, Do KH, Song JS, Song JW. Serial changes of Ct findings in patients with chronic hypersensitivity pneumonitis: imaging trajectories and predictors of fibrotic progression and acute exacerbation. *Eur Radiol*. (2021) 31:3993–4003. Epub 2020/11/27. doi: 10.1007/s00330-020-07469-2

18. MacIntyre N, Crapo RO, Viegi G, Johnson DC, van der Grinten CPM, Brusasco V, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J*. (2005) 26:720–35. doi: 10.1183/09031936.05.00034905

19. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. *Eur Respir J*. (2005) 26:511–22. doi: 10.1183/09031936.05.00035005

20. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J*. (2005) 26:319–38. doi: 10.1183/09031936.05.00034805

21. Crapo RO, Hankinson JL, Irvin C, MacIntyre NR, Voter K, Wise R, et al. Standardization of spirometry: 1994 update. *Am J Respir Crit Care Med*. (1995) 152:1107–36.

22. Society AT. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard Technique-1995 update. *Am J Respir Crit Care Med*. (1995) 152:2185–98. doi: 10.1164/ajrccm.152.6.8520796

23. Coates A, Peslin R, Rodenstein D, Stocks J. Measurement of lung volumes by plethysmography. *Eur Respir J*. (1997) 10:1415–27. doi: 10.1183/09031936.97.10061415

24. Laboratories ACoPSfCPF. Ats statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. (2002) 166:111–7. doi: 10.1164/ajrccm.166.1.at1102

25. Ryerson CJ, Vittinghoff E, Ley B, Lee JS, Mooney JJ, Jones KD, et al. Predicting survival across chronic interstitial lung disease: the Ild-gap model. *Chest*. (2014) 145:723–8. doi: 10.1378/chest.13-1474

26. Hansell DM, Bankier AA, MacMahon H, McLoud TC, Müller NL, Remy J. Fleischner society: glossary of terms for thoracic imaging. *Radiology*. (2008) 246:697–722. Epub 2008/01/16. doi: 10.1148/radiol.2462070712

27. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb)*. (2012) 22:276–82. Epub 2012/10/25. PMID: 23092060

28. Lynch DA, Sverzellati N, Travis WD, Brown KK, Colby TV, Galvin JR, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner society White paper. *Lancet Respir Med*. (2018) 6:138–53. Epub 2017/11/21. doi: 10.1016/s2213-2600(17)30433-2

29. Jacob J, Hirani N, van Moorsel CHM, Rajagopalan S, Murchison JT, van Es HW, et al. Predicting outcomes in rheumatoid arthritis related interstitial lung disease. *Eur Respir J*. (2019) 53 Epub 2018/11/30:1800869. doi: 10.1183/13993003.00869-2018

30. Unal I. Defining an optimal cut-point value in roc analysis: an alternative approach. *Comput Math Methods Med*. (2017) 2017:3762651. doi: 10.1155/2017/3762651

31. Kang L, Chen W, Petrick NA, Gallas BD. Comparing two correlated C indices with right-censored survival outcome: a one-shot nonparametric approach. *Stat Med*. (2015) 34:685–703. Epub 2014/11/18. doi: 10.1002/sim.6370

32. Lima MS, Coletta EN, Ferreira RG, Jasinowodolinski D, Arakaki JS, Rodrigues SC, et al. Subacute and chronic hypersensitivity pneumonitis: histopathological patterns and survival. *Respir Med*. (2009) 103:508–15. Epub 2009/01/31. doi: 10.1016/j.rmed.2008.12.016

33. Small JH, Flower CD, Traill ZC, Gleeson FV. Air-trapping in extrinsic allergic Alveolitis on computed tomography. *Clin Radiol*. (1996) 51:684–8. Epub 1996/10/01. doi: 10.1016/s0009-9260(96)80238-8

34. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of idiopathic pulmonary fibrosis. An official Ats/Ers/Jrs/Alat clinical practice guideline. *Am J Respir Crit Care Med*. (2018) 198:e44–68. doi: 10.1164/rccm.201807-1255ST

35. Tateishi T, Johkoh T, Sakai F, Miyazaki Y, Ogura T, Ichikado K, et al. High-resolution Ct features distinguishing usual interstitial pneumonia pattern in chronic hypersensitivity pneumonitis from those with idiopathic pulmonary fibrosis. *Jpn J Radiol*. (2020) 38:524–32. Epub 2020/02/28. doi: 10.1007/s11604-020-00932-6

36. Ohtani Y, Saiki S, Kitaichi M, Usui Y, Inase N, Costabel U, et al. Chronic bird Fancier's lung: histopathological and clinical correlation. An application of the 2002 Ats/Ers consensus classification of the idiopathic interstitial pneumonias. *Thorax*. (2005) 60:665–71. Epub 2005/08/03. doi: 10.1136/thx.2004.027326

37. Flaherty KR, Wells AU, Cottin V, Devaraj A, Walsh SL, Inoue Y, et al. Nintedanib in progressive Fibrosing interstitial lung diseases. *N Engl J Med*. (2019) 381:1718–27. doi: 10.1056/NEJMoa1908681

38. Gimenez A, Storrer K, Kuranishi L, Soares MR, Ferreira RG, Pereira CAC. Change in Fvc and survival in chronic fibrotic hypersensitivity pneumonitis. *Thorax*. (2018) 73:391–2. Epub 2017/09/09. doi: 10.1136/thoraxjnl-2017-210035

39. Mooney JJ, Elicker BM, Urbania TH, Agarwal MR, Ryerson CJ, Nguyen MLT, et al. Radiographic fibrosis score predicts survival in hypersensitivity pneumonitis. *Chest*. (2013) 144:586–92. Epub 2013/02/09. doi: 10.1378/chest.12-2623

40. Walsh SL, Sverzellati N, Devaraj A, Wells AU, Hansell DM. Chronic hypersensitivity pneumonitis: high resolution computed tomography patterns and pulmonary function indices as prognostic determinants. *Eur Radiol*. (2012) 22:1672–9. Epub 2012/04/03. doi: 10.1007/s00330-012-2427-0

41. Sharma A, Ferraro V, Renzoni EA, Morisset J. Chronic hypersensitivity pneumonitis: real world diagnostic criteria. *Curr Opin Pulm Med*. (2021) 27:414–21. Epub 2021/06/28. doi: 10.1097/MCP.0000000000000799

42. Walscher J, Gross B, Morisset J, Johannson KA, Vasakova M, Bruhwyler J, et al. Comorbidities and survival in patients with chronic hypersensitivity pneumonitis. *Respir Res*. (2020) 21:12. Epub 2020/01/11. doi: 10.1186/s12931-020-1283-8

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 24 March 2023
DOI 10.3389/fmed.2023.1155771

OPEN ACCESS

EDITED BY
Ivette Buendia-Roldan,
National Institute of Respiratory Diseases-Mexico (INER),
Mexico

REVIEWED BY
Francisco Paulin,
Hospital Fernández,
Argentina
Sergey N. Avdeev,
I.M. Sechenov First Moscow State Medical University,
Russia

*CORRESPONDENCE
Argyris Tzouvelekis
✉ atzouvelekis@upatras.gr

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 31 January 2023
ACCEPTED 06 March 2023
PUBLISHED 24 March 2023

CITATION
Kamenova A, Tzouvelekis A and Margaritopoulos GA (2023) Recent advances in the treatment of systemic sclerosis associated interstitial lung disease.
Front. Med. 10:1155771.
doi: 10.3389/fmed.2023.1155771

Recent advances in the treatment of systemic sclerosis associated interstitial lung disease

Antoniya Kamenova[1], Argyris Tzouvelekis[2]*and George A. Margaritopoulos[1]

[1]Interstitial Lung Disease Unit, London North West University Hospital HT, London, United Kingdom, [2]Respiratory Medicine Department, University of Patras, Patras, Greece

Connective tissue diseases (CTDs) are a heterogenous group of systemic inflammatory disorders. The development of connective tissue disease-associated interstitial lung disease (CTD-ILD) is a key complication associated with significant morbidity and mortality. The aim of this review is to explore the pathogenesis of CTD-ILD and summarize the recent evidence from clinical trials for novel treatment options, including the role of antifibrotics and immunomodulatory therapies with a focus on systemic sclerosis associated ILD. Further clinical trials are ongoing to explore combination therapies and more targeted therapeutic options. Clinicians remain faced with the difficult challenge of appropriately selecting patients who will benefit from the available therapies and timing the start of therapy at the most suitable part of the disease course.

KEYWORDS

interstitial lung disease, systemic sclerosis, cyclophosphamide, rituximab, mycophenolate mofetil, tocilizumab, nintedanib, pirfenidone

1. Introduction

Connective-tissue diseases (CTDs) are systemic conditions which are either caused by a specific genetic abnormality causing a defect in connective tissues or by an autoimmune reaction toward connective tissues, leading to symptoms affecting the skin, bone, cartilage, and blood vessels. The high amount of collagen and blood vessels in the lung make it a particularly susceptible target of autoimmune CTDs. Any component of the lung can be involved including airways, blood vessels, lung parenchyma, pleura or respiratory muscles (1). The development of interstitial lung disease (ILD) in autoimmune CTDs is a key complication, associated with significant morbidity and mortality (2). The types of autoimmune CTDs that are associated with the development of ILD are systemic sclerosis, rheumatoid arthritis, idiopathic inflammatory myositis (polymyositis-dermatomyositis and anti-synthetase syndrome), primary Sjogren syndrome, mixed connective tissue disease, and systemic lupus erythematosus, with various frequency (Table 1) (2). Gaining a clearer understanding of the pathogenesis of CTD-ILD paves the way for the development of targeted therapies and their timely application during the pathogenetic process. This review will focus on the pathogenesis of CTD-ILD, particularly SSc-ILD which is more commonly associated with ILD than other CTDs (3), and summarize the recent evidence from clinical trials.

2. Epidemiology of SSc-ILD

The prevalence of systemic sclerosis (SSc) is 50 to 300/million (4, 5) and SSc-ILD develops in more than 50% of cases. The types of SSc are limited cutaneous and diffuse cutaneous based on the degree of skin involvement. In limited cutaneous SSc (lcSSc) there is no skin involvement proximal to the elbows and the knees (with the exception of the face and neck areas that can be involved), while diffuse cutaneous SSc (dcSSc) affects the skin in proximal areas such as the trunk. Both lcSSc and dcSSc can be associated with ILD. ILD develops more frequently in dcSSc patients, but as lcSSc is the more common form of SSc, overall the prevalence of SSc-ILD is similar across both cohorts. The most common pattern of ILD associated with SSc is non-specific interstitial pneumonia (NSIP) (3). Another potential way to stratify the risk of developing SSc-ILD is the autoantibody profile. The risk of developing SSc-ILD is much higher with the presence of anti-topoisomerase antibodies (anti-Scl-70 autoantibodies) as opposed to anti-centromere antibodies which are more related with the development of pulmonary hypertension (5).

3. Pathogenesis – The transition from inflammation to fibrosis

The pathogenesis of CTD-ILD remains incompletely understood but is known to be a complex interplay between environmental and genetic factors. The first stage of the pathogenesis of CTD-ILD is inflammation and alveolar epithelial damage, which may be triggered by infection, by inhalation of irritating agents or by gastro-esophageal reflux disease in genetically susceptible individuals (3).

One of the major genes that have been implicated in the pathogenesis of idiopathic pulmonary fibrosis, *MUC5B*, has also been shown to play a role in the pathogenesis of CTD-ILD, particularly RA-ILD (6). The *MUC5B* promoter variant RS35705950, which has been found to be associated with RA-ILD (7), drives overexpression of MUC5B protein which affects the cilia clearance mechanisms of the lung (8). Mutations leading to telomere shortening have been found to be related to different types of pulmonary fibrosis, including CTD-ILD (9). Shorter leukocyte telomere length has been found to correlate with more rapid decline in lung function and shorter duration of transplant-free survival in patients with interstitial pneumonia with autoimmune features (IPAF) and CTD-ILD (10–12). One study identified that a polymorphism in the promoter of the connective-tissue growth factor gene is significantly more common in patients with systemic sclerosis suggesting a potential pathogenetic mechanism (13). Further genetic studies have implicated CD226 and IRF5 (14, 15). Genetic studies in different populations have identified several human leukocyte antigens (HLAs) that are associated with systemic sclerosis (16–20).

In these genetically susceptible individuals certain environmental factors trigger the pathogenetic process. These factors include gastro-esophageal reflux disease, infections, noxious chemicals and certain drugs (1). Gastro-esophageal reflux disease is associated with micro-aspirations that lead to repetitive lung injury (21). The noxious chemicals present in tobacco smoke are also known to cause inflammation and associated lung injury (22, 23), but its role in CTD-ILD is not clearly established. Among different types of CTD-ILD, smoking is most strongly associated with RA-ILD (24–26), but studies have also shown that patients with SSc who smoke heavily have increased prevalence of emphysema and decreased survival (27–29). Drugs are also implicated in the development of CTD-ILD (30). This can be challenging for the treating clinician particularly since anti-rheumatic medication such as penicillamine (31), gold (32, 33), sulfasalazine (34), and tacrolimus (35, 36) used to treat a particular type of CTD may lead to the development ILD. It is important to note, however, that particularly in the case of methotrexate, a growing body of evidence is accumulating to exonerate methotrexate in the pathogenesis of ILD (37). In fact methotrexate may protect from the development of ILD, as suggested by retrospective studies (38).

An environmental factor in a genetically susceptible individual acts to trigger the initial process of inflammation. This initial process of potentially reversible alveolitis eventually transforms into irreversible fibrosis. Therefore, it is key to identify markers of active alveolar inflammation that would identify reversible stages of the disease process (3). Surfactant Protein D (SP-D) and Krebs von den Lungen-6 (KL-6) are glycoproteins secreted by type II pneumocytes that can be used as markers of disease progression and also to signify the extent of lung involvement in scleroderma (39). This inflammatory process *via* the activation of various types of immune cells and the triggering of repair mechanisms eventually leads to the increased deposition of extra-cellular matrix, i.e., the development of fibrosis. It is important to note that in the early stages of the disease, inflammatory and pro-fibrotic processes may be occurring alongside each other, which has implications for the choice of therapeutic agents.

A key cell facilitating the increased turnover and eventual deposition of extra-cellular matrix is the resident interstitial pulmonary fibroblast. Interstitial pulmonary fibroblasts get activated *via* multiple pathways, including the transforming growth factor beta (TGF-β) pathway and recruit circulating fibroblasts and myofibroblasts. The crucial role played by the TGF-β pathway is demonstrated by a mouse model in which the TGF-β pathway is suppressed *via* the deletion of the high-affinity type II TGFβ receptor in resident fibroblasts. That mutation led to the complete absence of fibrosis after intrathecal administration of bleomycin (40).

Both innate and adaptive immunity play a role in the pathogenesis of CTD-ILD. A Th2-driven immune response has recently been elucidated, with the proinflammatory cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) playing a role in the pathogenesis (41). The active humoral response in CTD-ILD can also be illustrated by the multiple antibodies in the sera of patients with CTD-ILD. Different types of autoantibodies could be a marker for the different types of severity of lung fibrosis; anti-topoisomerase antibodies, anti-U11/U12 ribonucleoprotein (RNP) antibodies and anti-Th/To RNP antibodies are associated with an increased likelihood of developing clinically significant fibrosis (42, 43). It is not clear what the underlying pathophysiological mechanism is behind this association, but it is possible that autoantibodies can be used to risk stratify patients and may in the future prove to be suitable therapeutic targets (3). Another important role played by autoantibodies in the pathogenesis of systemic sclerosis is the link between autoantibody-mediated activation of the endothelium and vasculopathy. There are various functional autoantibodies in patients with systemic sclerosis secreted by dysregulated B cells that target endothelial cells, intercellular adhesion molecule 1 (ICAM-1), endothelin type A receptor (ETAR), angiotensin II type I receptor (AT1R) and platelet-derived growth factor receptor (PDGFR). This results in perturbation of the endothelial cells which in turn causes their activation and accelerated apoptosis. The activated

endothelial cells release a host of pro-inflammatory cytokines that stimulate the transformation of myofiobroblasts into fibroblasts leading to increased collagen deposition and subsequent tissue fibrosis (44).

4. Treatment

While immunosuppressant therapeutic options are well-established for the treatment of the systemic features of autoimmune CTDs, developing therapies that address the ILD manifestation of CTD, which is often the most severe component of the disease, is currently an area of active research. In addition to the considerable evidence base for the use of cyclophosphamide (45) and mycophenolate mofetil (46) in SSc-ILD, major breakthroughs have occurred in the last few years with new evidence accumulating from randomized controlled trials for the benefit of nintedanib (47, 48), rituximab (49), and tocilizumab (50) in SSc-ILD. The majority of clinical trials in CTD-ILD focused on SSc-ILD.

We will explore the findings of recent clinical trials for pharmacological options in CTD-ILD, summarized in Table 2.

Cyclophosphamide is a cytotoxic alkylating agent that is used in the treatment of certain oncological and autoimmune conditions (57). Retrospective studies indicated cyclophosphamide may be beneficial in the treatment of scleroderma-related ILD (SSc-ILD) (58, 59), but there was a need for prospective randomized-controlled trials to further test this hypothesis. Hoyles et al. designed a randomized controlled trial, the United Kingdom trial, in which 45 patients were randomized to receive either low dose prednisolone and 6 intravenous infusions of cyclophosphamide followed by oral azathioprine or to receive placebo. The trial was negative both for its primary endpoint, decline of forced vital capacity (FVC) and diffusing capacity for carbon monoxide (DLCO) at 12 months, as well as for its secondary end points, change of appearance in HRCT or dyspnea scores. There was, however, a trend toward statistical significance in the treatment arm of the trial, suggesting there likely was benefit of immunosuppressant therapy in SSc-ILD (51).

Further key evidence for the role of cyclophosphamide in the treatment of SSc-ILD came from the Scleroderma Lung Study-I (SLS-I) trial, the first positive trial in interstitial lung disease. The SLS-I trial was a 13-center double-blind, randomized, placebo-controlled trial in which 158 patients with symptomatic active alveolitis and SSc-ILD were randomized to receive either oral cyclophosphamide or placebo for 12 months. The patients were followed up for 12 more months after the end of the treatment period. The trial achieved its primary end point, demonstrating a statistically significant mean absolute difference in FVC at 12 months between the cyclophosphamide and the placebo arm of 2.53% in favor of the cyclophosphamide arm of the trial. In addition, the patients treated with cyclophosphamide had improvements in patient-centered outcomes including physiological improvements such as improved dyspnea, functional ability, health-related quality of life and reduced skin thickening (45). However, further subgroup analysis of the SLS-I trial patients showed that these improvements were not maintained at 24 months, except for a sustained improvement in dyspnea (60). This would suggest that ongoing immunosuppression is required in order to maintain the benefits of cyclophosphamide, but this needs to be balanced with the side effects of cyclophosphamide such as the risk of developing malignancy, infertility or hemorrhagic cystitis.

Interestingly, in the SLS-I trial there was a correlation between the extent of baseline fibrosis and the response to cyclophosphamide (45), which implies that patients with more active disease are a subgroup that would benefit more from treatment with cyclophosphamide. Given the significant side-effects associated with cyclophosphamide, it is important to balance the risk and the benefit of starting this medication.

Precisely the concern over cyclophosphamide-associated side effects prompted the design of the Scleroderma Lung Study 2 (SLS-II) trial, which compared oral cyclophosphamide against mycophenolate mofetil (MMF), an inosine monophosphate dehydrogenase inhibitor, which ultimately impairs lymphocyte proliferation and lymphocyte migration, for the treatment of SSc-ILD. A total of 142 patients with SSc-ILD that met specific criteria of breathlessness, lung function and HRCT findings were randomized to receive either MMF for 24 months or cyclophosphamide for 12 months followed by placebo for 12 months. The SLS-II trial failed to meet its primary end-point of change in forced vital capacity as a percent of the predicted normal values at 24 months. However, importantly it was noted that there was a higher drop-off rate from the cyclophosphamide arm of the trial and the time to stopping treatment was significantly shorter for the patients treated with cyclophosphamide. Side effects of leukopenia and thrombocytopenia were observed more frequently in the patients who received cyclophosphamide. However, a post-hoc analysis of the primary endpoint showed that there was substantial improvement in the adjusted FVC % from baseline to 24 months in both arms of the trial (46). Notably, SLS-II is the first trial demonstrating the effect of MMF in the treatment of SSc-ILD and suggested that the choice of therapy could be based on consideration of the side-effect profile.

The next development in the treatment options for SSc-ILD came from the SENSCIS (Safety and Efficacy of Nintedanib in Systemic Sclerosis) trial in which 576 patients with SSc-ILD with fibrosis affecting at least 10% of their lungs on HRCT were assigned to either receive the tyrosine kinase inhibitor nintedanib or placebo. The trial reached its primary end point with patients' annual rate of FVC decline being −52.4 mls in the nintedanib group versus −93.3 mls in the placebo group. Given that systemic sclerosis affects younger patients, this is a significant cumulative effect of reduced rate of FVC decline over time. Subgroup analysis showed there was no difference in the effect of nintedanib depending on the extent of underlying fibrosis, suggesting that patients could benefit from nintedanib irrespective of their degree of underlying fibrosis. While nintedanib was shown to reduce the rate of decline of FVC, it had no effect on extra-pulmonary manifestations of systemic sclerosis such as skin changes measured by the Rodnan skin score. The most frequently

TABLE 1 Autoimmune CTDs and frequency of associated ILD.

Connective tissue disease	Frequency of associated ILD (2)
Systemic sclerosis	+++
Polymyositis/dermatomyositis	+++
Rheumatoid arthritis	++
Primary Sjogren's disease	++
Systemic lupus erythematosus	++

TABLE 2 Summary of recent clinical trials for pharmacological therapies in CTD-ILD.

Trial	Year of completion	Study size	Study Design	Condition	Intervention	Primary Outcome	Result	References
UK Trial	2006	*N* = 45	RCT	SSc-ILD	Oral prednisolone, intravenous cyclophosphamide and oral azathioprine versus placebo	Decline in FVC and DLCO at 12 months	There is no statistically significant difference between combination therapy with oral prednisolone, intravenous cyclophosphamide and oral azathioprine versus placebo, but there was a trend toward lung function response with active therapy in SSc-ILD patients	(51)
SLS-I	2006	*N* = 158	RCT	SSc-ILD	Oral cyclophosphamide versus placebo	Decline in FVC at 12 months	There was a statistically significant reduction in FVC decline and in functional outcomes in SSc-ILD patients treated with oral cyclophosphamide, but the difference was not maintained at 24 months, apart from continuously improved dyspnoea	(45)
SLS-II	2016	*N* = 142	RCT	SSc-ILD	Oral cyclophosphamide versus MMF	Decline in FVC at 24 months	There was improvement in FVC with both cyclophosphamide and MMF and the drop-out rate was higher with cyclophosphamide. The first trial to show a benefit of MMF in SSc-ILD.	(46)
SENSCIS	2019	*N* = 576	RCT	SSc-ILD	Nintedanib versus placebo	Decline in FVC at 52 weeks	Nintedanib reduced the rate of decline of FVC compared to placebo in SSc-ILD.	(47)
INBUILD	2019	*N* = 170	RCT	progressing fibrosing autoimmune-ILD	Nintedanib versus placebo	Decline in FVC at 12 months	Nintedanib significantly slowed down the rate of FVC decline in progressing fibrosing autoimmune-ILD and this was maintained beyond 52 weeks. The effect was even more pronounced in patients with UIP-like ILD.	(48)
faSScinate	2016	*N* = 87	RCT	Progressive systemic sclerosis	Tocilizumab versus placebo	Decline in FVC at 48 weeks	Tocilizumab slowed FVC decline in patients with SSc-ILD, but the change was not statistically significant	(52)

(Continued)

TABLE 2 (Continued)

Trial	Year of completion	Study size	Study Design	Condition	Intervention	Primary Outcome	Result	References
focuSSced	2020	*N* = 210	RCT	dcSSc	Tocilizumab versus placebo	Change of mRRS from baseline to week 48	Failed to meet primary end point. Met secondary end point showing statistically significant reduction of the rate of decline of FVC in both the overall population and in the subgroup of patients with SSc-ILD.	(50)
STRATUS	2021	*N* = 40	RCT	SSc-ILD patients receiving stable MMF	Abituzumab versus placebo	Decline in FVC at 12 months	Trial terminated early due to small sample size	(53)
Sircar et al.	2018	*N* = 60	RCT	dcSSc	Rituximab versus cyclophosphamide	Change in FVC at 6 months	Statistically significant improvement in FVC at 6 months in the rituximab arm of the trial compared to decline in FVC in the cyclophosphamide arm of the trial. Serious adverse effects more common with cyclophosphamide.	(54)
RECITAL	2022	*N* = 101	RCT	CTD-ILD	Rituximab versus cyclophosphamide	Decline in FVC at 24 weeks	Reduced rate of decline of FVC at 24 weeks in both arms of the trial, but no statistically significant difference between rituximab and cyclophosphamide. Reduced rate of decline of FVC maintained at 48 weeks for both cyclophosphamide and rituximab.	(49)
SLS-III	2022 (expected)	*N* = 150	RCT	SSc-ILD	Pirfenidone and MMF versus placebo and MMF	Decline in FVC at 18 months	Results awaited	(55)
Wang et al.	2022	*N* = 111	RCT	CTD-ILD	Pirfenidone and immunosuppressants vs. placebo and immunosuppressants	Change in FVC at 24 weeks	Improvement in FVC at 24 weeks in patients with SSc-ILD and inflammatory myopathy-ILD	(56)

SLS-I, Scleroderma Lung Study I; SLS-II, Scleroderma Lung Study II; SLS-III, Scleroderma Lung Study III; SENSCIS, Safety and Efficacy of Nintedanib in Systemic Sclerosis; INBUILD, Efficacy and Safety of Nintedanib in Patients with Progressive Fibrosing Interstitial Lung Disease; APRIL, Abatacept in RA-ILD; faSScinate, A Study of RoActemra/Actemra (Tocilizumab) Versus Placebo in Patients With Systemic Sclerosis; focuSSced, A Study of the Efficacy and Safety of Tocilizumab in Participants with Systemic Sclerosis; STRATUS, Systemic Sclerosis Abituzumab Study; RECITAL, Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease; FVC, forced vital capacity; DLCO, diffusing capacity for carbon monoxide; MMF, mycophenolate mofetil; dcSSc, diffuse cutaneous scleroderma.

encountered side effects in the nintedanib group were gastrointestinal, including diarrhea and were generally tolerable to patients (47). The SENSCIS trial was continued as an open-label extension, the SENSCIS-ON trial (A Trial to Evaluate the Safety of Long Term Treatment With Nintedanib in Patients With Scleroderma Related Lung Fibrosis, NCT03313180) in order to study the long-term effects of nintedanib. Data from the SENSCIS-ON trial has shown that the effect of nintedanib on reducing the rate of FVC decline is maintained beyond 52 weeks (61).

Nintedanib's promise in the treatment of a broader range of non-IPF types of lung fibrosis was explored further by the landmark Efficacy and Safety of Nintedanib in Patients with Progressive

Fibrosing Interstitial Lung Disease (INBUILD) trial. The design of the trial was based on the premise that there is a common pathological mechanism in different types of pulmonary fibrosis that can be targeted irrespective of the underlying cause of the fibrosing lung disease. In this phase 3, double-blind, randomized, placebo-controlled trial, a total of 663 patients with pulmonary fibrosis that involved at least 10% of the lungs on HRCT and was progressing despite treatment over the past 24 months and were recruited. The patients were also selected according to pulmonary function test criteria of FVC at least 45% predicted and diffusing capacity for carbon monoxide (DLCO) in the range between 30 and 80 percent predicted. The trial reached its primary end point, demonstrating a significantly lower annual rate of decline of FVC in the patients treated with nintedanib: −80.8 ml per year for the treatment arm compared to – 187.8 ml per year for the placebo arm ($p < 0.001$). Furthermore, patients with a UIP-like fibrotic pattern were another primary population within the study and demonstrated an even more pronounced difference of −82.9 ml per in the treatment arm versus – 211.1 ml in the placebo arm ($p < 0.001$) (48).

A subgroup analysis of patients with autoimmune-related ILD from the INBUILD trial was conducted that demonstrated among 170 subjects with autoimmune disease-related ILDs, the rate of decline in FVC over 52 weeks was −75.9 ml/year with nintedanib versus −178.6 ml/year with placebo (62). The findings of the INBUILD trial raise an important point regarding whether a splitting or a lumping approach toward diagnosing ILD should be adopted, given that regardless of the cause in cases of progressive fibrosing ILD, the anti-fibrotic agent nintedanib can slow the rate of decline of FVC.

Further clinical trials were designed based on knowledge of the pathophysiology of systemic sclerosis. Increased levels of the cytokine interleukin-6 (IL-6) have been reported in patients with systemic sclerosis, particularly the cohort of patients that exhibit cutaneous involvement early (63, 64). It has also been identified as one of the markers of progression in patients with milder versions of SSc-ILD (FVC > 70%) as the serum level of IL-6 is correlated with the progression of SSc-ILD (65) as well as with increased decline in FVC and increased mortality (66). The central role of IL-6 in the pathogenesis of systemic sclerosis is hypothesized to be in driving the initial immune-mediated inflammation toward profibrotic processes (67, 68). Tocilizumab is an anti-IL-6 receptor antibody which has already been approved for the treatment of several autoimmune diseases including rheumatoid arthritis, juvenile idiopathic arthritis and giant cell arteritis (69). Initial promising results for tocilizumab in the treatment of systemic sclerosis were obtained from the phase 2 trial faSScinate (A Study of RoActemra/Actemra (Tocilizumab) Versus Placebo in Patients With Systemic Sclerosis) in which patients with progressive systemic sclerosis randomized to tocilizumab showed an improvement in skin thickness and a reduced rate of decline of FVC; however, both of these changes were not statistically significant, indicating the need for a larger-scale randomized controlled trial (52).

The potential role of tocilizumab in treating patients with SSc-ILD was explored in the focuSSced (A Study of the Efficacy and Safety of Tocilizumab in Participants with Systemic Sclerosis) trial, a phase 3 multicenter, randomized, double-blind, placebo-controlled trial in which 210 patients with diffuse cutaneous systemic sclerosis for 60 months or less and a modified Rodnan skin score (mRSS) of 10–35 at screening were randomized to receive either a weekly injection of tocilizumab or to placebo for 48 weeks. Importantly the patients in the trial were stratified according to their IL-6 levels. It is notable that the patient group in the focuSSced trial were highly selected; these were patients that have early disease, given the inclusion criteria was patients who exhibited their first non-Raynaud symptom no earlier than 60 months prior to recruitment in the trial. Furthermore, they had cutaneous involvement, which is known to be associated with more rapidly progressive ILD and their disease was assessed to be active based on at least one marker of inflammation among CRP, platelets and ESR. An exclusion criterion was the use of other background immunomodulatory therapies (50).

The focuSSced trial failed to meet its primary endpoint of difference in change from baseline to week 48 in mRSS. However, a key secondary end-point of change in FVC from baseline to week 48 was met. Patients in the tocilizumab arm of the trial maintained their FVC at 48 weeks, while the patients receiving placebo showed a significant decline in their FVC. Importantly, this outcome was demonstrated both in the overall patient population and in the approximately two thirds of patients that had SSc-ILD – patients with SSc-ILD in the placebo arm demonstrated a reduction of FVC by 257 mls, while patients with SSc-ILD in the tocilizumab arm demonstrated a reduction of only 20 mls. The observed adverse effects were in line with the known side effects of tocilizumab including infections and cardiac events (50). The focuSSced trial suggests that tocilizumab can prevent the decline of FVC in selected patients with scleroderma whose disease is at a high risk of progression. A continuation of the focuSSced trial as an open-label study explored the effects of tocilizumab at 96 weeks and demonstrated that the slowing of decline in FVC was preserved and the long-term side effects were in line with the known side effects of tocilizumab (70).

Another aspect of the pathogenesis of SSc-ILD was targeted in the STRATUS (Systemic Sclerosis Abituzumab Study) trial (53). The STRATUS trial studied the effect of abituzumab, an antibody against αVβ6 integrin (71, 72), which is involved with the activation of TGFβ (73), in patients with SSc-ILD. In this Phase II study the aim was to enroll 175 patients with SSc-ILD on stable MMF dose that would be randomized to either receive intravenous abituzumab every 4 weeks for 104 weeks or placebo and the primary end point was the annual rate of change in FVC. The trial was, however, terminated early due to low enrolment – 24 patients in total were enrolled, which made it impossible to draw meaningful conclusions from the data due to the small sample size (53).

Given the important role of humoral immunity in the development of CTD-ILD as signified by the association of certain antibodies with the development of ILD, it was hypothesized that rituximab, an anti-CD20 antibody that depletes B-cells (74), would have a beneficial effect for the treatment of CTD-ILD. A 2018 study by Sircar et al. included 60 patients with dcSSc who were randomized to receive either rituximab or cyclophosphamide. After 6 months patients in the rituximab arm of the trial had a statistically significant improvement in FVC while those in the cyclophosphamide arm of the trial showed a decline in FVC. Furthermore, there were more adverse events observed in the patients taking cyclophosphamide (54). In the RECITAL (Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease) trial 101 patients with CTD-ILD were randomized to either receive cyclophosphamide or rituximab. The primary end-point was the rate of change of FVC at 24 weeks, which improved in both arms of the trial. There was a slightly greater improvement of 38 mls in FVC in the cyclophosphamide arm of the trial by 24 weeks, but this did not reach statistical significance ($p = 0.493$). The observed improvement in FVC was again maintained at 48 weeks in both arms of the trial, but without statistically significant difference (49).

In summary, both immunomodulatory and antifibrotic therapies play an important role in the treatment of CTD-ILD, with immunomodulatory therapies likely being more effective in earlier inflammatory stages of the disease and antifibrotic therapies in later fibrotic stages. There is significant potential for combining immunomodulatory and antifibrotic therapies, which is explored by ongoing trials (55).

5. Discussion

5.1. When treatment should be initiated

The management challenge in SSc-ILD stems from the highly variable course of the condition, which ranges from subclinical to life-threatening (75). The treatment dilemma arises from the fact that there is a subset of patients whose disease may be relatively stable and who could develop medication related side effects without associated benefits. On the other hand, early initiation of treatment in the appropriate clinical setting could halt the progression of fibrosis in other patients. The SSc-ILD is most likely to progress during the first 4 years of systemic disease and especially in the first 2 years and in a small subset of patients, in whom lung disease precedes the cutaneous manifestations of SSc[3]. The presence of a mild to moderate ILD early in the course of the systemic disease or the evidence of recent ILD progression from the clinical, lung function and imaging point of view, should lower the threshold for treatment initiation. A factor that plays an important role in the introduction of treatment is disease severity (76). A staging system has been devised (United Kingdom Raynauds and Scleroderma Association Staging System, UKRSA) that uses HRCT and FVC data to stratify the severity of disease and has been validated for prognostication (77). When ILD extent was obviously less than, or obviously more than, 20% of the total lung volume on rapid assessment, ILD can be defined as "mild" or "extensive," respectively. In cases with an "indeterminate" disease extent (i.e., expert HRCT scoring would be required to classify disease), the FVC threshold of 70% can be used to define the ILD as mild or extensive. The distinction between mild and extensive lung disease was shown to be strongly predictive of mortality and subsequent disease progression. Treatment decisions can only be undertaken on a case-by-case basis, taking into account the views of the patient especially in mild forms of ILD. In case of mild disease in which often the introduction of treatment is not required, a strategy of close monitoring with repeat lung function tests every 4 months for at least 2 years could be applied to assess the behavior of ILD. An alternative approach highlighted by the focuSSced trial is early initiation of treatment in patients with mild forms of SSc-ILD who are deemed at high risk of disease progression based on a combination of demographic factors, lung function tests, radiological data and serum markers. Clinically reliable biomarkers to predict disease progression are still being developed and this is a promising approach for the future once these biomarkers become more reliable (78). On the other hand, in extensive disease treatment introduction is warranted to prevent progression of ILD. In any case, discussion in a dedicated multidisciplinary team (MDT) meeting should take place to decide the initial management approach (either observation or treatment), the change of medication in case of treatment failure and the most appropriate non-pharmacological approach.

5.2. Future directions

More refined understanding of the pathogenesis of CTD-ILD has aided the design of further clinical trials. Future possibilities for the treatment of CTD-ILD arise from elucidating the role Th2-driven immune response plays in the pathogenesis of systemic sclerosis. Romilkimab, an anti-IL-4 and anti-IL-13 antibody (79, 80), has shown promising results in animal models and is currently undergoing Phase II trials (Effectiveness and Safety of SAR156597 in Treating Diffuse Systemic Sclerosis, NCT02921971). Aside from using immunomodulatory therapies on their own, future directions for combined antifibrotic and immunomodulatory therapies in CTD-ILD will hopefully come from the Scleroderma Lung Study-3 (SLS-3) trial, which will compare the effect of the combination of pirfenidone and mycophenolate with mycophenolate and placebo on FVC at 18 months[55]. There is already promising data for pirfenidone from a recent randomized control trial conducted by Wang et al. who demonstrated that patients with SSc-ILD who receive both pirfenidone and immunosuppressants have a statistically significant improvement in FVC at 24 weeks compared to patients receiving only immunosuppressants (56). Furthermore, the LOTUSS study in which patients with SSc-ILD received pirfenidone for either 2 weeks or for 4 weeks demonstrated that pirfenidone has an acceptable side effect and safety profile, so pirfenidone is a promising antifibrotic therapy to continue exploring for patients with CTD-ILD (81). Another method of combining antifibrotic and anti-inflammatory therapies may be derived from the use of Janus kinase inhibitors (JAKi), which have both antifibrotic and anti-inflammatory actions and are currently being investigated for their potential role in the treatment of SSc-ILD (82).

5.3. Conclusion

CTD-ILD is a heterogenous group of conditions with a variable clinical course, which leads to challenges in their diagnosis and subsequent management. The ILD component of CTD is a major determinant of morbidity and mortality in CTDs and new developments from clinical trials have started yielding results to fill the urgent need for new therapies for CTD-ILD. The therapies for CTD-ILD remain an area of active research and hopefully there will soon be an even greater array of targeted therapies to choose from. Clinicians are still faced with the challenge of carefully selecting the most appropriate patients whose disease is likely to progress to start therapy on and balancing the risks and benefits of commencing therapy at the most appropriate time.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Shao T, Shi X, Yang S, Zhang W, Li X, Shu J, et al. Interstitial lung disease in connective tissue disease: a common lesion with heterogeneous mechanisms and treatment considerations. *Front Immunol*. (2021) 12:684699. doi: 10.3389/fimmu.2021.684699

2. Fischer A, Richeldi L. Cross-disciplinary collaboration in connective tissue disease-related lung disease. *Semin Respir Crit Care Med*. (2014) 35:159–5. doi: 10.1055/s-0034-1371530

3. Wells AU, Denton CP. Interstitial lung disease in connective tissue disease—mechanisms and management. *Nat Rev Rheumatol*. (2014) 10:728–9. doi: 10.1038/nrrheum.2014.149

4. Chifflot H, Fautrel B, Sordet C, Chatelus E, Sibilia J. Incidence and prevalence of systemic sclerosis: a systematic literature review. *Semin Arthritis Rheum*. (2008) 37:223–5. doi: 10.1016/j.semarthrit.2007.05.003

5. Wells AU, Margaritopoulos GA, Antoniou KM, Denton C. Interstitial lung disease in systemic sclerosis. *Semin Respir Crit Care Med*. (2014) 35:213–1. doi: 10.1055/s-0034-1371541

6. Juge PA, Lee JS, Ebstein E, Furukawa H, Dobrinskikh E, Gazal S, et al. MUC5B promoter variant and rheumatoid arthritis with interstitial lung disease. *N Engl J Med*. (2018) 379:2209–19. doi: 10.1056/NEJMoa1801562

7. Newton CA, Oldham JM, Ley B, Anand V, Adegunsoye A, Liu G, et al. Telomere length and genetic variant associations with interstitial lung disease progression and survival. *Eur Respir J*. (2019) 53:1801641. doi: 10.1183/13993003.01641-2018

8. Evans CM, Fingerlin TE, Schwarz MI, Lynch D, Kurche J, Warg L, et al. Idiopathic pulmonary fibrosis: a genetic disease that involves Mucociliary dysfunction of the peripheral airways. *Physiol Rev*. (2016) 96:1567–91. doi: 10.1152/physrev.00004.2016

9. Newton CA, Batra K, Torrealba J, Kozlitina J, Glazer CS, Aravena C, et al. Telomere-related lung fibrosis is diagnostically heterogeneous but uniformly progressive. *Eur Respir J*. (2016) 48:1710–20. doi: 10.1183/13993003.00308-2016

10. Stuart BD, Lee JS, Kozlitina J, Noth I, Devine MS, Glazer CS, et al. Effect of telomere length on survival in patients with idiopathic pulmonary fibrosis: an observational cohort study with independent validation. *Lancet Respir Med*. (2014) 2:557–5. doi: 10.1016/S2213-2600(14)70124-9

11. Dai J, Cai H, Li H, Zhuang Y, Min H, Wen Y, et al. Association between telomere length and survival in patients with idiopathic pulmonary fibrosis. *Respirology*. (2015) 20:947–2. doi: 10.1111/resp.12566

12. Snetselaar R, van Batenburg AA, van Oosterhout MFM, Kazemier KM, Roothaan SM, Peeters T, et al. Short telomere length in IPF lung associates with fibrotic lesions and predicts survival. *PLoS One*. (2017) 12:e0189467. doi: 10.1371/journal.pone.0189467

13. Fonseca C, Lindahl GE, Ponticos M, Sestini P, Renzoni EA, Holmes AM, et al. A polymorphism in the CTGF promoter region associated with systemic sclerosis. *N Engl J Med*. (2007) 357:1210–20. doi: 10.1056/NEJMoa067655

14. Bossini-Castillo L, Simeon CP, Beretta L, Broen JC, Vonk MC, Ríos-Fernández R, et al. A multicenter study confirms CD226 gene association with systemic sclerosis-related pulmonary fibrosis [internet]. *Arthritis Res Ther*. (2012) 14:R85. doi: 10.1186/ar3809

15. Gorlova O, Martin JM, Rueda B, Koeleman BPC, Ying J, Teruel M, et al. Identification of novel genetic markers associated with the clinical phenotypes of systemic sclerosis through a genome wide association strategy. *J Transl Med*. (2010) 8:O1. doi: 10.1186/1479-5876-8-S1-O1

16. Gladman DD, Kung TN, Siannis F, Pellett F, Farewell VT, Lee P. HLA markers for susceptibility and expression in scleroderma. *J Rheumatol*. (2005) 32:1481–7.

17. Simeón CP, Fonollosa V, Tolosa C, Palou E, Selva A, Solans R, et al. Association of HLA class II genes with systemic sclerosis in Spanish patients. *J Rheumatol*. (2009) 36:2733–6. doi: 10.3899/jrheum.090377

18. Tikly M, Rands A, McHugh N, Wordsworth P, Welsh K. Human leukocyte antigen class II associations with systemic sclerosis in south Africans. *Tissue Antigens*. (2004) 63:487–0. doi: 10.1111/j.0001-2815.2004.00199.x

19. Wang J, Guo X, Yi L, Guo G, Tu W, Wu W, et al. Association of HLA-DPB1 with scleroderma and its clinical features in Chinese population. *PLoS One*. (2014) 9:e87363. doi: 10.1371/journal.pone.0087363

20. Odani T, Yasuda S, Ota Y, Fujieda Y, Kon Y, Horita T, et al. Up-regulated expression of HLA-DRB5 transcripts and high frequency of the HLA-DRB5*01:05 allele in scleroderma patients with interstitial lung disease. *Rheumatology*. (2012) 51:1765–74. doi: 10.1093/rheumatology/kes149

21. Johannson KA, Strâmbu I, Ravaglia C, Grutters JC, Valenzuela C, Mogulkoc N, et al. Antacid therapy in idiopathic pulmonary fibrosis: more questions than answers? *Lancet Respir Med*. (2017) 5:591–8. doi: 10.1016/S2213-2600(17)30219-9

22. Zanetti F, Giacomello M, Donati Y, Carnesecchi S, Frieden M, Barazzone-Argiroffo C. Nicotine mediates oxidative stress and apoptosis through cross talk between NOX1 and Bcl-2 in lung epithelial cells. *Free Radic Biol Med*. (2014) 76:173–4. doi: 10.1016/j.freeradbiomed.2014.08.002

23. Stevenson CS, Docx C, Webster R, Battram C, Hynx D, Giddings J, et al. Comprehensive gene expression profiling of rat lung reveals distinct acute and chronic responses to cigarette smoke inhalation. *Am J Physiol Lung Cell Mol Physiol*. (2007) 293:L1183–93. doi: 10.1152/ajplung.00105.2007

24. Criswell LA, Merlino LA, Cerhan JR, Mikuls TR, Mudano AS, Burma M, et al. Cigarette smoking and the risk of rheumatoid arthritis among postmenopausal women: results from the Iowa Women's health study. *Am J Med*. (2002) 112:465–1. doi: 10.1016/S0002-9343(02)01051-3

25. Stolt P, Bengtsson C, Nordmark B, Lindblad S, Lundberg I, Klareskog L, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. *Ann Rheum Dis*. (2003) 62:835–1. doi: 10.1136/ard.62.9.835

26. Liu X, Tedeschi SK, Barbhaiya M, Leatherwood CL, Speyer CB, Lu B, et al. Impact and timing of smoking cessation on reducing risk of rheumatoid arthritis among women in the nurses' health studies [internet]. *Arthritis Care Res*. (2019) 71:914–4. doi: 10.1002/acr.23837

27. Antoniou KM, Margaritopoulos GA, Goh NS, Karagiannis K, Desai SR, Nicholson AG, et al. Combined pulmonary fibrosis and emphysema in scleroderma-related lung disease has a major confounding effect on lung physiology and screening for pulmonary hypertension. *Arthritis Rheumatol*. (2016) 68:1004–12. doi: 10.1002/art.39528

28. Hudson M, Lo E, Lu Y, Hercz D, Baron M, Steele R, et al. Cigarette smoking in patients with systemic sclerosis. *Arthritis Rheum*. (2011) 63:230–8. doi: 10.1002/art.30071

29. Hissaria P, Roberts-Thomson PJ, Lester S, Ahern MJ, Smith MD, Walker JG. Cigarette smoking in patients with systemic sclerosis reduces overall survival: comment on the article by Hudson et al [internet]. *Arthritis Rheum*. (2011) 63:1758–9. Available from:. doi: 10.1002/art.30352

30. Schwaiblmair M, Behr W, Haeckel T, Märkl B, Foerg W, Berghaus T. Drug induced interstitial lung disease. *Open Respir Med J*. (2012) 6:63–74. doi: 10.2174/1874306401206010063

31. Scott DL, Bradby GV, Aitman TJ, Zaphiropoulos GC, Hawkins CF. Relationship of gold and penicillamine therapy to diffuse interstitial lung disease. *Ann Rheum Dis*. (1981) 40:136–1. doi: 10.1136/ard.40.2.136

32. Sinha A, Silverstone EJ, O'Sullivan MM. Gold-induced pneumonitis: computed tomography findings in a patient with rheumatoid arthritis. *Rheumatology*. (2001) 40:712–4. doi: 10.1093/rheumatology/40.6.712

33. Lansdown ABG. GOLD: human exposure and update on toxic risks. *Crit Rev Toxicol*. (2018) 48:596–614. doi: 10.1080/10408444.2018.1513991

34. Hamadeh MA, Atkinson J, Smith LJ. Sulfasalazine-induced pulmonary disease. *Chest*. (1992) 101:1033–7. doi: 10.1378/chest.101.4.1033

35. Koike R, Tanaka M, Komano Y, Sakai F, Sugiyama H, Nanki T, et al. Tacrolimus-induced pulmonary injury in rheumatoid arthritis patients. *Pulm Pharmacol Ther*. (2011) 24:401–6. doi: 10.1016/j.pupt.2011.01.016

36. Sasaki T, Nakamura W, Inokuma S, Matsubara E. Characteristic features of tacrolimus-induced lung disease in rheumatoid arthritis patients. *Clin Rheumatol*. (2016) 35:541–5. doi: 10.1007/s10067-015-2865-6

37. Wells AU. New insights into the treatment of CTD-ILD. *Nat Rev Rheumatol*. (2021) 17:79–80. doi: 10.1038/s41584-020-00567-x

38. Juge PA, Lee JS, Lau J, Kawano-Dourado L, Rojas Serrano J, Sebastiani M, et al. Methotrexate and rheumatoid arthritis associated interstitial lung disease. *Eur Respir J*. (2021) 57:2000337. doi: 10.1183/13993003.00337-2020

39. Hant FN, Ludwicka-Bradley A, Wang HJ, Li N, Elashoff R, Tashkin DP, et al. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. *J Rheumatol*. (2009) 36:773–0. doi: 10.3899/jrheum.080633

40. Hoyles RK, Derrett-Smith EC, Khan K, Shiwen X, Howat SL, Wells AU, et al. An essential role for resident fibroblasts in experimental lung fibrosis is defined by lineage-specific deletion of high-affinity type II transforming growth factor β receptor. *Am J Respir Crit Care Med*. (2011) 183:249–1. doi: 10.1164/rccm.201002-0279OC

41. Pechkovsky DV, Prasse A, Kollert F, Engel KMY, Dentler J, Luttmann W, et al. Alternatively activated alveolar macrophages in pulmonary fibrosis—mediator production and intracellular signal transduction. *Clin Immunol*. (2010) 137:89–1. doi: 10.1016/j.clim.2010.06.017

42. Nihtyanova SI, Schreiber BE, Ong VH, Rosenberg D, Moinzadeh P, Coghlan JG, et al. Prediction of pulmonary complications and long-term survival in systemic sclerosis. *Arthritis Rheumatol*. (2014) 66:1625–35. doi: 10.1002/art.38390

43. Nihtyanova SI, Denton CP. Autoantibodies as predictive tools in systemic sclerosis. *Nat Rev Rheumatol*. (2010) 6:112–6. doi: 10.1038/nrrheum.2009.238

44. Furue M, Mitoma C, Mitoma H, Tsuji G, Chiba T, Nakahara T, et al. Pathogenesis of systemic sclerosis—current concept and emerging treatments. *Immunol Res*. (2017) 65:790–7. doi: 10.1007/s12026-017-8926-y

45. Tashkin DP, Elashoff R, Clements PJ, Goldin J, Roth MD, Furst DE, et al. Cyclophosphamide versus placebo in scleroderma lung disease. *N Engl J Med*. (2006) 354:2655–66. doi: 10.1056/NEJMoa055120

46. Tashkin DP, Roth MD, Clements PJ, Furst DE, Khanna D, Kleerup EC, et al. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir Med*. (2016) 4:708–9. doi: 10.1016/S2213-2600(16)30152-7

47. Distler O, Highland KB, Gahlemann M, Azuma A, Fischer A, Mayes MD, et al. Nintedanib for systemic sclerosis-associated interstitial lung disease. *N Engl J Med*. (2019) 380:2518–28. doi: 10.1056/NEJMoa1903076

48. Flaherty KR, Wells AU, Cottin V, Devaraj A, Walsh SLF, Inoue Y, et al. Nintedanib in progressive Fibrosing interstitial lung diseases. *N Engl J Med*. (2019) 381:1718–27. doi: 10.1056/NEJMoa1908681

49. Maher TM, Tudor VA, Saunders P, Gibbons MA, Fletcher SV, Denton CP, et al. Rituximab versus intravenous cyclophosphamide in patients with connective tissue disease-associated interstitial lung disease in the UK (RECITAL): a double-blind, double-dummy, randomised, controlled, phase 2b trial. *Respir Med*. (2023) 11:45–54. doi: 10.1016/S2213-2600(22)00359-9

50. Khanna D, Lin CJF, Furst DE, Goldin J, Kim G, Kuwana M, et al. Tocilizumab in systemic sclerosis: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med*. (2020) 8:963–4. doi: 10.1016/S2213-2600(20)30318-0

51. Hoyles RK, Ellis RW, Wellsbury J, Lees B, Newlands P, Goh NSL, et al. A multicenter, prospective, randomized, double-blind, placebo-controlled trial of corticosteroids and intravenous cyclophosphamide followed by oral azathioprine for the treatment of pulmonary fibrosis in scleroderma. *Arthritis Rheum*. (2006) 54:3962–70. doi: 10.1002/art.22204

52. Khanna D, Denton CP, Jahreis A, van Laar JM, Frech TM, Anderson ME, et al. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. *Lancet*. (2016) 387:2630–40. doi: 10.1016/S0140-6736(16)00232-4

53. Khanna D, Tashkin DP, Wells AU, Seibold JR, Wax S, Vazquez-Mateo C, et al. STRATUS: a phase II study of Abituzumab in patients with systemic sclerosis–associated interstitial lung disease. *J Rheumatol*. (2021) 48:1295–8. doi: 10.3899/jrheum.191365

54. Sircar G, Goswami RP, Sircar D, Ghosh A, Ghosh P. Intravenous cyclophosphamide vs rituximab for the treatment of early diffuse scleroderma lung disease: open label, randomized, controlled trial. *Rheumatology*. (2018) 57:2106–13. doi: 10.1093/rheumatology/key213

55. Roth M. Scleroderma lung study III–combining Pirfenidone with mycophenolate (SLSIII). (2023) ClinicalTrials gov Identifier NCT03221257 Los Angeles, University of California.

56. Wang J, Wang X, Qi X, Sun Z, Zhang T, Cui Y, et al. The efficacy and safety of Pirfenidone combined with immunosuppressant therapy in connective tissue disease-associated interstitial lung disease: a 24-week prospective controlled cohort study. *Front Med*. (2022) 9:871861. doi: 10.3389/fmed.2022.871861

57. de Jonge ME, de Jonge ME, Huitema ADR, Rodenhuis S, Beijnen JH. Clinical pharmacokinetics of cyclophosphamide [internet]. *Clin Pharmacokinet*. (2005) 44:1135–64. doi: 10.2165/00003088-200544110-00003

58. White B, Moore WC, Wigley FM, Xiao HQ, Wise RA. Cyclophosphamide is associated with pulmonary function and survival benefit in patients with scleroderma and alveolitis. *Ann Intern Med*. (2000) 132:947–4. doi: 10.7326/0003-4819-132-12-200006200-00004

59. Latsi PI, Wells AU. Evaluation and management of alveolitis and interstitial lung disease in scleroderma. *Curr Opin Rheumatol*. (2003) 15:748–5. doi: 10.1097/00002281-200311000-00011

60. Tashkin DP, Elashoff R, Clements PJ, Roth MD, Furst DE, Silver RM, et al. Effects of 1-year treatment with cyclophosphamide on outcomes at 2 years in scleroderma lung disease. *Am J Respir Crit Care Med*. (2007) 176:1026–34. doi: 10.1164/rccm.200702-326OC

61. Allanore Y, Vonk MC, Distler O, Azuma A, Mayes MD, Gahlemann M, et al. Continued treatment with nintedanib in patients with systemic sclerosis-associated interstitial lung disease: data from SENSCIS-ON. *Ann Rheum Dis*. (2022) 81:1722–9. doi: 10.1136/ard-2022-222564

62. Matteson EL, Kelly C, Distler JHW, Hoffmann-Vold AM, Seibold JR, Mittoo S, et al. Nintedanib in patients with autoimmune disease-related progressive Fibrosing interstitial lung diseases: subgroup analysis of the INBUILD trial. *Arthritis Rheumatol*. (2022) 74:1039–47. doi: 10.1002/art.42075

63. Sato S, Hasegawa M, Takehara K. Serum levels of interleukin-6 and interleukin-10 correlate with total skin thickness score in patients with systemic sclerosis. *J Dermatol Sci*. (2001) 27:140–6. doi: 10.1016/S0923-1811(01)00128-1

64. Matsushita T, Hasegawa M, Hamaguchi Y, Takehara K, Sato S. Longitudinal analysis of serum cytokine concentrations in systemic sclerosis: association of interleukin 12 elevation with spontaneous regression of skin sclerosis. *J Rheumatol*. (2006) 33:275–4.

65. Lauretis AD, De Lauretis A, Sestini P, Pantelidis P, Hoyles R, Hansell DM, et al. Serum interleukin 6 is predictive of early functional decline and mortality in interstitial lung disease associated with systemic sclerosis [internet]. *J Rheumatol*. (2013) 40:435–6. doi: 10.3899/jrheum.120725

66. Khan K, Xu S, Nihtyanova S, Derrett-Smith E, Abraham D, Denton CP, et al. Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. *Ann Rheum Dis*. (2012) 71:1235–42. doi: 10.1136/annrheumdis-2011-200955

67. Nihtyanova SI, Denton CP. Pathogenesis of systemic sclerosis associated interstitial lung disease. *J Scleroderma Relat Disord*. (2020) 5:6–16. doi: 10.1177/2397198320903867

68. Gao X, Jia G, Guttman A, DePianto DJ, Morshead KB, Sun KH, et al. Osteopontin links myeloid activation and disease progression in systemic sclerosis. *Cell Rep Med*. (2020) 1:100140. doi: 10.1016/j.xcrm.2020.100140

69. Sheppard M, Laskou F, Stapleton PP, Hadavi S, Dasgupta B. Tocilizumab (Actemra). *Hum Vaccin Immunother*. (2017) 13:1972–88. doi: 10.1080/21645515.2017.1316909

70. Khanna D, Lin CJF, Furst DE, Wagner B, Zucchetto M, Raghu G, et al. Long-term safety and efficacy of tocilizumab in early systemic sclerosis–interstitial lung disease: open-label extension of a phase 3 randomized controlled trial. *Am J Respir Crit Care Med*. (2022) 205:674–4. doi: 10.1164/rccm.202103-0714OC

71. Élez E, Kocáková I, Höhler T, Martens UM, Bokemeyer C, Van Cutsem E, et al. Abituzumab combined with cetuximab plus irinotecan versus cetuximab plus irinotecan alone for patients with KRAS wild-type metastatic colorectal cancer: the randomised phase I/II POSEIDON trial. *Ann Oncol*. (2015) 26:132–40. doi: 10.1093/annonc/mdu474

72. Hussain M, Le Moulec S, Gimmi C, Bruns R, Straub J, Miller K, et al. Differential effect on bone lesions of targeting Integrins: randomized phase II trial of Abituzumab in patients with metastatic castration-resistant prostate cancer. *Clin Cancer Res*. (2016) 22:3192–00. doi: 10.1158/1078-0432.CCR-15-2512

73. Katsumoto TR, Violette SM, Sheppard D. Blocking TGFβ via inhibition of the αvβ6 integrin: a possible therapy for systemic sclerosis interstitial lung disease. *Int J Rheumatol*. (2011) 2011:1–7. doi: 10.1155/2011/208219

74. Perosa F, Prete M, Racanelli V, Dammacco F. CD20-depleting therapy in autoimmune diseases: from basic research to the clinic [internet]. *J Intern Med*. (2010) 267:260–7. doi: 10.1111/j.1365-2796.2009.02207.x

75. Hoffmann-Vold AM, Allanore Y, Alves M, Brunborg C, Airó P, Ananieva LP, et al. Progressive interstitial lung disease in patients with systemic sclerosis-associated interstitial lung disease in the EUSTAR database. *Ann Rheum Dis*. (2021) 80:219–7. doi: 10.1136/annrheumdis-2020-217455

76. Steen VD, Lanz JK, Conte C, Owens GR, Medsger TA. Therapy for severe interstitial lung disease in systemic sclerosis. A retrospective study. *Arthritis Rheum*. (1994) 37:1290–6. doi: 10.1002/art.1780370904

77. Goh NSL, Desai SR, Veeraraghavan S, Hansell DM, Copley SJ, Maher TM, et al. Interstitial lung disease in systemic sclerosis: a simple staging system. *Am J Respir Crit Care Med*. (2008) 177:1248–54. doi: 10.1164/rccm.200706-877OC

78. Khanna D, Lescoat A, Roofeh D, Bernstein EJ, Kazerooni EA, Roth MD, et al. Systemic sclerosis-associated interstitial lung disease: how to incorporate two Food and Drug Administration-approved therapies in clinical practice. *Arthritis Rheumatol*. (2022) 74:13–27. doi: 10.1002/art.41933

79. Raghu G, Richeldi L, Crestani B, Wung P, Bejuit R, Esperet C, et al. SAR156597 in idiopathic pulmonary fibrosis: a phase 2 placebo-controlled study (DRI11772). *Eur Respir J*. (2018) 52:1801130. doi: 10.1183/13993003.01130-2018

80. Allanore Y, Wung P, Soubrane C, Esperet C, Frederic M, Bejuit R, et al. OP0250 efficacy and safety of Romilkimab in diffuse cutaneous systemic sclerosis (dcssc): randomized, double-blind, placebo-controlled, 24-week, proof of concept study [internet]. *Ann Rheum Dis*. (2020) 79:157–8. doi: 10.1136/annrheumdis-2020-eular.4830

81. Khanna D, Albera C, Fischer A, Khalidi N, Raghu G, Chung L, et al. An open-label, phase II study of the safety and tolerability of Pirfenidone in patients with scleroderma-associated interstitial lung disease: the LOTUSS trial. *J Rheumatol*. (2016) 43:1672–9. doi: 10.3899/jrheum.151322

82. Fiorentini E, Bonomi F, Peretti S, Orlandi M, Lepri G, Matucci Cerinic M, et al. Potential role of JAK inhibitors in the treatment of systemic sclerosis-associated interstitial lung disease: a narrative review from pathogenesis to real-life data. *Life*. (2022) 12:2101. doi: 10.3390/life12122101

frontiers | Frontiers in Medicine

TYPE Review
PUBLISHED 06 April 2023
DOI 10.3389/fmed.2023.1160755

OPEN ACCESS

EDITED BY
Theodoros Karampitsakos,
University of South Florida, United States

REVIEWED BY
Ilias C. Papanikolaou,
General Hospital of Corfu "Agia Eirini", Greece
Ourania Papaioannou,
General University Hospital of Patras, Greece

*CORRESPONDENCE
Nadera Sweiss
✉ nsweiss@uic.edu

†These authors have contributed equally to this work and share first authorship

‡These authors have contributed equally to this work and share second authorship

§These authors have contributed equally to this work and share third authorship

SPECIALTY SECTION
This article was submitted to Pulmonary Medicine, a section of the journal Frontiers in Medicine

RECEIVED 07 February 2023
ACCEPTED 20 March 2023
PUBLISHED 06 April 2023

CITATION
Sweis JJG, Sweis NWG, Alnaimat F, Jansz J, Liao T-WE, Alsakaty A, Azam A, Elmergawy H, Hanson HA, Ascoli C, Rubinstein I and Sweiss N (2023) Immune-mediated lung diseases: A narrative review. *Front. Med.* 10:1160755. doi: 10.3389/fmed.2023.1160755

Immune-mediated lung diseases: A narrative review

Jaleel Jerry G. Sweis[1†], Nabil W. G. Sweis[1†], Fatima Alnaimat[2‡], Jacqueline Jansz[3‡], Ting-Wei Ernie Liao[4,5‡], Alaa Alsakaty[6§], Abeera Azam[7§], Hesham Elmergawy[6§], Hali A. Hanson[8§], Christian Ascoli[9], Israel Rubinstein[9,10] and Nadera Sweiss[6*]

[1]School of Medicine, The University of Jordan, Amman, Jordan, [2]Division of Rheumatology, Department of Internal Medicine, The University of Jordan, Amman, Jordan, [3]Department of Medicine, University of Illinois Chicago, Chicago, IL, United States, [4]School of Medicine, Faculty of Medicine, National Yang Ming Chiao Tung University, Taipei City, Taiwan, [5]Division of Cardiology, Department of Medicine, Taipei Veterans General Hospital, Taipei City, Taiwan, [6]Division of Rheumatology, Department of Medicine, University of Illinois Chicago, Chicago, IL, United States, [7]Department of Internal Medicine, The University of Texas Health Science Center at Tyler, Tyler, TX, United States, [8]UIC College of Pharmacy, University of Illinois Chicago, Chicago, IL, United States, [9]Division of Pulmonary, Critical Care, Sleep and Allergy, Department of Medicine, University of Illinois Chicago, Chicago, IL, United States, [10]Research Service, Jesse Brown VA Medical Center, Chicago, IL, United States

The role of immunity in the pathogenesis of various pulmonary diseases, particularly interstitial lung diseases (ILDs), is being increasingly appreciated as mechanistic discoveries advance our knowledge in the field. Immune-mediated lung diseases demonstrate clinical and immunological heterogeneity and can be etiologically categorized into connective tissue disease (CTD)-associated, exposure-related, idiopathic, and other miscellaneous lung diseases including sarcoidosis, and post-lung transplant ILD. The immunopathogenesis of many of these diseases remains poorly defined and possibly involves either immune dysregulation, abnormal healing, chronic inflammation, or a combination of these, often in a background of genetic susceptibility. The heterogeneity and complex immunopathogenesis of ILDs complicate management, and thus a collaborative treatment team should work toward an individualized approach to address the unique needs of each patient. Current management of immune-mediated lung diseases is challenging; the choice of therapy is etiology-driven and includes corticosteroids, immunomodulatory drugs such as methotrexate, cyclophosphamide and mycophenolate mofetil, rituximab, or other measures such as discontinuation or avoidance of the inciting agent in exposure-related ILDs. Antifibrotic therapy is approved for some of the ILDs (e.g., idiopathic pulmonary fibrosis) and is being investigated for many others and has shown promising preliminary results. A dire need for advances in the management of immune-mediated lung disease persists in the absence of standardized management guidelines.

KEYWORDS

immune-mediated lung diseases, interstitial lung disease, connective tissue diseases, post-COVID-19, idiopathic pulmonary fibrosis, sarcoidosis, drug-induced lung injury, post-lung transplant

1. Introduction

The immune system is a heterogeneous collection of physical, cellular, and biochemical components that defend against microbial organisms and non-microbial foreign antigens. Based on their antigen specificity and promptness of response, the immune system's components can be divided into innate and adaptive immune arms (1). Innate immunity represents the early response system to foreign body entry. It comprises cutaneous and mucosal barriers, phagocytic cells (e.g., macrophages and neutrophils), soluble factors (e.g., complement system), and receptors (e.g., pattern recognition receptors). The rapidity of the response of the innate arm is a crucial feature conferred by the capacity of its receptors to recognize pre-determined structural patterns common to a vast array of foreign pathogens, which accounts for its lack of specificity (2). The delayed, more antigen-specific adaptive immune response ensues, generating cells and/or mediators specifically tailored to the invading pathogen or substance, producing a more efficient response. The adaptive immune system can be further divided into humoral immunity, effectuated by B lymphocytes and their secreted antibodies, and cellular immunity, effectuated by various subsets of T lymphocytes. The adaptive response also generates memory cells for a more rapid anamnestic response against the same foreign antigen should re-exposure occur (3). As convenient as the classification may seem, the innate and adaptive arms are not mutually exclusive; a substantial degree of crosstalk occurs between both systems *via* cells (e.g., dendritic cells) and an intricate collection of mediators orchestrates and fine-tunes the immune response (4).

The respiratory tract merits special consideration in the discussion of immunity. By virtue of its gas exchange function, the respiratory tract is a stage for constant clashes between the immune system and foreign exposures that manage to trickle into the airways with each breath (4). As such, the respiratory system is equipped with a remarkable set of defense lines, from the mucociliary apparatus and respiratory epithelium to the effector immune cells lingering in pulmonary tissue. Small particles that overcome the mechanical barrier are initially faced with resident innate immune cells, namely, the alveolar macrophages, alongside a group of secreted enzymes, immunoglobulins, and antimicrobial factors (5). Recruitment of other cells from the innate and adaptive systems occurs for more effective clearance of the foreign substance. Despite affording great protection to the lungs in particular and the body in general, the respiratory immune system may prove detrimental in conditions where it is dysregulated or generates an exaggerated response. In such settings, a wide range of pulmonary pathologies can emerge and have been described quite extensively (6). Nevertheless, the specific mechanisms that link immunity to lung injury in many of these disease entities remain poorly defined and are integral to devising targeted therapeutic interventions, especially for the diffuse parenchymal lung diseases.

Diffuse parenchymal lung diseases, also referred to as interstitial lung diseases (ILDs), are a diverse set of illnesses grouped according to comparable clinical, radiological, physiological, or pathologic characteristics (7–9). The word "interstitial" is used to describe the pathologic appearance that the aberration starts in the interstitium. Still, it is slightly misleading because most of these disorders are also linked to significant changes in the alveolar and pulmonary architecture. Among the various ILDs, sarcoidosis, connective tissue disease (CTD)–associated ILDs, and idiopathic pulmonary fibrosis (IPF) are the most common fibrotic ILDs, with an estimated prevalence of 30.2, 12.1, and 8.2 cases per 100,000, respectively (10). A multidisciplinary approach, typically involving pulmonologists, radiologists, and pathologists, is necessary for the differential diagnosis of ILDs (11). Clinical presentation, a thorough history review (medications, infectious and occupational exposures), smoking status, changes in lung function, the findings of serological tests, imaging, and, if necessary, a lung biopsy are all part of an evaluation (9, 12, 13). ILD is first suspected when a patient experiences progressive shortness with exertion (dyspnea), a prolonged non-productive cough, and/or pulmonary symptoms linked to another illness, including rheumatic disease. Regular laboratory testing is frequently non-specific. High-resolution computed tomography (HRCT) of the chest is the primary diagnostic tool for ILD (13). The underlying diagnosis and the progression of the disease serve as the basis for decisions regarding pharmacologic treatment. Antifibrotic medications, such as pirfenidone or nintedanib, are indicated for IPF patients (14). In most cases of fibrosing ILD other than IPF, immunomodulation with glucocorticoids, immunosuppressive therapy, or both are appropriate and are often utilized as first-line therapy if there is a suspicion of inflammation-driven disease (15–17).

The association between ILD and immunity in general and autoimmunity in particular is evolving. This review summarizes the current understanding of immunity and lung injury observed in CTD-associated, exposure-related, idiopathic, and other miscellaneous lung diseases (**Figure 1**). Also, we will shed light on common clinical, radiologic, and pathologic findings in each section. It is crucial to make an early diagnosis, identify triggers and consider immunosuppressive therapy when indicated (6). Other ILDs, including idiopathic interstitial pneumonia and ILDs with cysts and/or airspace filling (18), were not discussed in this review.

2. Connective tissue disease-associated lung diseases

Connective tissue disease -associated lung diseases are summarized in **Table 1**.

2.1. ILD in systemic sclerosis (SSc-ILD)

Systemic sclerosis (SSc) is a chronic systemic immune disease characterized by fibrosis of the skin, lungs, and other vital organs. SSc is characterized by persistent collagen overproduction and connective tissue deposition in various organs. The exact etiology is unknown but involves genetic, environmental, and autoimmune factors. SSc is classified as a class II Human Leukocyte Antigen (HLA) disease, and the HLA genes strongly influence SSc predisposition (19, 20). Humoral and cellular immunity alterations associated with specific autoantibodies are a feature of SSc and occur very early in the disease process.

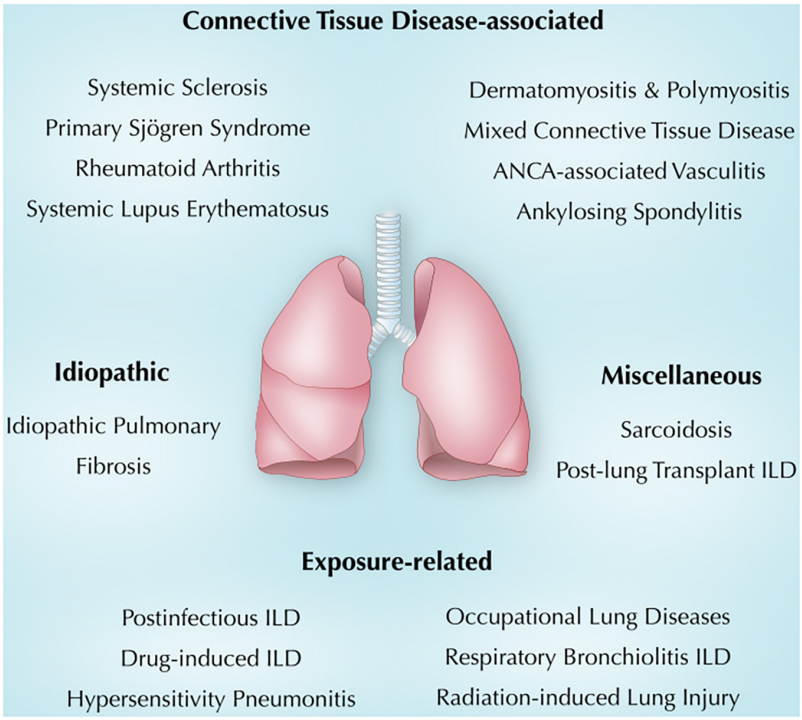

FIGURE 1
Overview of immune-mediated lung diseases discussed in this review. ANCA, anti-neutrophil cytoplasmic antibodies; ILD, interstitial lung disease.

The initial phase in the pathophysiology of SSc ILD is thought to originate in response to a chronic insult to the alveolar epithelial and endothelial cells caused by local inflammation or an environmental stimulus (21). This injury stimulates the recruitment of several inflammatory cells, which infiltrate the alveolar spaces and activate existing immune cells in the lungs (22, 23). Evidence of immune cell activation has been validated in studies demonstrating that CCL2 and other chemokines are released after the epithelial cell injury and influence inflammation and the migration of leukocytes, particularly the activation of M1 (pro-inflammatory) and M2 (profibrotic) macrophages (24). SP-D and KL-6 are glycoproteins that are released from injured epithelial cells and have been found to be elevated in the serum of SSc patients with ILD as opposed to those without ILD supporting the premise of chronic epithelial cell injury (22), further emphasizing the essential role of epithelial cell injury in the SSc-ILD (25).

Adaptive immune system activation leads to the infiltration of T- and B-cells, inducing profibrotic factors and the stimulation of transforming growth factor beta (TGF-β) from its latent state (21), the latter being the principal controller of the immune response (26).

In the absence of effective tissue repair, the resident lung fibroblasts differentiate into myofibroblasts culminating in parenchymal fibrosis. Other potential sources of the myofibroblast include circulating fibrocytes derived from the bone marrow and a biochemical process called the epithelial-mesenchymal transition (EMT) (27, 28). In EMT, alveolar epithelial cells' polarity is lost, and they lay down an increased extracellular matrix and acquire mesenchymal characteristics (28). However, this theory is not yet proven and has been challenged by more recent research (29).

2.1.1. Clinical manifestations

Patients often present with Raynaud's phenomenon and may also present with swollen fingers, sclerodactyly (tightening and thickening of the skin), arthralgia or arthritis, malaise, gastrointestinal, renal, cardiac, or pulmonary symptoms (30). The disease can be limited, formerly known as CREST syndrome, or diffuse. Antinuclear antibodies (ANA) are detected in most SSc patients (19, 31). Patients who lack detectable ANA tend to be males and have a reduced frequency of vasculopathy manifestations (32). Several specific antinuclear antibodies are present in systemic sclerosis, including the anti-topoisomerase I (ATA; anti-Scl70), the anti-centromere (ACA), and the anti-RNA polymerase antibodies. These antibodies act as major disease markers, are mutually exclusive and define clinical presentation (31).

Interstitial lung disease is a serious organ involvement in SSc patients and is the principal cause of death (33). Pulmonary involvement occurs in 80% of SSc (34). Traditionally, ILD is linked with diffuse SSc compared to the limited form. However, it is thought that the presence of ATA carries the highest risk of ILD regardless of the cutaneous form (35). Inflammation and fibrosis findings on radiological imaging by HRCT and histopathology characterize SSc-ILD. Non-specific interstitial pneumonia (NSIP) is the major histopathologic type of SSc ILD, while the usual interstitial pneumonia (UIP) form occurs in fewer patients. Moreover, NSIP is characterized by mostly inflammatory cellular/lymphocytic infiltrates and minimal damage or fibrosis (36).

2.1.2. Treatment

Effective SSc-ILD treatment remains a challenge despite the development of new therapeutics.

TABLE 1 Summary of CTD-associated lung diseases.

	Systemic sclerosis	Primary Sjögren syndrome	Rheumatoid arthritis	Systemic lupus erythematosus (SLE)	Dermatomyositis (DM) and polymyositis (PM)	MCTD	Systemic (ANCA-associated) vasculitis	Ankylosing pondylitis
Clinical manifestations	Raynaud's phenomenon, MSS (arthralgia or arthritis), skin (tightening, thickening), GIS, RS (ILD, PAH), renal system (renal crisis), CVS (arrhythmia, pericardial disease, HF).	Dry eyes, mouth, and mucous membrane, parotitis, MSS (arthritis), parotitis; RS (ILD), skin, renal system, CNS, lymph nodes may rarely be affected	MSS (most common), rarely RS (ILD) and vascular system (vasculitis), skin (pyoderma, rheumatoid nodules), eyes (scleritis, keratitis)	MSS, renal system (lupus nephritis), skin, mucous membranes, RS (ILD), CVS (pericardial disease, MI), CNS (seizures, psychosis), hematological abnormalities (cytopenia)	MSS (proximal muscle weakness), skin in DM (Symmetric erythematous rash), RS (ILD), CVS (myocarditis, HF). Increased risk of malignancy	Raynaud's phenoumenon; MSS (arthralgia, myalgia), RS (ILD, PAH), CVS (pericarditis), Renal system, and CNS may be affected	Vascular system (wall thickening), skin (purpura), RS (ILD), CNS (headache, seizures, ischemia), MSS (arthralgia), renal system (glomerulonephritis)	MSS (enthesitis, pain in the sacroiliac joints and spine), eyes (uveitis), CVS (aortitis), RS (ILD, restrictive lung disease)
Laboratory data and pathological findings	ANA, anti-topoisomerase I, anti-centromere and others). Excess matrix deposition, fibrosis, epithelial and endothelial cells dysfunction and death	ANA, anti-Ro/SSA, anti-La/SSB, rheumatoid factor. B-cell and T-cell infiltration	Rheumatoid factor and anti-CCP. Swollen synovium, presence of fibroblast-like and macrophage-like synoviocytes, macrophages and B-cells	ANA, anti-dsDNA, anti-Smith, anti-Ro/SSA, anti-La/SSB, anti-histone (most commonly in drug induced SLE), antiphospholipid antibodies, hypocomplementemia, anemia, lymphocytopenia immune complex formation and deposition, producing inflammation and vasculitis	Serum muscle enzymes (such as creatine kinase and aldolase), an ANA test, anti-Mi-2 antibodies, MDA-5 antibodies and anti-Jo-1 antibody. Capillary abnormalities and loss, perimysial abnormalities, and peri fascicular atrophy	ANA and anti-U1 ribonucleoprotein (RNP). Activation of B-and T-cell, autoantigen modifications	ANCA. ANCA staining pattern on neutrophils and monocytes; patchy infiltrates, vessel wall granulomas, fibrous tissue	HLA-B27, acute inflammatory markers (CRP and ESR) Bone mineralization, capsular fibrosis
Pulmonary involvement	NSIP is the most common followed by OP, DAD/AIP, UIP, and DAH	NSIP and LIP are the most common followed by OP, DAD/AIP and UIP	UIP is the most common followed by NSIP, OP, DAD/AIP, LIP and DAH	NSIP, DAD/AIP, and DAH are the most common followed by OP, LIP and UIP	NSIP and OP are the most common followed by DAD/AIP, UIP and DAH	NSIP is the most common followed by OP, DAD/AIP, UIP, and DAH	UIP is the most common followed by NSIP and OP	Bilateral apical fibrosis
Treatment of pulmonary involvement	CYC and MMF. Autologous stem cell transplantation	Rituximab, antifibrotic drug nintedanib (for gradual fibrosing phenotype)	CS DMARDs (e.g., methotrexate)	High-dose CS in alongside CYC and rituximab in severe cases	CS (mainstay). MMF, AZA and IVIG may be used conjugation.	CS monotherapy or a combination of CS and CYC	CS and CYC or rituximab	Treat secondary infections. Anti-TNF therapy may impact respiratory symptoms

AIP, acute interstitial pneumonia; ANA, antinuclear antibody; ANCA, anti-neutrophil cytoplasmic antibodies; AZA, azathioprine; CNS, central nervous system; CS, corticosteroid; CVS, cardiovascular system; CYC, cyclophosphamide; DAD, diffuse alveolar damage; DAH, diffuse alveolar hemorrhage; DMARDs, disease-modifying antirheumatic drugs; GIS, gastrointestinal system; HF, heart failure; HLA, human leukocyte antigen ILD, interstitial lung disease; IVIG, intravenous immunoglobulin; LIP, lymphoid interstitial pneumonia; MCTD, mixed connective tissue disease; MDA-5, melanoma differentiation-associated gene 5; MMF, mycophenolate mofetil; MSS, musculoskeletal system; NSIP, non-specific interstitial pneumonia; OP, organizing pneumonia; PAH, pulmonary arterial hypertension; RS, respiratory system; TNF, tumor necrosis factor; UIP, usual interstitial pneumonia.

10.3389/fmed.2023.1160755

Currently, SSc-ILD is being treated mostly with immunosuppressive medications like cyclophosphamide (CYC), an alkylating agent that cross-links strands of DNA and RNA and inhibits protein synthesis, or mycophenolate mofetil (MMF) an inhibitor of inosine monophosphate dehydrogenase (IMPDH). Furthermore, autologous stem cell transplantation has also been used as a treatment modality (36). Toxicity remains a major issue in these treatment modalities, although MMF was more tolerable and linked to reduced toxicity (37, 38).

Patients with SSc manifest a variety of B-cell abnormalities, among which is persistent memory B-cell hyperreactivity that may be attributed to overexpression of CD19. This theory is supported by the observation that SSc patients express 20% more CD19 than the normal controls as measured by flow cytometry examination (39). Goswami et al. (40) published a systematic review and meta-analysis in 2021 on using rituximab, an anti-CD20 monoclonal antibody, in treating SSc-ILD that comprised a total of 20 trials. Researchers demonstrated that Rituximab improved pulmonary function results after 6 and 12 months and may be an alternative to MMF and CYC.

The IL-6 receptor monoclonal antibody tocilizumab was licensed by the food and drug administration (FDA) in 2021 to treat SSc-ILD. It was shown to have a persistent favorable effect in the extension phase of the focuSSced trial (41).

Janus kinases (JAK) are a family of cytoplasmic tyrosine kinases occurring in diverse tissues and cells that interact with the Signal Transducers and Activators of Transcription (STATs) transcription factor family (42). When activated by cytokines or growth factors, these transcription factors lead to the differentiation of immune cells and regulate macrophage polarization and the production of a variety of cytokines. JAK inhibitors interfere with the JAK/STAT pathway and halt genetic transcription (43). JAK inhibitors simultaneously exert anti-inflammatory and antifibrotic effects (24) and are promising therapeutic options for SSc-ILD with a favorable outcome, as reported in a recent non-systematic review by Fiorentini et al. (23).

Nintedanib is a triple kinase inhibitor that substantially inhibits the receptors for the fibroblast, vascular endothelial, and platelet-derived growth factors (25). After initial approval and wide-scale use in treating idiopathic pulmonary fibrosis, nintedanib was approved by the FDA in 2019 in SSc-ILD based on the positive results of the SENSCIS study (44). Based on the Phase III trials, the level of evidence supporting nintedanib was high (44, 45) or moderate (46), indicating high confidence in the conclusion that nintedanib significantly slows the annual decline in forced vital capacity (FVC) in SSc-ILD or progressive fibrosing ILD including SSc-ILD. In addition, it has been demonstrated that nintedanib, both by itself and in conjunction with MMF, can slow the deterioration of pulmonary function (47).

Several clinical trials are looking into potential future SSc-ILD therapies. Phase II research examining MMF in combination with pirfenidone is being conducted in the SLS III trial (NCT03221257). MMF with or without the proteasome inhibitor bortezomib is being investigated in another phase II placebo-controlled trial of MMF combination therapy (NCT02370693) (47).

2.2. ILD in Sjögren syndrome

SS is an autoimmune disease with sicca symptoms as a hallmark feature due to exocrine gland B-and T-cell infiltration along with focal lymphocytic sialadenitis leading to tissue fibrosis and glandular dysfunction. Similar to other CTD-ILDs, the pathogenesis of SS-ILD involves injury to the alveolar epithelium with impaired healing, which causes fibroblasts to be activated and produce an increased extracellular matrix, resulting in an imbalance between collagen formation and degradation and alveolar fibrosis due to collagen accumulation (48).

Autoreactive effector T-cells originate and reinforce pathogenic B-cell responses. Interleukin 2 (IL-2) is the key stimulator of CD4+ T-cells and controls regulatory T-cells (Tregs) (49). The function of Treg cells was observed to be disrupted in patients with primary SS (50).

Low-dose IL-2 therapy significantly improved disease activity scores and immunological effects in 60 primary SS patients (49). However, the potential role of this therapy on ILD in SS is not well-defined.

In the presence of cytokines, notably IL-21, B-cells get activated through B-cell receptor (BCR) activation and CD40 ligation. IL-21 is a key cytokine that influences B-cell function and is found in higher levels in Sjögren patients than in healthy subjects (51).

Type I IFN system is hyperactivated in primary SS, leading to overproduction of the B-cell–activating factor of the TNF family (BAFF), also known as B Lymphocyte Stimulator (BLyS). BAFF is a potent B-cell activator and plays a crucial function in B-cell proliferation, and differentiation and augments the B-cell migration mediated by CXCL13 (52). It has been suggested that BAFF may be a more useful marker for discovering individuals with high disease activity in the early stages of SS (53).

2.2.1. Clinical manifestations

SS presents with sicca symptoms as a hallmark feature due to exocrine glandular dysfunction (54). Patients can present with dry, itchy eyes, dry mouth with accelerated teeth decay, parotid gland swelling, nasal dryness, chronic dry cough, and dry skin (55). Some patients can have systemic extra-glandular manifestations such as joints, kidneys, nervous system, skin vasculitis, and pulmonary manifestations (56). SS may develop as a primary condition or a result of another connective tissue disorder. Lung involvement in SS manifests mostly as interstitial pneumonitis and dryness of airway mucosal surfaces, but the disease can also result in pleurisy, pulmonary arterial hypertension, pulmonary lymphoma, and amyloidosis (57). SS is distinguished by a range of autoantibodies, specifically anti-Ro/SSA, which is part of the most recent 2016 ACR/EULAR criteria for the classification of Sjögren syndrome (58). ANA, rheumatoid factor (RF), and anti-La/SSB can also be found in SS, but up to 18% of the patients are seronegative (59). Autoantibodies can predate the disease's clinical manifestation by up to 18–20 years (60).

Interstitial lung diseases can be seen in 20% of SS patients (61). However, the prevalence rises when patients are systematically screened for it regardless of respiratory symptoms. In one report, up to 50% of SS patients had high-resolution computed tomography abnormalities (62). SS patients with ILD were shown

to have a lower quality of life and worse prognosis than those without ILD (63).

The most prevalent ILD pattern in individuals with primary SS is NSIP. Less frequent patterns include usual interstitial pneumonia, lymphocytic interstitial pneumonia, and organizing pneumonia, and some patients exhibit a combination of these patterns (64). Patients with advanced age, male gender, or smoking and those with anti-Ro52 antibodies were found to be more likely to be affected by ILD (65, 66).

2.2.2. Treatment

Unfortunately, the only treatments currently available for SS are symptomatic and primarily experimental. Rituximab is a promising treatment for SS-ILD, with small studies showing improvement in clinical symptoms and stabilization of radiological changes for patients treated with rituximab (67, 68).

Belimumab is a BLyS (BAFF) antibody and is an approved treatment for systemic lupus erythematosus. A systematic review on the use of belimumab in autoimmune diseases (69) suggested that the drug has a potential role in SS in general. Still, the study did not address ILD in particular, and no randomized trials were available to include in the review.

Patients with SS can manifest significant BAL lymphocytosis, but this was not proven to be an early sign of severe lung fibrosis (70). Type II pneumocytes in patients with ILD produce a mucin-like glycoprotein called KL-6 (71). The serum level of KL-6 correlates significantly with ILD prognosis and disease severity as defined by HRCT and is considered a potentially useful diagnostic marker for detecting asymptomatic SS-ILD patients (71–73).

Some patients with SS-ILD develop a gradual fibrosing phenotype associated with increased mortality and reduced quality of life. Such patients are eligible for the targeted antifibrotic drug nintedanib (74).

2.3. ILD in rheumatoid arthritis (RA)

Rheumatoid arthritis is an autoimmune disease that is characterized by inflammatory arthritis. The arthritis is often symmetrical, and if left untreated, usually results in joint damage from the erosion of bone and cartilage, which results in abnormalities of the joints (75). Numerous cell types are involved, including T-cells, B-cells, macrophages, and fibrocyte-like and macrophage-like synoviocytes. Extra-articular involvement, including lung pathology in the form of ILD, is a well-established complication of the disease (76). There are many identified risk factors for ILD and RA; smoking is one common overlapping risk factor (76). One hypothesis is that rheumatoid arthritis autoimmunity can be generated at non-articular sites, including the lung. Rheumatoid factor (RF) and anti–cyclic citrullinated peptide (anti-CCP) antibody are found in smokers without evidence of rheumatoid arthritis joint involvement. This is important because smoking is the only known modifiable risk factor for RA-ILD. Other studies looking at non-articular sites include gut dysbiosis as a possible mechanism for the development of RA (77). The commonality between these two hypotheses is that these mucosal sites decrease self-tolerance to citrullinated autoantigens.

Numerous noxious substances, including smoke particles, silica particles, textile dust, and bacteria, can harm the lungs and produce immunological reactions linked to RA when combined with susceptibility genes. Dendritic cells (DCs), macrophages, and B-cells are stimulated by toll-like receptor (TLR) stimuli from tobacco smoke, silica dust, textile dust, or from elements of an aberrant microbial flora inside the lungs (78–80). Peptidyl-arginine deiminase (PAD) activation and local extracellular citrullination may result from this mechanism, which could cause the emergence of neo-antigens linked to RA. Furthermore, another effect could be the differentiation of B-cells, which can result in the initial somatic mutation of the immunoglobulin genes and allow B-cells to bind and deliver post-translationally changed autoantigens to T-cells (81). Early in the development of RA-related immune responses and disease, the lungs produce structures resembling germinal centers called induced bronchus-associated lymphoid tissue (iBALT). A local generation of antibodies to citrullinated protein antigens (ACPAs) is encouraged in the lungs by T-cell-dependent B-cell activation. T-cell receptor; APC, antigen-presenting cell (82–84).

2.3.1. Clinical manifestations

Rheumatoid arthritis commonly presents with stiffness and swelling in the hands and feet. While joint involvement is the pre-dominant feature used in the ACR/EULAR criteria for RA, RA also affects other body parts, including the lungs and blood vessels (vasculitis), skin (pyoderma, rheumatoid nodules), eyes (scleritis, keratitis). The most common form of lung involvement in rheumatoid arthritis is ILD (85).

Approximately 10% of patients with rheumatoid arthritis will develop clinically significant ILD (85). However, some studies suggest that this number may be higher, and there is radiographic evidence on CT before patients become symptomatic (86). For some patients, ILD precedes the diagnosis of rheumatoid arthritis (87). One study by Hyldgaard et al. (88, 89) found that 14% of patients with RA-ILD had been diagnosed with ILD 1–5 years before their rheumatoid arthritis diagnosis. It is important to identify and treat RA-ILD as early as possible because it is the second most common cause of death in patients with RA after cardiovascular disease (86).

2.3.2. Treatment

According to case series and clinical evidence, systemic glucocorticoids and/or disease-modifying antirheumatic drugs (DMARDs) may be beneficial in some patients with RA-ILD (7). Data to guide the choice of conventional DMARDs such as methotrexate or mycophenolate, or biologic DMARDs such as abatacept, in RA-ILD is still evolving (90). Known for producing a favorable articular response in resistant RA, biologic treatment with rituximab has also been shown to stabilize RA-ILD in the majority of patients in several reports, and even improve lung status in a small patient subset (91). Among those, a report of 700 RA patients treated with rituximab demonstrated stabilization or improvement of lung disease in 30 out of 44 patients with concurrent RA-ILD, with the remaining 14 patients experiencing ILD progression (92). In that study, a radiologic pattern of UIP and more severe ILD prior to rituximab initiation conferred a poorer response to rituximab and an increased risk of ILD deterioration. Concerns have been raised over rituximab-induced ILD and the general side effect profile of rituximab although the data remains limited in RA patients.

The treatment of RA-ILD has proved to be challenging for many clinicians. Adding to this challenge is the concern that the mainstay treatment for RA with DMARDs increases the risk of pulmonary toxicity. Additionally, little evidence suggests that DMARDs are an effective treatment for ILD (91). Thus, the treatment for joint and lung involvement may need to be evaluated separately. There is evidence for using antifibrotic medications such as nintedanib from the INBUILD trial. This trial demonstrated that in patients who received nintedanib, the annual rate of decline in the FVC was significantly lower than in those who received the placebo (46). Additional research and clinical trials are still needed to determine the optimal treatment for RA-ILD.

2.4. ILD in systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder of unknown etiology that involves a complex interplay of environmental and genetic factors. Much of the pathology in SLE is believed to stem from failure of self-tolerance, dysregulated apoptosis, formation of autoantibodies, and immune complex formation and deposition, producing inflammation and vasculitis that culminate in the multisystemic manifestations of SLE. Among the systems involved, pulmonary involvement is common in the form of pleuritis and pleural effusions and, to a lesser extent, ILD (93).

The underlying mechanisms of lung involvement in SLE, particularly ILD, also remain poorly defined, and whether it arises from progression of lung inflammation and consequent parenchymal damage and fibrosis or not is also unclear. Lung inflammation has been associated with circulating immune complexes, increased levels of certain cytokines, and neutrophils (94). The initial inflammatory insult damages and activates epithelial and endothelial cells in the lung, with production of mediators that attract various cells including neutrophils. The role of neutrophils in perpetuating the inflammatory response in SLE has been highlighted through the process of NETosis in which released DNA and nuclear antigens, which are primary autoantigens in SLE, further drive inflammation (94). Additionally, the role of certain cytokines in the development of pulmonary pathology has been proposed on the basis of observations demonstrating augmented systemic levels of IFN-gamma, IL-6, and IL-8 in comparison to SLE patients without lung involvement (95, 96). The exact mechanisms that govern progression to fibrosis, however, are poorly understood.

2.4.1. Clinical manifestations

Like other autoimmune disorders, SLE primarily affects women. It has a variety of clinical presentations and is sometimes referred to as the "great mimicker" because of this heterogeneity. Common clinical manifestations include a malar rash, mouth ulcers, hematologic abnormalities, cardiovascular abnormalities, neuropsychiatric abnormalities, synovitis, joint tenderness or stiffness, and renal involvement.

Pulmonary involvement is a common finding in SLE. Although only 5% of patients have pulmonary involvement as their presenting symptom, nearly half of all patients with SLE will develop it in the future (97). There are two major categories of lung conditions in lupus: acute lupus pneumonitis and ILD. Interstitial pneumonitis is a less frequent occurrence in SLE than other CTDs and has received less attention than other CTD-ILDs, particularly SSc-ILD, but it shares similar pathogenic mechanisms (98). The most frequent histopathology types are acute interstitial pneumonia and NSIP, and high-resolution CT of the lung shows ground glass opacities (99, 100).

Patients with ILD secondary to SLE are more likely to present at an earlier age than those with ILD who do not have an autoimmune condition. One study by Hussain et al. (101) found that the average age at ILD diagnosis in a patient with SLE was 59.28 compared to 72.32 years in those without autoimmune disease. The same study also looked at the racial distribution and found that African American populations with ILD, compared to other races, were more likely to have an underlying autoimmune disease, including SLE, mixed connective tissue disease, myositis, or scleroderma. A multicenter study of 513 patients with SLE found that ILD was present in 1% at the time of onset, 4% of patients within the following year, and 8% by 12 years. ILD seemed to increase with the duration of disease and also with age (102, 103).

Immunologic criteria for SLE include antinuclear antibody (ANA) and SLE-specific antibodies: anti-dsDNA and anti-Smith antibody (104, 105). None has shown a good correlation with development of ILD (106). Other antibodies in SLE include anti-histone antibodies (which are associated with drug-induced lupus), anti-Ro/SSA, anti-La/SSB, as well as antiphospholipid antibodies. Additional laboratory testing may show anemia, lymphocytopenia, and hypocomplementemia (107).

Similar to other diffuse CTD-ILDs, the most typical symptoms include non-productive coughing, pleuritic chest discomfort, and persistent exertional dyspnea. Physical examination may reveal bibasilar crackles, cyanosis, and fever. As opposed to idiopathic ILD, clubbing is less frequent in SLE.

Biopsies collected from people with ILD related to SLE have been shown in histological examinations to have lymphocytic, mononuclear interstitial, and peribronchiolar infiltrates (108). A study done by Chen et al. (109) found that among Chinese patients with SLE, there were several statistical differences compared to those without ILD: including older age, increased illness duration, lower levels of anti-dsDNA, high C3 levels, increased ratios of Raynaud's phenomenon, moist rales and tachypnea. This poses the question of whether there should be increased screening for ILD in patients with these biomarkers or with Raynauds.

2.4.2. Treatment

Treatment of SLE-ILD is primarily based on case reports, case series, or expert opinion and there are varying degrees of consensus on treatment since there are few randomized controlled studies to support the current treatments. Current therapies frequently involve high-dose corticosteroids in conjunction with medications like cyclophosphamide and rituximab in severe instances (100, 110). In milder cases or to preserve long-term disease control, steroid-sparing medications like MMF and azathioprine may be utilized (111, 112). Belimumab is an approved treatment for SLE (69), but data on its utility for SLE-ILD is limited, with few case reports of its favorable effect (113). In addition, as with other autoimmune-related ILD, those patients who develop fibrosing

phenotype are eligible for treatment with the targeted antifibrotic drug nintedanib (74).

2.5. ILD in dermatomyositis and polymyositis

Dermatomyositis (DM) and polymyositis (PM), which are categorized under idiopathic inflammatory myopathies, are immune-mediated myopathies characterized by the involvement of the muscles as well as extramuscular symptoms (114). The exact pathogenic mechanisms responsible for DM are still unknown. Application of genomic technologies to DM skin and muscle samples has led to the hypothesis that DM pathology is caused by exposure to a type 1 interferon (IFN) that damages keratinocytes, myofibers, and capillaries (115–117). The microscopic pathology of DM, which includes perifascicular atrophy, capillary abnormalities and loss, and perimysial abnormalities, is distinct from all other muscle illnesses. Perifascicular myofibers, which are situated at the borders between fascicles and perimysial connective tissue, are preferentially harmed. These myofibers are atrophic and exhibit neonatal myosin (MHY8), vimentin, and natural cell adhesion molecule (NCAM), which are often linked to regeneration (118, 119). However, it seems improbable that these myofibers are regenerating. Moreover, PM is thought of as a condition in which myofibers are invaded by lymphocytes, particularly CD8+ cytotoxic T-cells. A protein called melanoma differentiation-associated gene 5 (MDA-5) serves as an intracellular pattern-recognition receptor that can identify danger signals in double-stranded RNA. Type I interferons are produced in considerable quantities after MDA-5 is activated the reason behind this remains unknown (120). It is hypothesized that this leads to the activation of non-inflammatory macrophages which produce IL-10 and TGF-β and are involved in the progression of lung fibrosis (121, 122).

2.5.1. Clinical manifestations

Both DM and PM have a wide range of clinical symptoms and are multisystem diseases. The majority of individuals show symptoms of muscular inflammation and proximal skeletal muscle weakening (118, 123). DM is characterized by a number of distinctive skin eruptions, such as Gottron papules and the heliotrope eruption (124). Patients suffering from myositis-ILD commonly experience shortness of breath and non-productive cough. However, some patients may not show any symptoms and ILD is only discovered through an abnormal lung examination or chest X-ray (125). ILD occurs in 20–80% of cases with DM and PM patients and is a common cause of death among them (126, 127). DM and PM have been linked to a number of ILD histologic patterns with NSIP and OP being the most common pattern (128). A complete blood count and differential, hepatic and renal function assessments, and a brain natriuretic peptide (BNP) or N-terminal-proBNP test are frequently included in initial laboratory evaluations. Additionally, serum muscle enzymes (such as creatine kinase and aldolase), an antinuclear antibody test, anti-Mi-2, and an anti-Jo-1 antibody are done if the diagnosis of DM or PM has not yet been established (129). The clinical examination and outcomes of the preliminary tests serve as the basis for further testing (e.g., further antisynthetase antibodies, MDA-5 antibodies which are associated with rapidly progressive ILD, and testing for an overlap syndrome) (128, 130, 131). The diagnosis of ILD in patients with a history of DM and PM can typically be confirmed through a combination of their symptoms, chest imaging (e.g., HRCT), and pulmonary function tests (132). It is recommended that all individuals diagnosed with myositis, regardless of their respiratory condition, undergo complete pulmonary function tests (PFTs) at the time of diagnosis and then annually afterward (127). Furthermore, a lung biopsy is not typically needed. In cases where the symptoms or imaging appear atypical (for example, accompanied by a fever, rapidly worsening, or occurring during immunosuppressive treatment), bronchoscopy with BAL may be performed to rule out an infection or drug-related cause (133). If the diagnosis is still uncertain, a lung biopsy may be necessary.

Karampitsakos et al. (128) reported that ILD is a common feature of inflammatory myopathies, including those without muscle involvement, and may occur before the onset of muscle symptoms. Consequently, physicians should be aware that cases of organizing pneumonia without obvious muscle involvement may be related to myopathy. When patients with bilateral organizing pneumonia do not respond to antibiotics, physicians should thoroughly evaluate them for myositis-associated ILD, especially if they have certain autoimmune markers like anti-MDA5, which can have a negative impact on prognosis (128).

2.5.2. Treatment

Diagnosing and treating myositis-ILD presents challenges that are best addressed through a collaborative effort between experienced rheumatologists and pulmonologists (134). There are currently no standard guidelines for managing patients with myositis-ILD. Treatment plans are based on the expertise of medical professionals and can vary between medical centers. Corticosteroids (CS) are used as a baseline therapy for all patients with myositis-ILD due to their rapid onset of action and availability. The starting dose is determined based on the severity of the patient's disease and any underlying health conditions, such as diabetes or osteoporosis (127, 135). Because of the potential long-term consequences of taking prednisone and the risk of disease flare-ups, a steroid-sparing agent, such as MMF or AZA, is usually prescribed concurrently with prednisone to allow for a gradual reduction in prednisone use (127, 136). Although there is no clear evidence supporting the use of one steroid-sparing agent over the other, MMF is preferred due to a lower risk of gastrointestinal symptoms and lab abnormalities. IVIG is used as adjunctive therapy for patients with refractory disease, severe skin involvement, or severe myositis (127). Regarding tacrolimus, it is used only for patients who do not show improvement or continue to have declining pulmonary function after 3 months of treatment with steroids and an anti-metabolite (MMF or AZA). In cases of severe combined ILD and myositis, tacrolimus may be chosen as a first-line steroid-sparing agent. However, when possible, MMF or AZA is favored due to the potential for long-term side effects and the need for monitoring drug levels in patients taking tacrolimus. Rituximab is also used as an additional therapy for patients with persistent or progressive disease despite treatment with prednisone and an anti-metabolite or calcineurin inhibitor. CYC is rarely used due to concerns about side effects and lack of evidence that it is more effective than other immunosuppressants for ILD. Despite treatment with immunomodulatory therapy, some

patients with myositis-ILD may develop a progressive fibrotic form of ILD. In cases where there is radiographic evidence of worsening bronchiectasis or the development of honeycomb changes, anti-fibrotic therapy with nintedanib or pirfenidone is added (127).

2.6. ILD in mixed connective tissue disease (MCTD)

Mixed connective tissue disease is a rare autoimmune disorder affecting multiple body systems, including the joints, skin, lungs, blood vessels, and muscles. It is characterized by the presence of antibodies to a protein called ribonucleoprotein (RNP) and symptoms of overlap between SLE, scleroderma, and polymyositis (137, 138). The exact cause of MCTD is unknown, but it is believed to be a combination of genetic and environmental factors. It is widely known that autoimmunity plays a part in ILD linked to CTD. Increased levels of proinflammatory and profibrotic mediators have been linked to the development of ILD and might play an important role in MCTD-ILD. These mediators include lipids, prostanoids, growth factors, chemokines, and cytokines (99). In MCTD-ILD, the immune complex complements C3 factor, anti-U1-RNP, and CH50 are all substantially expressed. Gunnarsson et al. (139) reported that anti-Ro52 antibodies are associated with MCTD-ILD.

2.6.1. Clinical manifestations

Mixed connective tissue disease might present with non-specific early clinical symptoms including general malaise, arthralgias, myalgias, and low-grade fever (140). Raynaud phenomenon and the absence of severe renal and central nervous system disease help differentiate MCTD from SSc and SLE (141–143). Dyspnea, dry cough, and pleuritic chest pain are early signs of pulmonary involvement in MCTD that should be taken seriously (144). One of the most serious complications of MCTD is ILD. ILD is thought to be caused by the immune system attacking the lung tissue. ILD can occur in about up to 50% of patients with MCTD. Furthermore, a sensitive diagnostic test to identify ILD is HRCT. Septal thickening, ground-glass opacities, non-septal linear opacities, and peripheral or lower lobe pre-dominance are the most prevalent HRCT results and are most comparable to the SSc findings (145, 146).

2.6.2. Treatment

As there have been no randomized clinical trials to help direct treatment, it is believed that MCTD is an incurable condition. The treatment is mostly determined by the proven effectiveness of certain medications for conditions that are similar to SLE, scleroderma, or polymyositis. In the early inflammatory stage of ILD associated with MCTD, CS monotherapy or a combination of CS and CYP may be beneficial in stopping the progression of ILD and the development of fibrosis (147).

2.7. ILD in antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis

Antineutrophil cytoplasmic antibody-associated vasculitis (AAV) is a systemic vasculitis that mainly affects small blood vessels. It consists of three different clinical syndromes: microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (EGPA) (148). Association between ANCA-associated vasculitis (AAV) and interstitial lung disease has been increasingly reported over the past 3 decades (148). The significant pre-dominance of myeloperoxidase (MPO) or (P-ANCA) related AAV-ILD was reported compared to other AAV subtypes (149). The prevalence rate of ILD was reported in up to 45% of MPA patients and 23% of GPA patients (148). Mucin 5B (MUC5B) promotor has a role in airway clearance and host defense against bacteria, with one of its variants rs35705950T identified (148) and was found to increase the risk of IPF in AAV-ILD patients in a case-control study from Japan (148, 150). The impact of ANCA is based on the activation status of neutrophils. Once primed, neutrophils can be bound by ANCA to relevant antigens on the cell membrane. This leads to abnormal activation through crosslinking of MPO or PR3 or by binding to Fc receptors. ANCA's binding to neutrophils can increase the interaction between neutrophils and endothelial cells, causing microvascular damage, which supports ANCA's potential role in causing systemic vasculitis (151, 152).

The exact cause of ILD in AAV is not well understood. However, Kagiyama et al. (153) postulated that repeated episodes of alveolar hemorrhage due to pulmonary capillaritis may lead to the development of pulmonary fibrosis. Also, Guilpain et al. (154) suggested that oxidative stress, particularly the production of hypochlorous acid (HOCl) through the interaction of MPO with anti-MPO antibodies, could drive the fibrotic process observed in MPA. Pathology of ILD associated with ANCA-positive vasculitis was evaluated in a few studies. In a case series study of 9 patients with MPO-ANCA idiopathic interstitial pneumonia (IIP), small airway disease was found in all the patients, lymphoid follicles in seven patients, UIP with areas of NSIP in eight patients, UIP and diffuse alveolar damage (DAD in two patients and no vasculitis in any patients (155).

In another study of 18 patients evaluated with surgical lung biopsy, 65% were noted to have UIP patterns and 48% with UIP and other atypical findings of UIP related to IPF (148, 156).

2.7.1. Clinical manifestations

Individuals who have both AVV usually exhibit general symptoms such as fever, feeling unwell, loss of appetite, weight loss, muscle aches, and joint pain. These early warning signs can persist for several weeks to months without showing specific signs of organ damage. Later, specific organ problems may become evident such as the Vascular system (wall thickening), skin (purpura), nervous system (mononeuritis), and renal system (glomerulonephritis) (157).

Pulmonary involvement in AVV can manifest as pulmonary nodules/consolidation, more common with GPA, diffuse alveolar hemorrhage that is often associated with renal vasculitis, tracheobronchial stenosis, fibrosing interstitial disease, and IPF (158). Although the median age at onset of MPA vasculitis is 55 years, MPA-associated ILD usually presents in patients 65 years and older, with a higher incidence in males (148).

In a retrospective study on 745 north American patients with IPF, 4.0% of the patients were positive for ANCAs at the time of diagnosis and 25–33% of them were diagnosed with vasculitis during a median duration of follow-up 18 months (148, 159).

However, isolated IPF cases with positive ANCA in the absence of any AAV manifestations (148, 160).

Interstitial lung disease in AAV, especially anti-MPO ILD with or without systemic manifestations, is becoming more recognized, ANCA is not typically included in ILD workup and standardized criteria for diagnosis and classification of autoimmune-ILD are not available. More studies are needed to evaluate the association between ANCA positivity and ILD, its impact on disease course and prognosis, and potentially beneficial treatment options.

In a multicenter study in Japan that included 150 patients with MPA, 66% had interstitial lung abnormality (148, 161). The most common radiologic pattern with ANCA/AAV-related ILD is UIP (78%), NSIP (13–64%), less common patterns include organizing pneumonia and desquamative interstitial pneumonia (148, 161, 162). Other described radiologic patterns include ground glass opacities (GGO), honeycombing, nodular pattern, consolidation, reticulation, and interlobular septal thickening (148, 156, 163, 164).

2.7.2. Treatment

There are numerous studies about ANCA-associated systemic vasculitis, but few studies address ANCA-associated ILD, with less defined treatment for ANCA-associated ILD in the absence of systemic vasculitis (148). Retrospective case reports and limited data, especially with non-UIP patterns revealed an improvement in PFT in patients treated with immunosuppressive agents (148, 165). However, a study on 62 patients showed no improvement in the prognosis with immunosuppressive treatment (166). A study on 49 patients who were treated with glucocorticoids and cyclophosphamide or rituximab as induction therapy showed higher 1- and 5-year survival rates compared to those who were treated with glucocorticoids alone (148, 164).

As with all other CTD-ILD, the INBUILD study suggested a benefit of nintedanib in the progressive fibrosis-ILD (PF-ILD) subtypes (148, 167). Moreover, antifibrotic rather than anti-inflammatory therapy should be prioritized in patients with a high risk for infection, a UIP phenotype and no systemic vasculitis features (167).

2.8. Ankylosing spondylitis (AS)-associated lung disease

Ankylosing spondylitis (AS) is a chronic inflammatory disease that is part of the broader entity termed axial spondyloarthropathies. It is pre-dominantly a disease of the axial skeleton, including the sacroiliac joints and spine, and less commonly, peripheral joints (168). Extra-articular involvement may occur and includes ocular (e.g., uveitis), cardiovascular (e.g., aortitis), and pulmonary manifestations (e.g., ILD) (169–171). In 85–90% of AS cases, there is an association with HLA-B27, an MHC variant presumed to contribute to disease pathogenesis *via* aberrant presentation of unidentified antigens that trigger inflammation in the axial joints (172). Cytokines such as TNF-alpha, IL-17, and IL-23 are important mediators of the disease and serve as therapeutic targets for AS (173, 174).

Pulmonary disease in AS primarily follows a restrictive pattern of changes that is largely attributed to musculoskeletal disease, and to a lesser extent, parenchymal lung involvement (171, 175). Spinal and costovertebral joint fusion and rigidity, enthesitis in the anterior chest wall and the ensuing decline in chest wall mobility restrict chest wall expansion (171). Other pulmonary changes, including apical fibrosis, have no clear pathophysiology but are possibly related to altered mechanical stresses and diminished ventilation in the apices of the lung, chronic inflammation, and recurrent aspiration pneumonitis secondary to esophageal dysfunction (171).

2.8.1. Clinical manifestations

The characteristic presenting symptom of AS is insidious-onset inflammatory back pain in an adult below the age of 40 that may or may not be associated with peripheral articular and extra-articular symptoms at presentation. Testing for suspected AS will include conventional radiography of the sacroiliac joints, revealing sclerotic to erosive changes as well as joint space narrowing indicative of sacroiliitis, in addition to a spine X-ray, revealing loss of lumbar lordosis, bony growths (i.e., syndesmophytes) and a bamboo spine in more advanced stages of the disease. More advanced imaging with CT and MRI proves useful in cases of equivocal sacroiliac X-ray findings with clinical suspicion of AS. Laboratory testing will likely reveal HLA-B27 positivity, along with elevation of non-specific inflammatory markers such as CRP and ESR (169, 176).

Pulmonary disease in ankylosing spondylitis is a rare and late occurrence, although onset has been reported to vary but occurs at an average of 15 years following the onset of articular symptoms (171, 177, 178). Pulmonary disease in AS follows an insidious course. Among the most common pulmonary changes observed in AS is a restrictive pattern demonstrated on pulmonary function tests, including mildly to moderately diminished vital capacity. This can be due to isolated musculoskeletal disease or concurrent parenchymal lung pathology which can occur in the form of ILD, apical fibrosis, emphysema and bronchiectasis (179).

In 1.3–15% of patients, plain radiography may demonstrate apical fibrosis but is often an asymptomatic finding (175, 180, 181). Nevertheless, colonization and superinfection of apical cysts or cavities may occur, in which Aspergillus fumigatus is the most common culprit, followed by mycobacteria, and may produce symptoms of fever, hemoptysis and dysnpea. Additionally, apical fibrobullous disease may pre-dispose to spontaneous pneumothorax (171). HRCT better reveals parenchymal lung changes as well as pleural thickening, cavitation and bronchiectasis (175). Histopathologic lung specimens of AS patients with pulmonary involvement may reveal patchy pneumonia, scarring, bullae formation, bronchial dilatation, bronchiolitis obliterans or organizing pneumonia (177, 178, 182–184).

2.8.2. Treatment

The main way to manage respiratory issues related to ankylosing spondylitis is by treating any secondary infections with either systemic or locally administered antibacterial or antifungal agents (171). Anti-TNF-α therapy has been proven effective both clinically and in imaging studies for treating ankylosing spondylitis and is an option for patients who are unresponsive to non-steroidal anti-inflammatory drugs (185). Although drugs with anti-inflammatory properties, such as infliximab, etanercept, and adalimumab, are utilized to treat ankylosing spondylitis, it remains uncertain how they impact respiratory symptoms (171, 185, 186).

Also, there is no evidence that any form of therapy can change the progression of apical fibrobullous disease.

3. Exposure-related lung diseases

Most of the diseases listed in this section are discussed briefly.

3.1. Post-infectious lung diseases

Post-infectious immune-mediated ILD is a condition that arises when infectious agents stimulate an immune response that attacks the lungs or when the healing process becomes abnormal following an infection (187). Post-infectious ILD has been described following various infectious agents, and interest has grown dramatically with the advent of coronavirus disease 2019 (COVID-19).

Infections may contribute to the development of ILD (187). It is postulated that the pathogenesis of infection-induced ILD involves either direct epithelial injury followed by an aberrant healing process or activation of the immune system and the subsequent generation of pro-fibrotic and pro-inflammatory cytokines, both of which may cause pulmonary fibrosis (188).

Viral infections and their association with ILDs have received significant attention in recent years, particularly during the Coronavirus disease 2019 (COVID-19) pandemic, despite the fact that the mechanisms behind virus-induced ILDs remain unknown. Alterations of fibrosis-related pathways and mechanisms, such as decreased expression of angiotensin-converting enzyme 2 (ACE2), overexpression of the TGF-β/Smad pathway, increased production of pro-fibrotic and pro-inflammatory cytokines, as well as increased stress in the endoplasmic reticulum, have been shown to contribute to epithelial-mesenchymal transformation, a major mechanism of pulmonary fibrosis (188).

Infection with influenza may cause lung fibrosis by activating the TGF-β/Smad pathway, which can trigger the overexpression of pro-fibrotic gene. Jolly et al. (189) reported in a murine study that influenza A infection induced αvβ6 integrin-mediated TGF-β activity in epithelial cells through activation of toll-like receptor 3 (TLR3), leading to the death of epithelial cells and an increase in collagen deposition. Shatskaya et al. (190) discovered, in influenza A-infected mouse models, that an increase in TGF-β expression may affect the balance of activation and inhibition of SMAD proteins, leading to epithelial-mesenchymal transition and progression to fibrosis.

Herpesviruses have been shown to cause pulmonary fibrosis. Malizia et al. (191) found that Epstein–Barr virus (EBV)-infected human alveolar epithelial cells had increased TGF-β1 expression, which was decreased by Ganciclovir treatment. Mora et al. (192) discovered that infection of IFN-γ receptor-deficient mice with Murine Gammaherpesvirus 68 (MHV-68) led to epithelial injury, myofibroblast transformation, enhanced TGF-β expression, and ultimately deposition of interstitial collagen. Cook et al. (193) observed aberrant TNF-alpha expression by polymerase chain reaction (PCR) and a substantial increase in lung fibrosis in murine Cytomegalovirus (CMV) reactivation models 2 weeks after infection, compared to uninfected controls. In a meta-analysis including 1,287 patients, Sheng et al. (194) found that the presence of EBV, CMV, Human betaherpesvirus 7 (HHV-7), and Human Herpesvirus-8 (HHV-8) significantly increased the risk of developing idiopathic pulmonary fibrosis.

Limited information is available about the role of bacteria and fungi in developing ILDs. Molyneaux et al. (195) found that a higher bacterial load in bronchoalveolar lavage was predictive of a decline in lung function and increased mortality in idiopathic pulmonary fibrosis (IPF) patients and that *Haemophilus*, *Streptococcus*, *Neisseria*, and *Veillonella* spp. were more prevalent in IPF patients than in control subjects. In addition, a significant prevalence of *Pneumocystis jirovecii*, a fungus, was seen among immunocompetent ILD patients (196). However, more clinical and translational research is needed to understand the pathogenesis of ILD caused by these infectious agents.

It can be challenging to establish the connection between infection and interstitial lung disease (ILD) in human clinical studies because the infection may exacerbate existing ILD or cause new-onset ILD through other mechanisms. Therefore, many animal studies are needed to help clarify how different infections contribute to developing ILD. As mentioned earlier, there are numerous subtypes of ILD with varying degrees of inflammation and fibrosis. Still, current animal studies have primarily focused on elucidating the pathogenesis of lung fibrotic changes. Further research is needed to examine the immune system pathways involved in the various subtypes of ILD. In the future, when uncontrolled infection is suspected as a possible cause of ILD, infection-induced ILD should be considered as a differential diagnosis to allow for early treatment and improved prognosis.

3.1.1. Clinical manifestations

The clinical presentation of post-infectious immune-mediated ILD can be diverse. It may be either acute or chronic, with the clinical course differing between different pathogens and being unpredictable in some cases (187). Many patients with certain infectious diseases have also had long-term residual radiological abnormalities, such as evidence of inflammatory and persistent fibrotic-like changes visible on chest CT scans (188). The most common pathological finding in post-infectious ILD is the infiltration of immune cells and the development of fibrotic changes, which can manifest as various forms of interstitial pneumonia, including diffuse alveolar damage, pleuroparenchymal fibroelastosis, granulomatous inflammation, or eosinophilic pneumonia upon histopathological examination (187).

3.2. Post-COVID-19 ILD

Coronavirus disease 2019 (COVID-19) has been the leading cause of morbidity and mortality since the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in 2019 (197, 198).

Coronaviruses are currently being extensively studied for their role in the underlying pathogenesis of ILDs. Kim et al.

(199) infected human dipeptidyl peptidase 4 (hDPP4)-transgenic mice with Middle East Respiratory Syndrome-Coronavirus (MERS-CoV) and identified histological indicators of progressive pulmonary fibrosis, increased production of pro-fibrotic cytokines, and inflammatory response in the lungs.

Characterized by acute respiratory distress syndrome (ARDS) like hyperinflammation and endothelial dysfunction, these post-COVID changes can be summarized as a fibroproliferative response that can persist for a long period. Lung injury in severe COVID-19 disease is associated with an increased risk of progression to ILD. ILD has a broad spectrum combining inflammatory and fibrotic features. It is crucial to understand the primary contributors of ILD in COVID-19, how to mitigate the risk factors involved, and management strategies to approach the long-term consequences.

Fibrotic ILD/pulmonary fibrosis is the most common long-term pulmonary sequela of COVID-19 as suggested by fibrotic features seen on the histopathology of COVID-19 patients (200). Pathogenesis of ILD secondary to COVID-19 is thought to be caused by multiple etiologies, including the development of cytokine storm and abnormal repair mechanisms (201). According to Ravaglia et al. (202), the immune component of underlying pathology is determined by transbronchial lung cryo-biopsy along with CT imaging (203) suggestive of residual lung disease. Different morphological patterns can be observed in three different groups: (1) chronic fibrosis, (2) organizing pneumonia and fibrosing interstitial pneumonia, and (3) diffuse vascular injury within normal parenchyma. Immunophenotypical changes are also seen due to abnormal expression of STAT3 in hyperplastic pneumocytes and PD-L1, IDO, and STAT3 in endothelial cells.

3.2.1. Clinical manifestations

Lungs are the primary target organ of SARS-CoV-2, which manifests as critical pneumonia, often with long-term pulmonary complications ranging from impaired respiratory physiology, and residual shortness of breath to vascular compromise (204).

3.2.2. Treatment

Being a relatively new condition, pharmacological therapies for COVID-19-associated ILD are still being studied. Currently, high-dose corticosteroid (dexamethasone) has been proven to be the first line of management (200, 203). Prior to COVID-19, antifibrotic drugs like pirfenidone were used for IPF (205). Since fibrosis is a key feature of post-COVID disease, such classes of drugs can be explored for the management of COVID-associated ILD. Ntatsoulis et al. (206) suggest therapeutic targeting of Autotaxin (ATX), a lysophospholipase D responsible for extracellular production of lysophosphatidic acid (LPA), a signaling lysophospholipid that effects pulmonary and immune cells. It is shown that COVID-19 increases the production of IL-6 as well as ATX, hence anti-ATX therapy could be an effective treatment for COVID-19. Treatment for prolonged COVID-19 symptoms continues to evolve, and more research should be promoted to further understand underlying etiologies and pathogenesis, which lead toward targeted therapies (207). The role of immunomodulatory or anti-fibrotic therapies in COVID-associated ILD is still questionable. Immunomodulators may potentially promote the reversal of "inflammatory" changes whereas anti-fibrotic (e.g., nintedanib used in progressive-fibrosing ILD) may reduce "fibrotic-like" changes (208).

In a recent retrospective report evaluating post-COVID-19 ILD using machine learning radiographic models, 13 out of 232 patients followed after severe COVID-19 infection developed fibrotic-like changes on HRCT with persistent functional impairment at 6 months of follow-up. After antifibrotic therapy with pirfenidone or nintedniib, improvement was observed on imaging and PFTs in these patients. Interestingly, given the unvaccinated status of the majority of the cohort in their study and in all of the 13 patients with fibrotic-like changes receiving antifibrotic therapy, this raises the possibility that vaccination against COVID-19 might also prevent the sequelae of COVID-19 infection including post-COVID-19 ILD, and further research is needed to substantiate these observations (209).

3.3. Drug-induced ILD

When exposure to a drug causes inflammation and eventually fibrosis of the lung interstitium, it is known as drug-induced interstitial lung disease (DIILD) (210). Over 350 drugs may cause DIILD, and patient presentation and imaging vary vastly between drugs and between patients on the same drug. Risk factors for DIILD include age, smoking, sex and any underlying lung condition (210). All available tests for DIILD lack specificity, thus it is a diagnosis of exclusion and is made based on establishing a temporal relationship between symptoms and drug exposure. This poses a significant challenge for treating physicians. A recent systematic review of observational studies was conducted and found that cancer drugs were the leading cause of DIILD, followed by DMARDs, antibiotics, non-steroidal anti-inflammatory agents, psychiatric medications, and anti-arrhythmic agents. In rheumatology, it is especially difficult to analyze DIILD given the background prevalence of ILD and the immunosuppressive nature of DMARDs, and associated risk of infections that often present as respiratory symptoms. The lack of diagnostic biomarkers for DIILD has limited treatment options and drug development. Though there have been several attempts to find an association between treatment with methotrexate, leflunomide, and biologic DMARDs, definitive causation and the underlying mechanism that rheumatic drugs may induce alveolar inflammation, interstitial inflammation, and/or interstitial fibrosis remains unknown (211). DIILD and other ILDs are difficult to distinguish clinically from one another. Also, the same drug may produce varying imaging patterns and vice versa (210).

3.3.1. Treatment

Treatment of DIILD requires empiric drug discontinuation (210). The reported efficacy of glucocorticoid (GC) therapy to treat DIILD varies widely, and its use is generally reserved for patients with rapidly progressive or more severe pulmonary toxicity. Although there is currently no consistent evidence on which to base recommendations for either drug withdrawal or GC use in DIILD, clinical experience and available literature demonstrating improvement or resolution with this treatment modality suggests a link between immunity and ILD.

3.4. Hypersensitivity pneumonitis (HP)

Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is a complex syndrome with variable severity, clinical presentation, and natural history. Agricultural dusts, bioaerosols, microbes (fungal, bacterial, or protozoal), and certain reactive chemical species are just a few of the many inciting agents that have been identified. HP can be classified as acute, subacute, or chronic (212, 213). However, guidelines from the American Thoracic Society, Japanese Respiratory Society, and Asociación Latinoamericana de Tórax (ATS/JRS/ALAT) now classify HP into two subtypes: non-fibrotic and fibrotic (214). These subtypes are easier to differentiate and have a stronger correlation with clinical outcomes. Regardless of whether HP is non-fibrotic or fibrotic, common symptoms include difficulty breathing and coughing. During a physical examination, crackles, mid-inspiratory squeaks, and occasionally wheezes may be detected, and clubbing of the fingers may be observed (215). The clinical, radiologic, and histopathologic characteristics of HP overlap with those of other ILDs, making a diagnosis difficult. Additionally, it may not be feasible to pinpoint the exposure that caused the disease. The prognosis and treatment are significantly impacted by the presence of lung fibrosis on HRCT (214, 216). Individuals with HP should undergo routine monitoring to determine whether their condition is progressing. The inciting antigen must be avoided. Although frequently used to treat HP, immunosuppression has not been proven to delay the development of fibrotic disease. For fibrotic HP with a progressive phenotype, the tyrosine kinase inhibitor nintedanib is an approved treatment option (216).

3.5. Occupational lung diseases

Occupational lung diseases include pneumoconioses (interstitial lung diseases). Pneumoconioses, accumulation of mineral dust and inorganic dust, followed by lung response, can be classified as fibrogenic (caused by silica, coal, talc, or asbestos), innocuous or inert (caused by iron, tin, or barium), granulomatous (caused by beryllium), or giant cell pneumonia linked to inhalation of hard metals (e.g., cobalt) (217, 218). Previous exposure history and suggestive radiographic findings help in the diagnosis. Tissue biopsy is typically not necessary to confirm the diagnosis when the exposure history and radiographic pattern are characteristic due to the typical radiographic appearance of the most prevalent pneumoconioses. Specific imaging patterns in pneumoconiosis include focal nodules and masses, diffuse lung disease and pleural plaques (219). Occupational asthma is the most frequently diagnosed occupational lung disease. In the past, research on occupational lung diseases has primarily focused on diseases resulting from exposure to hazards relevant to high-income countries and obvious hazardous occupations such as coal mining-induced silicosis. But, in 2019, peer-reviewed publications have expanded the scope to include low- and middle-income countries and previously neglected occupations such as dry cleaning and animal husbandry. Thanks to technological advancements and a better understanding of the causes of the disease, researchers and clinicians can now implement improved risk analysis, screening, and mitigation strategies not only to treat occupational diseases but also to identify at-risk populations and establish measures to prevent or reduce the negative effects of workplace hazards. As occupational lung diseases are increasingly recognized as a global threat in various occupations, research is progressing, leading to the development of better treatments and preventative measures, which promote workers' rights and ensure their continued good health (220). There is currently no specific cure for most occupational lung diseases. Therefore, a multidisciplinary approach is required for the prevention and control of occupational lung disease progression.

3.6. Respiratory bronchiolitis-ILD

Respiratory bronchiolitis (RB) is a medical condition found in cigarette smokers that does not have any noticeable symptoms. It is characterized by the buildup of macrophages that are tan or yellow in color in the lumens of bronchioles (9). This accumulation is accompanied by chronic inflammation and fibrosis that extends from the respiratory bronchioles to the alveolar walls. When RB is accompanied by clinical evidence of ILD, it is referred to as respiratory bronchiolitis-interstitial lung disease (RB-ILD), which is a distinct form of ILD. The difference between RB and RB-ILD is mainly based on the presence of clinical evidence of ILD, such as symptoms, chest imaging abnormalities, and pulmonary function test results, rather than the extent of fibrosis in the alveolar walls as observed through histopathology (221). A person is usually suspected of having RB-ILD if they have non-specific respiratory symptoms like dyspnea or cough and an abnormal chest radiograph (222). Findings on HRCT are non-specific and include air entrapment, centrilobular nodules, and diffuse or patchy ground-glass opacities. The diagnosis of RB-ILD may be made based on a firm clinical suspicion, compatible pulmonary function test and HRCT results, and mild respiratory impairment (223, 224). Invasive tests may not be necessary if a patient's condition stabilizes or improves after quitting smoking. Lung biopsy is frequently performed to corroborate the diagnosis and rule out other treatable interstitial lung diseases in cases of more serious or progressive respiratory impairment despite smoking cessation. The most crucial diagnostic and therapeutic stage is giving up smoking and avoiding exposure to cigarette smoke (222, 225).

3.7. Radiation-induced lung injury

Radiation Pneumonitis (RP), the initial stage of radiotherapy-induced lung injury (RILI), is defined by lung tissue inflammation brought on by radiation exposure. Radiation Fibrosis (RF), the second stage of RILI, is a clinical condition brought on by persistent pulmonary tissue damage. Currently, a diagnosis is usually made by excluding other potential causes using clinical examination and radiological imaging. Pulmonary function tests are helpful in determining the state of the patient's lung function and in identifying any possible side effects or toxicity during radiotherapy. Although systemic corticosteroids are frequently used to treat pneumonitis complications, their use needs to be standardized and taken into consideration for prophylactic reasons due to the potentially fatal consequences of this adverse event (226).

4. Idiopathic lung diseases

While the etiology of immune-mediated lung disorders is not clearly defined, most of these disorders could be classified under idiopathic lung disease. This review will focus on IPF, the prototype of idiopathic lung diseases where lung injury leads to fibrosis.

4.1. Idiopathic pulmonary fibrosis (IPF)

Idiopathic pulmonary fibrosis, the most common type of spontaneously occurring diffuse parenchymal lung disease, is a chronic progressive and eventually lethal disease with a poor prognosis characterized by irreversible diffuse lung fibrosis and impaired lung function likely caused by a multitude of cycles of epithelial cell injury and uncontrolled repair (12, 227). IPF's histopathological features include aberrant mesenchymal cell proliferation, various degrees of fibrosis, overproduction, and disordered collagen and extracellular matrix deposition (228). Furthermore, the pathogenesis of IPF is complex and not fully known; however, several likely contributors have been recognized in the literature; injured alveolar epithelial cells secrete growth factors, especially TGF-β, which help in the recruitment of fibroblasts and subsequent differentiation into myofibroblasts expressing features of both fibroblasts and smooth muscle cells. The myofibroblasts cause excessive collagen and extracellular matrix (ECM) deposition leading to scar formation and distortion of the alveolar structure. It is possible that multiple micro-injuries to alveolar epithelial cells induce a fibrotic environment (229). Alpha-smooth muscle actin (SMA), which is expressed by myofibroblasts, helps to distinguish them from smooth muscle cells and fibroblasts (230). Myofibroblasts secrete collagen after being recruited to the lungs or differentiating from local fibroblasts. Collagen accumulates as a result of an imbalance between interstitial collagenases and their tissue inhibitors (231).

Over the past decade, research efforts regarding immunity and IPF have reached important milestones. IPF involves both innate and adaptive inflammatory processes (205). Since neutrophils are important in the acute phase of inflammation, their aggregation in response to lung injury may intensify tissue remodeling and fibrosis, presumably *via* neutrophil elastase. B-cells build up in the lungs of people with IPF, and humoral autoimmunity against epithelial auto-antigens can fuel and sustain persistent inflammation there (205). In addition, T-cells have been shown to be involved in IPF. The Th2 cytokines IL-4 and IL-13 are extremely pro-fibrotic, whereas the Th1 cytokines IFN- and IL-12 prevent the development of tissue fibrosis. Th1 and Th2 cytokines play conflicting roles in fibrosis (232).

The risk of IPF is significantly increased by a single nucleotide polymorphism (rs35705950) in the promoter region of MUC5B (233). MUC5B is a gene that codes for mucin 5B, a glycoprotein important for innate immune responses against bacterial and airway clearance. Some researchers have proposed that abnormal mucociliary clearance may cause changes in the lung microbiota and innate immune responses that support IPF. However, the exact mechanism relating to mucin 5B overexpression and IPF risk is yet to be known (195, 234).

4.1.1. Clinical manifestations

Patients usually present with worsening cough, dyspnea, and impaired quality of life. The diagnostic approach initially involves the exclusion of other interstitial lung diseases or overlapping conditions such as hypersensitivity pneumonitis, pulmonary sarcoidosis, or an underlying autoimmune disease. IPF demonstrates "honeycombing" on high-resolution CT scan (subpleural cystic airspaces with well-defined walls). In addition, lung biopsy may be considered in some patients (13, 235). IPF is frequently misdiagnosed, and is improperly treated with immunosuppressive medicine. Recent treatments can halt the progression of the disease (235).

4.1.2. Treatment

Results from previous negative landmark studies imply that the inflammatory changes found in IPF happen independently of the main fibrotic remodeling mechanism. Nevertheless, current medications (such as pirfenidone and nintedanib) or new medications that have shown promise in Phase-II trials for IPF (like PMR151 and GLPG1690) also control inflammatory processes (205). It is crucial to understand that treating lung injury frequently requires treating the inflammation (e.g., infections, physical trauma). Given that immune cells constitute a typical component of the human lungs' normal structure and operation, this problem may be particularly relevant to inflammatory illnesses of the respiratory system (236). Consequently, there is a critical need for stratified medicine based on genomes, biomarkers, and also inflammatory profiles to identify IPF patients who may benefit from combining standard-of-care "anti-fibrotic" medication with co-treatment with anti-inflammatory/immunomodulatory therapy. For IPF patients, this precision medicine should result in accurate health advice, a precise diagnosis, and a unique treatment strategy (205).

Recent studies have shown that most Interleukin 17 (IL-17) isoforms are involved in acute and chronic inflammation *via* innate and adaptive immunity (237). Cipolla et al. (238) reported that Interleukin 17A (IL-17A) and complement (C') activation play an important role in the pathogenesis of IPF. IL-17A activates profibrotic signaling pathways and C' by regulating mRNA and protein expression of the C' components (238). Consequently, C3a, C5a, and TGF-β mediate alveolar epithelial injury *via* p38MAPK activation (239). Although there is currently no direct evidence that IL-17F contributes to the development of IPF, these findings on IL-17A and inflammatory responses imply that IL-17F may be a useful therapeutic target (237).

Pentraxin 2, also known as serum amyloid P, can prevent the development of fibrocytes that promote fibrosis and inflammatory macrophages. Patients with IPF have lower levels of Pentraxin 2 in their plasma compared to healthy individuals (240). In a phase 2 trial, 111 patients with IPF received either intravenous recombinant human Pentraxin 2 (at a dose of 10 mg/kg) or placebo every 4 weeks for 24 weeks. Most of the patients were already being treated with an anti-fibrotic agent. The Pentraxin 2 group had a slower decline in FVC compared to the placebo group (241). The most common side effects were cough and fatigue. In a follow-up study lasting 76 weeks, patients who were switched from placebo to Pentraxin 2 showed a slower decline in lung function and distance walked in 6 min, which was similar to the initial study (242). These

findings were consistent even after 128 weeks (243). Pentraxin 2 was generally well-tolerated by patients.

Phosphodiesterase 4 (PDE4) inhibitor (BI 1015550), which prevents the degradation of cyclic adenosine monophosphate, has antifibrotic and immunomodulatory effects (244). In a placebo-controlled trial to test the efficacy of BI 1015550 in preventing a decline in lung function in patients with idiopathic pulmonary fibrosis. The use of BI 1015550, either alone or in combination with an antifibrotic agent, was successful in preventing a decrease in lung function (245).

In a phase IIb randomized clinical study to assess the safety and efficacy of anti-αvβ6 monoclonal antibody (BG00011). The results did not support the ongoing clinical advancement of BG00011 (246). Additional investigation is necessary to pinpoint better therapeutic methods that can alter the inflammatory and fibrotic pathways in IPF.

5. Miscellaneous lung diseases

5.1. Sarcoidosis

Sarcoidosis is a multisystem inflammatory disorder of unknown etiology that can affect virtually any organ system but classically involves the lungs and hilar lymph nodes. At the center of sarcoidosis pathogenesis is the non-caseating granuloma, a cellular aggregation orchestrated by a complex ensemble of innate and adaptive immune cells and inflammatory mediators. The perplexing interplay of these immune components is yet to be fully delineated and is of prodigious value in revealing therapeutic avenues for sarcoidosis management (247, 248).

The inciting event in sarcoidosis pathogenesis is thought to be exposure to a yet unidentified antigen detected by antigen-presenting cells (APCs). Putative antigens may be broadly classified as microbial (e.g., mycobacteria and propionibacteria) or non-microbial (e.g., autoantigens such as vimentin, or exogenous antigens such as occupational exposures) (249). The antigen is delivered *via* dendritic cells and presented *via* major histocompatibility complex class II molecules to T cells, promoting their differentiation into various T-cell subsets, including T helper (Th) 1 cells. Early phases of sarcoidosis are characterized by a Th1-promoting milieu of chemokines such as IL-2 and IFN-gamma, which led to sarcoidosis being classically viewed as a Th1-polarized disease (250). Th1 cells elaborate cytokines, including IFN-gamma, which primarily serve to activate macrophages to ingest or destroy the target antigen and amplify the granulomatous response. More recently, however, a shift in the understanding of sarcoidosis pathogenesis occurred with recognition of Th17 cells as new players in the disease. Recent studies have suggested that Th17.1 cells to the pre-dominant source of IFN-gamma in sarcoidosis bronchoalveolar lavage fluid, putting into question the classical Th1 paradigm (251). Another contributing factor to sarcoidosis immunopathogenesis is believed to be an imbalance between the immunosuppressive effects of regulatory T (Treg) cells and the proinflammatory effects of other T-cell subsets (252) interest in the Treg population of cells is growing, and whether disease activity may correlate with the balance between Treg cell activity and other T-cell subset activity would be of great value to the sarcoidosis community.

Although much of sarcoidosis pathogenesis focuses on T-cell-mediated immunity, humoral mechanisms have been suggested to play a role on the basis of several observations, including hypergammaglobulinemia, autoantibodies, circulating immune complexes (253–255). Nevertheless, anti-B-cell therapy with rituximab has shown conflicting results in refractory disease, and further studies are certainly needed to elucidate the true significance of humoral immunity in sarcoidosis immunopathogenesis (256–258). Additionally, the innate arm is being increasingly recognized as an important contributor to sarcoidosis pathogenesis, with derangements in toll-like receptor (TLR) and nucleotide oligomerization domain receptor (NLR) signaling being implicated (249).

Progression to fibrotic pulmonary sarcoidosis is challenging to understand given the pre-dominance of the antifibrotic Th1 cytokine, IFN-gamma, in the milieu of sarcoidosis granulomas. It has therefore been postulated that persistent inflammation may induce a switch from an antifibrotic Th1-predominant state to a profibrotic Th2-predominant state, transitioning into fibrotic pulmonary sarcoidosis (259). Additionally, Treg cells may acquire the capacity to inhibit the Th2 cells and reduce fibrosis (260). Decreased Treg cell numbers in bronchoalveolar lavage fluid, and Treg cell dysfunction have been observed in sarcoidosis and may promote fibrosis (261, 262). Roles for other cells, including Th17 cells and macrophages may also contribute (263). Finally, genetic polymorphisms in various genes, such as TGF-β and GREM1, have also been linked to fibrotic pulmonary sarcoidosis (263).

5.1.1. Clinical manifestations

Sarcoidosis can affect virtually any organ system of the body, but pulmonary involvement is characteristic and occurs in up to 90% of cases (264). Bilateral hilar lymphadenopathy detected incidentally on chest radiography is a typical scenario in an asymptomatic patient and confers an excellent prognosis, with the majority of patients achieving remission within 3 years following the diagnosis (265). In the presence of concurrent parenchymal lung disease which commonly appears in the form of reticulonodular pulmonary infiltrates, respiratory symptoms of dry cough, dyspnea, and chest pain are possible presentations (265). Long-standing pulmonary disease may progress into pulmonary fibrosis, in which case chronic cough and dyspnea are prominent complaints, alongside potential physical exam findings of clubbing, wheezing and end-inspiratory crackles (266). Regardless of organ involvement, constitutional symptoms including fatigue, fever, and night sweats are relatively common (267, 268). Alternatively, classical sarcoidosis syndromes have been described including Lofgren syndrome (bilateral hilar lymphadenopathy, migratory polyarthritis, and erythema nodosum) as well as Heerfrodt syndrome (uveitis, parotitis, and facial nerve palsy), and the presence of either of these syndromes has substantial diagnostic relevance (265). Pulmonary hypertension is a potential complication of sarcoidosis and incidence rates vary from 5 to 70% of sarcoidosis patients depending on the study population. Sarcoidosis-associated pulmonary hypertension (SAPH) patients are almost invariably dyspneic and have a reduced 6-min walk distance when screened for SAPH. SAPH is a significant cause of morbidity and mortality in sarcoidosis patients and

confers a poorer prognosis (266). Other pulmonary pathologies in sarcoidosis include pleural disease in the form of pleural effusion or pneumothorax but are relatively uncommon findings in sarcoidosis (264, 266). Extrapulmonary sarcoidosis may coexist with pulmonary disease or occur in isolation, and can involve the skin (e.g., erythema nodosum, lupus pernio), eyes (e.g., uveitis), musculoskeletal system (e.g., arthritis), and nervous systems (e.g., facial nerve palsy, meningitis), heart (e.g., arrhythmias, heart failure) among others (269).

The diagnosis of sarcoidosis is often challenging and emerges in the context of clinical, radiographic, and histopathological evidence of the disease, all in the absence of an alternative explanation for the clinical findings (270). This often necessitates comprehensive evaluation in order to eliminate other sarcoidosis mimics. Chest X-rays are the initial imaging modality of choice and aid in thoracic sarcoidosis staging. Typical findings include bilateral hilar lymphadenopathy and/or pulmonary infiltrates. Computed tomography scans are usually sought for further evaluation of the disease as they demonstrate superior sensitivity in detecting lymphadenopathy and parenchymal lung changes, in addition to better characterizing the presence of pulmonary fibrosis as compared to chest radiographs (265, 271). An absolute diagnosis in many cases, however, calls for a tissue biopsy from the most accessible site. This would ideally be from a cutaneous lesion, but in the absence of peripheral involvement, endobronchial or transbronchial biopsy are the gold standard. Demonstration of non-caseating granulomatous inflammation is the hallmark pathology of sarcoidosis. Laboratory evaluation is complementary and may assist in monitoring disease activity. Classic laboratory findings include hypercalcemia, elevated ACE levels, serum soluble interleukin-2 receptor, rheumatoid factor and elevated inflammatory markers (270, 272).

5.1.2. Treatment

The mainstay of management of pulmonary sarcoidosis remains to be oral glucocorticoid therapy. Immunosuppressive therapy with methotrexate, azathioprine, and leflunomide are second-line options used in glucocorticoid-resistant disease, or as steroid-sparing therapy in those who cannot tolerate steroids or need long-term management. Those who are also refractory to the aforementioned agents may benefit from anti-TNF-alpha therapy with infliximab or adalimumab which are considered third-line options for sarcoidosis (273–276). Numerous other therapies remain investigational, including rituximab, cyclophosphamide, and tocilizumab (256–258, 277). End-stage fibrotic disease is not responsive to immunosuppressive therapy and in such cases, lung transplantation may be the sole option (278, 279). Antifibrotic therapy for progressive pulmonary fibrosis in sarcoidosis remains investigational. Nintedanib was shown to slow disease progression in a recent report, and other antifibrotic agents such as pirfenidone are also under investigation (266).

5.2. Post-lung transplant ILD

Post-lung transplant immune-mediated ILD is a condition that can present as either a recurrent episode of the primary disease or as a *de novo* disease after lung transplantation. Due to the rarity of cases, the pathogenesis of recurring or *de novo* ILDs after lung transplantation remains incompletely understood. However, it is crucial for clinicians to be aware that various subtypes of ILD might reoccur after a transplant and to consider this diagnosis in situations with deteriorating respiratory symptoms. Further research is required to gain comprehensive knowledge of the factors that may contribute to the development of ILD after transplantation.

The most common clinical presentation of this condition is the deterioration of respiratory function that requires clinical management or intervention, which can frequently be mistaken for rejection episodes and should be considered by physicians following up on patients despite being relatively rare compared to rejection. To confirm the cause of the sudden deterioration in clinical manifestations, lung biopsies may be necessary. Histopathological examination of lung specimens from patients with post-transplant immune-mediated ILD often reveals one of the patterns of a subtype of ILDs, as well as infiltration of immune cells and fibrotic changes (280).

Lung transplantation can improve quality of life and survival for patients with terminal lung diseases that are resistant to other treatment options. However, in rare cases, primary diseases, including various subtypes of interstitial lung disease (ILD), have been reported to recur after transplantation (280). Collins et al. (281) reported 15 occurrences of primary disease recurrence among 1,394 lung transplant recipients at six medical centers.

Following lung transplantation, sarcoidosis was the most commonly reported cause of recurrence of the primary disease (280–282). Schultz et al. (283) found that about 30% of lung transplant recipients with sarcoidosis experienced recurrence of sarcoid granulomas. Ionescu et al. (284) employed DNA analysis methods and suggested that the origin of recurrent granulomas in the graft is associated with the recipient. Banga et al. (285) found that 7 out of 30 cases experienced recurrence of sarcoidosis over an 18-year period in a single center and determined that the presence of granulomas on explanted lungs was the sole predictor of recurrence Additionally, they observed that sarcoidosis recurrence did not appear to affect 1–5-year survival rates. Although the recurrence of granulomas in transplanted lungs is generally not associated with significant deterioration in allograft function or patient survival, it is important for clinicians to consider arranging appropriate surveillance strategies such as biopsy and high-resolution computed tomography during the post-transplant follow-up period to monitor for the recurrence of sarcoidosis (282).

Idiopathic interstitial pneumonia, including desquamative interstitial pneumonia (DIP), NSIP, has been shown to recur after lung transplantation, with varying time frames reported in the literature (280). King et al. (286) reported a case of DIP recurrence 1 month after transplantation in a 50-year-old female patient, leading to Cytomegalovirus and Nocardia infections, respiratory failure, and ultimately, mortality 8 months after transplantation despite aggressive treatment with high-dose steroids, antibiotics, and mechanical ventilation Verleden et al. (287) reported a case of DIP recurrence in a 51-year-old male patient more than 1 year after his lung transplantation. The patient fully recovered after a course of corticosteroid therapy and a gradual decrease in dosage. No symptoms of dyspnea or coughing were reported for at least 2 years following treatment until the patient was lost to follow-up (287). Bhatt et al. (288) reported a case of a 42-year-old woman who experienced a decline in respiratory function 8 weeks after receiving a bilateral lung transplant for NSIP. Biopsy

findings at 6 months post-transplant revealed acute rejection with mixed desquamative and NSIP findings, similar to the patient's pre-transplant pathology. The study also observed that recipient macrophages began accumulating in the lungs as early as 2 months after transplantation and continued to build up, contributing to the development of interstitial fibrosis.

Connective tissue disease-associated interstitial lung diseases (CTD-ILDs) have been reported to recur or occur *de novo* following lung transplantation. Arboleda et al. (289) described a case of a 15-year-old female patient who received a bilateral lung transplant for polymyositis-related UIP and developed recurrent UIP 8 months post-transplant, ultimately leading to respiratory failure and death 16 days after receiving mechanical ventilation and extracorporeal membrane oxygenation (ECMO) treatment. A post-mortem investigation of the graft revealed advanced pulmonary fibrosis with UIP, indicating a recurrence of the primary disease. Scallan et al. (290) described a case of a 51-year-old female patient who developed recurrent idiopathic fibrotic non-specific interstitial pneumonia (iNSIP-F) and new onset antisynthetase syndrome (anti-SS) 30 months after undergoing bilateral lung transplantation. As of the time this case was reported in 2020, the patient had been 10 years post-transplant and continued to have clinical and serologic features of anti-SS with slowly progressive fibrotic changes, and had also developed pulmonary hypertension that responded to treatment with sildenafil. A meta-analysis of outcomes after lung transplantation found that non-myositis CTD-ILDs had a similar survival rate to IPF, while myositis CTD-ILDs were associated with a poorer survival outcome (291).

6. Conclusion

Immune-mediated lung diseases are a heterogeneous group of disorders that pose a diagnostic and therapeutic challenge. Timely diagnosis of immune-mediated lung diseases is important. Moreover, frequent follow-up to assess disease progression is necessary. A personalized approach to treatment is required given the heterogeneity of the presentation and overlap among multiple disorders. CS remains the mainstay as initial treatment. However, the dose and duration vary depending on the primary disease, systemic involvement, inflammatory lung changes and the presence or absence of lung fibrosis. In patients who experience CS toxicity or where CS lacks efficacy, alternatives may be helpful. In cases where there may be concomitant fibrosis, anti-fibrotic agents may be considered. There are no standardized management guidelines, thus a collaborative treatment team should consider a personalized approach according to the underlying lung disease, rate of disease progression, and disease severity. Special attention should be paid to the risk of infection and to proper vaccination. Advanced research technologies combined with artificial intelligence will allow us to identify potential therapeutic targets for inflammatory and fibrotic phenotypes. Furthermore, it will allow us to design translational clinical trials that will offer therapeutic options and may potentially decrease the progression to severe lung disease.

Author contributions

JS, NWS, and NS were built the table. NWS designed the figure. All authors contributed to the scientific writing of the manuscript and approved the submitted version.

Funding

This research was supported by a gracious donation from the Bernie Mac Foundation and Professor Robert Barish the Vice Chancellor for Health Affairs at the University of Illinois Chicago. Also, this material is the result of work supported with resources and the use of facilities at the Jesse Brown VA Medical Center, Chicago, IL.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Author disclaimer

The views expressed in this article are those of the authors and do not necessarily reflect the position or policy of the Department of Veterans Affairs or the United States government.

References

1. Parkin J, Cohen B. An overview of the immune system. *Lancet.* (2001) 357: 1777–89.

2. Delves P, Roitt I. The immune system. First of two parts. *N Engl J Med.* (2000) 343:37–49. doi: 10.1056/NEJM20000706343 0107

3. Bonilla F, Oettgen H. Adaptive immunity. *J Allergy Clin Immunol.* (2010) 125(Suppl. 2):S33–40. doi: 10.1016/j.jaci.2009.09.017

4. Clark R, Kupper T. Old meets new: the interaction between innate and adaptive immunity. *J Invest Dermatol.* (2005) 125:629–37. doi: 10.1111/j.0022-202X.2005. 23856.x

5. Bals R, Hiemstra P. Innate immunity in the lung: how epithelial cells fight against respiratory pathogens. *Eur Respir J.* (2004) 23:327–33. doi: 10.1183/09031936.03.00098803

6. Jacobs K, Kligerman S. Immune-Mediated Lung Diseases. *Semin Ultrasound CT MR.* (2019) 40:213–28. doi: 10.1053/j.sult.2018.11.011

7. Wells A, Hirani N. 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.* (2008) 63(Suppl. 5):v1–58. doi: 10.1136/thx.2008.101691

8. Mittoo S, Gelber A, Christopher-Stine L, Horton M, Lechtzin N, Danoff S. Ascertainment of collagen vascular disease in patients presenting with interstitial lung disease. *Respir Med.* (2009) 103:1152–8. doi: 10.1016/j.rmed.2009.02.009

9. Travis W, Costabel U, Hansell D, King T, Lynch D, Nicholson A, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med.* (2013) 188:733–48. doi: 10.1164/rccm.201308-1483ST

10. Duchemann B, Annesi-Maesano I, Jacobe de Naurois C, Sanyal S, Brillet P, Brauner M. Prevalence and incidence of interstitial lung diseases in a multi-ethnic county of Greater Paris. *Eur Respir J.* (2017) 50:1602419. doi: 10.1183/13993003.02419-2016

11. De Sadeleer L, Meert C, Yserbyt J, Slabbynck H, Verschakelen J, Verbeken E, et al. Diagnostic ability of a dynamic multidisciplinary discussion in interstitial lung diseases: a retrospective observational study of 938 cases. *Chest.* (2018) 153:1416–23. doi: 10.1016/j.chest.2018.03.026

12. Raghu G, Collard H, Egan J, Martinez F, Behr J, Brown K, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med.* (2011) 183:788–824. doi: 10.1164/rccm.2009-040GL

13. Martinez F, Collard H, Pardo A, Raghu G, Richeldi L, Selman M, et al. Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers.* (2017) 3:17074. doi: 10.1038/nrdp.2017.74

14. Raghu G, Rochwerg B, Zhang Y, Garcia C, Azuma A, Behr J, et al. An Official ATS/ERS/JRS/ALAT Clinical Practice Guideline: treatment of Idiopathic Pulmonary Fibrosis. An Update of the 2011 Clinical Practice Guideline. *Am J Respir Crit Care Med.* (2015) 192:e3–19. doi: 10.1164/rccm.201506-1063ST

15. Wijsenbeek M, Kreuter M, Olson A, Fischer A, Bendstrup E, Wells C, et al. Progressive fibrosing interstitial lung diseases: current practice in diagnosis and management. *Curr Med Res Opin.* (2019) 35:2015–24. doi: 10.1080/03007995.2019.1647040

16. George P, Spagnolo P, Kreuter M, Altinisik G, Bonifazi M, Martinez F, et al. Progressive fibrosing interstitial lung disease: clinical uncertainties, consensus recommendations, and research priorities. *Lancet Respir Med.* (2020) 8:925–34. doi: 10.1016/S2213-260030355-6

17. Maher T, Wuyts W. Management of Fibrosing Interstitial Lung Diseases. *Adv Ther.* (2019) 36:1518–31. doi: 10.1007/s12325-019-00992-9

18. Raghu G, Remy-Jardin M, Richeldi L, Thomson C, Inoue Y, Johkoh T, et al. Idiopathic Pulmonary Fibrosis (an Update) and Progressive Pulmonary Fibrosis in Adults: an Official ATS/ERS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med.* (2022) 205:e18–47. doi: 10.1164/rccm.202202-0399ST

19. Gourh P, Safran S, Alexander T, Boyden S, Morgan N, Shah A, et al. HLA and autoantibodies define scleroderma subtypes and risk in African and European Americans and suggest a role for molecular mimicry. *Proc Natl Acad Sci U.S.A.* (2020) 117:552–62. doi: 10.1073/pnas.1906593116

20. Arnett F, Gourh P, Shete S, Ahn C, Honey R, Agarwal S, et al. Major histocompatibility complex (MHC) class II alleles, haplotypes and epitopes which confer susceptibility or protection in systemic sclerosis: analyses in 1300 Caucasian, African-American and Hispanic cases and 1000 controls. *Ann Rheum Dis.* (2010) 69:822–7. doi: 10.1136/ard.2009.111906

21. Nihtyanova S, Denton C. Pathogenesis of systemic sclerosis associated interstitial lung disease. *J Scleroderma Relat Disord.* (2020) 5(2 Suppl):6–16. doi: 10.1177/2397198320903867

22. Hant F, Ludwicka-Bradley A, Wang H, Li N, Elashoff R, Tashkin D, et al. Surfactant protein D and KL-6 as serum biomarkers of interstitial lung disease in patients with scleroderma. *J Rheumatol.* (2009) 36:773–80. doi: 10.3899/jrheum.080633

23. Fiorentini E, Bonomi F, Peretti S, Orlandi M, Lepri G, Matucci Cerinic M, et al. Potential Role of JAK Inhibitors in the Treatment of Systemic Sclerosis-Associated Interstitial Lung Disease: a Narrative Review from Pathogenesis to Real-Life Data. *Life.* (2022) 12:2101. doi: 10.3390/life12122101

24. Lescoat A, Lelong M, Jeljeli M, Piquet-Pellorce C, Morzadec C, Ballerie A, et al. Combined anti-fibrotic and anti-inflammatory properties of JAK-inhibitors on macrophages in vitro and in vivo: perspectives for scleroderma-associated interstitial lung disease. *Biochem Pharmacol.* (2020) 178:114103. doi: 10.1016/j.bcp.2020.114103

25. Hilberg F, Roth G, Krssak M, Kautschitsch S, Sommergruber W, Tontsch-Grunt U, et al. BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. *Cancer Res.* (2008) 68:4774–82. doi: 10.1158/0008-5472.CAN-07-6307

26. Lafyatis R. Transforming growth factor β–at the centre of systemic sclerosis. *Nat Rev Rheumatol.* (2014) 10:706–19. doi: 10.1038/nrrheum.2014.137

27. Willis B, Borok Z. TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. *Am J Physiol Lung Cell Mol Physiol.* (2007) 293:L525–34. doi: 10.1152/ajplung.00163.2007

28. Kalluri R, Weinberg R. The basics of epithelial-mesenchymal transition. *J Clin Invest.* (2009) 119:1420–8. doi: 10.1172/JCI39104

29. Rock J, Barkauskas C, Cronce M, Xue Y, Harris J, Liang J, et al. Multiple stromal populations contribute to pulmonary fibrosis without evidence for epithelial to mesenchymal transition. *Proc Natl Acad Sci U.S.A.* (2011) 108:E1475–83. doi: 10.1073/pnas.1117988108

30. Denton C, Khanna D. Systemic sclerosis. *Lancet.* (2017) 390:1685–99. doi: 10.1016/S0140-673630933-9

31. Stochmal A, Czuwara J, Trojanowska M, Rudnicka L. Antinuclear Antibodies in Systemic Sclerosis: an Update. *Clin Rev Allergy Immunol.* (2020) 58:40–51. doi: 10.1007/s12016-018-8718-8

32. Salazar G, Assassi S, Wigley F, Hummers L, Varga J, Hinchcliff M, et al. Antinuclear antibody-negative systemic sclerosis. *Semin Arthritis Rheum.* (2015) 44:680–6. doi: 10.1016/j.semarthrit.2014.11.006

33. Tyndall A, Bannert B, Vonk M, Airò P, Cozzi F, Carreira P, et al. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis.* (2010) 69:1809–15. doi: 10.1136/ard.2009.114264

34. Ferri C, Valentini G, Cozzi F, Sebastiani M, Michelassi C, La Montagna G, et al. Systemic sclerosis: demographic, clinical, and serologic features and survival in 1,012 Italian patients. *Medicine.* (2002) 81:139–53. doi: 10.1097/00005792-200203000-00004

35. Nihtyanova S, Sari A, Harvey J, Leslie A, Derrett-Smith E, Fonseca C, et al. Using Autoantibodies and Cutaneous Subset to Develop Outcome-Based Disease Classification in Systemic Sclerosis. *Arthritis Rheumatol.* (2020) 72:465–76. doi: 10.1002/art.41153

36. Bouros D, Wells A, Nicholson A, Colby T, Polychronopoulos V, Pantelidis P, et al. Histopathologic subsets of fibrosing alveolitis in patients with systemic sclerosis and their relationship to outcome. *Am J Respir Crit Care Med.* (2002) 165:1581–6. doi: 10.1164/rccm.2106012

37. Tashkin D, Elashoff R, Clements P, Goldin J, Roth M, Furst D, et al. Cyclophosphamide versus placebo in scleroderma lung disease. *N Engl J Med.* (2006) 354:2655–66. doi: 10.1056/NEJMoa055120

38. Tashkin D, Roth M, Clements P, Furst D, Khanna D, Kleerup E, et al. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial. *Lancet Respir Med.* (2016) 4:708–19. doi: 10.1016/S2213-260030152-7

39. Sato S, Fujimoto M, Hasegawa M, Takehara K, Tedder T. Altered B lymphocyte function induces systemic autoimmunity in systemic sclerosis. *Mol Immunol.* (2004) 41:1123–33. doi: 10.1016/j.molimm.2004.06.025

40. Goswami R, Ray A, Chatterjee M, Mukherjee A, Sircar G, Ghosh P. Rituximab in the treatment of systemic sclerosis-related interstitial lung disease: a systematic review and meta-analysis. *Rheumatology.* (2021) 60:557–67. doi: 10.1093/rheumatology/keaa550

41. Khanna D, Lin C, Furst D, Wagner B, Zucchetto M, Raghu G, et al. Long-Term Safety and Efficacy of Tocilizumab in Early Systemic Sclerosis-Interstitial Lung Disease: open-Label Extension of a Phase 3 Randomized Controlled Trial. *Am J Respir Crit Care Med.* (2022) 205:674–84. doi: 10.1164/rccm.202103-0714OC

42. Montero P, Milara J, Roger I, Cortijo J. Role of JAK/STAT in Interstitial Lung Diseases; Molecular and Cellular Mechanisms. *Int J Mol Sci.* (2021) 22:6211. doi: 10.3390/ijms22126211

43. Gadina M, Johnson C, Schwartz D, Bonelli M, Hasni S, Kanno Y, et al. Translational and clinical advances in JAK-STAT biology: the present and future of jakinibs. *J Leukoc Biol.* (2018) 104:499–514. doi: 10.1002/JLB.5RI0218-084R

44. Distler O, Highland K, Gahlemann M, Azuma A, Fischer A, Mayes M, et al. Nintedanib for Systemic Sclerosis-Associated Interstitial Lung Disease. *N Engl J Med.* (2019) 380:2518–28. doi: 10.1056/NEJMoa1903076

45. Seibold J, Maher T, Highland K, Assassi S, Azuma A, Hummers L, et al. Safety and tolerability of nintedanib in patients with systemic sclerosis-associated interstitial lung disease: data from the SENSCIS trial. *Ann Rheum Dis.* (2020) 79:1478–84. doi: 10.1136/annrheumdis-2020-217331

46. Flaherty K, Wells A, Cottin V, Devaraj A, Walsh S, Inoue Y, et al. Nintedanib in Progressive Fibrosing Interstitial Lung Diseases. *N Engl J Med.* (2019) 381:1718–27. doi: 10.1056/NEJMoa1908681

47. Vonk M, Smith V, Sfikakis P, Cutolo M, Del Galdo F, Seibold J. Pharmacological treatments for SSc-ILD: systematic review and critical appraisal of the evidence. *Autoimmun Rev.* (2021) 20:102978. doi: 10.1016/j.autrev.2021.102978

48. Jindal S, Agarwal R. Autoimmunity and interstitial lung disease. *Curr Opin Pulm Med.* (2005) 11:438–46. doi: 10.1097/01.mcp.0000170522.71497.61

49. He J, Chen J, Miao M, Zhang R, Cheng G, Wang Y, et al. Efficacy and Safety of Low-Dose Interleukin 2 for Primary Sjögren Syndrome: a Randomized Clinical Trial. *JAMA Netw Open.* (2022) 5:e2241451. doi: 10.1001/jamanetworkopen.2022.41451

50. Karlsen M, Jonsson R, Brun J, Appel S, Hansen T. TLR-7 and -9 Stimulation of Peripheral Blood B Cells Indicate Altered TLR Signalling in Primary Sjögren's Syndrome Patients by Increased Secretion of Cytokines. *Scand J Immunol.* (2015) 82:523–31. doi: 10.1111/sji.12368

51. Ettinger R, Sims G, Fairhurst A, Robbins R, da Silva Y, Spolski R, et al. IL-21 induces differentiation of human naive and memory B cells into antibody-secreting plasma cells. *J Immunol.* (2005) 175:7867–79. doi: 10.4049/jimmunol.175.12.7867

52. Badr G, Borhis G, Lefevre E, Chaoul N, Deshayes F, Dessirier V, et al. BAFF enhances chemotaxis of primary human B cells: a particular synergy between BAFF and CXCL13 on memory B cells. *Blood.* (2008) 111:2744–54. doi: 10.1182/blood-2007-03-081232

53. Loureiro-Amigo J, Franco-Jarava C, Perurena-Prieto J, Palacio C, Martínez-Valle F, Soláns-Laqué R. Serum CXCL13, BAFF, IL-21 and IL-22 levels are related to disease activity and lymphocyte profile in primary Sjögren's syndrome. *Clin Exp Rheumatol.* (2021) 39(Suppl. 133):131–9. doi: 10.55563/clinexprheumatol/fp741f

54. Goules A, Kapsogeorgou E, Tzioufas A. Insight into pathogenesis of Sjögren's syndrome: dissection on autoimmune infiltrates and epithelial cells. *Clin Immunol.* (2017) 182:30–40. doi: 10.1016/j.clim.2017.03.007

55. Kassan S, Moutsopoulos H. Clinical manifestations and early diagnosis of Sjögren syndrome. *Arch Intern Med.* (2004) 164:1275–84. doi: 10.1001/archinte.164.12.1275

56. Fox RI. Sjögren's syndrome. *Lancet (Lond Engl).* (2005) 366:321–31.

57. Quismorio F. Pulmonary involvement in primary Sjögren's syndrome. *Curr Opin Pulm Med.* (1996) 2:424–8. doi: 10.1097/00063198-199609000-00013

58. Shiboski C, Shiboski S, Seror R, Criswell L, Labetoulle M, Lietman T, et al. 2016 American College of Rheumatology/European League Against Rheumatism Classification Criteria for Primary Sjögren's Syndrome: a Consensus and Data-Driven Methodology Involving Three International Patient Cohorts. *Arthritis Rheumatol.* (2017) 69:35–45. doi: 10.1002/art.39859

59. He J, Jiang J, Baumgart K. Candidate autoantibodies for primary Sjögren's syndrome: where are they now? *Clin Exp Rheumatol.* (2021) 40:2387–94.

60. Theander E, Jonsson R, Sjöström B, Brokstad K, Olsson P, Henriksson G. Prediction of Sjögren's Syndrome Years Before Diagnosis and Identification of Patients With Early Onset and Severe Disease Course by Autoantibody Profiling. *Arthritis Rheumatol.* (2015) 67:2427–36. doi: 10.1002/art.39214

61. Sambataro G, Ferro F, Orlandi M, Sambataro D, Torrisi S, Quartuccio L, et al. Clinical, morphological features and prognostic factors associated with interstitial lung disease in primary Sj?gren's syndrome: a systematic review from the Italian Society of Rheumatology. *Autoimmun Rev.* (2020) 19:102447. doi: 10.1016/j.autrev.2019.102447

62. Matsuyama N, Ashizawa K, Okimoto T, Kadota J, Amano H, Hayashi K. Pulmonary lesions associated with Sjögren's syndrome: radiographic and CT findings. *Br J Radiol.* (2003) 76:880–4. doi: 10.1259/bjr/18937619

63. Palm O, Garen T, Berge Enger T, Jensen J, Lund M, Aaløkken T, et al. Clinical pulmonary involvement in primary Sjogren's syndrome: prevalence, quality of life and mortality–a retrospective study based on registry data. *Rheumatology.* (2013) 52:173–9. doi: 10.1093/rheumatology/kes311

64. Luppi F, Sebastiani M, Silva M, Sverzellati N, Cavazza A, Salvarani C, et al. Interstitial lung disease in Sjögren's syndrome: a clinical review. *Clin Exp Rheumatol.* (2020) 38(Suppl. 126):291–300.

65. Wang Y, Hou Z, Qiu M, Ye Q. Risk factors for primary Sjögren syndrome-associated interstitial lung disease. *J Thorac Dis.* (2018) 10:2108–17. doi: 10.21037/jtd.2018.03.120

66. Buvry C, Cassagnes L, Tekath M, Artigues M, Pereira B, Rieu V, et al. Anti-Ro52 antibodies are a risk factor for interstitial lung disease in primary Sjögren syndrome. *Respir Med.* (2020) 163:105895. doi: 10.1016/j.rmed.2020.105895

67. Chen M, Chen C, Chou H, Chen M, Tsai C, Chang D. Rituximab therapy in primary Sjögren's syndrome with interstitial lung disease: a retrospective cohort study. *Clin Exp Rheumatol.* (2016) 34:1077–84.

68. van Vollenhoven R, Furie R, Morand E, Tummala R, Maho E, Lindholm C. POS0709 SUSTAINED BICLA AND BILAG ORGAN DOMAIN RESPONSES IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) RECEIVING ANIFROLUMAB IN TWO PHASE 3 TRIALS. *Ann Rheum Dis.* (2022) 81(Suppl. 1):635–6.

69. Kaegi C, Steiner U, Wuest B, Crowley C, Boyman O. Systematic review of safety and efficacy of belimumab in treating immune-mediated disorders. *Allergy.* (2021) 76:2673–83. doi: 10.1111/all.14704

70. Dalavanga Y, Voulgari P, Georgiadis A, Leontaridi C, Katsenos S, Vassiliou M, et al. Lymphocytic alveolitis: a surprising index of poor prognosis in patients with primary Sjogren's syndrome. *Rheumatol Int.* (2006) 26:799–804. doi: 10.1007/s00296-005-0092-1

71. Weng L, Chen Y, Liang T, Lin Y, Liu D, Yu C, et al. Biomarkers of interstitial lung disease associated with primary Sjögren's syndrome. *Eur J Med Res.* (2022) 27:199. doi: 10.1186/s40001-022-00828-3

72. Kim Y, Choe J, Moon S, Song J. Blood KL-6 predicts prognosis in primary Sjögren's syndrome-associated interstitial lung disease. *Sci Rep.* (2022) 12:5343. doi: 10.1038/s41598-022-09283-w

73. Chiu Y, Lu C, Liu F, Tang S, Chu S, Kuo S, et al. FRI0228 KL-6 AS A BIOMARKER OF DEVELOPING INTERSTITIAL LUNG DISEASE IN PATIENTS WITH SJÖGREN SYNDROME. *Ann Rheum Dis.* (2019) 78:793.

74. Cottin V, Wollin L, Fischer A, Quaresma M, Stowasser S, Harari S. Fibrosing interstitial lung diseases: knowns and unknowns. *Eur Respir Rev.* (2019) 28:180100. doi: 10.1183/16000617.0100-2018

75. Lee D, Weinblatt M. Rheumatoid arthritis. *Lancet.* (2001) 358:903–11. doi: 10.1016/S0140-673606075-5

76. McInnes I, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med.* (2011) 365:2205–19. doi: 10.1056/NEJMra1004965

77. Horta-Baas G, Romero-Figueroa M, Montiel-Jarquín A, Pizano-Zárate M, García-Mena J, Ramírez-Durán N. Intestinal dysbiosis and rheumatoid arthritis: a link between gut microbiota and the pathogenesis of rheumatoid arthritis. *J Immunol Res.* (2017) 2017:4835189. doi: 10.1155/2017/4835189

78. Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. *Arthritis Rheum.* (2004) 50:3085–92. doi: 10.1002/art.20553

79. Stolt P, Yahya A, Bengtsson C, Källberg H, Rönnelid J, Lundberg I, et al. Silica exposure among male current smokers is associated with a high risk of developing ACPA-positive rheumatoid arthritis. *Ann Rheum Dis.* (2010) 69:1072–6. doi: 10.1136/ard.2009.114694

80. Too C, Muhamad N, Ilar A, Padyukov L, Alfredsson L, Klareskog L, et al. Occupational exposure to textile dust increases the risk of rheumatoid arthritis: results from a Malaysian population-based case-control study. *Ann Rheum Dis.* (2016) 75:997–1002. doi: 10.1136/annrheumdis-2015-208278

81. Malmström V, Catrina A, Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. *Nat Rev Immunol.* (2017) 17:60–75. doi: 10.1038/nri.2016.124

82. Reynisdottir G, Karimi R, Joshua V, Olsen H, Hensvold A, Harju A, et al. Structural changes and antibody enrichment in the lungs are early features of anti-citrullinated protein antibody-positive rheumatoid arthritis. *Arthritis Rheumatol.* (2014) 66:31–9. doi: 10.1002/art.38201

83. Reynisdottir G, Olsen H, Joshua V, Engström M, Forsslund H, Karimi R, et al. Signs of immune activation and local inflammation are present in the bronchial tissue of patients with untreated early rheumatoid arthritis. *Ann Rheum Dis.* (2016) 75:1722–7. doi: 10.1136/annrheumdis-2015-208216

84. Demoruelle M, Weisman M, Simonian P, Lynch D, Sachs P, Pedraza I, et al. Brief report: airways abnormalities and rheumatoid arthritis-related autoantibodies in subjects without arthritis: early injury or initiating site of autoimmunity? *Arthritis Rheum.* (2012) 64:1756–61. doi: 10.1002/art.34344

85. Olson A, Swigris J, Sprunger D, Fischer A, Fernandez-Perez E, Solomon J, et al. Rheumatoid arthritis-interstitial lung disease-associated mortality. *Am J Respir Crit Care Med.* (2011) 183:372–8. doi: 10.1164/rccm.201004-0622OC

86. Robles-Pérez A, Luburich P, Bolivar S, Dorca J, Nolla J, Molina-Molina M, et al. A prospective study of lung disease in a cohort of early rheumatoid arthritis patients. *Sci Rep.* (2020) 10:15640. doi: 10.1038/s41598-020-72768-z

87. Gizinski A, Mascolo M, Loucks J, Kervitsky A, Meehan R, Brown K, et al. Rheumatoid arthritis (RA)-specific autoantibodies in patients with interstitial lung disease and absence of clinically apparent articular RA. *Clin Rheumatol.* (2009) 28:611–3. doi: 10.1007/s10067-009-1128-9

88. Bendstrup E, Møller J, Kronborg-White S, Prior T, Hyldgaard C. Interstitial Lung Disease in Rheumatoid Arthritis Remains a Challenge for Clinicians. *J Clin Med.* (2019) 8:2038. doi: 10.3390/jcm8122038

89. Hyldgaard C, Hilberg O, Pedersen A, Ulrichsen S, Løkke A, Bendstrup E, et al. A population-based cohort study of rheumatoid arthritis-associated interstitial lung disease: comorbidity and mortality. *Ann Rheum Dis.* (2017) 76:1700–6. doi: 10.1136/annrheumdis-2017-211138

90. Dai Y, Wang W, Yu Y, Hu S. Rheumatoid arthritis-associated interstitial lung disease: an overview of epidemiology, pathogenesis and management. *Clin Rheumatol.* (2021) 40:1211–20. doi: 10.1007/s10067-020-05320-z

91. Cassone G, Manfredi A, Vacchi C, Luppi F, Coppi F, Salvarani C, et al. Treatment of rheumatoid arthritis-associated interstitial lung disease: lights and shadows. *J Clin Med.* (2020) 9:1082. doi: 10.3390/jcm9041082

92. Md Yusof M, Kabia A, Darby M, Lettieri G, Beirne P, Vital E, et al. Effect of rituximab on the progression of rheumatoid arthritis-related interstitial lung disease: 10 years' experience at a single centre. *Rheumatology.* (2017) 56:1348–57. doi: 10.1093/rheumatology/kex072

93. Kaul A, Gordon C, Crow M, Touma Z, Urowitz M, van Vollenhoven R, et al. Systemic lupus erythematosus. *Nat Rev Dis Primers.* (2016) 2:16039. doi: 10.1038/nrdp.2016.39

94. Shin J, Lee K, Park S, Yang J, Kim H, Song K, et al. Systemic lupus erythematosus and lung involvement: a comprehensive review. *J Clin Med.* (2022) 11:6714. doi: 10.3390/jcm11226714

95. Kamen D, Strange C. Pulmonary manifestations of systemic lupus erythematosus. *Clin Chest Med.* (2010) 31:479–88. doi: 10.1016/j.ccm.2010.05.001

96. Memet B, Ginzler E. Pulmonary manifestations of systemic lupus erythematosus. *Semin Respir Crit Care Med.* (2007) 28:441–50. doi: 10.1055/s-2007-985665

97. Torre O, Harari S. Pleural and pulmonary involvement in systemic lupus erythematosus. *Presse Med.* (2011) 40:e19–29. doi: 10.1016/j.lpm.2010.11.004

98. Hannah J, D'Cruz D. Pulmonary Complications of Systemic Lupus Erythematosus. *Semin Respir Crit Care Med.* (2019) 40:227–34. doi: 10.1055/s-0039-1685537

99. Castelino F, Varga J. Interstitial lung disease in connective tissue diseases: evolving concepts of pathogenesis and management. *Arthritis Res Ther.* (2010) 12:213. doi: 10.1186/ar3097

100. Okada M, Suzuki K, Matsumoto M, Nakashima M, Nakanishi T, Takada K, et al. Intermittent intravenous cyclophosphamide pulse therapy for the treatment of active interstitial lung disease associated with collagen vascular diseases. *Mod Rheumatol.* (2007) 17:131–6. doi: 10.1007/s10165-007-0554-2

101. Hussain A, Desai D, Khandwala P, Desai D. POS1413 INTERSTITIAL LUNG DISEASE(ILD) PREVALENCE AND TRENDS IN SYSTEMIC LUPUS ERYTHEMATOSUS(SLE), RHEUMATOID ARTHRITIS(RA), SCLERODERMA(SCL), MYOSITIS AND MIXED CONNECTIVE TISSUE DISEASES(MCTD) ALONG WITH COMPARISON OF LENGTH OF HOSPITALIZATION STAY(LOS), COST AND RACIAL PREDISPOSITION AMONG THESE PATIENTS. *Ann Rheum Dis.* (2022) 81:20–33.

102. Cheema G, Quismorio F. Interstitial lung disease in systemic lupus erythematosus. *Curr Opin Pulm Med.* (2000) 6:424–9. doi: 10.1097/00063198-200009000-00007

103. Jacobsen S, Petersen J, Ullman S, Junker P, Voss A, Rasmussen J, et al. A multicentre study of 513 Danish patients with systemic lupus erythematosus. I. Disease manifestations and analyses of clinical subsets. *Clin Rheumatol.* (1998) 17:468–77. doi: 10.1007/BF01451282

104. Rekvig O, Putterman C, Casu C, Gao H, Ghirardello A, Mortensen E, et al. Autoantibodies in lupus: culprits or passive bystanders? *Autoimmun Rev.* (2012) 11:596–603. doi: 10.1016/j.autrev.2011.10.021

105. Cojocaru M, Cojocaru I, Silosi I, Vrabie C. Manifestations of systemic lupus erythematosus. *Maedica.* (2011) 6:330–6. doi: 10.1016/j.oftale.2021.12.001

106. Yee C, Hussein H, Skan J, Bowman S, Situnayake D, Gordon C. Association of damage with autoantibody profile, age, race, sex and disease duration in systemic lupus erythematosus. *Rheumatology.* (2003) 42:276–9. doi: 10.1093/rheumatology/keg078

107. Yu H, Nagafuchi Y, Fujio K. Clinical and Immunological Biomarkers for Systemic Lupus Erythematosus. *Biomolecules.* (2021) 11:928. doi: 10.3390/biom11070928

108. Vivero M, Padera R. Histopathology of lung disease in the connective tissue diseases. *Rheum Dis Clin North Am.* (2015) 41:197–211. doi: 10.1016/j.rdc.2014.12.002

109. Chen Y, Wang Y, Chen X, Liang H, Yang X. Association of Interstitial Lung Disease With Clinical Characteristics of Chinese Patients With Systemic Lupus Erythematosus. *Arch Rheumatol.* (2020) 35:239–46. doi: 10.46497/ArchRheumatol.2020.7583

110. Lim S, Gillis D, Smith W, Hissaria P, Greville H, Peh C. Rituximab use in systemic lupus erythematosus pneumonitis and a review of current reports. *Intern Med J.* (2006) 36:260–2. doi: 10.1111/j.1445-5994.2006.01055.x

111. Disler R, Gallagher R, Davidson P, Sun S, Chen L, Zhou M, et al. Factors impairing the postural balance in COPD patients and its influence upon activities of daily living. *Eur Respir J.* (2019) 15:142–8.

112. Swigris J, Olson A, Fischer A, Lynch D, Cosgrove G, Frankel S, et al. Mycophenolate mofetil is safe, well tolerated, and preserves lung function in patients with connective tissue disease-related interstitial lung disease. *Chest.* (2006) 130:30–6. doi: 10.1378/chest.130.1.30

113. Mwangi J, Litteken C, Gorthi R, Attoti Y, Atluri R. Belimumab in the treatment of connective tissue disease-associated interstitial lung disease: case report and literature review. *Cureus.* (2021) 13:e19218. doi: 10.7759/cureus.19218

114. Hervier B, Uzunhan Y. Inflammatory myopathy-related interstitial lung disease: from pathophysiology to treatment. *Front Med.* (2020) 6:326. doi: 10.3389/fmed.2019.00326

115. Greenberg S. Type 1 interferons and myositis. *Arthritis Res Ther.* (2010) 12 Suppl 1:S4. doi: 10.1186/ar2885

116. Greenberg S. Dermatomyositis and type 1 interferons. *Curr Rheumatol Rep.* (2010) 12:198–203. doi: 10.1007/s11926-010-0101-6

117. Greenberg S. Proposed immunologic models of the inflammatory myopathies and potential therapeutic implications. *Neurology.* (2007) 69:2008–19. doi: 10.1212/01.WNL.0000291619.17160.b8

118. Dalakas M, Hohlfeld R. Polymyositis and dermatomyositis. *Lancet.* (2003) 362:971–82. doi: 10.1016/S0140-673614368-1

119. Dalakas M. Inflammatory disorders of muscle: progress in polymyositis, dermatomyositis and inclusion body myositis. *Curr Opin Neurol.* (2004) 17:561–7. doi: 10.1097/00019052-200410000-00006

120. Rodero M, Crow Y. Type I interferon-mediated monogenic autoinflammation: The type I interferonopathies, a conceptual overview. *J Exp Med.* (2016) 213:2527–38. doi: 10.1084/jem.20161596

121. Prasse A, Pechkovsky D, Toews G, Jungraithmayr W, Kollert F, Goldmann T, et al. A vicious circle of alveolar macrophages and fibroblasts perpetuates pulmonary fibrosis via CCL18. *Am J Respir Crit Care Med.* (2006) 173:781–92. doi: 10.1164/rccm.200509-1518OC

122. Zhang L, Wang Y, Wu G, Xiong W, Gu W, Wang C. Macrophages: friend or foe in idiopathic pulmonary fibrosis? *Respir Res.* (2018) 19:170. doi: 10.1186/s12931-018-0864-2

123. Bendewald M, Wetter D, Li X, Davis M. Incidence of dermatomyositis and clinically amyopathic dermatomyositis: a population-based study in Olmsted County, Minnesota. *Arch Dermatol.* (2010) 146:26–30. doi: 10.1001/archdermatol.2009.328

124. Dugan E, Huber A, Miller F, Rider L. Review of the classification and assessment of the cutaneous manifestations of the idiopathic inflammatory myopathies. *Dermatol Online J.* (2009) 15:2.

125. Fathi M, Dastmalchi M, Rasmussen E, Lundberg I, Tornling G. Interstitial lung disease, a common manifestation of newly diagnosed polymyositis and dermatomyositis. *Ann Rheum Dis.* (2004) 63:297–301. doi: 10.1136/ard.2003.006122

126. Ye S, Chen X, Lu X, Wu M, Deng Y, Huang W, et al. Adult clinically amyopathic dermatomyositis with rapid progressive interstitial lung disease: a retrospective cohort study. *Clin Rheumatol.* (2007) 26:1647–54. doi: 10.1007/s10067-007-0562-9

127. Hallowell R, Paik J. Myositis-associated interstitial lung disease: a comprehensive approach to diagnosis and management. *Clin Exp Rheumatol.* (2022) 40:373–83. doi: 10.55563/clinexprheumatol/brvl1v

128. Karampitsakos T, Tzilas V, Papaioannou O, Chrysikos S, Vasarmidi E, Juge P, et al. Clinical features and outcomes of patients with myositis associated-interstitial lung disease. *Front Med.* (2023) 9:1096203. doi: 10.3389/fmed.2022.1096203

129. Richards T, Eggebeen A, Gibson K, Yousem S, Fuhrman C, Gochuico B, et al. Characterization and peripheral blood biomarker assessment of anti-Jo-1 antibody-positive interstitial lung disease. *Arthritis Rheum.* (2009) 60:2183–92. doi: 10.1002/art.24631

130. Moghadam-Kia S, Oddis C, Sato S, Kuwana M, Aggarwal R. Antimelanoma Differentiation-associated Gene 5 Antibody: Expanding the Clinical Spectrum in North American Patients with Dermatomyositis. *J Rheumatol.* (2017) 44:319–25. doi: 10.3899/jrheum.160682

131. Chen L, Somers A, Liu J. ANTISYNTHETASE SYNDROME AND PLEURAL EFFUSION: A CASE REPORT. *Chest.* (2020) 158:A1991–2. doi: 10.2169/internalmedicine.0302-17

132. Hayashi S, Tanaka M, Kobayashi H, Nakazono T, Satoh T, Fukuno Y, et al. High-resolution computed tomography characterization of interstitial lung diseases in polymyositis/dermatomyositis. *J Rheumatol.* (2008) 35:260–9.

133. Wallaert B, Rossi G, Sibille Y. Clinical guidelines and indications for bronchoalveolar lavage (BAL): collagen-vascular diseases. *Eur Respir J.* (1990) 3:961–9.

134. Sambataro D, Sambataro G, Pignataro F, Zanframundo G, Codullo V, Fagone E, et al. Patients with Interstitial Lung Disease Secondary to Autoimmune Diseases: How to Recognize Them? *Diagnostics.* (2020) 10:208. doi: 10.3390/diagnostics10040208

135. Jablonski R, Bhorade S, Strek M, Dematte J. Recognition and Management of Myositis-Associated Rapidly Progressive Interstitial Lung Disease. *Chest.* (2020) 158:252–63. doi: 10.1016/j.chest.2020.01.033

136. Yamakawa H, Hagiwara E, Kitamura H, Iwasawa T, Otoshi R, Aiko N, et al. Predictive Factors for the Long-Term Deterioration of Pulmonary Function in Interstitial Lung Disease Associated with Anti-Aminoacyl-tRNA Synthetase Antibodies. *Respiration.* (2018) 96:210–21. doi: 10.1159/000488358

137. Sharp G, Irvin W, Tan E, Gould R, Holman H. Mixed connective tissue disease–an apparently distinct rheumatic disease syndrome associated with a specific antibody to an extractable nuclear antigen (ENA). *Am J Med.* (1972) 52:148–59. doi: 10.1016/0002-934390064-2

138. Cappelli S, Bellando Randone S, Martinoviæ D, Tamas M, Pasaliæ K, Allanore Y, et al. To be or not to be, ten years after: evidence for mixed connective tissue disease as a distinct entity. *Semin Arthritis Rheum.* (2012) 41:589–98. doi: 10.1016/j.semarthrit.2011.07.010

139. Gunnarsson R, El-Hage F, Aaløkken T, Reiseter S, Lund M, Garen T, et al. Associations between anti-Ro52 antibodies and lung fibrosis in mixed connective tissue disease. *Rheumatology.* (2016) 55:103–8. doi: 10.1093/rheumatology/kev300

140. Alves M, Isenberg D. Mixed connective tissue disease: a condition in search of an identity. *Clin Exp Med.* (2020) 20:159–66. doi: 10.1007/s10238-020-00606-7

141. Maldonado M, Perez M, Pignac-Kobinger J, Marx E, Tozman E, Greidinger E, et al. Clinical and immunologic manifestations of mixed connective tissue disease in a

Miami population compared to a Midwestern US Caucasian population. *J Rheumatol.* (2008) 35:429–37.

142. Sen S, Sinhamahapatra P, Choudhury S, Gangopadhyay A, Bala S, Sircar G, et al. Cutaneous manifestations of mixed connective tissue disease: study from a tertiary care hospital in eastern India. *Indian J Dermatol.* (2014) 59:35–40. doi: 10.4103/0019-5154.123491

143. Kitridou R, Akmal M, Turkel S, Ehresmann G, Quismorio F, Massry S. Renal involvement in mixed connective tissue disease: a longitudinal clinicopathologic study. *Semin Arthritis Rheum.* (1986) 16:135–45. doi: 10.1016/0049-017290047-8

144. Bull T, Fagan K, Badesch D. Pulmonary vascular manifestations of mixed connective tissue disease. *Rheum Dis Clin North Am.* (2005) 31:451–64.

145. Kozuka T, Johkoh T, Honda O, Mihara N, Koyama M, Tomiyama N, et al. Pulmonary involvement in mixed connective tissue disease: high-resolution CT findings in 41 patients. *J Thorac Imaging.* (2001) 16:94–8. doi: 10.1097/00005382-200104000-00005

146. Barnett J, Devaraj A. Computed Tomographic Imaging in Connective Tissue Diseases. *Semin Respir Crit Care Med.* (2019) 40:159–72. doi: 10.1055/s-0039-1685165

147. Bodolay E, Szekanecz Z, Dévényi K, Galuska L, Csípo I, Vègh J, et al. Evaluation of interstitial lung disease in mixed connective tissue disease (MCTD). *Rheumatology.* (2005) 44:656–61. doi: 10.1093/rheumatology/keh575

148. Kadura S, Raghu G. Antineutrophil cytoplasmic antibody-associated interstitial lung disease: a review. *Eur Respir Rev.* (2021) 30:210123. doi: 10.1183/16000617.0123-2021

149. Ha J, Pyo J, Ahn S, Song J, Park Y, Lee S. Incidence and Patterns of Interstitial Lung Disease and Their Clinical Impact on Mortality in Patients with Antineutrophil Cytoplasmic Antibody-Associated Vasculitis: Korean Single-Centre Observational Study. *J Immunol Res.* (2022) 2022:2499404. doi: 10.1155/2022/2499404

150. Namba N, Kawasaki A, Sada K, Hirano F, Kobayashi S, Yamada H, et al. Association of MUC5B promoter polymorphism with interstitial lung disease in myeloperoxidase-antineutrophil cytoplasmic antibody-associated vasculitis. *Ann Rheum Dis.* (2019) 78:1144–6. doi: 10.1136/annrheumdis-2018-214263

151. Ciavatta D, Yang J, Preston G, Badhwar A, Xiao H, Hewins P, et al. Epigenetic basis for aberrant upregulation of autoantigen genes in humans with ANCA vasculitis. *J Clin Invest.* (2010) 120:3209–19. doi: 10.1172/JCI40034

152. Yang J, Pendergraft W, Alcorta D, Nachman P, Hogan S, Thomas R, et al. Circumvention of normal constraints on granule protein gene expression in peripheral blood neutrophils and monocytes of patients with antineutrophil cytoplasmic autoantibody-associated glomerulonephritis. *J Am Soc Nephrol.* (2004) 15:2103–14. doi: 10.1097/01.ASN.0000135058.46193.72

153. Kagiyama N, Takayanagi N, Kanauchi T, Ishiguro T, Yanagisawa T, Sugita Y. Antineutrophil cytoplasmic antibody-positive conversion and microscopic polyangiitis development in patients with idiopathic pulmonary fibrosis. *BMJ Open Respir Res.* (2015) 2:e000058. doi: 10.1136/bmjresp-2014-000058

154. Guilpain P, Chéreau C, Goulvestre C, Servettaz A, Montani D, Tamas N, et al. The oxidation induced by antimyeloperoxidase antibodies triggers fibrosis in microscopic polyangiitis. *Eur Respir J.* (2011) 37:1503–13. doi: 10.1183/09031936.00148409

155. Tanaka T, Otani K, Egashira R, Kashima Y, Taniguchi H, Kondoh Y, et al. Interstitial pneumonia associated with MPO-ANCA: clinicopathological features of nine patients. *Respir Med.* (2012) 106:1765–70. doi: 10.1016/j.rmed.2012.08.024

156. Baqir M, Yi E, Colby T, Cox C, Ryu J, Specks U. Radiologic and pathologic characteristics of myeloperoxidase-antineutrophil cytoplasmic antibody-associated interstitial lung disease: a retrospective analysis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2019) 36:195–201. doi: 10.36141/svdld.v36i3.8053

157. Kitching A, Anders H, Basu N, Brouwer E, Gordon J, Jayne D, et al. ANCA-associated vasculitis. *Nat Rev Dis Primers.* (2020) 6:71. doi: 10.1038/s41572-020-0204-y

158. Bell P, Sheehy R, Droney L, Prain K, Wong R, Keir G. Pulmonary involvement in Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis: a single centre case series. *Respirol Case Rep.* (2022) 10:e01058. doi: 10.1002/rcr2.1058

159. Liu G, Ventura I, Achtar-Zadeh N, Elicker B, Jones K, Wolters P, et al. Prevalence and Clinical Significance of Antineutrophil Cytoplasmic Antibodies in North American Patients With Idiopathic Pulmonary Fibrosis. *Chest.* (2019) 156:715–23. doi: 10.1016/j.chest.2019.05.014

160. Hozumi H, Enomoto N, Oyama Y, Kono M, Fujisawa T, Inui N, et al. Clinical Implication of Proteinase-3-antineutrophil Cytoplasmic Antibody in Patients with Idiopathic Interstitial Pneumonias. *Lung.* (2016) 194:235–42. doi: 10.1007/s00408-016-9851-x

161. Yamagata M, Ikeda K, Tsushima K, Iesato K, Abe M, Ito T, et al. Prevalence and Responsiveness to Treatment of Lung Abnormalities on Chest Computed Tomography in Patients With Microscopic Polyangiitis: a Multicenter, Longitudinal, Retrospective Study of One Hundred Fifty Consecutive Hospital-Based Japanese Patients. *Arthritis Rheumatol.* (2016) 68:713–23. doi: 10.1002/art.39475

162. Suzuki A, Sakamoto S, Kurosaki A, Kurihara Y, Satoh K, Usui Y, et al. Chest High-Resolution CT Findings of Microscopic Polyangiitis: a Japanese First Nationwide Prospective Cohort Study. *AJR Am J Roentgenol.* (2019) 213:104–14. doi: 10.2214/AJR.18.20967

163. Kwon M, Lee A, Mira-Avendano I, Rojas C, Grage R, Abril A. Interstitial Lung Disease in Antineutrophil Cytoplasmic Antibody-Associated Vasculitis Patients: comparison With Idiopathic Pulmonary Fibrosis. *J Clin Rheumatol.* (2021) 27:324–30. doi: 10.1097/RHU.0000000000001357

164. Comarmond C, Crestani B, Tazi A, Hervier B, Adam-Marchand S, Nunes H, et al. Pulmonary fibrosis in antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis: a series of 49 patients and review of the literature. *Medicine.* (2014) 93:340–9. doi: 10.1097/MD.0000000000000217

165. Juman S, Oldroyd A, Chaudhuri N, Haque S. AB0593 INTERSTITIAL LUNG DISEASE ASSOCIATED WITH ANCA POSITIVITY: A RETROSPECTIVE ANALYSIS. *Ann Rheum Dis.* (2019) 78(Suppl 2):1757–8.

166. Maillet T, Goletto T, Beltramo G, Dupuy H, Jouneau S, Borie R, et al. Usual interstitial pneumonia in ANCA-associated vasculitis: A poor prognostic factor. *J Autoimmun.* (2020) 106:102338. doi: 10.1016/j.jaut.2019.102338

167. Yamakawa H, Toyoda Y, Baba T, Kishaba T, Fukuda T, Takemura T, et al. Anti-Inflammatory and/or Anti-Fibrotic Treatment of MPO-ANCA-Positive Interstitial Lung Disease: A Short Review. *J Clin Med.* (2022) 11:3835. doi: 10.3390/jcm11133835

168. Sepriano A, Ramiro S, van der Heijde D, van Gaalen F, Hoonhout P, Molto A, et al. What is axial spondyloarthritis? A latent class and transition analysis in the SPACE and DESIR cohorts. *Ann Rheum Dis.* (2020) 79:324–31. doi: 10.1136/annrheumdis-2019-216516

169. Taurog J, Chhabra A, Colbert R. Ankylosing Spondylitis and Axial Spondyloarthritis. *N Engl J Med.* (2016) 374:2563–74. doi: 10.1056/NEJMra1406182

170. Lautermann D, Braun J. Ankylosing spondylitis–cardiac manifestations. *Clin Exp Rheumatol.* (2002) 20(6 Suppl 28):S11–5.

171. Kanathur N, Lee-Chiong T. Pulmonary manifestations of ankylosing spondylitis. *Clin Chest Med.* (2010) 31:547–54. doi: 10.1016/j.ccm.2010.05.002

172. Bowness P. HLA-B27. *Annu Rev Immunol.* (2015) 33:29–48. doi: 10.1146/annurev-immunol-032414-112110

173. Ranganathan V, Gracey E, Brown M, Inman R, Haroon N. Pathogenesis of ankylosing spondylitis - recent advances and future directions. *Nat Rev Rheumatol.* (2017) 13:359–67. doi: 10.1038/nrrheum.2017.56

174. Shamji M, Bafaquh M, Tsai E. The pathogenesis of ankylosing spondylitis. *Neurosurg Focus.* (2008) 24:E3. doi: 10.3171/FOC/2008/24/1/E3

175. Quismorio F. Pulmonary involvement in ankylosing spondylitis. *Curr Opin Pulm Med.* (2006) 12:342–5. doi: 10.1097/01.mcp.0000239551.47702.f4

176. Sieper J, Rudwaleit M, Baraliakos X, Brandt J, Braun J, Burgos-Vargas R, et al. The Assessment of SpondyloArthritis international Society (ASAS) handbook: a guide to assess spondyloarthritis. *Ann Rheum Dis.* (2009) 68(Suppl 2):ii1–44. doi: 10.1136/ard.2008.104018

177. Gupta S, Johnston W. Apical pulmonary disease in ankylosing spondylitis. *N Z Med J.* (1978) 88:186–8.

178. Rumancik W, Firooznia H, Davis M, Leitman B, Golimbu C, Rafii M, et al. Fibrobullous disease of the upper lobes: an extraskeletal manifestation of ankylosing spondylitis. *J Comput Tomogr.* (1984) 8:225–9. doi: 10.1016/0149-936x90066-3

179. Mercieca C, van der Horst-Bruinsma I, Borg A. Pulmonary, renal and neurological comorbidities in patients with ankylosing spondylitis; implications for clinical practice. *Curr Rheumatol Rep.* (2014) 16:434. doi: 10.1007/s11926-014-0434-7

180. Kiris A, Ozgocmen S, Kocakoc E, Ardicoglu O, Ogur E. Lung findings on high resolution CT in early ankylosing spondylitis. *Eur J Radiol.* (2003) 47:71–6. doi: 10.1016/s0720-048x00085-2

181. Boushea D, Sundstrom W. The pleuropulmonary manifestations of ankylosing spondylitis. *Semin Arthritis Rheum.* (1989) 18:277–81. doi: 10.1016/0049-017290049-8

182. Wiedemann H, Matthay R. Pulmonary manifestations of the collagen vascular diseases. *Clin Chest Med.* (1989) 10:677–722.

183. Turner J, Enzenauer R. Bronchiolitis obliterans and organizing pneumonia associated with ankylosing spondylitis. *Arthritis Rheum.* (1994) 37:1557–9. doi: 10.1002/art.1780371025

184. Wolson A, Rohwedder J. Upper lobe fibrosis in ankylosing spondylitis. *Am J Roentgenol Radium Ther Nucl Med.* (1975) 124:466–71. doi: 10.2214/ajr.124.3.466

185. Braun J, Brandt J, Listing J, Zink A, Alten R, Golder W, et al. Treatment of active ankylosing spondylitis with infliximab: a randomised controlled multicentre trial. *Lancet.* (2002) 359:1187–93. doi: 10.1016/s0140-673608215-6

186. Davis J, Van Der Heijde D, Braun J, Dougados M, Cush J, Clegg D, et al. Recombinant human tumor necrosis factor receptor (etanercept) for treating ankylosing spondylitis: a randomized, controlled trial. *Arthritis Rheum.* (2003) 48:3230–6. doi: 10.1002/art.11325

187. Azadeh N, Limper A, Carmona E, Ryu J. The Role of Infection in Interstitial Lung Diseases: A Review. *Chest.* (2017) 152:842–52. doi: 10.1016/j.chest.2017.03.033

188. Huang W, Tang X. Virus infection induced pulmonary fibrosis. *J Transl Med.* (2021) 19:496. doi: 10.1186/s12967-021-03159-9

189. Jolly L, Stavrou A, Vanderstoken G, Meliopoulos V, Habgood A, Tatler A, et al. Influenza promotes collagen deposition via αvβ6 integrin-mediated transforming growth factor β activation. *J Biol Chem.* (2014) 289:35246–63. doi: 10.1074/jbc.M114.582262

190. Shatskaya E, Kovner A, Potapova O, Cherdantseva L, Shkurupy V, Shestopalov A. Study of SMAD-Dependent Signal Pathway in the Development of Early Pulmonary Fibrosis in Mice Infected with Influenza A/H1N1 Virus. *Bull Exp Biol Med.* (2017) 162:647–9. doi: 10.1007/s10517-017-3678-1

191. Malizia A, Keating D, Smith S, Walls D, Doran P, Egan J. Alveolar epithelial cell injury with Epstein-Barr virus upregulates TGFbeta1 expression. *Am J Physiol Lung Cell Mol Physiol.* (2008) 295:L451–60. doi: 10.1152/ajplung.00376.2007

192. Mora A, Woods C, Garcia A, Xu J, Rojas M, Speck S, et al. Lung infection with gamma-herpesvirus induces progressive pulmonary fibrosis in Th2-biased mice. *Am J Physiol Lung Cell Mol Physiol.* (2005) 289:L711–21. doi: 10.1152/ajplung.00007.2005

193. Cook C, Zhang Y, Sedmak D, Martin L, Jewell S, Ferguson R. Pulmonary cytomegalovirus reactivation causes pathology in immunocompetent mice. *Crit Care Med.* (2006) 34:842–9. doi: 10.1097/01.ccm.0000201876.11059.05

194. Sheng G, Chen P, Wei Y, Yue H, Chu J, Zhao J, et al. Viral Infection Increases the Risk of Idiopathic Pulmonary Fibrosis: a Meta-Analysis. *Chest.* (2020) 157:1175–87. doi: 10.1016/j.chest.2019.10.032

195. Molyneaux P, Willis-Owen S, Cox M, James P, Cowman S, Loebinger M, et al. Host-Microbial Interactions in Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med.* (2017) 195:1640–50. doi: 10.1164/rccm.201607-1408OC

196. Vidal S, de la Horra C, Martín J, Montes-Cano M, Rodríguez E, Respaldiza N. Pneumocystis jirovecii colonisation in patients with interstitial lung disease. *Clin Microbiol Infect.* (2006) 12:231–5. doi: 10.1111/j.1469-0691.2005.01337.x

197. Ambardar S, Hightower S, Huprikar N, Chung K, Singhal A, Collen J. Post-COVID-19 Pulmonary Fibrosis: novel Sequelae of the Current Pandemic. *J Clin Med.* (2021) 10:2452. doi: 10.3390/jcm10112452

198. Lazar M, Barbu E, Chitu C, Tiliscan C, Stratan L, Arama S, et al. Interstitial Lung Fibrosis Following COVID-19 Pneumonia. *Diagnostics.* (2022) 12:2028. doi: 10.3390/diagnostics12082028

199. Kim J, Yang Y, Jeong Y, Jang Y. Middle East Respiratory Syndrome-Coronavirus Infection into Established hDPP4-Transgenic Mice Accelerates Lung Damage Via Activation of the Pro-Inflammatory Response and Pulmonary Fibrosis. *J Microbiol Biotechnol.* (2020) 30:427–38. doi: 10.4014/jmb.1910.10055

200. Valenzuela C, Waterer G, Raghu G. Interstitial lung disease before and after COVID-19: a double threat? *Eur Respir J.* (2021) 58:2101956. doi: 10.1183/13993003.01956-2021

201. Uemasu K, Yasuda Y, Hirayama Y, Arasawa S, Iwashima D, Takahashi K. Post-COVID-19 interstitial lung disease presenting with profound hypoxemia: Report of three cases demonstrating a good response to high-dose corticosteroid therapy. *J Infect Chemother.* (2022) 28:321–5. doi: 10.1016/j.jiac.2021.11.010

202. Ravaglia C, Doglioni C, Chilosi M, Piciucchi S, Dubini A, Rossi G, et al. Clinical, radiological and pathological findings in patients with persistent lung disease following SARS-CoV-2 infection. *Eur Respir J.* (2022) 60:2102411. doi: 10.1183/13993003.02411-2021

203. Cottin V, Lafitte C, Sénéchal A, Traclet J. Interstitial Lung Disease after COVID-19. *Am J Respir Crit Care Med.* (2021) 203:1314–5. doi: 10.1164/rccm.202006-2466IM

204. Al-Jahdhami I, Al-Mawali A, Bennji S. Respiratory Complications after COVID-19. *Oman Med J.* (2022) 37:e343. doi: 10.5001/omj.2022.52

205. Heukels P, Moor C, von der Thüsen J, Wijsenbeek M, Kool M. Inflammation and immunity in IPF pathogenesis and treatment. *Respir Med.* (2019) 147:79–91. doi: 10.1016/j.rmed.2018.12.015

206. Ntatsoulis K, Karampitsakos T, Tsitoura E, Stylianaki E, Matralis A, Tzouvelekis A, et al. Commonalities Between ARDS, Pulmonary Fibrosis and COVID-19: the Potential of Autotaxin as a Therapeutic Target. *Front Immunol.* (2021) 12:687397. doi: 10.3389/fimmu.2021.687397

207. Galal I, Hussein A, Amin M, Saad M, Zayan H, Abdelsayed M, et al. Determinants of persistent post-COVID-19 symptoms: value of a novel COVID-19 symptom score. *The Egypt J Bronchol.* (2021) 15:10.

208. Mehta P, Rosas I, Singer M. Understanding post-COVID-19 interstitial lung disease (ILD): a new fibroinflammatory disease entity. *Intensive Care Med.* (2022) 48:1803–6. doi: 10.1007/s00134-022-06877-w

209. Karampitsakos T, Sotiropoulou V, Katsaras M, Tsiri P, Georgakopoulou V, Papanikolaou I, et al. Post-COVID-19 interstitial lung disease: insights from a machine learning radiographic model. *Front Med.* (2023) 9:1083264. doi: 10.3389/fmed.2022.1083264

210. Skeoch S, Weatherley N, Swift A, Oldroyd A, Johns C, Hayton C, et al. Drug-Induced Interstitial Lung Disease: a Systematic Review. *J Clin Med.* (2018) 7:356. doi: 10.3390/jcm7100356

211. Roubille C, Haraoui B. Interstitial lung diseases induced or exacerbated by DMARDS and biologic agents in rheumatoid arthritis: a systematic literature review. *Semin Arthritis Rheum.* (2014) 43:613–26. doi: 10.1016/j.semarthrit.2013.09.005

212. Rose C, King T. Controversies in hypersensitivity pneumonitis. *Am Rev Respir Dis.* (1992) 145:1–2. doi: 10.1164/ajrccm/145.1.1

213. Costabel U, Miyazaki Y, Pardo A, Koschel D, Bonella F, Spagnolo P, et al. Hypersensitivity pneumonitis. *Nat Rev Dis Primers.* (2020) 6:65. doi: 10.1038/s41572-020-0191-z

214. Raghu G, Remy-Jardin M, Ryerson C, Myers J, Kreuter M, Vasakova M, et al. Diagnosis of Hypersensitivity Pneumonitis in Adults. An Official ATS/JRS/ALAT Clinical Practice Guideline. *Am J Respir Crit Care Med.* (2020) 202:e36–69. doi: 10.1164/rccm.202005-2032ST

215. Hanak V, Golbin J, Ryu J. Causes and presenting features in 85 consecutive patients with hypersensitivity pneumonitis. *Mayo Clin Proc.* (2007) 82:812–6. doi: 10.4065/82.7.812

216. Hamblin M, Prosch H, Vašáková M. Diagnosis, course and management of hypersensitivity pneumonitis. *Eur Respir Rev.* (2022) 31:210169. doi: 10.1183/16000617.0169-2021

217. Akira M. Uncommon pneumoconioses: CT and pathologic findings. *Radiology.* (1995) 197:403–9. doi: 10.1148/radiology.197.2.7480684

218. Choi J, Lee K, Chung M, Han J, Chung M, Park J. Giant cell interstitial pneumonia: high-resolution CT and pathologic findings in four adult patients. *AJR Am J Roentgenol.* (2005) 184:268–72. doi: 10.2214/ajr.184.1.01840268

219. Leung C, Yu I, Chen W. Silicosis. *Lancet.* (2012) 379:2008–18. doi: 10.1016/S0140-673660235-9

220. Vlahovich K, Sood A. A 2019 update on occupational lung diseases: a narrative review. *Pulm Ther.* (2021) 7:75–87. doi: 10.1007/s41030-020-00143-4

221. Fraig M, Shreesha U, Savici D, Katzenstein A. Respiratory bronchiolitis: a clinicopathologic study in current smokers, ex-smokers, and never-smokers. *Am J Surg Pathol.* (2002) 26:647–53. doi: 10.1097/00000478-200205000-00011

222. Portnoy J, Veraldi K, Schwarz M, Cool C, Curran-Everett D, Cherniack R, et al. Respiratory bronchiolitis-interstitial lung disease: long-term outcome. *Chest.* (2007) 131:664–71. doi: 10.1378/chest.06-1885

223. Holt R, Schmidt R, Godwin J, Raghu G. High resolution CT in respiratory bronchiolitis-associated interstitial lung disease. *J Comput Assist Tomogr.* (1993) 17:46–50. doi: 10.1097/00004728-199301000-00007

224. Caminati A, Cavazza A, Sverzellati N, Harari S. An integrated approach in the diagnosis of smoking-related interstitial lung diseases. *Eur Respir Rev.* (2012) 21:207–17. doi: 10.1183/09059180.00003112

225. Myers J, Veal C, Shin M, Katzenstein A. Respiratory bronchiolitis causing interstitial lung disease. a clinicopathologic study of six cases. *Am Rev Respir Dis.* (1987) 135:880–4. doi: 10.1164/arrd.1987.135.4.880

226. Arroyo-Hernández M, Maldonado F, Lozano-Ruiz F, Muñoz-Montaño W, Nuñez-Baez M, Arrieta O. Radiation-induced lung injury: current evidence. *BMC Pulm Med.* (2021) 21:9. doi: 10.1186/s12890-020-01376-4

227. Navaratnam V, Fleming K, West J, Smith C, Jenkins R, Fogarty A, et al. The rising incidence of idiopathic pulmonary fibrosis in the U.K. *Thorax.* (2011) 66:462–7. doi: 10.1136/thx.2010.148031

228. Katzenstein Anna-Luise A, Myers Jeffrey L. Idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* (1998) 157:1301–15.

229. Frangogiannis N. Transforming growth factor–ß in tissue fibrosis. *J Exp Med.* (2020) 217:e20190103.

230. Rao KB, Malathi N, Narashiman S, Rajan S. Evaluation of myofibroblasts by expression of alpha smooth muscle actin: a marker in fibrosis, dysplasia and carcinoma. *J Clin Diagn Res.* (2014) 8:ZC14–7. doi: 10.7860/JCDR/2014/7820.4231

231. Chuliá-Peris L, Carreres-Rey C, Gabasa M, Alcaraz J, Carretero J, Pereda J. Matrix Metalloproteinases and Their Inhibitors in Pulmonary Fibrosis: EMMPRIN/CD147 Comes into Play. *Int J Mol Sci.* (2022) 23:6894. doi: 10.3390/ijms23136894

232. Wick G, Backovic A, Rabensteiner E, Plank N, Schwentner C, Sgonc R. The immunology of fibrosis: innate and adaptive responses. *Trends Immunol.* (2010) 31:110–9. doi: 10.1016/j.it.2009.12.001

233. Seibold M, Wise A, Speer M, Steele M, Brown K, Loyd J, et al. A common MUC5B promoter polymorphism and pulmonary fibrosis. *N Engl J Med.* (2011) 364:1503–12. doi: 10.1056/NEJMoa1013660

234. Morris A, Gibson K, Collman R. The lung microbiome in idiopathic pulmonary fibrosis. What does it mean and what should we do about it? *Am J Respir Crit Care Med.* (2014) 190:850–2. doi: 10.1164/rccm.201409-1626ED

235. Lederer D, Martinez F. Idiopathic Pulmonary Fibrosis. Longo DL, editor. *New England J Med.* (2018) 378:1811–23.

236. Nature Immunology. The lungs at the frontlines of immunity. *Nat Immunol.* (2015) 16:17. doi: 10.1038/ni.3069

237. Nie Y, Wu S, Xuan Y, Yan G. Role of IL-17 family cytokines in the progression of IPF from inflammation to fibrosis. *Mil Med Res.* (2022) 9:21. doi: 10.1186/s40779-022-00382-3

238. Cipolla E, Fisher A, Gu H, Mickler E, Agarwal M, Wilke C, et al. IL-17A deficiency mitigates bleomycin-induced complement activation during lung fibrosis. *FASEB J.* (2017) 31:5543–56. doi: 10.1096/fj.201700289R

239. Gu H, Mickler E, Cummings O, Sandusky G, Weber D, Gracon A, et al. Crosstalk between TGF-β1 and complement activation augments epithelial injury in pulmonary fibrosis. *FASEB J.* (2014) 28:4223–34. doi: 10.1096/fj.13-247650

240. Murray L, Chen Q, Kramer M, Hesson D, Argentieri R, Peng X, et al. TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. *Int J Biochem Cell Biol.* (2011) 43:154–62. doi: 10.1016/j.biocel.2010.10.013

241. Raghu G, van den Blink B, Hamblin M, Brown A, Golden J, Ho L, et al. Effect of Recombinant Human Pentraxin 2 vs. placebo on change in forced vital capacity in patients with idiopathic pulmonary fibrosis: a randomized clinical trial. *JAMA.* (2018) 319:2299–307. doi: 10.1001/jama.2018.6129

242. Raghu G, van den Blink B, Hamblin M, Brown A, Golden J, Ho L, et al. Long-term treatment with recombinant human pentraxin 2 protein in patients with idiopathic pulmonary fibrosis: an open-label extension study. *Lancet Respir Med.* (2019) 7:657–64. doi: 10.1016/S2213-260030172-9

243. Raghu G, Hamblin M, Brown A, Golden J, Ho L, Wijsenbeek M, et al. Long-term evaluation of the safety and efficacy of recombinant human pentraxin-2 (rhPTX-2) in patients with idiopathic pulmonary fibrosis (IPF): an open-label extension study. *Respir Res.* (2022) 23:129. doi: 10.1186/s12931-022-02047-0

244. Herrmann F, Hesslinger C, Wollin L, Nickolaus P. BI 1015550 is a PDE4B Inhibitor and a Clinical Drug Candidate for the Oral Treatment of Idiopathic Pulmonary Fibrosis. *Front Pharmacol.* (2022) 13:838449. doi: 10.3389/fphar.2022.838449

245. Seedat U. Trial of a preferential phosphodiesterase 4B inhibitor for idiopathic pulmonary fibrosis. *African J Thoracic Crit Care Med.* (2022) 28:135.

246. Raghu G, Mouded M, Chambers D, Martinez F, Richeldi L, Lancaster L, et al. A Phase IIb Randomized Clinical Study of an Anti-αvβ6 Monoclonal Antibody in Idiopathic Pulmonary Fibrosis. *Am J Respir Crit Care Med.* (2022) 206:1128–39. doi: 10.1164/rccm.202112-2824OC

247. Culver D. Sarcoidosis. *Immunol Allergy Clin North Am.* (2012) 32:487–511. doi: 10.1016/j.iac.2012.08.005

248. Drent M, Crouser E, Grunewald J. Challenges of Sarcoidosis and Its Management. *N Engl J Med.* (2021) 385:1018–32. doi: 10.1056/NEJMra2101555

249. Patterson K, Chen E. The Pathogenesis of Pulmonary Sarcoidosis and Implications for Treatment. *Chest.* (2018) 153:1432–42. doi: 10.1016/j.chest.2017.11.030

250. Facco M, Cabrelle A, Teramo A, Olivieri V, Gnoato M, Teolato S, et al. Sarcoidosis is a Th1/Th17 multisystem disorder. *Thorax.* (2011) 66:144–50. doi: 10.1136/thx.2010.140319

251. Ramstein J, Broos C, Simpson L, Ansel K, Sun S, Ho M, et al. IFN-γ-Producing T-Helper 17.1 Cells Are Increased in Sarcoidosis and Are More Prevalent than T-Helper Type 1 Cells. *Am J Respir Crit Care Med.* (2016) 193:1281–91. doi: 10.1164/rccm.201507-1499OC

252. Zhang H, Costabel U, Dai H. The Role of Diverse Immune Cells in Sarcoidosis. *Front Immunol.* (2021) 12:788502. doi: 10.3389/fimmu.2021.788502

253. Lee N, Barber L, Akula S, Sigounas G, Kataria Y, Arce S. Disturbed homeostasis and multiple signaling defects in the peripheral blood B-cell compartment of patients with severe chronic sarcoidosis. *Clin Vaccine Immunol.* (2011) 18:1306–16. doi: 10.1128/CVI.05118-11

254. Dall'Aglio P, Pesci A, Bertorelli G, Brianti E, Scarpa S. Study of immune complexes in bronchoalveolar lavage fluids. *Respiration.* (1988) 54(Suppl. 1):36–41. doi: 10.1159/000195495

255. Ando M, Goto A, Takeno Y, Yamasue M, Komiya K, Umeki K, et al. Significant elevation of the levels of B-cell activating factor (BAFF) in patients with sarcoidosis. *Clin Rheumatol.* (2018) 37:2833–8. doi: 10.1007/s10067-018-4183-2

256. Cinetto F, Compagno N, Scarpa R, Malipiero G, Agostini C. Rituximab in refractory sarcoidosis: a single centre experience. *Clin Mol Allergy.* (2015) 13:19. doi: 10.1186/s12948-015-0025-9

257. Krause M, Cooper L, Chareonthaitawee P, Amin S. Successful use of rituximab in refractory cardiac sarcoidosis. *Rheumatology.* (2016) 55:189–91. doi: 10.1093/rheumatology/kev309

258. Sweiss N, Lower E, Mirsaeidi M, Dudek S, Garcia J, Perkins D, et al. Rituximab in the treatment of refractory pulmonary sarcoidosis. *Eur Respir J.* (2014) 43:1525–8. doi: 10.1183/09031936.00224513

259. Moller D. Pulmonary fibrosis of sarcoidosis. New approaches, old ideas. *Am J Respir Cell Mol Biol.* (2003) 29(3 Suppl):S37–41.

260. Josefowicz S, Lu L, Rudensky A. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol.* (2012) 30:531–64. doi: 10.1146/annurev.immunol.25.022106.141623

261. Sakthivel P, Grunewald J, Eklund A, Bruder D, Wahlström J. Pulmonary sarcoidosis is associated with high-level inducible co-stimulator (ICOS) expression on lung regulatory T cells–possible implications for the ICOS/ICOS-ligand axis in disease course and resolution. *Clin Exp Immunol.* (2016) 183:294–306. doi: 10.1111/cei.12715

262. Oswald-Richter K, Richmond B, Braun N, Isom J, Abraham S, Taylor T, et al. Reversal of global CD4+ subset dysfunction is associated with spontaneous clinical resolution of pulmonary sarcoidosis. *J Immunol.* (2013) 190:5446–53. doi: 10.4049/jimmunol.1202891

263. Weeratunga P, Moller D, Ho L. Immune mechanisms in fibrotic pulmonary sarcoidosis. *Eur Respir Rev.* (2022) 31:220178. doi: 10.1183/16000617.0178-2022

264. Judson M. The clinical features of sarcoidosis: a comprehensive review. *Clin Rev Allergy Immunol.* (2015) 49:63–78. doi: 10.1007/s12016-014-8450-y

265. Thillai M, Atkins C, Crawshaw A, Hart S, Ho L, Kouranos V, et al. BTS Clinical Statement on pulmonary sarcoidosis. *Thorax.* (2021) 76:4–20. doi: 10.1136/thoraxjnl-2019-214348

266. Gupta R, Baughman R. Advanced Pulmonary Sarcoidosis. *Semin Respir Crit Care Med.* (2020) 41:700–15. doi: 10.1055/s-0040-1709495

267. Sharma O. Fatigue and sarcoidosis. *Eur Respir J.* (1999) 13:713–4. doi: 10.1034/j.1399-3003.1999.13d01.x

268. Drent M, Lower E, De Vries J. Sarcoidosis-associated fatigue. *Eur Respir J.* (2012) 40:255–63. doi: 10.1183/09031936.00002512

269. Sève P, Pacheco Y, Durupt F, Jamilloux Y, Gerfaud-Valentin M, Isaac S, et al. Sarcoidosis: A Clinical Overview from Symptoms to Diagnosis. *Cells.* (2021) 10:766. doi: 10.3390/cells10040766

270. Judson M. The diagnosis of sarcoidosis. *Curr Opin Pulm Med.* (2019) 25:484–96. doi: 10.1097/MCP.0000000000000596

271. Spagnolo P, Rossi G, Trisolini R, Sverzellati N, Baughman R, Wells A. Pulmonary sarcoidosis. *Lancet Respir Med.* (2018) 6:389–402. doi: 10.1016/S2213-260030064-X

272. Grutters J, Fellrath J, Mulder L, Janssen R, van den Bosch J, van Velzen-Blad H. Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. *Chest.* (2003) 124:186–95. doi: 10.1378/chest.124.1.186

273. Baughman R, Grutters J. New treatment strategies for pulmonary sarcoidosis: antimetabolites, biological drugs, and other treatment approaches. *Lancet Respir Med.* (2015) 3:813–22. doi: 10.1016/S2213-260000199-X

274. Korsten P, Strohmayer K, Baughman R, Sweiss N. Refractory pulmonary sarcoidosis - proposal of a definition and recommendations for the diagnostic and therapeutic approach. *Clin Pulm Med.* (2016) 23:67–75. doi: 10.1097/CPM.0000000000000136

275. Sweiss N, Welsch M, Curran J, Ellman M. Tumor necrosis factor inhibition as a novel treatment for refractory sarcoidosis. *Arthritis Rheum.* (2005) 53:788–91. doi: 10.1002/art.21468

276. Rahaghi F, Baughman R, Saketkoo L, Sweiss N, Barney J, Birring S, et al. Delphi consensus recommendations for a treatment algorithm in pulmonary sarcoidosis. *Eur Respir Rev.* (2020) 29:190146. doi: 10.1183/16000617.0146-2019

277. Sharp M, Donnelly S, Moller D. Tocilizumab in sarcoidosis patients failing steroid sparing therapies and anti-TNF agents. *Respir Med X.* (2019) 1:100004. doi: 10.1016/j.yrmex.2019.100004

278. Milman N, Burton C, Andersen C, Carlsen J, Iversen M. Lung transplantation for end-stage pulmonary sarcoidosis: outcome in a series of seven consecutive patients. *Sarcoidosis Vasc Diffuse Lung Dis.* (2005) 22:222–8.

279. Shlobin O, Nathan S. Management of end-stage sarcoidosis: pulmonary hypertension and lung transplantation. *Eur Respir J.* (2012) 39:1520–33. doi: 10.1183/09031936.00175511

280. Rama Esendagli D, Ntiamoah P, Kupeli E, Bhardwaj A, Ghosh S, Mukhopadhyay S, et al. Recurrence of primary disease following lung transplantation. *ERJ Open Res.* (2022) 8:00038–2022. doi: 10.1183/23120541.00038-2022

281. Collins J, Hartman M, Warner T, Müller N, Kazerooni E, McAdams H, et al. Frequency and CT findings of recurrent disease after lung transplantation. *Radiology.* (2001) 219:503–9. doi: 10.1148/radiology.219.2.r01ma12503

282. Meyer K. Lung transplantation for pulmonary sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2019) 36:92–107. doi: 10.36141/svdld.v36i2.7163

283. Schultz H, Andersen C, Steinbruuchel D, Perch M, Carlsen J, Iversen M. Recurrence of sarcoid granulomas in lung transplant recipients is common and does not affect overall survival. *Sarcoidosis Vasc Diffuse Lung Dis.* (2014) 31:149–53.

284. Ionescu D, Hunt J, Lomago D, Yousem S. Recurrent sarcoidosis in lung transplant allografts: granulomas are of recipient origin. *Diagn Mol Pathol.* (2005) 14:140–5. doi: 10.1097/01.pas.0000176765.26047.6f

285. Banga A, Sahoo D, Lane C, Farver C, Budev M. Disease recurrence and acute cellular rejection episodes during the first year after lung transplantation among patients with sarcoidosis. *Transplantation.* (2015) 99:1940–5. doi: 10.1097/TP.0000000000000673

286. King M, Jessurun J, Hertz M. Recurrence of desquamative interstitial pneumonia after lung transplantation. *Am J Respir Crit Care Med.* (1997) 156:2003–5. doi: 10.1164/ajrccm.156.6.9703001

287. Verleden G. Recurrence of desquamative interstitial pneumonia after lung transplantation. *Am J Respir Crit Care Med.* (1998) 157:1349–50. doi: 10.1164/ajrccm.157.4.corres2

288. Bhatt S, Parekh K, Geist L, Weydert J, Klesney-Tait J. Rapid Recurrence of Interstitial Fibrosis Following Lung Transplantation. *Chest.* (2010) 138:44A.

289. Arboleda R, Gonzalez O, Cortes M, Perez-Cerda F. Recurrent polymyositis-associated lung disease after lung transplantation. *Interact Cardiovasc Thorac Surg.* (2015) 20:560–2.

290. Scallan C, Venado A, Han L, Xu H, Mulligan M, Raghu G. Recurrent Pulmonary Fibrosis in a Lung Allograft Secondary to De Novo Antisynthetase Syndrome. *Ann Am Thorac Soc.* (2020) 17:901–4. doi: 10.1513/AnnalsATS.202002-126RL

291. Yang X, Wei D, Liu M, Wu B, Zhang J, Xu H, et al. Survival and outcomes after lung transplantation for connective tissue disease-associated interstitial lung diseases. *Clin Rheumatol.* (2021) 40:3789–95. doi: 10.1007/s10067-021-05704-9

frontiers | Frontiers in Medicine

TYPE Perspective
PUBLISHED 10 May 2023
DOI 10.3389/fmed.2023.1174518

OPEN ACCESS

EDITED BY
Theodoros Karampitsakos,
University of South Florida, United States

REVIEWED BY
Ourania Papaioannou,
General University Hospital of Patras, Greece
Carole Y. Perrot,
University of South Florida, United States
Dominique Valeyre,
Université Sorbonne Paris Nord, France
Shu-Yi Liao,
National Jewish Health, United States

*CORRESPONDENCE
Spyros A. Papiris
✉ papiris@otenet.gr

RECEIVED 26 February 2023
ACCEPTED 05 April 2023
PUBLISHED 10 May 2023

CITATION
Papiris SA, Kolilekas L, Rivera N, Spanos M, Li G, Gokulnath P, Chatterjee E, Georgakopoulos A, Kallieri M, Papaioannou AI, Raptakis T, Apollonatou V, Antonogiannaki E-M, Gialafos E, Chatziioannou S, Grunewald J and Manali ED (2023) From Karl Wurm and Guy Scadding's staging to ^{18}F-FDG PET/CT scan phenotyping and far beyond: perspective in the evading history of phenotyping in sarcoidosis. *Front. Med.* 10:1174518. doi: 10.3389/fmed.2023.1174518

From Karl Wurm and Guy Scadding's staging to ^{18}F-FDG PET/CT scan phenotyping and far beyond: perspective in the evading history of phenotyping in sarcoidosis

Spyros A. Papiris[1]*, Lykourgos Kolilekas[2], Natalia Rivera[3], Michail Spanos[4], Guoping Li[4], Priyanka Gokulnath[4], Emeli Chatterjee[4], Alexandros Georgakopoulos[5], Maria Kallieri[1], Andriana I. Papaioannou[6], Thomas Raptakis[1], Vasiliki Apollonatou[1], Elvira-Markela Antonogiannaki[7], Elias Gialafos[8,9], Sofia Chatziioannou[5,10], Johan Grunewald[3] and Effrosyni D. Manali[1]

[1]2nd Pulmonary Medicine Department, Medical School, General University Hospital Attikon, National and Kapodistrian University of Athens, Athens, Greece, [2]7th Pulmonary Department, Athens Chest Hospital "Sotiria", Athens, Greece, [3]Respiratory Medicine Division, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden, [4]Cardiovascular Research Center, Simches 3 Massachusetts General Hospital, Boston, MA, United States, [5]2nd Department of Radiology, Nuclear Medicine Section, Medical School, General University Hospital "Attikon", National and Kapodistrian University of Athens, Athens, Greece, [6]1st Respiratory Medicine Department, Athens Medical School, Sotiria Chest Hospital of Athens, National and Kapodistrian University of Athens, Athens, Greece, [7]4th Pulmonary Department, Athens Chest Hospital "Sotiria", Athens, Greece, [8]Department of Cardiology, Medical School, General University Hospital "Attikon", National and Kapodistrian University of Athens, Athens, Greece, [9]First Department of Neurology, Medical School, Aeginition Hospital, National and Kapodistrian University of Athens, Athens, Greece, [10]Division of Nuclear Medicine, Biomedical Research Foundation of the Academy of Athens, Athens, Greece

Sarcoidosis is an inflammatory granulomatous disease of unknown etiology involving any organ or tissue along with any combination of active sites, even the most silent ones clinically. The unpredictable nature of the sites involved in sarcoidosis dictates the highly variable natural history of the disease and the necessity to cluster cases at diagnosis based on clinical and/or imaging common characteristics in an attempt to classify patients based on their more homogeneous phenotypes, possibly with similar clinical behavior, prognosis, outcome, and therefore with therapeutic requirements. In the course of the disease's history, this attempt relates to the availability of a means of detection of the sites involved, from the Karl Wurm and Guy Scadding's chest x-ray staging through the ACCESS, the WASOG Sarcoidosis Organ Assessment Instruments, and the GenPhenReSa study to the ^{18}F-FDG PET/CT scan phenotyping and far beyond to new technologies and/or the current "omics." The hybrid molecular imaging of the ^{18}F-FDG PET/CT scan, by unveiling the glucose metabolism of inflammatory cells, can identify high sensitivity inflammatory active granulomas, the hallmark of sarcoidosis—even in clinically and physiologically silent sites—and, as recently shown, is successful in identifying an unexpected ordered stratification into four phenotypes: (I) hilar–mediastinal nodal, (II) lungs and hilar–mediastinal nodal, (III) an extended nodal supraclavicular, thoracic, abdominal, inguinal, and (IV) all the above in addition to systemic organs and tissues, which is

therefore the ideal phenotyping instrument. During the "omics era," studies could provide significant, distinct, and exclusive insights into sarcoidosis phenotypes linking clinical, laboratory, imaging, and histologic characteristics with molecular signatures. In this context, the personalization of treatment for sarcoidosis patients might have reached its goal.

KEYWORDS

sarcoidosis, chest roentgenogram staging, ^{18}F-FDG PET/CT scan phenotyping, omics, personalized treatment

Introduction

Sarcoidosis is a systemic inflammatory disease of unknown etiology, which occurs in populations worldwide and involves the lungs and the intrathoracic lymph nodes (1–3). The etiologically implicated antigen "still eludes us," and the disease is considered a dysregulated, immune-mediated response due to its presence, persistence, and failure to clear, leading to tissue granulomas formation (4, 5). The histological hallmark of the disease indeed constitutes well-formed, non-caseating granulomas that may localize in any organ or tissue without boundaries and with any combination patterns of active sites of involvement in most of the cases, notably, even in the clinically silent (6, 7) cases. The detection of granulomas with the abovementioned characteristics is never pathognomonic for the disease (8). Therefore, it is necessary to ensure diagnosis in most cases is associated with the combination of compatible clinical, laboratory, and imaging characteristic patterns with histologic findings, as well as the exclusion of any other etiology of granulomatous inflammation (1, 9).

However, clinical compatibility for sarcoidosis in most cases—beyond the well-known and quite pathognomonic clinical syndromes (phenotypes), such as Löfgren's syndrome and Heerfordt's syndrome—constitutes primarily the unpredictable and "anarchic" tissue distribution of the disease in combination with some characteristic imaging features from the lungs, in addition to the asymptomatic or oligosymptomatic disease's first appearance (1, 10–12). Due to the unpredictability of the sites involved in sarcoidosis, the disease has a highly variable natural history, and since every sarcoidosis patient represents a distinctive case of theirown, individual management strategies may be imperative (13–20).

Clustering or better phenotyping patients with the disease at diagnosis on the basis of clinical and/or imaging common characteristics is an old attempt to assign patients with an unpredictable disease to more or less homogeneous groups possibly with similar clinical behavior, prognosis, and outcome and therefore with similar therapeutics requirements (21–30). In the course of the history of the disease, this attempt relates to the availability of the current means of detection of any site of involvement in a systemic disease, from the chest x-ray of Karl Wurm and Guy Scadding's staging and through the ACCESS study, the WASOG Sarcoidosis Organ Assessment Instrument, and the GenPhenReSa study to the ^{18}F-fluoro-2-deoxyglucose (^{18}F-FDG) positron emission tomography (PET) computed tomography (CT) scan phenotyping and far beyond to new technologies and/or the current "omics" availability (31–41). Stratification of sarcoidosis patients based on T-cell count in Bronchoalveolar Lavage (BAL) and gallium scan, the presence of impaired physiology, fibrosis, and pulmonary hypertension, as well as clinical activity, as reflected by acute or non-acute disease in onset, treatment, and long-term treatment requirements, and the clinical outcome status of the disease have also been described (24, 42–48). Successful phenotyping, to be clinically useful by the physicians in everyday clinical practice, should be easy, simple, reliable in unraveling most, if not all, sites of disease involvement, less expensive, reproducible worldwide in different populations with the disease, and able to offer information concerning the clinical behavior of the individual phenotypes, thus providing valuable information for the decision-making process (49). The corpus of this research article has been focused on the initial evaluation of the sarcoidosis patient.

Ordering the unpredictable

Sometimes sarcoidosis creates order within itself. Sven Löfgren was the first to associate erythema nodosum and bilateral hilar lymphadenopathy with sarcoidosis—an early or acute manifestation of the disease (fever and polyarthritis commonly coexist) that is fairly distinct from tuberculosis, with good prognosis—an assumption that maintains its value till present (50–52). Cristian Heerfordt was the first to describe that, in sarcoidosis, the "febris uveoparotidea subchronica" (fever, parotid enlargement, and uveitis) was occasionally associated with the seventh nerve palsy and other rare manifestations of the disease (53). Both the above two phenotypes though fairly uncommon maintain a significant diagnostic value not necessitating histologic confirmation in most if not in all patients. Prognosis is excellent in Löfgren's, and indeterminate in the Heerfordt's syndrome (1, 8). However, despite the fact that the abovementioned phenotypes are narrow in terms of clinical manifestations and are relatively homogeneous, nothing is known about other silent systemic sites of active disease; better detection and definitive identification of these silent systemic sites are possible with the application of new technologies (7). As disease evolved, technology and research followed its lead.

In the beginning it was Wilhelm Conrad Röntgen

In the beginning, it was Wilhelm Conrad Röntgen (recipient of Nobel Prize in Physics in 1901) who was unaware of sarcoidosis, and by discovering the electromagnetic radiation known as Röntgen rays (X-rays), he offered the availability of chest roentgenogram to the pioneers of the disease (Ernest Besnier, Caesar Boeck, and Jörgen Schaumann, who were the men behind the sarcoidosis disease and the disease was named after them in early days) rendering them aware that the skin, eyes and joints disease in front of them was part of an internal organ, systemic disease involving almost always the lungs and the intrathoracic lymph nodes: the indispensable first encounter of every physician with any sarcoidosis patient to this day (54–56).

Karl Wurm and Guy Scadding's staging first step through chest radiology

The first chest röentgenogram (x-rays) classification of sarcoidosis, which was designed by Karl Wrum, was divided into three stages (stage I bilateral hilar–mediastinal lymphadenopathy, stage II lungs reversible involvement and stage III lungs fibrosis) and was the first attempt to draw prognostic considerations of the disease, and accordingly arriving at therapeutic decisions for sarcoidosis patients (31, 32). It belongs to Guy Scadding the current chest radiological staging of sarcoidosis in "four groups" as firstly reported, [group 1) enlarged hilar lymph-nodes, group 2) hilar nodes and lung shadowing, group 3) lung shadowing and group 4) fibrosis, though not always "sharply demarcated"] and belong to him the seminal conclusions regarding the prognostic significance of the above radiologic grouping as well as the fundamental clinical advice that corticosteroids are necessary only in a minority of patients (33) (Figure 1A), all of which were determined by Guy Scadding. Moreover, Guy Scadding also confirmed previous observations reporting the benign clinical course and spontaneous resolution of the Löfgren's syndrome phenotype. Several years later, Johan Grunewald and Anders Eklund provided additional significant information regarding the influence of the genetic background, human leukocytes antigen (HLA) polymorphisms, particularly the *HLA-DRB1*03* allele on the outcome of this phenotype—a new step in the effort to draw information about phenotypes from the science advancement adding to the clinical information the results of the "bench" (11, 23, 57–61). During the early Scadding's times, the awareness of extrathoracic manifestations of sarcoidosis was mostly limited to the skin, eyes, parotids, superficial lymph nodes, joints, and few others, and there were no reliable conclusions that could be added regarding the clinicians' influence on disease's clinical behavior, prognosis, and outcome. In modern times, the necessity to evolve from Karl Wurm and Guy Scadding's staging was unavoidable and possible because of the advancement of technology and scientific thinking (49).

The ACCESS study for the definition of organ involvement in sarcoidosis

To solve the problem of the elusive identification of the etiologically implicated antigen in sarcoidosis, the National Heart, Lung, and Blood Institute (NHLBI) set up the A Case Control Etiologic Study of Sarcoidosis (ACCESS) multicenter study (34–36). In present times, the ACCESS study proposed an instrument to assess and define organ involvement in sarcoidosis, a clear step that has been taken ahead to evolve from Karl Wurm and Guy Scadding's staging. It was successful in identifying differences in HLA gene associations with sarcoidosis among European and African Americans; however, for several reasons, the instrument failed to address all possible sites of the disease's activity, which is indispensable to identifying phenotypes (62). Furthermore, the development of new technologies made the ACCESS instrument outdated, and the necessity for organ assessment in sarcoidosis resulted in developing new instruments.

The WASOG Sarcoidosis Organ Assessment Instrument

The WASOG Sarcoidosis Organ Assessment Instrument was developed as an update of the previous ACCESS tool to establish reliable criteria for the probability of any organ being involved in the disease in a sarcoidosis patient, in the new technology era. The probability of organ involvement from sarcoidosis was based on two criteria: granulomatous inflammation and compatible clinical manifestation, excluding in both cases alternative etiologies. Based on the above findings, clinical manifestations were graded as (1) highly probable, 90% likelihood, (2) probable, 50–90% likelihood, and (3) possible, <50% likelihood. For each manifestation, an agreement of 70% was needed for consensus (Delphi study methodology). Indeterminate probability was defined when consensus was not reached (37).

GenPhenReSa consortium

The GenPhenReSa consortium is the first attempt, entirely utilizing the WASOG Sarcoidosis Organ Assessment Instrument to identify almost all sites of involvement by the disease, in order firstly to identify reliable and homogeneous phenotypes of sarcoidosis appearance, useful cohorts for further biomedical studies and secondly to attempt possible genotype–phenotype associations were detected. The results of the phenotype module of the GenPhenReSa as evidenced by the cluster analysis of 2,163 Caucasian patients phenotyped in 31 different sarcoidosis expert study centers regard the identification of five "distinct" subgroups identified from organ involvement: (1) abdominal organ involvement, (2) ocular-cardiac-cutaneous-central nervous system disease involvement, (3) musculoskeletal-cutaneous involvement, (4) pulmonary and intrathoracic lymph node involvement, and (5) extrapulmonary involvement, defined by the authors as "homogeneous cohorts useful for further biomedical studies" (38). However, some concerns arise at first glance regarding distinctness and homogeneity since the same authors acknowledge that "new

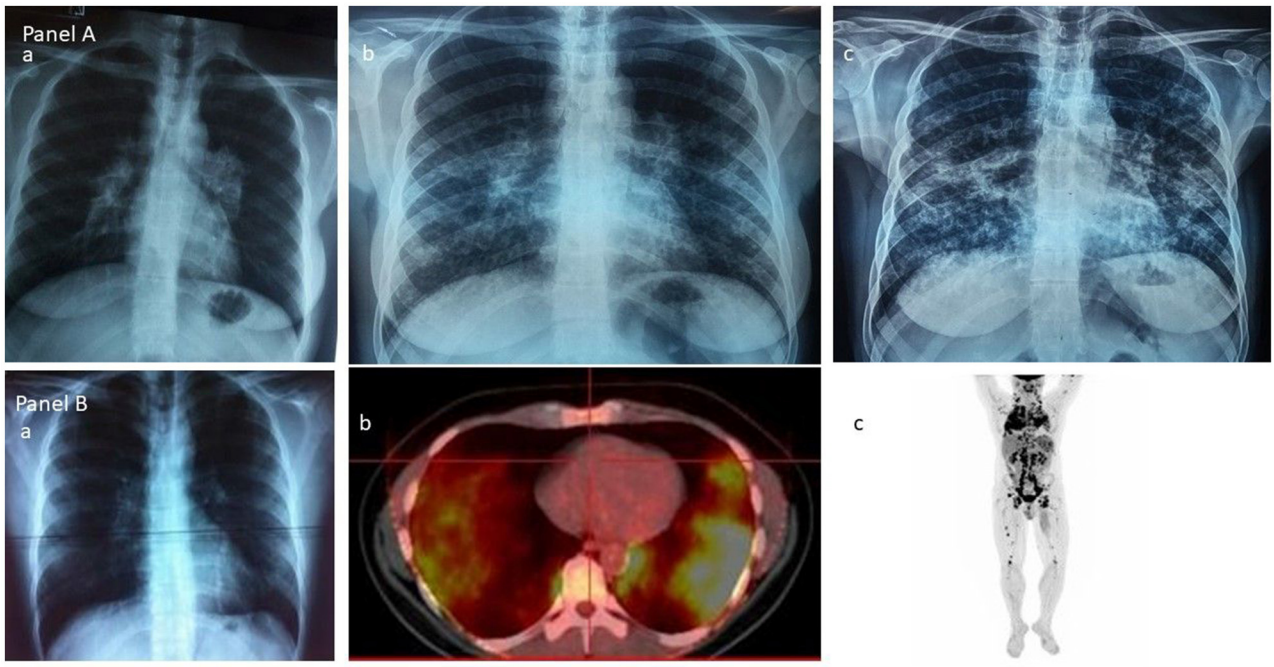

FIGURE 1
(A) The Scadding's vision of sarcoidosis through the chest roentgenogram: group 1—enlarged hilar lymph-nodes; group 2—hilar nodes and lung shadowing; and group 3—lung shadowing. **(B)** The current vision of sarcoidosis through the ^{18}F-FDG PET/CT scan: (a) and (b) the lungs; (c) the whole body.

technologies will enable better detection of organ involvement"; therefore, frequencies and clusters of organ involvement are likely to change over time. Indeed, using the abovementioned system to define organ involvement appears laborious in terms of the necessity for several tests and examinations as well as somewhat controversial in terms of the homogeneity of clusters since phenotyping appears to overlap and is somewhat ambiguous.

^{18}F-FDG PET/CT scan phenotyping

The hybrid molecular imaging of the ^{18}F-FDG PET/CT scan by unveiling glucose metabolism of inflammatory cells is able to identify, with high sensitivity, inflammatory active granulomas, the hallmark of sarcoidosis, even in clinically and physiologically silent sites, providing simultaneously whole-body PET and CT images (63, 64). Therefore, the ^{18}F-FDG PET/CT scan appears to be the ideal instrument for the assessment of organs involved in sarcoidosis in detecting their intrinsic inflammatory activity (65–67) (Figure 1B) in the most beneficial fashion. Through the ^{18}F-FDG PET/CT scan, investigators may attain a far better understanding of sarcoidosis physical history, behavior, unveiling patterns of disease expression, subdividing patients by clusters, and identifying phenotypes. Recently, Papiris et al. by implementing an ^{18}F-FDG PET/CT scan in newly diagnosed, especially in the treatment-naïve patients with sarcoidosis, identified, despite the random distribution of the disease, by a statistical method called hierarchical cluster analysis, an unexpected ordered stratification into four phenotypes: (I) hilar–mediastinal nodal, (II) lungs and hilar–mediastinal nodal, (III) an extended nodal supraclavicular, thoracic, abdominal, inguinal, and (IV) all the above in addition to systemic organs and tissues such as muscles–bones–spleen and skin (39) (Figure 2). Although this approach represents a simplified assessment of sarcoidosis where the use of only one investigative tool and in "one shot" depicts the universal expression of the disease by its active presence, the approach indisputably offered a much logical picture of the disease much closer to the perception of caring clinicians. Furthermore, this "one-shop-stop" in the acquisition of data offers "an open book" approach to the clinicians in getting themselves exposed to every single "page written by the disease" by scientists and experts who have in-depth knowledge and to decide on therapeutic requirements. The organ clusters (phenotypes) identified in this analysis remain far better than anyone else's analysis carried out before and are in accordance with the existing knowledge of sarcoidosis. The phenotypes I and II appeared to coincide with the familial Scadding's first two stages; however, by applying ^{18}F-FDG PET/CT scan, all other sites of disease involvement were surely excluded, a sort of "pure organs Scadding's stages I and II," while in Scadding's staging the other coexisting sites of disease involvement slowly got away from the disease scenario and went unidentified. The phenotype III disclosed was rather suspected in sarcoidosis patients and occasionally observed clinically and by other means such as computerized tomography (CT) scans and echocardiogram (ECHO). However, for the first time, the disease involvement was clearly identified in all possible extensions of the actual site, and a lot of work must be done to identify its prognostic significance and therapeutic requirements (apparently necessitating none). Finally, the phenotype IV appears familial to the clinician-caring sarcoidosis patients since it appears

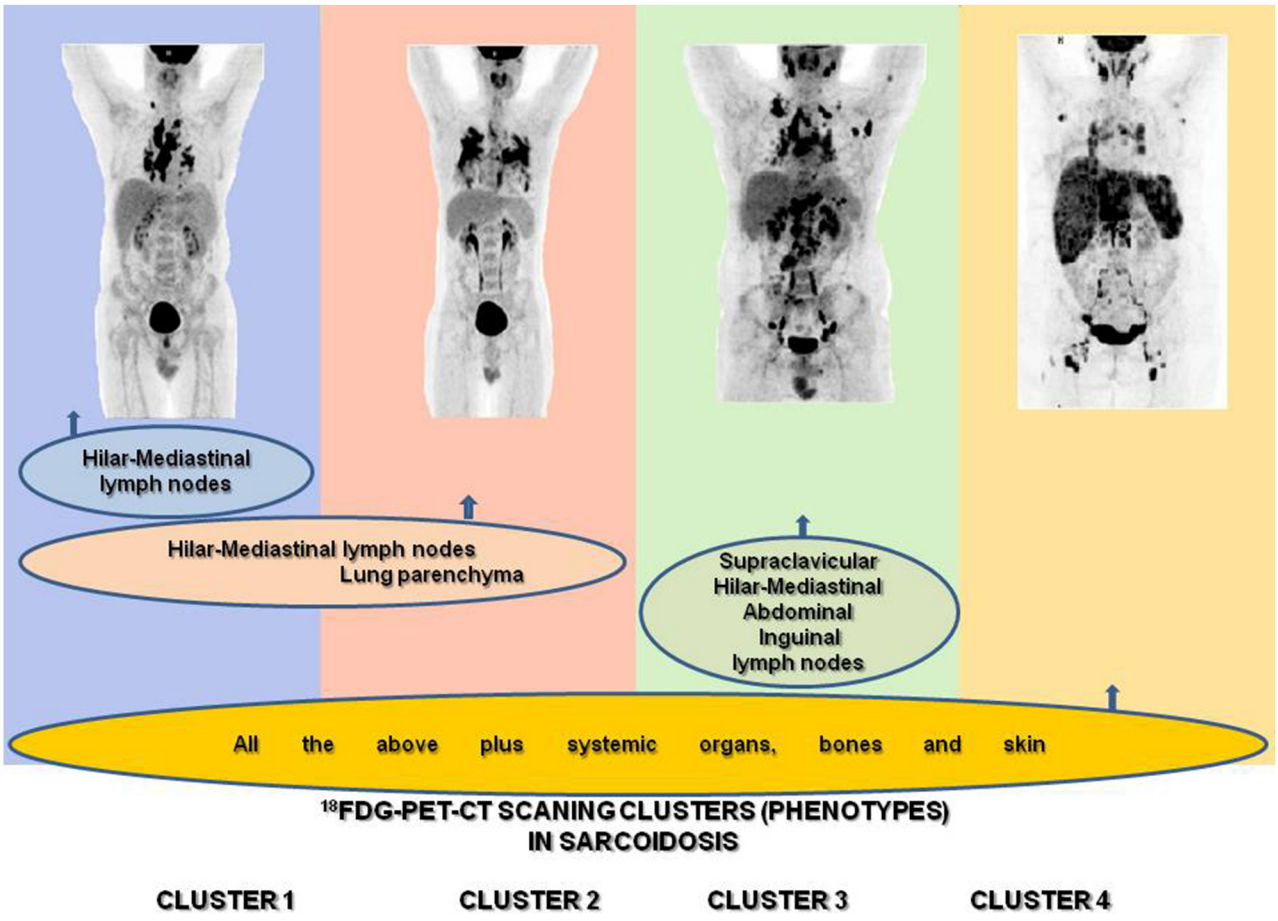

FIGURE 2
Identification of sarcoidosis ^{18}F-FDG PET/CT scan phenotypes according to organ involvement using two-step cluster analysis: (1) hilar–mediastinal nodal, (2) lungs and hilar–mediastinal nodal, (3) an extended nodal supraclavicular, thoracic, abdominal, inguinal, and (4) all the above in addition to systemic organs and tissues such as muscles–bones–spleen and skin.

to be satisfying to the current knowledge of the systemic nature of the disease, probably relating to the systemic spreading, from the lungs and hilar–mediastinal lymph nodes to the whole body, of the etiologically implicated antigen. Therefore, by clustering investigations of sarcoidosis through the ^{18}F-FDG PET/CT scan, clinically logical phenotypes were identified. However, like all other clustering investigations, some patients may not respect the boundaries but also Scadding's staging by the simplest of the means of investigation, the chest roentgenogram, which clarifies that stages may not be "sharply demarcated," and we feel right to confirm his elaboration concerning clustering. Based on hierarchical cluster analysis of the study population and implementing adequate preparation with low carbohydrate diet for 24 h followed by 18-h fasting to suppress radiotracer's uptake by normal myocardial cell, no significant difference was detected between the clusters regarding myocardial involvement, suggesting that heart disease could be detected and should be evaluated in any cluster. Given the important prognostic and treatment implications of cardiac involvement, the role of ^{18}F-FDG PET/CT scan has been examined extensively. Although this modality requires a specific protocol including diet restriction and being more prone than cardiac magnetic resonance (CMR) to provide false positive results, it is a technique much more sensitive than transthoracic echocardiogram (70 vs. 25%) that should be used as complementary to CMR for the detection of myocardial inflammation and fibrosis, respectively (16, 39, 68, 69). Certainly, the low specificity of ^{18}F-FDG PET/CT scan poses several concerns regarding the differentiation with any neoplasm or other inflammatory etiology, but in the patient diagnosed with sarcoidosis, further approach in unusual or suspected sites investigated by additional investigative tools including biopsy should be warranted (70). The ^{18}F-FDG PET/CT scan molecular imaging by its high sensibility in detecting organs' intrinsic inflammatory activity, its worldwide availability, the "relatively low" radiation impact, the reasonable cost, and its reliability to evaluate treatment response appears the ideal instrument for phenotyping in sarcoidosis. However, the ^{18}F-FDG PET/CT is unable to "see" the eye; therefore, to obtain a complete phenotype, an ophthalmologic examination is mandatory (71–77). Neurological and endocrinology evaluations are also indispensable for thoroughness to detect neurosarcoidosis, small-fiber neuropathy, and abnormal calcium metabolism (78–81). Until further well-designed multicenter studies are performed to confirm the abovementioned findings as universal patterns of disease behavior, we do not consider this study an ideal destination

for sarcoidosis and its assessment but rather treat it as just another journey ahead. ^{18}F-FDG PET/CT should be used rationally and wisely, its radiation exposure that is comparable to whole-body diagnostic CT should also be taken into consideration and should be balanced by its higher sensitivity for both thoracic and extrathoracic disease. The unveiling of the abovementioned phenotypes, especially in treatment naïve patients at the first glance on diagnosis, appears useful in designing future studies with more homogeneous cohorts, toward the acquisition in sarcoidosis patients of a more personalized medicine approach (39). Currently, relevant studies are lacking; whether ^{18}F-FDG PET/CT should be recommended to all patients and whether ^{18}F-FDG PET/CT phenotyping could provide additional information on outcome, treatment indications, silent lung disease, such as asymptomatic radiographic stage I included, should be further validated.

The "omics era"

"Omics" is a high-throughput technology that allows for the comprehensive profiling of various biochemical molecules in a context-dependent manner in multiple organisms. "Omics" studies include genetics, epigenetics, transcriptomics, proteomics, lipidomics, metabolomics, and microbiomics. Given that sarcoidosis is a complex, polygenic disease of unknown cause with diverse clinical phenotypes, the "omics" approach adds another layer of information by delving into the molecular signatures that could provide important insights on the pathogenesis of this disease (4, 5, 41, 82–88). Genetic predisposition and immune dysregulation, inherent to sarcoidosis, are investigated by characterizing both previously known and newly discovered immune-cell-specific pathways, gene expression, and epigenetic modifications that may differ between specific tissues and compartments as well as between progressive and non-progressive disease manifestations (41, 89–94). Through "omics," we have currently gained access to a very advanced group of techniques that can provide conceptual, mechanistic, and molecular interpretations of phenotypes irrespective of treatment. For instance, genome-wide association studies (GWAS) examined the genetic composition of many genomes to identify variants associated with specific traits or diseases. GWASs provide insight into phenotypic biology, predict clinical outcomes, and reveal causal relationships between risk factors and health outcomes. Recent GWAS in European, African American, and Asian populations identified a non-synonymous single-nucleotide polymorphism (SNP), rs1049550, within the annexin A11 (ANXA11) gene as being associated with susceptibility to sarcoidosis (95). The RNA-sequencing (RNA-seq) technologies, such as bulk and single-cell RNA-sequencing (scRNA-seq), permit unbiased interrogation of the whole transcriptome and deep immunophenotyping of heterogeneous single cells suspensions, respectively. The RNA-seq data analysis for differential gene expression has become a standard method for comparing gene expression among healthy and diseased individuals, tissues, and cell types and can provide valuable information about dysregulated pathways and inciting disease mechanisms. ScRNA-seq can then be combined with spatial transcriptomics and cellular proteomics to provide a "transcriptometabolomic" map of the cell's active status in healthy and diseased conditions. Using this approach, a recent study examined the immunostructural stoichiometry of sarcoidosis patients' granulomas, revealing that granulomas hijack the transcriptional programs that regulate normal lymphoid organ development and alter cytokine and chemokine pathways (96). These findings may explain the increased inflammatory activity and 18F-FDG uptake observed in those tissues and aid in identifying potentially targetable molecules for therapeutic development. Another method that combines single-cell transcriptomics with proteomics, which is called cellular indexing of transcriptomes and epitopes (CITE-Seq), is a sequencing-based method that allows simultaneous quantification of cell surface protein and single-cell sequencing data, enabling both surface marker and transcriptomic phenotyping of immune cells that are dysregulated in various diseases (97). Since cell surface proteins are indicators of cell phenotype and functional status, CITE-seq may provide a superior method for profiling immune cells in sarcoidosis when compared with scRNA-seq or proteomic analysis alone (98). While "omics" cannot be directly applied to everyday clinical practice, they could provide significant, distinct, and exclusive insights into the described phenotypes of sarcoidosis by combining clinical, laboratory, imaging, and histologic characteristics with molecular signatures in a sort of "reverse phenotyping" approach. Eventually, the future may see an algorithm combining all the aforementioned methods to efficiently diagnose, phenotype, and eventually lead to efficient sarcoidosis treatment. However, larger studies need to be conducted to arrive to robust conclusions that can be developed further into clinical applications.

Perspective in the evading history of phenotyping in sarcoidosis

Discussion

As we critically reviewed the history of phenotyping in sarcoidosis, we realized that we have constantly been making progress from what could initially be observed by the inquiring minds of scientists only through the naked eye to what we are currently able to observe using the recent great advances in technology, and the progress seems enormous (99–101). For example, the contribution of ^{18}F-FDG PET/CT scan in enriching our ability to screen patients with sarcoidosis for all organs and sites involved, even the most silent ones, in "one shot" is indisputable as well as the ability of -omics studies to provide unbiased insights about pathophysiological and molecular signatures that could be representative of specific sarcoidosis phenotypes, is still unperceivable to clinical observation (39, 41). However, to this day, clinical observation and judgment are the first means of capturing and validating the existence of the phenotypes associated with sarcoidosis. Defining and evolving disease phenotypes is perpetually motivated by the desire to better understand the disease and its many obscure aspects (4). The utility of phenotypes becomes increasingly relevant as studies that intend to elucidate disease mechanisms underlying sarcoidosis pathogenesis and to identify biomarkers that can be used for diagnosis, prediction of outcomes, and optimization of disease management can only be carried out in well-phenotyped populations of multiple ethnic origins; therefore, an initiative requiring international collaboration efforts,

such as the Multi-Ethnic Sarcoidosis Genomics Consortium (MESARGEN), may bring together many scientists and clinicians from around the world toward this goal.[1]

Due to its high sensitivity in detecting intrinsic inflammatory activity, ^{18}F-FDG PET/CT scanning appears to be the most appropriate tool for guiding and orchestrating our efforts in phenotyping in sarcoidosis in the future. In the era of "omics", the research could provide unique insights into sarcoidosis phenotypes through the association of clinical, laboratory, imaging, and histologic characteristics with molecular signatures. In this context, the personalization of treatment for sarcoidosis patients might have reached its goal.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

SP: concept and design of the study (text and figures), analysis and interpretation of all data, and wrote the manuscript. LK, NR, MS, GL, PG, and EC: interpretation of the data, wrote parts of the manuscript, and revised the work critically for very important intellectual content. AG and MK: produced the figures and revised the work critically for important intellectual content. AP, TR, VA, E-MA, EG, SC, and JG: major contribution in the interpretation of the data and revised the work critically for important intellectual content. EM: major contribution to the concept of the study, to the acquisition, analysis and interpretation of data, had access to all data, supervised the accuracy and integrity of any part of the work, and wrote the final version of the manuscript with SP. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

1 Available online at: https://mesargen.org/.

References

1. Grunewald J, Grutters JC, Arkema EV, Saketkoo LA, Moller DR, Müller-Quernheim J. Sarcoidosis. *Nat Rev Dis Primers.* (2019) 5:45. doi: 10.1038/s41572-019-0096-x

2. Arkema EV, Cozier YC. Sarcoidosis epidemiology: recent estimates of incidence, prevalence and risk factors. *Curr Opin Pulm Med.* (2020) 26:527–34. doi: 10.1097/MCP.0000000000000715

3. Ma X, Zhu L, Kurche JS, Xiao H, Dai H, Wang C. Global and regional burden of interstitial lung disease and pulmonary sarcoidosis from 1990 to 2019: results from the global burden of disease study 2019. *Thorax.* (2022) 77:596–605. doi: 10.1136/thoraxjnl-2020-216732

4. Judson MA. A primer on the clinical aspects of sarcoidosis for the basic and translational scientist. *J Clin Med.* (2021) 10:2857. doi: 10.3390/jcm10132857

5. Grunewald J, Spagnolo P, Wahlström J, Eklund A. Immunogenetics of disease-causing inflammation in sarcoidosis. *Clin Rev Allergy Immunol.* (2015) 49:19–35. doi: 10.1007/s12016-015-8477-8

6. Tana C, Donatiello I, Caputo A, Tana M, Naccarelli T, Mantini C, et al. Clinical features, histopathology and differential diagnosis of sarcoidosis. *Cells.* (2021) 11:59. doi: 10.3390/cells11010059

7. Judson MA. Screening sarcoidosis patients for occult disease. *Semin Respir Crit Care Med.* (2020) 41:741–57. doi: 10.1055/s-0040-1709496

8. Judson MA. The diagnosis of sarcoidosis. *Curr Opin Pulm Med.* (2019) 25:484–96. doi: 10.1097/MCP.0000000000000596

9. Judson MA. Granulomatous sarcoidosis mimics. *Front Med.* (2021) 8:680989. doi: 10.3389/fmed.2021.680989

10. Palmucci S, Torrisi SE, Caltabiano DC, Puglisi S, Lentini V, Grassedonio E, et al. Clinical and radiological features of extra-pulmonary sarcoidosis: a pictorial essay. *Insights Imaging.* (2016) 7:571–87. doi: 10.1007/s13244-016-0495-4

11. Karakaya B, Kaiser Y, van Moorsel CHM, Grunewald J. Löfgren's syndrome: diagnosis, management, and disease pathogenesis. *Semin Respir Crit Care Med.* (2017) 38:463–76. doi: 10.1055/s-0037-1602380

12. Dua A, Manadan A. Images in clinical medicine. Heerfordt's syndrome, or uveoparotid fever. *N Engl J Med.* (2013) 369:458. doi: 10.1056/NEJMicm1303454

13. Kolilekas L, Triantafillidou C, Manali E, Rontogianni D, Chatziioannou S, Papiris S. The many faces of sarcoidosis: asymptomatic muscle mass mimicking giant-cell tumor. *Rheumatol Int.* (2009) 29:1389–90. doi: 10.1007/s00296-009-0989-1

14. Froudarakis ME, Bouros D, Voloudaki A, Papiris S, Kottakis Y, Constantopoulos SH, et al. Pneumothorax as a first manifestation of sarcoidosis. *Chest.* (1997) 112:278–80. doi: 10.1378/chest.112.1.278

15. Papadavid E, Dalamaga M, Stavrianeas N, Papiris SA. Subcutaneous sarcoidosis masquerading as cellulitis. *Dermatology.* (2008) 217:212–4. doi: 10.1159/000142945

16. Crouser ED, Maier LA, Wilson KC, Bonham CA, Morgenthau AS, Patterson KC, et al. Diagnosis and detection of sarcoidosis. an official American thoracic society clinical practice guideline. *Am J Respir Crit Care Med.* (2020) 201:e26–51. doi: 10.1164/rccm.202002-0251ST

17. Baughman RP, Valeyre D, Korsten P, Mathioudakis AG, Wuyts WA, Wells A, et al. ERS clinical practice guidelines on treatment of sarcoidosis. *Eur Respir J.* (2021) 58:2004079. doi: 10.1183/13993003.04079-2020

18. Gupta R, Judson MA, Baughman RP. Management of advanced pulmonary sarcoidosis. *Am J Respir Crit Care Med.* (2022) 205:495–506. doi: 10.1164/rccm.202106-1366CI

19. Trivieri MG, Spagnolo P, Birnie D, Liu P, Drake W, Kovacic JC, et al. Challenges in cardiac and pulmonary sarcoidosis: Jacc State-of-the-Art review. *J Am Coll Cardiol.* (2020) 76:1878–901. doi: 10.1016/j.jacc.2020.08.042

20. Calandriello L, D'Abronzo R, Pasciuto G, Cicchetti G, Del Ciello A, Farchione A, et al. Novelties in imaging of thoracic sarcoidosis. *J Clin Med.* (2021) 10:2222. doi: 10.3390/jcm10112222

21. Lin NW, Arbet J, Mroz MM, Liao SY, Restrepo CI, Mayer AS, et al. Clinical phenotyping in sarcoidosis using cluster analysis. *Respir Res.* (2022) 23:88. doi: 10.1186/s12931-022-01993-z

22. Loddenkemper R, Kloppenborg A, Schoenfeld N, Grosser H, Costabel U. Clinical findings in 715 patients with newly detected pulmonary sarcoidosis–results of a cooperative study in former West Germany and Switzerland. Watl Study Group. Wissenschaftliche Arbeitsgemeinschaft Für Die Therapie Von Lungenkrankheitan. *Sarcoidosis Vasc Diffuse Lung Dis.* (1998) 15:178–82.

23. Scadding JG. Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years' observation. *Br Med J.* (1961) 2:1165–72. doi: 10.1136/bmj.2.5261.1165

24. Prasse A, Katic C, Germann M, Buchwald A, Zissel G, Müller-Quernheim J. Phenotyping sarcoidosis from a pulmonary perspective. *Am J Respir Crit Care Med.* (2008) 177:330–6. doi: 10.1164/rccm.200705-742OC

25. Lundkvist A, Kullberg S, Arkema EV, Cedelund K, Eklund A, Grunewald J, et al. Differences in disease presentation between men and women with sarcoidosis: a cohort study. *Respir Med.* (2022) 191:106688. doi: 10.1016/j.rmed.2021.106688

26. Abo Al Hayja M, Wahlström J, Kullberg S, Darlington P, Eklund A, Grunewald J. Bronchoalveolar lavage fluid cell subsets associate with the disease course in Löfgren's and Non-Löfgren's sarcoidosis patients. *Respir Med.* (2021) 186:106521. doi: 10.1016/j.rmed.2021.106521

27. Rossides M, Grunewald J, Eklund A, Kullberg S, Di Giuseppe D, Askling J, et al. Familial aggregation and heritability of sarcoidosis: a Swedish nested case-control study. *Eur Respir J.* (2018) 52:1800385. doi: 10.1183/13993003.00385-2018

28. Lhote R, Annesi-Maesano I, Nunes H, Launay D, Borie R, Sacré K, et al. Clinical phenotypes of extrapulmonary sarcoidosis: an analysis of a French, multi-ethnic, multicentre cohort. *Eur Respir J.* (2021) 57:2001160. doi: 10.1183/13993003.01160-2020

29. Spyropoulos G, Domvri K, Manika K, Fouka E, Kontakiotis T, Papakosta D. Clinical, imaging and functional determinants of sarcoidosis phenotypes in a greek population. *J Thorac Dis.* (2022) 14:1941–9. doi: 10.21037/jtd-21-1760

30. Crouser ED, Amin EN. Severe sarcoidosis phenotypes: an occupational hazard? *Chest.* (2016) 150:263–5. doi: 10.1016/j.chest.2016.02.663

31. Meier G, Wurm K. On the prognosis of sarcoidosis (Boeck's disease). *Beitr Klin Tuberk Spezif Tuberkuloseforsch.* (1960) 123:90–7. doi: 10.1007/BF02142474

32. Wurm K. The significance of stage classification of sarcoidosis (Boeck's disease). *Dtsch Med Wochenschr.* (1960) 85:1541–8. doi: 10.1055/s-0028-1112616

33. Scadding JG. The late stages of pulmonary sarcoidosis. *Postgrad Med J.* (1970) 46:530–6. doi: 10.1136/pgmj.46.538.530

34. Baughman RP, Teirstein AS, Judson MA, Rossman MD, Yeager H Jr, Bresnitz EA, et al. Clinical characteristics of patients in a case control study of sarcoidosis. *Am J Respir Crit Care Med.* (2001) 164:1885–9. doi: 10.1164/ajrccm.164.10.2104046

35. Rossman MD, Kreider ME. Lesson learned from access (a case controlled etiologic study of sarcoidosis). *Proc Am Thorac Soc.* (2007) 4:453–6. doi: 10.1513/pats.200607-138MS

36. Judson MA, Baughman RP, Teirstein AS, Terrin ML, Yeager H Jr. Defining organ involvement in sarcoidosis: the access proposed instrument. access research group. a case control etiologic study of sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (1999) 16:75–86.

37. Judson MA, Costabel U, Drent M, Wells A, Maier L, Koth L, et al. The WASOG sarcoidosis organ assessment instrument: an update of a previous clinical tool. *Sarcoidosis Vasc Diffuse Lung Dis.* (2014) 31:19–27.

38. Schupp JC, Freitag-Wolf S, Bargagli E, Mihailović-Vučinić V, Rottoli P, Grubanovic A, et al. Phenotypes of organ involvement in sarcoidosis. *Eur Respir J.* (2018) 51:1700991. doi: 10.1183/13993003.00991-2017

39. Papiris SA, Georgakopoulos A, Papaioannou AI, Pianou N, Kallergi M, Kelekis NL, et al. Emerging phenotypes of sarcoidosis based on 18F-FDG PET/CT: a hierarchical cluster analysis. *Expert Rev Respir Med.* (2020) 14:229–38. doi: 10.1080/17476348.2020.1684902

40. Garman L, Montgomery CG, Rivera NV. Recent advances in sarcoidosis genomics: epigenetics, gene expression, and gene by environment (G × E) interaction studies. *Curr Opin Pulm Med.* (2020) 26:544–53. doi: 10.1097/MCP.0000000000000719

41. Bhargava M, Liao SY, Crouser ED, Maier LA, Leach SM. The landscape of transcriptomic and proteomic studies in sarcoidosis. *ERJ Open Res.* (2022) 8:00621–2021. doi: 10.1183/23120541.00621-2021

42. Pinkston P, Bitterman PB, Crystal RG. Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. *N Engl J Med.* (1983) 308:793–800. doi: 10.1056/NEJM198304073081401

43. Schoenberger CI, Line BR, Keogh BA, Hunninghake GW, Crystal RG. Lung inflammation in sarcoidosis: comparison of serum angiotensin-converting enzyme levels with bronchoalveolar lavage and gallium-67 scanning assessment of the T lymphocyte alveolitis. *Thorax.* (1982) 37:19–25. doi: 10.1136/thx.37.1.19

44. Balbi B, Moller DR, Kirby M, Holroyd KJ, Crystal RG. Increased numbers of T lymphocytes with gamma delta-positive antigen receptors in a subgroup of individuals with pulmonary sarcoidosis. *J Clin Invest.* (1990) 85:1353–61. doi: 10.1172/JCI114579

45. Walsh SL, Wells AU, Sverzellati N, Keir GJ, Calandriello L, Antoniou KM, et al. An integrated clinicoradiological staging system for pulmonary sarcoidosis: a case-cohort study. *Lancet Respir Med.* (2014) 2:123–30. doi: 10.1016/S2213-2600(13)70276-5

46. Shlobin OA, Kouranos V, Barnett SD, Alhamad EH, Culver DA, Barney J, et al. physiological predictors of survival in patients with sarcoidosis-associated pulmonary hypertension: results from an international registry. *Eur Respir J.* (2020) 55:1901747. doi: 10.1183/13993003.01747-2019

47. Kirkil G, Lower EE, Baughman RP. Predictors of mortality in pulmonary sarcoidosis. *Chest.* (2018) 153:105–13. doi: 10.1016/j.chest.2017.07.008

48. Baughman RP, Nagai S, Balter M, Costabel U, Drent M, du Bois R, et al. Defining the clinical outcome status (COS) in sarcoidosis: results of WASOG task force. *Sarcoidosis Vasc Diffuse Lung Dis.* (2011) 28:56–64.

49. Culver DA, Baughman RP. It's time to evolve from scadding: phenotyping sarcoidosis. *Eur Respir J.* (2018) 51:1800050. doi: 10.1183/13993003.00050-2018

50. Lofgren S, Lundback H. The bilateral hilar lymphoma syndrome; a study of the relation to tuberculosis and sarcoidosis in 212 cases. *Acta Med Scand.* (1952) 142:265–73. doi: 10.1111/j.0954-6820.1952.tb13865.x

51. Lofgren S. Primary pulmonary sarcoidosis. II. Clinical course and prognosis. *Acta Med Scand.* (1953) 145:465–74. doi: 10.1111/j.0954-6820.1953.tb07044.x

52. Lofgren S. The concept of sarcoidosis. *Am Rev Respir Dis.* (1961) 84:17–9.

53. Theobald GD, Wilder HL. Heerfordt's syndrome. *Trans Am Acad Ophthalmol Otolaryngol.* (1953) 57:332–3.

54. Röntgen WC. On a new kind of rays. *Science.* (1896) 3:227–31. doi: 10.1126/science.3.59.227

55. Young RC Jr, Rachal RE, Cowan CL Jr. Sarcoidosis–the beginning: historical highlights of personalities and their accomplishments during the early years. *J Natl Med Assoc.* (1984) 76:887–96.

56. Spagnolo P. Sarcoidosis: a critical review of history and milestones. *Clin Rev Allergy Immunol.* (2015) 49:1–5. doi: 10.1007/s12016-015-8480-0

57. Wolin A, Lahtela EL, Anttila V, Petrek M, Grunewald J, van Moorsel CHM, et al. SNP variants in major histocompatibility complex are associated with sarcoidosis susceptibility-a joint analysis in four european populations. *Front Immunol.* (2017) 8:422. doi: 10.3389/fimmu.2017.00422

58. Kaiser Y, Lepzien R, Kullberg S, Eklund A, Smed-Sörensen A, Grunewald J. Expanded lung T-bet+RORγT+ CD4+ T-cells in sarcoidosis patients with a favourable disease phenotype. *Eur Respir J.* (2016) 48:484–94. doi: 10.1183/13993003.00092-2016

59. Darlington P, Gabrielsen A, Sörensson P, Tallstedt L, Padyukov L, Eklund A, et al. Hla-Alleles associated with increased risk for extra-pulmonary involvement in sarcoidosis. *Tissue Antigens.* (2014) 83:267–72. doi: 10.1111/tan.12326

60. Grunewald J, Eklund A. Löfgren's syndrome: human leukocyte antigen strongly influences the disease course. *Am J Respir Crit Care Med.* (2009) 179:307–12. doi: 10.1164/rccm.200807-1082OC

61. Grunewald J, Brynedal B, Darlington P, Nisell M, Cederlund K, Hillert J, et al. Different HLA-DRB1 Allele distributions in distinct clinical subgroups of sarcoidosis patients. *Respir Res.* (2010) 11:25. doi: 10.1186/1465-9921-11-25

62. Rossman MD, Thompson B, Frederick M, Maliarik M, Iannuzzi MC, Rybicki BA, et al. HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. *Am J Hum Genet.* (2003) 73:720–35. doi: 10.1086/378097

63. Adams H, van Rooij R, van Moorsel CHM, Spee-Dropkova M, Grutters JC, Keijsers RG. Volumetric FDG pet analysis of global lung inflammation: new tool for precision medicine in pulmonary sarcoidosis? *Sarcoidosis Vasc Diffuse Lung Dis.* (2018) 35:44–54. doi: 10.36141/svdld.v35i1.5807

64. Keijsers RG, van den Heuvel DA, Grutters JC. Imaging the inflammatory activity of sarcoidosis. *Eur Respir J.* (2013) 41:743–51. doi: 10.1183/09031936.00088612

65. Vagts C, Ascoli C, Fraidenburg DR, Baughman RP, Huang Y, Edafetanure-Ibeh R, et al. Unsupervised clustering reveals sarcoidosis phenotypes marked by a reduction in lymphocytes relate to increased inflammatory activity on 18FDG-PET/CT. *Front Med.* (2021) 8:595077. doi: 10.3389/fmed.2021.595077

66. Papiris SA, Manali ED, Papaioannou AI, Georgakopoulos A, Kolilekas L, Pianou NK, et al. Prevalence, distribution and clinical significance of joints, muscles and bones in sarcoidosis: an F-FDG-PET/CT study. *Expert Rev Respir Med.* (2020) 14:957–64. doi: 10.1080/17476348.2020.1775587

67. Papiris SA, Manali ED, Pianou NK, Kallergi M, Papaioannou AI, Georgakopoulos A, et al. 18F-FDG PET/CT in pulmonary sarcoidosis:quantifying inflammation by the TLG index. *Expert Rev Respir Med.* (2020) 14:103–10. doi: 10.1080/17476348.2020.1682997

68. Vita T, Okada DR, Veillet-Chowdhury M, Bravo PE, Mullins E, Hulten E, et al. Complementary value of cardiac magnetic resonance imaging and positron emission tomography/computed tomography in the assessment of cardiac sarcoidosis. *Circ Cardiovasc Imaging.* (2018) 11:e007030. doi: 10.1161/CIRCIMAGING.117.007030

69. Alvi RM, Young BD, Shahab Z, Pan H, Winkler J, Herzog E, et al. Repeatability and optimization of FDG positron emission tomography for evaluation of cardiac sarcoidosis. *JACC Cardiovasc Imaging.* (2019) 12:1284–7. doi: 10.1016/j.jcmg.2019.01.011

70. Chen X, Xu X, Chrysikos S, Zhao M, Zhou Y. Value of 18-fluorodeoxyglucose positron emission tomography/computed tomography (18F-FDG PET/CT) in

the differential diagnosis of sarcoidosis and lung cancer with lymph node metastasis: a retrospective study. *Transl Lung Cancer Res.* (2022) 11:1926–35. doi: 10.21037/tlcr-22-611

71. Jung RS, Mittal BR, Maturu NV, Kumar R, Bhattacharya A, Gupta D. Ocular sarcoidosis: does F-FDG PET/CT have any role? *Clin Nucl Med.* (2014) 39:464–6. doi: 10.1097/RLU.0000000000000419

72. Quinn B, Dauer Z, Pandit-Taskar N, Schoder H, Dauer LT. Radiation dosimetry of 18F-FDG PET/CT: incorporating exam-specific parameters in dose estimates. *BMC Med Imaging.* (2016) 16:41. doi: 10.1186/s12880-016-0143-y

73. Nishiyama Y, Yamamoto Y, Fukunaga K, Takinami H, Iwado Y, Satoh K, et al. Comparative evaluation of 18F-FDG PET and 67GA scintigraphy in patients with sarcoidosis. *J Nucl Med.* (2006) 47:1571–6.

74. Braun JJ, Kessler R, Constantinesco A, Imperiale A. 18F-FDG PET/CT in sarcoidosis management: review and report of 20 cases. *Eur J Nucl Med Mol Imaging.* (2008) 35:1537–43. doi: 10.1007/s00259-008-0770-9

75. Bakker ALM, Mathijssen H, Azzahhafi J, Swaans MJ, Veltkamp M, Keijsers RGM, et al. Effectiveness and safety of infliximab in cardiac sarcoidosis. *Int J Cardiol.* (2021) 330:179–85. doi: 10.1016/j.ijcard.2021.02.022

76. Sgard B, Brillet PY, Bouvry D, Djelbani S, Nunes H, Meune C, et al. Evaluation of FDG PET combined with cardiac MRI for the diagnosis and therapeutic monitoring of cardiac sarcoidosis. *Clin Radiol.* (2019) 74:81.e9–18. doi: 10.1016/j.crad.2018.09.015

77. Maturu VN, Rayamajhi SJ, Agarwal R, Aggarwal AN, Gupta D, Mittal BR. Role of serial F-18 FDG PET/CT scans in assessing treatment response and predicting relapses in patients with symptomatic sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2016) 33:372–80.

78. Raasing L, Vogels OJM, Veltkamp M, Grutters JC. Infliximab decreases inflammatory activity but has no effect on small fiber neuropathy related symptoms in dutch patients with sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis.* (2022) 39:e2022033. doi: 10.36141/svdld.v39i4.12031

79. Gosselin J, Roy-Hewitson C, Bullis SSM, DeWitt JC, Soares BP, Dasari S, et al. Neurosarcoidosis: phenotypes, approach to diagnosis and treatment. *Curr Rheumatol Rep.* (2022) 24:371–82. doi: 10.1007/s11926-022-01089-z

80. Walker MD, Shane E. Hypercalcemia: a review. *JAMA*. (2022) 328:1624–36. doi: 10.1001/jama.2022.18331

81. Gwadera Ł, Białas AJ, Iwański MA, Górski P, Piotrowski WJ. Sarcoidosis and calcium homeostasis disturbances-do we know where we stand? *Chron Respir Dis.* (2019) 16:1479973119878713. doi: 10.1177/1479973119878713

82. Kaiser Y, Eklund A, Grunewald J. Moving target: shifting the focus to pulmonary sarcoidosis as an autoimmune spectrum disorder. *Eur Respir J.* (2019) 54:1802153. doi: 10.1183/13993003.021532018

83. Barna BP, Judson MA, Thomassen MJ. Inflammatory pathways in sarcoidosis. *Adv Exp Med Biol.* (2021) 1304:39–52. doi: 10.1007/978-3-030-68748-9_3

84. Lin NW, Maier LA, Mroz MM, Jacobson S, MacPhail K, Liu S, et al. Genomic biomarkers in chronic beryllium disease and sarcoidosis. *Respir Med.* (2021) 187:106390. doi: 10.1016/j.rmed.2021.106390

85. Spagnolo P, Maier LA. Genetics in sarcoidosis. *Curr Opin Pulm Med.* (2021) 27:423–9. doi: 10.1097/MCP.0000000000000798

86. Rivera NV, Patasova K, Kullberg S, Diaz-Gallo LM, Iseda T, Bengtsson C, et al. A gene-environment interaction between smoking and gene polymorphisms provides a high risk of two subgroups of sarcoidosis. *Sci Rep.* (2019) 9:18633. doi: 10.1038/s41598-019-54612-1

87. Rivera NV, Ronninger M, Shchetynsky K, Franke A, Nöthen MM, Müller-Quernheim J, et al. High-density genetic mapping identifies new susceptibility variants in sarcoidosis phenotypes and shows genomic-driven phenotypic differences. *Am J Respir Crit Care Med.* (2016) 193:1008–22. doi: 10.1164/rccm.201507-1372OC

88. Gialafos E, Triposkiadis F, Kouranos V, Rapti A, Kosmas I, Manali E, et al. Relationship between tumor necrosis factor-? (TNFA) gene polymorphisms and cardiac sarcoidosis. *In Vivo.* (2014) 28:1125–9.

89. Konigsberg IR, Lin NW, Liao SY, Liu C, MacPhail K, Mroz MM, et al. Multi-omic signatures of sarcoidosis and progression in bronchoalveolar lavage cells. *bioRxiv [Preprint].* (2023). doi: 10.1101/2023.01.26.525601

90. Jiang Y, Jiang D, Costabel U, Dai H, Wang C. A transcriptomics-based meta-analysis identifies a cross-tissue signature for sarcoidosis. *Front Med.* (2022) 9:960266. doi: 10.3389/fmed.2022.960266

91. Liu J, Ma P, Lai L, Villanueva A, Koenig A, Bean GR, et al. Transcriptional and immune landscape of cardiac sarcoidosis. *Circ Res.* (2022) 131:654–69. doi: 10.1161/CIRCRESAHA.121.320449

92. Vukmirovic M, Yan X, Gibson KF, Gulati M, Schupp JC, DeIuliis G, et al. Transcriptomics of bronchoalveolar lavage cells identifies new molecular endotypes of sarcoidosis. *Eur Respir J.* (2021) 58:2002950. doi: 10.1183/13993003.02950-2020

93. Häggmark A, Hamsten C, Wiklundh E, Lindskog C, Mattsson C, Andersson E, et al. Proteomic profiling reveals autoimmune targets in sarcoidosis. *Am J Respir Crit Care Med.* (2015) 191:574–83. doi: 10.1164/rccm.201407-1341OC

94. Rybicki BA, Sinha R, Iyengar S, Gray-McGuire C, Elston RC, Iannuzzi MC. Genetic linkage analysis of sarcoidosis phenotypes: the sarcoidosis genetic analysis (SAGA) study. *Genes Immun.* (2007) 8:379–86. doi: 10.1038/sj.gene.6364396

95. Karakaya B, van der Vis JJ, Veltkamp M, Biesma DH, Grutters JC, van Moorsel CHM. ANXA11 rs1049550 associates with Löfgren's syndrome and chronic sarcoidosis patients. *Cells.* (2022) 11:1557. doi: 10.3390/cells11091557

96. Krausgruber T, Redl A, Barreca D, Doberer K, Romanovskaia D, Dobnikar L, et al. Single-cell and spatial transcriptomics reveal aberrant lymphoid developmental programs driving granuloma formation. *Immunity.* (2023) 56:289–306.e7. doi: 10.1016/j.immuni.2023.01.014

97. Su Y, Chen D, Yuan D, Lausted C, Choi J, Dai CL, et al. Multi-omics resolves a sharp disease-state shift between mild and moderate COVID-19. *Cell.* (2020) 183:1479–95.e20. doi: 10.1016/j.cell.2020.10.037

98. Magallon RE, Knapp JR, Harmacek LD, Tu T-HC, Vestal B, Gillespie M, et al. Comparative profiling of the immune system in sarcoidosis via CITE-Seq and flow cytometry. *J Immunol.* (2020) 204:224.24-.24. doi: 10.4049/jimmunol.204.Supp.224.24

99. Liu A, Sharma L, Yan X, Dela Cruz CS, Herzog EL, Ryu C. Emerging insights in sarcoidosis: moving forward through reverse translational research. *Am J Physiol Lung Cell Mol Physiol.* (2022) 322:L518–l25. doi: 10.1152/ajplung.00266.2021

100. Miedema JR, Bonella F, Grunewald J, Spagnolo P. Looking into the future of sarcoidosis: what is next for treatment? *Curr Opin Pulm Med.* (2020) 26:598–607. doi: 10.1097/MCP.0000000000000709

101. Fischer A, Grunewald J, Spagnolo P, Nebel A, Schreiber S, Müller-Quernheim J. Genetics of sarcoidosis. *Semin Respir Crit Care Med.* (2014) 35:296–306. doi: 10.1055/s-0034-1376860

frontiers | Frontiers in Medicine

TYPE Original Research
PUBLISHED 07 September 2023
DOI 10.3389/fmed.2023.1195501

OPEN ACCESS

EDITED BY
Hyungsoon Im,
Massachusetts General Hospital and Harvard Medical School, United States

REVIEWED BY
Michael Adam O'Reilly,
University of Rochester, United States
Miho Jeong,
Massachusetts General Hospital, United States

*CORRESPONDENCE
Vassilis Aidinis
✉ V.Aidinis@Fleming.gr

RECEIVED 28 March 2023
ACCEPTED 07 August 2023
PUBLISHED 07 September 2023

CITATION
Galaris A, Fanidis D, Tsitoura E, Kanellopoulou P, Barbayianni I, Ntatsoulis K, Touloumi K, Gramenoudi S, Karampitsakos T, Tzouvelekis A, Antoniou K and Aidinis V (2023) Increased lipocalin-2 expression in pulmonary inflammation and fibrosis.
Front. Med. 10:1195501.
doi: 10.3389/fmed.2023.1195501

Increased lipocalin-2 expression in pulmonary inflammation and fibrosis

Apostolos Galaris[1], Dionysios Fanidis[1], Eliza Tsitoura[2], Paraskevi Kanellopoulou[1], Ilianna Barbayianni[1], Konstantinos Ntatsoulis[1], Katerina Touloumi[1], Sofia Gramenoudi[1], Theodoros Karampitsakos[3], Argyrios Tzouvelekis[3], Katerina Antoniou[2] and Vassilis Aidinis[1]*

[1]Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center Alexander Fleming, Athens, Greece, [2]Department of Respiratory Medicine, School of Medicine, University of Crete, Heraklion, Greece, [3]Department of Respiratory Medicine, School of Medicine, University of Patras, Patras, Greece

Introduction: Idiopathic Pulmonary Fibrosis (IPF) is a chronic, progressive interstitial lung disease with dismal prognosis. The underlying pathogenic mechanisms are poorly understood, resulting in a lack of effective treatments. However, recurrent epithelial damage is considered critical for disease initiation and perpetuation, via the secretion of soluble factors that amplify inflammation and lead to fibroblast activation and exuberant deposition of ECM components. Lipocalin-2 (LCN2) is a neutrophil gelatinase-associated lipocalin (NGAL) that has been suggested as a biomarker of kidney damage. LCN2 has been reported to modulate innate immunity, including the recruitment of neutrophils, and to protect against bacterial infections by sequestering iron.

Methods: *In silico* analysis of publicly available transcriptomic datasets; ELISAs on human IPF patients' bronchoalveolar lavage fluids (BALFs); bleomycin (BLM)-induced pulmonary inflammation and fibrosis and LPS-induced acute lung injury (ALI) in mice: pulmonary function tests, histology, Q-RT-PCR, western blot, and FACS analysis.

Results and discussion: Increased *LCN2* mRNA expression was detected in the lung tissue of IPF patients negatively correlating with respiratory functions, as also shown for BALF LCN2 protein levels in a cohort of IPF patients. Increased *Lcn2* expression was also detected upon BLM-induced pulmonary inflammation and fibrosis, especially at the acute phase correlating with neutrophilic infiltration, as well as upon LPS-induced ALI, an animal model characterized by neutrophilic infiltration. Surprisingly, and non withstanding the limitations of the study and the observed trends, *Lcn2*$^{-/-}$ mice were found to still develop BLM- or LPS-induced pulmonary inflammation and fibrosis, thus questioning a major pathogenic role for *Lcn2* in mice. However, LCN2 qualifies as a surrogate biomarker of pulmonary inflammation and a possible indicator of compromised pulmonary functions, urging for larger studies.

KEYWORDS

idiopathic pulmonary fibrosis (IPF), bleomycin (BLM), acute lung injury, transcriptomics, lipocalin-2 (LCN2)

Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease characterized by the exuberant deposition of collagens and other ECM components by lung fibroblasts, leading to the distortion of lung architecture and the impairment of respiratory functions. The underlying mechanisms of the disease are poorly understood, resulting in a

lack of effective treatments. However, epithelial damage is considered a key event initiating the pathogenesis of IPF, where repeated injury and/or abnormal repair of the epithelium trigger a cascade of signaling events that result in the recruitment and activation of immune cells, as well as the activation and accumulation of lung fibroblasts (1, 2).

Expression profiling of human IPF samples has been instrumental in the discovery of novel pathogenic genes and cellular pathways (3), that some were validated in animal models and some were translated into the clinic (4). In this context, we have recently developed Fibromine, a database and data mining tool, hosting all publicly available IPF transcriptomic (and proteomic) datasets (5), thus allowing the further exploitation of legacy data. Comparative analysis selected several hundred genes as differentially expressed in IPF, while an explainable machine learning phenotype classification algorithm prioritized 76 genes that include previously identified IPF expression hallmarks (e.g., Col1a1), IPF biomarkers (e.g., MMP7), as well as many genes previously shown to be involved in the pathophysiology of IPF (e.g., SPP1) (6). Among the novel, commonly identified deregulated genes in IPF was Lipocalin-2 (LCN2), also known as neutrophil gelatinase B-associated lipocalin (NGAL), as it was initially identified in neutrophilic granules in association with matrix metalloproteinase 9 (MMP9; gelatinase B) (7, 8). However, LCN2 secretion from other immune cells, as well as epithelial cells, has been reported (9, 10). LCN2 is considered an acute-phase protein, and increased LCN2 expression has been reported in different pathophysiological situations, including heart failure, kidney disease, and gut inflammation (10).

In the lung, increased LCN2 expression has been reported in subclinical pulmonary emphysema (11), chronic obstructive pulmonary disease (COPD) (12, 13), acute respiratory distress syndrome (ARDS) (14), as well as in patients with influenza A and SARS-CoV-2 virus infections (15). Not surprisingly, given their commonalities (16), higher LCN2 expression in bronchial epithelial cells of IPF patients has been also reported (17). Moreover, and more intriguingly, LCN2 has been suggested to mediate innate immune responses to bacterial infection by sequestrating iron (18), whereas both iron homeostasis (19), as well as microbiome regulation (20), have been linked with IPF pathogenesis. Therefore, in this report, we investigated a possible role for LCN2 in pulmonary inflammation and fibrosis, by using *in silico* analysis of publicly available transcriptomic datasets, examination of LCN2 protein levels in IPF patients, as well as *in vivo* mouse models of pulmonary inflammation and fibrosis.

Materials and methods

Datasets

All analyzed bulk-sequencing datasets (Supplementary Table S1) were sourced from Fibromine (5). scRNA sequencing (scRNAseq) datasets used in the study are detailed in Supplementary Table S2.

TABLE 1 Demographics and clinical characteristics of IPF patients.

Characteristic	IPF ($n = 26$)
Age (yr) (Mean ± SD)	72.8 ± 7.3
Gender, *n* (%)	
Male	25 (96.1%)
Female	1 (3.9%)
Pulmonary function tests (mean ± SD)	
DLCO%	56.2 ± 19.4
FEV1/FVC%	85.4 ± 4.7
KCO	94.1 ± 21.5
Hematological analysis (%) (mean ± SD)	
Macrophages	83.1 ± 9.8
Lymphocytes	7.6 ± 7.6*
Polymorphonuclear	7.5 ± 6.4
Eosinophils	1.3 ± 1.61
BALF LCN2 (ng/mL) eosinophils	58.9 ± 52.3

*FVC%, Forced vital capacity percent predicted; DLCO%, Carbon monoxide diffusing capacity percent predicted; FEV1%, Forced expiratory volume in 1-s percent predicted.

Human patients

All studies were performed in accordance with the Declaration of Helsinki principles at the Department of Thoracic Medicine, University Hospital of Heraklion, and the demographics and clinical characteristics of the IPF patients can be found in Table 1. The diagnosis of IPF was based on ATS/ERS criteria or multidisciplinary discussion according to the Fleischer criteria (2, 21). Patients were anti-fibrotic naïve. All patients were evaluated with complete pulmonary function tests (PFTs) within 1 month of bronchoscopy. Lung volumes were measured using body plethysmography and the diffusion capacity (DLco, corrected for hemoglobin) using the single breath technique, and a computerized system (Jaeger 2.12; MasterLab, Würzburg, Germany). Patients were classified as non-smokers, current smokers, or former smokers (defined as having smoked a minimum of one cigarette a day for a minimum of 1 year, and stopping at least 6 months before presentation). All patients provided written informed consent. The study was approved by the Ethics Committees of the University Hospital of Heraklion (IRB numbers: 1045 and 17030).

Mice

All mice were bred at the animal facilities of the Alexander Fleming Biomedical Sciences Research Center under specific pathogen-free conditions. Mice were housed at 20–22°C, 55 ± 5% humidity, and a 12-h light–dark cycle; water and food were given *ad libitum*. Mice were bred and maintained in their respective genetic backgrounds for more than 10 generations. All experiments performed on mice for this project were in line with the ARRIVE guidelines and were approved by the Institutional Animal Ethical Committee (IAEC) of the Biomedical Sciences Research Center

"Alexander Fleming" (#373/375), as well as the Veterinary service and Fishery Department of the local governmental prefecture (#5508). Lipocalin-2 deficient mice (*Lcn2*$^{-/-}$) were procured from the Jackson Laboratory (#024630) and were maintained in a C57Bl6 genetic background for over 10 generations; genotyping was performed as previously published (18). Mice were humanely euthanized in a gradually filled CO_2 chamber.

BLM-induced pulmonary fibrosis

Pulmonary fibrosis was induced through the administration of bleomycin (BLM, 0.8 U/Kg of body weight; Nippon Kayaku) to anesthetized mice (intraperitoneal; ketamine/xylazine/atropine, 100/10/0.05 mg/Kg, respectively) via the oropharyngeal (OA) track, as previously described (22). In brief, mice were carefully placed on a plastic wall upon anesthesia. Their tongue was pulled out with forceps to get a better view of the trachea. The nares were blocked to force inhalation, and the bleomycin, diluted in normal saline (~50 μl for each mouse), was directly delivered to the oropharyngeal cavity using a conventional pipette tip. Normal saline was administered in the same way to littermate mice used as controls 3, 7, and 14 days after bleomycin (or saline) administration, at the peak of BLM-induced disease (which spontaneously resolves at d21).

Lipopolysaccharide (LPS)-induced acute lung injury (ALI)

The acute lung injury (ALI) model was performed using LPS delivered by inhalation, as previously described (23). In brief, bacterial lipopolysaccharides (LPS) from *Pseudomonas aeruginosa* (serotype 10, Sigma, St. Louis, MO, USA) were dissolved in normal saline at a concentration of 2 mg/ml. A total of 5 ml of this solution was administered into a chamber containing 5–7 mice via a custom-made nebulizer at an oxygen flow rate of 4 lt/min for 25 min. Normal saline was administered to the control mice. All measures were taken to minimize animal suffering; however, during the protocol, no anesthetics were used as no invasive or painful techniques were performed. After the induction of ALI, the condition of the animals was checked every 2 h during the light period. Mice were euthanized 24 h after the induction of ALI.

Respiratory functions

The respiratory functions were examined with FlexiVent (Scireq), following the manufacturer's instructions and as previously published (22).

Analyses of samples

Blood was collected through the portal vein and placed into tubes containing 0.5 M EDTA at a concentration of 10%$^{v}/_{v}$. Then, it was centrifuged for 20 min at 2.000 *g* at 4°C, and the plasma was transferred in new siliconized tubes. BALF was obtained by lavaging the airways with 3 ml of normal saline using a cannula through the trachea (three times; 1 mL each). Then, BALF was centrifuged for 15 min at 1.200 *g* at 4°C. The first 1 ml of the BALF was transferred without the cells into a new siliconized tube. The other 2 ml were discarded; the cells were pooled and treated with GEYS solution for 10 min in ice. Then, they were centrifuged for 10 min at 1.200 *g* at 4°C, the suspension was discarded, the cell pellet was re-suspended in fresh PBS, and the cells were counted under an inverted microscope using a Neubauer chamber. The left lung lobe was cut and instantly transferred into liquid nitrogen for RNA and protein extraction. The remaining lobes were filled with formalin (143091.1214, AppliChem), to be later mounted into paraffin. Additionally, total protein concentration was estimated in the BALF using Bradford reagent (Cat.no.: 39222.03, SERVA) following the manufacturer's instructions.

Flow cytometry

Mice were euthanized under deep anesthesia followed by exsanguination. Then, BALF was collected via tracheotomy by injecting and slowly withdrawing 3 ml (3 times; 1 mL each) of phosphate-buffered saline (PBS). The cells were collected via 10 min centrifugation at 1,200 rpm at 4°C, and they were treated with 1 mL of Gey's Solution for 2–3 min. The Gey's Solution was removed after a 10 min centrifugation at 1,200 rpm at 4°C, and the cells were resuspended in 1XPBS/1 %FBS and counted manually under a reversed light microscope using an improved Neubauer hemacytometer according to common procedures. Next, the cells were centrifuged at 1,200 rpm for 10 min at 4°C. The cell pellets were resuspended in 50 μl blocking buffer (1XPBS with 1% FBS and 1:400 CD16/32) for 10 min. Then, 100 μL of PBS was added to each sample, and the cells were collected via 5 min centrifugation at 1200 rpm at 4°C. The cells were resuspended and stained in the desired concentrations of antibodies in 1XPBS + 1%FBS for 30 min. Then, 100 μL of PBS was added to each sample, and the cells were collected via 5 min centrifugation at 1200 rpm at 4°C. Finally, the cells were resuspended in 250 μl filtered PBS, and data were acquired on a BD FACSCanto TM II flow cytometer using BD FACSDiva software (BD Biosciences). The analyses of the RAW data were performed with the FlowJo software (TreeStar, Ashland, OR).

RNA extraction and real-time PCR

The upper half of the left lobe isolated from the animals was homogenized in 1 mL of Trizol (TR118, Molecular Research Center) followed by total RNA extraction according to the manufacturer's instructions. A total of 2 μg of total RNA were used for cDNA construction using M-MLV reverse transcriptase (28025-013, Invitrogen) according to the manufacturer's instructions. Real-time polymerase chain reaction (RT-PCR) was performed using SoFAst EvaGreen Supermix on a Bio-Rad CFX96 Touch™ real-time PCR detection system (Bio-Rad Laboratories Ltd, CA, USA). Values were normalized to β2-microglobulin (B2M) and the

primers used are: Lcn2 (F: 5′-GGG AAA TAT GCA CAG GTA TCC TC-3′; R: 5′- CAT GGC GAA CTG GTT GTA GTC-3′) and B2M (F: 5′-TTC TGG TGC TTG TCT CAC TGA-3′; R: 5′-CAG TAT GTT CGG CTT CCC ATT C-3′).

Protein extraction and Western blot analysis

The lower half of the left lobe was homogenized in 100 μL of homemade RIPA cell lysis buffer (20 mM Tris-HCl pH = 7.5, 150 mM 5M NaCl, 2 mM 0.5 M EDTA, 1 mM 0.5 M EGTA, 0.5% Sodium Deoxycholate, 0.1% SDS, 1% N-P40) containing protease inhibitor mixture (Cat. No: 11836170001, Roche) using a manual tissue grinder, and lysates were spun n at 10.000 rpm for 10 min at 4°C. Protein concentration was estimated by Bradford reagent (Cat. no.: 39222.03, SERVA), and 10 μg of total protein was prepared for immunoblotting in the final volume of 15 μl. In detail, protein mixtures were incubated at 100°C for 5 min, and they were immediately spun and electrophoresed in SDS-PAGE gel. Proteins were then transferred onto nitrocellulose blotting membrane (GE10600002, Amersham, Germany), and the membranes were incubated in 1% BSA-1‰ Tween20 PBS in 1:1200 rabbit anti-mouse lipocalin-2 antibody (ab63929, Abcam) and 1:1,200 goat anti-mouse actin antibody (sc-1615, Santa Cruz Biotechnology) O/N at 4°C. The next day, the membranes were washed in 1‰ Tween20 PBS followed by incubation with 1:20.000 secondary antibodies (anti-rabbit: 925-68073, LI-COR; anti-goat: 925-32214, LI-COR) in 1% BSA-1‰ Tween20 PBS. The blot was visualized in an Odyssey DLx Imaging System (LI-COR).

Immunohistochemistry

Fixed lung tissues were mounted into paraffin; 4 μm slices were cut and placed on slides. Then, hematoxylin/eosin (H&E) staining was performed as previously described (22). In brief, the slices were deparaffinized at 60°C for 2 h followed by xylene washes and hydrated in gradual ethanol concentrations. The slices were stained against Lcn2 (ab63929, Abcam) in 1:200 concentration. Peroxidase conjugated secondary antibody (4010-05, Southern Biotech) and DAB kit (SK-4100, Vector Laboratories, Inc.) were used to visualize Lcn2 in the lung tissue slices.

ELISA

LCN2 levels were estimated in human and murine BALF using a commercially available ELISA kit (EA100541, OriGene Technologies Inc.), according to the manufacturer's instructions.

In silico analyses

Differential gene expression analysis results produced during Fibromine creation (5) were used for volcano plot creation. Respective boxplots summarize *LCN2* expression in terms of log2 fold change, while depicted datasets had a statistically significant difference between the compared groups (IPF_vs_Ctrl; Bleomycin_vs_Ctrl). Absolute fold change (FC) of at least 1.2 and FDR-corrected $p < 0.05$ were selected as thresholds for differential expression. The correlation of *LCN2* expression values with those of spirometry measurements was examined using Spearman's correlation test. An absolute rho value of at least 0.5 was considered the threshold of a strong relationship, while a $p < 0.05$ was required for a relationship to be deemed significant. Visualizations were performed using packages ggplot2 (v.3.3.5) and ggrepel (v.0.9.1).

Single-cell RNA-seq data were found at GSE136831 (24), (GSE135893_ILD_annotated_fullsize.rds.gz) (25), as well as in the GitHub repositories (26, 27). All downstream described processes were completed with the R package Seurat (4.0.5) (28, 29).

For GSE136831, already filtered data were log normalized using a scaling factor of ten thousand (*NormalizeData*), and highly variable features (HVG) were retrieved (*FindVariableFeatures*) and scaled (*ScaleData*). Linear dimensionality reduction (PCA) (*RunPCA*) was followed by the creation of the closest neighborhood graph (*FindNeighbors*) using the first 7 principal components, as proposed by the median of all findPC methods output (30). Clusters were identified using Louvain clustering with a resolution of 1.3 (*FindClusters*). Cell typing information provided along with the count data was adopted. Non-linear dimensionality reduction was performed using Uniform Manifold Approximation and Projection (UMAP) (*RunUMAP*). As its name implies, UMAP is a non-linear method for reducing the dimensions of a dataset based on manifold calculation (31). Although not developed for scRNA-seq data *per se*, it is a method of choice for the analysis of such data yielding reproducible results in fast running times (32). Taking into consideration the same number of principal components as above results in a visualization very similar to that of the initial publication. Batch correction of any kind was not performed as proven unnecessary during the original data analysis.

From the GSE135893 object, IPF and control originating cells were maintained, while read counts were log normalized with a scaling factor of ten thousands before any downstream analysis (*NormalizeData* function). Mayr et al. lung dataset object was analyzed for IPF and control cells only, while barcodes that were assigned an "empty" cell type were removed. Log normalization with a 10,000 scaling was applied (*NormalizeData* function). Similarly, barcodes assigned a "NA" or "Low-Quality Cells" cell type were removed from the Strunz et al. whole lung dataset object before downstream analysis.

For all single-cell data differential expression analyses, the Wilcoxon rank-sum test was applied (*FindMarkers*), while an absolute FC of at least 1.2 and a Bonferroni-corrected $p < 0.05$ were set as significant thresholds.

Statistics

Statistical analysis was performed using the GraphPad Prism software (v8.0, GraphPad, San Diego, California, USA), as explicitly indicated in each figure legend.

Results

Increased *LCN2* expression in IPF patients negatively correlates with respiratory functions

To explore a possible involvement of *LCN2* in IPF, *LCN2* expression was interrogated in IPF transcriptomic datasets (Supplementary Table S1), sourced from Fibromine (www.fibromine.com), a database and data mining tool for target discovery in IPF (5). Using absolute fold change of at least 1.2 and FDR-corrected $p < 0.05$, widely accepted thresholds for the selection of differentially expressed genes, the expression of *LCN2* was found to be significantly increased in most datasets interrogating gene expression in the lungs of IPF patients in comparison with control individuals (Supplementary Table S1; Figure 1A). Indicatively, *LNC2* presented with a natural scale fold change of 2.3, 4.5, and 3.9 in three of the largest ones (Figure 1B; Supplementary Table S1). Importantly, *LCN2* expression negatively correlated with the respiratory functions (DLCO, FVC, and FEV1) of IPF patients in the same datasets (Figure 1C; Supplementary Figures S1A, B).

To examine the cell specificity of *LCN2* expression in fibrotic lungs, we re-analyzed data from three publicly available single-cell RNA seq (scRNAseq) datasets of human origin (Supplementary Table S2) (24–26). *LCN2* was found in all three data collections (Figure 1D; Supplementary Figures S1C, D), primarily expressed in epithelial cells, including goblet, ciliated, basal, club, and aberrant basaloid cells (Figures 1D, E; Supplementary Figures S1E, F). Comparing cell types between phenotypes (IPF and control), *LCN2* was found over-expressed mostly in alveolar type 1 and 2 cells (AT1 and AT2) (Figure 1F; Supplementary Figures S1G, H). However, *LCN2* expression from neutrophils, as shown in other pathological contexts summarized by the CellMarker2.0 database (33) (Supplementary Table S3), cannot be excluded, given the low representation of neutrophils in human IPF scRNAseq datasets.

To validate the *in silico* findings, we estimated LCN2 levels in the bronchoalveolar lavage fluid of 26 IPF patients (Table 1), with a commercially available ELISA kit. As shown *in silico* for mRNA levels in the lung tissue of IPF patients (Figure 1), LCN2 BALF levels of IPF patients negatively correlated with their respiratory functions (FEV1/FVC, TLCO, and KCO) (Table 1; Figures 2B, C).

Therefore, IPF is associated with increased *LCN2* expression, predominantly in pulmonary epithelial cells, negatively correlating with impaired lung functions.

Increased *Lcn2* expression upon pulmonary inflammation and fibrosis in mice

To examine *Lcn2* expression in the lungs of mice post-bleomycin (BLM)-induced pulmonary inflammation and fibrosis, a widely used animal model of pulmonary fibrosis (4, 22, 34), we mined the relative transcriptomic datasets from Fibromine (Supplementary Table S1), as in IPF patients. *Lcn2* was found over-expressed in most datasets when comparing the fibrotic phase of the model to control samples (Supplementary Table S1; Figure 3A), with indicative natural scale fold change scores of 5.4, 3, and 4.2 (Figure 3B). Moreover, re-analysis of a publicly available murine scRNAseq dataset (Supplementary Table S2) (27) indicated that, as in the human lung, *Lcn2* is highly expressed mainly by epithelial cells, as well as neutrophils (Figures 3C, D). More specifically, classical and activated AT2 cells, neutrophils, goblet, and activated mesothelial cells, as well as lymphatic endothelial cells (LECs), were marked by *Lcn2* expression (Figures 3C, D). Similar results were revealed from the CellMarker2.0 database query (33), where *Lcn2* was defined as a marker of murine lung neutrophils and AT2 cells (Supplementary Table S3). Importantly, the highest *Lcn2* expression was detected during the earlier inflammatory phase of the model (Figures 3E–G), which is characterized by epithelial damage and neutrophilic inflammation.

To validate the *in silico* mouse results, we examined *Lcn2* expression during the development of BLM-induced pulmonary inflammation and fibrosis. To this end, BLM (0.8 U/Kg) was administered by oropharyngeal aspiration to 8–10-wk-old C57Bl6 mice, which were then euthanized 3, 7, and 14 days post-BLM administration, timepoints corresponding to the inflammatory (3, 7) and fibrotic (14) phases of the disease (which resolves at 21 d; not shown). As expected, BLM administration resulted in the vascular leak and pulmonary edema, as indicated by the total protein concentration of the bronchoalveolar lavage fluid (BALF), determined with the Bradford assay (Figure 4A), as well as in inflammation, as indicated by the inflammatory cells in the (BALF) (Figure 4B). Soluble collagen levels in the BALF, as determined with the Direct Red assay, were also found gradually increasing in fibrotic lungs (Figure 1C). The H&E staining performed in lung sections of murine lungs post-BLM administration revealed the increasing presence of peribronchiolar and parenchymal fibrotic regions (Figure 4D). Moreover, the development of pulmonary fibrosis was reflected in the impairment of respiratory functions, as quantified with FlexiVent (Figures 4E–J). The development of BLM-induced pulmonary fibrosis and the impairment of respiratory functions were associated with increased lung tissue *Lcn2* mRNA expression, as detected with Q-RT-PCR, in all phases of the disease, but especially in the acute inflammatory phase (Figure 4K). A similar profile was detected in the Lcn2 protein concentrations in the BALF (Figure 4L), while the increased concentration in the serum of the same mice was only detected in the acute phase. To possibly correlate Lcn2 levels with immune cell populations in the BALF post-BLM administration, a multicolor FACS analysis was performed, quantifying 10 distinct immune cell types; the employed gating strategy is described in detail in Supplementary Figure S2. FACS indicated an abundance of neutrophils in the acute inflammatory phase post-BLM (Figure 4O), at the peak of Lcn2 expression. However, increased Lcn2 protein levels could still be detected in the fibrotic lung tissue 14 d post-BLM (Figures 4P, Q), while Lcn2 immunostaining was localized in epithelial cells and fibrotic regions, constitutive expression was detected from the bronchial epithelium (Figure 4R).

To confirm Lcn2 as a marker of pulmonary inflammation, we then examined Lcn2 levels upon LPS-induced acute lung injury (ALI). LPS was administered (5 mL; 2 mg/mL) via a nebulizer (flowrate 4 lt/min) to WT C57Bl6 mice, that were euthanized 24 h later. The development of ALI, as indicated by the vascular leak (Figure 5A) and the infiltration of inflammatory cells (Figure 5B;

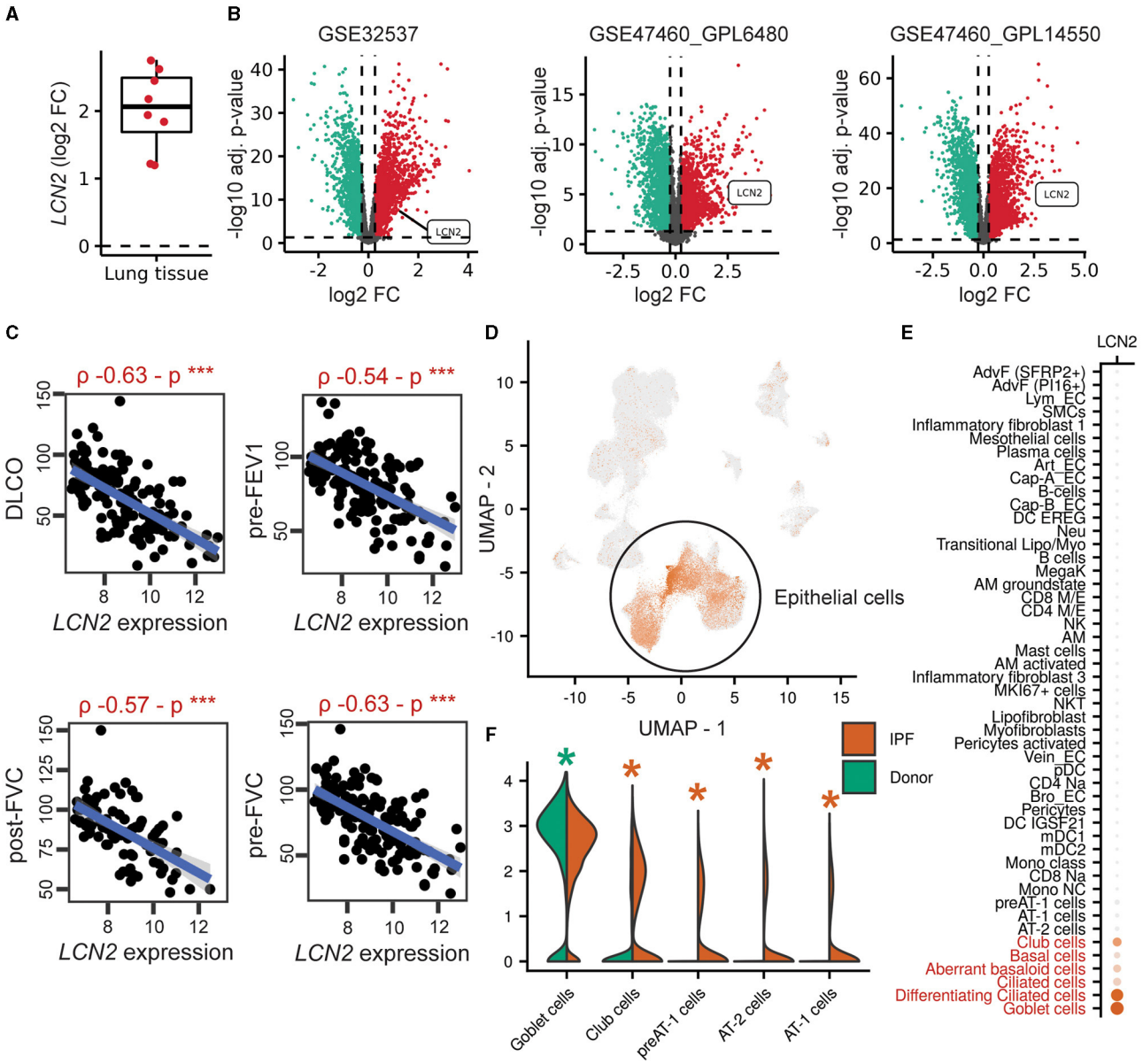

FIGURE 1
Increased *LCN2* mRNA expression in lung epithelial cells of IPF patients, negatively correlating with their respiratory functions. **(A)** Mean differential *LCN2* mRNA expression in IPF patients' cohorts/datasets, detailed in Supplementary Table S1. **(B)** Volcano plots of differentially expressed genes (FC > 1.2; FDR adjusted $p < 0.05$) in the three largest transcriptomics datasets of **(A)** interrogating the expression of 115/44, 28/15, 84/75 IPF patients and controls, respectively (Supplementary Table S1). **(C)** Spearman's correlation plots of *LCN2* expression with spirometry measurements from the GSE47460_GPL14550 cohort (***$p < 0.01$). **(D)** Dimensionality reduction plot localizing *LCN2* expression in pulmonary epithelial cells originating from 4 IPF patients and 6 controls (26). **(E)** Dot plot of the same dataset depicting cell type-specific *LCN2* expression. The Wilcoxon rank-sum test comparing each cell type with the rest validated *LCN2* as a marker gene of the cell types marked in red font (FC > 1.2; Bonferroni adjusted $p < 0.05$). **(F)** Per cell type differential expression analysis between cells of different phenotype (IPF vs. control origin) using the Wilcoxon rank-sum test (*FC ≥ 1.2; Bonferroni-corrected $p < 0.05$; *upregulated in IPF; *downregulated in IPF).

mostly neutrophils; data not shown) (35), was associated with increased *Lcn2* mRNA (Figure 5C) and protein levels (Figures 5D, E) in the lung tissue. The increased Lcn2 expression upon ALI was also reflected in the BALF and sera of the same mice (Figures 5F, G).

Therefore, Lcn2 is a marker of pulmonary inflammation in mice, correlating with epithelial damage and neutrophilic infiltration.

Genetic dissection of the role of Lcn2 in pulmonary inflammation and fibrosis in mice

To dissect a possible role for Lcn2 in pulmonary inflammation and fibrosis, we then investigated the effects of BLM-induced pulmonary inflammation and fibrosis on *Lcn2* ubiquitous knockout mice (KO); the lack of Lcn2 expression in KO mice was verified

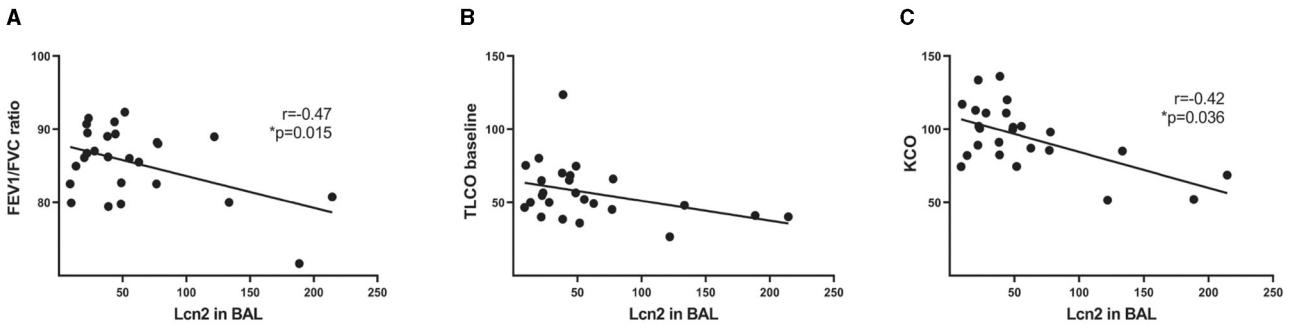

FIGURE 2
LCN2 BALF levels of IPF patients negatively correlate with their respiratory functions. **(A–C)** Spearman's correlation plots of LCN2 levels, as measured using a commercially available ELISA, with the ratio of forced expiratory volume (FEV)/forced vital capacity (FVC) **(A)**, with a transfer capacity of the lung for the uptake of carbon monoxide (TLCO) **(B)** and with carbon monoxide transfer coefficient (KCO) **(C)**. Statistical significance was assessed with Spearman's r = −0.47, −0.42 as indicated; * denotes $p < 0.05$.

using Q-RT-PCR and Western blot analysis (Figures 6A, B). The BLM-induced weight loss, an indicator of overall systemic health, did not reach statistical significance in *Lcn2*$^{-/-}$ mice (Figure 6C); however, no statistically significant changes were detected in vascular leak (Figure 6D), inflammation (Figure 6E), or soluble BALF collagen (Figure 6F). Accordingly, no major statistically significant differences were detected in the associated distortion of lung architecture (Figures 6G, H). However, BLM-induced impairment of respiratory functions did not reach statistical significance in *Lcn2*$^{-/-}$ mice (Figures 6I, J), suggesting again, as the human data, a possible negative correlation of Lcn2 expression with respiratory functions.

Given the suggested role of Lcn2 in metabolic disorders and obesity (36) and the correlation between IPF and obesity in patients (37), the effect of obesity-driven microbiome changes in the lungs (38), as well as the suggested role of Lcn2 in iron sequestration and microbiome regulation, we next investigated the role of Lcn2 in the pathogenesis of pulmonary fibrosis in obese mice, following the high-fat diet (HFD) feeding for 13 weeks, in comparison with mice fed a matched control diet. No statistically significant changes in disease severity were observed either, although a clear trend of disease attenuation was observed (Supplementary Figures S3A–D), as opposed to lean mice.

Moreover, given the increased *Lcn2* expression in the acute phase post-BLM administration (Figure 4), as well as following LPS-induced ALI (Figure 5), we then examined a possible role of Lcn2 in acute inflammation by administering LPS in *Lcn2*$^{-/-}$ and control wt mice. *Lcn2*$^{-/-}$ mice presented with increased pulmonary edema (Figure 7A), but no significant changes in inflammation (Figures 7B, C).

Therefore, despite observed trends in *Lcn2*$^{-/-}$ mice, no solid conclusions on the role of Lcn2 in pulmonary inflammation and fibrosis could be drawn upon disease modeling in mice, in these settings.

Discussion

In this report, increased *LCN2* mRNA expression has been detected *in silico* in most available transcriptomics datasets at Fibromine.com (Figure 1; Supplementary Table S1). The *in silico* approach, given the availability of datasets in Fibromine, emerges as a valuable surrogate tool for the identification of the expression levels of genes under investigation. Moreover, given the multiple available datasets/human samples, the method is more practical and valuable than the usual practice, i.e., individual RT-PCRs in a limited number of IPF samples.

LCN2 mRNA expression levels in IPF patients negatively correlated with respiratory functions (Figure 1); accordingly, LCN2 BALF levels negatively correlated with patients' respiratory functions (FEV1/FVC, TLCO, and KCO) of a cohort (n=26) of IPF patients (Figure 2), in agreement with a previous study (17). However, much larger clinical studies will be needed to possibly associate LCN2 expression levels, in both sera and BALF, with respiratory functions and other specific pathophysiological disease attributes. A meta-analysis of publicly available scRNAseq datasets indicated the lung epithelium as the major source of LCN2 in the fibrotic lung (Figure 1), as previously shown with immunocytochemistry (17). Recurrent epithelial damage is considered the initiating insult of IPF pathogenesis, and acute exacerbation of IPF is characterized by increased alveolar epithelial cell injury, suggesting that future studies on LCN2 and IPF should include the evaluation of LCN2 levels in patients with acute IPF exacerbation and the correlation with other epithelial injury markers.

Similar results were obtained in BLM-induced pulmonary fibrosis in mice (Figures 3, 4), further indicating higher *Lcn2* expression in the acute phase of the disease, following BLM-induced epithelial damage and correlating with neutrophilic inflammation. Lcn2 levels declined at the fibrotic phase, although remained higher than controls, as is the case for various inflammatory markers, e.g., TNF (39). Moreover, and in agreement with an acute role for Lcn2, neutrophilic infiltration upon LPS-induced ALI was also correlated with higher Lcn2 expression (Figure 5).

However, despite the increased Lcn2 expression upon BLM- or LPS-induced lung damage, no statistically significant changes were observed upon BLM or LPS administration to *Lcn2*$^{-/-}$ mice (Figures 6, 7; Supplementary Figure S3), suggesting that either Lcn2 does not have a major pathogenic role or Lcn-2 can

10.3389/fmed.2023.1195501

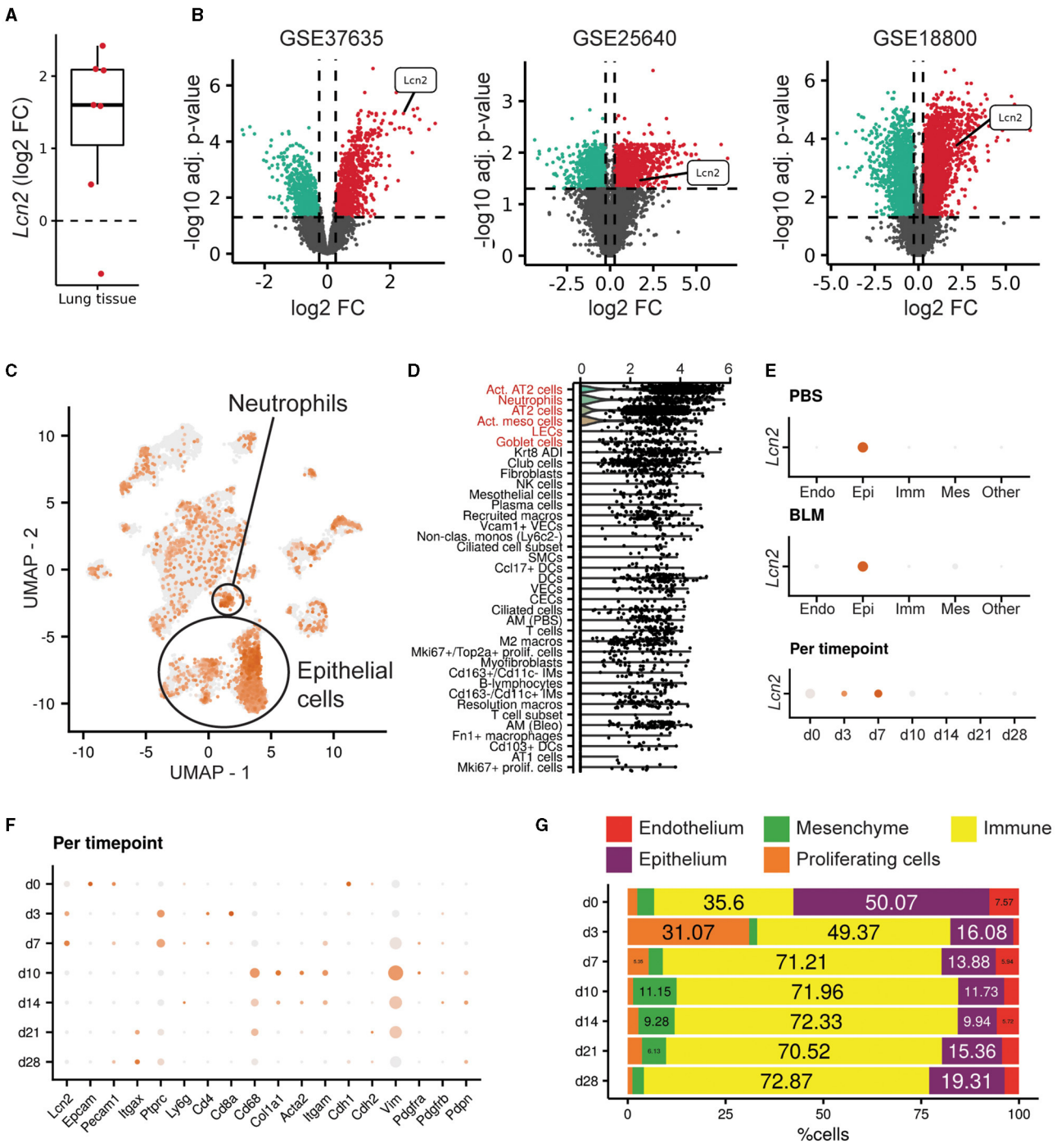

FIGURE 3
Increased *Lcn2* expression in mouse lungs post-BLM-induced pulmonary fibrosis. **(A)** Mean differential *Lcn2* mRNA expression in different transcriptomics BLM datasets (Supplementary Table S1). **(B)** Volcano plots of the three major datasets of **(A)**. **(C)** Feature plot showing *Lcn2* expression in the mouse lung (27). **(D)** Dot plot revealing the cell type expression pattern of *Lcn2* (in decreasing order of importance). The Wilcoxon rank-sum test comparing each cell type with the rest validated *Lcn2* as a marker gene of the cells types marked in red font (FC > 1.2; Bonferroni adjusted $p < 0.05$). **(E)** Separate examination of the control (PBS) and fibrotic (BLM) cells further supports the epithelial origin of *Lcn2*. **(F)** Per timepoint examination of cell population markers. **(G)** Bar plot depicting the changes in the mouse lung major cell populations, as defined by scRNAseq analysis clustering and cell typing, across timepoints of BLM administration.

have different roles in different cell populations, masked in the ubiquitous knockout mice, and that a cell-specific Lcn2 deletion could be more informative. Moreover, it is also possible that a pathogenic role for Lcn2 cannot be efficiently dissected in animal models, as has been shown for many other genes (4). In this context, a very possible role of Lcn2 in iron sequestration and microbiome regulation (18) cannot be likely examined in modeled mice, given their sterile and controlled living conditions, as well as due to the

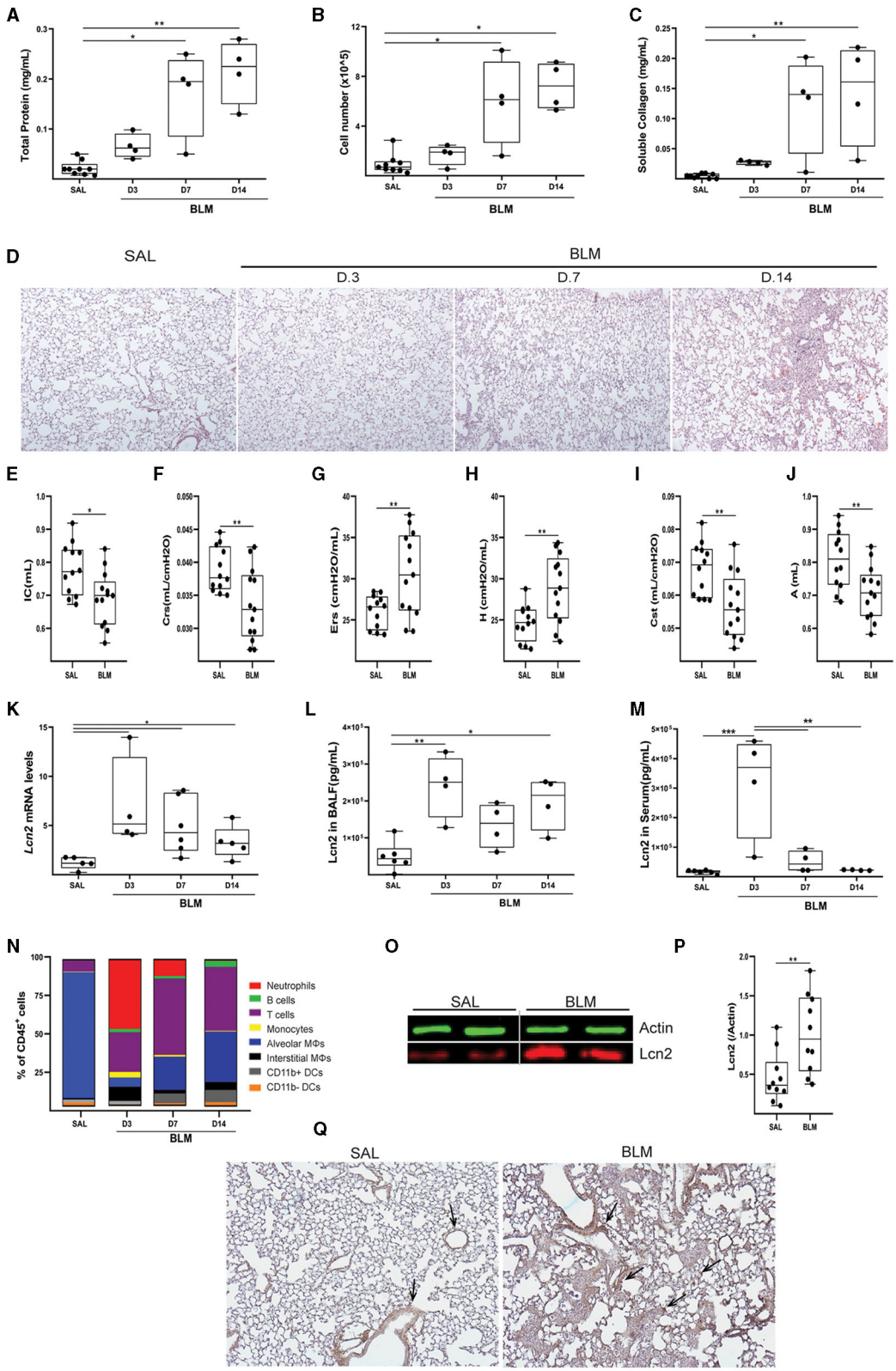

FIGURE 4
Increased *Lcn2* expression in mouse lungs during the development of BLM-induced pulmonary inflammation and fibrosis. **(A)** Total protein concentration in BALFs, as determined using the Bradford assay. **(B)** Inflammatory cell numbers in BALFs, as counted with a hemacytometer. **(C)** Soluble collagen levels in the BALFs as detected with the Direct Red assay. Statistical significance was assessed with one-way ANOVA; */** denote $p < 0.05/0.01$ respectively. **(D)** Representative images from H&E-stained lung sections of murine lungs at 3, 7, and 14 d post-BLM administration (×10). **(E–J)** Respiratory functions were measured with FlexiVent, 14 days post-BLM; mean respiratory system compliance (Crs); mean respiratory system elastance (Ers); mean tissue elastance (H); mean static lung compliance (Cst); mean total lung capacity (A). Cumulative results from three

(Continued)

FIGURE 4 (Continued)
independent experiments; statistical significance was assessed using the Mann–Whitney test; */** denote $p < 0.05/0.01$, respectively. **(K)** *Lcn2* mRNA expression was interrogated using Q-RT-PCR; Values were normalized over the expression of the housekeeping gene *B2m* and presented as fold change over control. **(L, M)** Lcn2 concentration in BALF **(L)** and serum **(M)** of mice at 3, 7, and 14 d post-BLM administration. Lcn2 levels were measured using a commercially available ELISA kit; Statistical significance was assessed with one-way ANOVA, */**/*** denote $p < 0.05/0.01/0.001$ respectively. **(N)** Bar plot showing the percentage of immune cell populations in the murine lung post-BLM; the employed gating strategy is described in Supplementary Figure S2. **(O)** Representative Western blot of Lcn2 expression (red) in fibrotic lungs, 14 d post-BLM. **(P)** Densitometry analysis of Lcn2 expression, normalized to the expression of Actin (green); cumulative result from two independent experiments; statistical significance was assessed with unpaired *t*-test; ** denotes $p < 0.01$. **(Q)** Representative images of two independent experiments, from immunohistochemistry for Lcn2 in control (SAL) and fibrotic (BLM) murine lung tissue (×10).

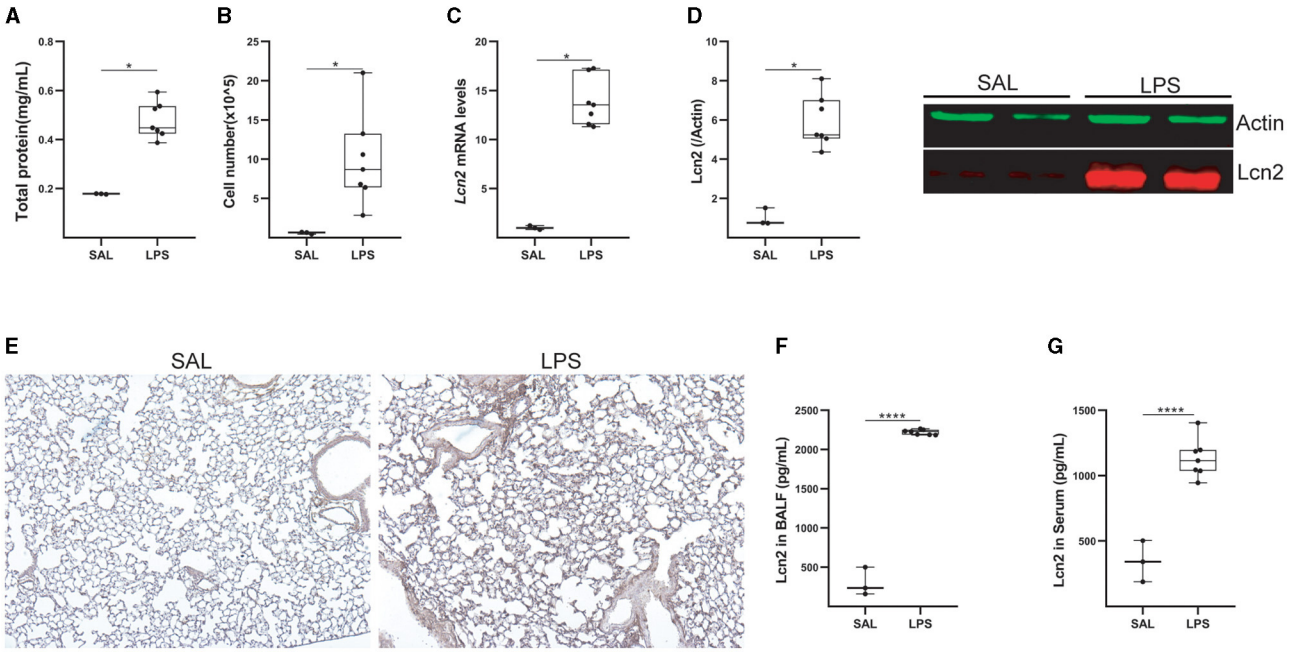

FIGURE 5
Lcn2 expression is upregulated during LPS-induced Acute Lung Injury (ALI). **(A)** Total protein concentration in BALF, as determined using the Bradford assay. **(B)** Inflammatory cell numbers in BALF, from saline and LPS-treated mice, as counted with a hemacytometer. **(C)** *Lcn2* mRNA expression was interrogated with Q-RT-PCR; Values were normalized over the expression of the housekeeping gene *B2m* and presented as fold change over control; representative results from three independent experiments. **(D)** Western blot of Lcn2 expression (red) in lungs from mice with LPS-induced ALI, followed by densitometry analysis of Lcn2 expression, normalized to the expression of Actin (green). **(E)** Representative images from immunohistochemistry for Lcn2 in lungs from control (SAL) and LPS-treated mice (×10). **(F, G)** Lcn2 levels in BALF **(F)** and serum **(G)** of mice were estimated using ELISA; statistical significance was assessed using the Mann–Whitney test; */**** denote $p < 0.05/0.0001$.

species populating the lung that are not amenable to the suggested bacteriostatic functions of Lcn2 (38, 40). However, a role for LCN2 in microbiome regulation in humans remains likely and should be pursued in future clinical studies, especially since increased airway microbiota has been associated with a more rapid disease progression and a higher risk of mortality across different patient cohorts and quantification platforms (20, 41, 42).

Moreover, microbiome differences in different animal houses could explain the contradictory results on the role of Lcn2 in inflammation in mice. For example, Lcn2 has been suggested to mediate the recruitment of neutrophils and thus to stimulate pro-inflammatory signaling; however, anti-inflammatory effects have also been suggested, including M2 polarization and T_{Regs} expansion (10). We reported here no major role for Lcn2 in LPS-induced ALI, while it was recently reported that *Lcn2*$^{-/-}$ mice had relatively increased survival than control mice following intratracheal administration of LPS (43); the contradiction could be due to experimental design, dose, and species of administered LPS, as well as the local microbiome of the animal houses. In the same context, systemic administration of LPS in *Lcn2*$^{-/-}$ mice was reported to result in exacerbated neuroinflammatory responses (44), although an opposite role in neuroinflammation has been also suggested promoting macrophage M1 polarization (45). However, in the lungs, LCN2 was reported to deactivate macrophages resulting in impaired immune responses following pneumococcal pneumonia (46).

As an acute phase response protein, secreted by epithelial cells upon damage, and/or infiltrating neutrophils, it is conceivable that LCN2 may contribute to chronic damage responses via the lung epithelium in IPF patients through the amplification of neutrophil recruitment. Increased neutrophils were detected in the IPF cohort examined here (Table 1; $p = 0.038$), while BAL neutrophilia has been proposed as an independent predictor of early mortality in IPF patients (47).

10.3389/fmed.2023.1195501

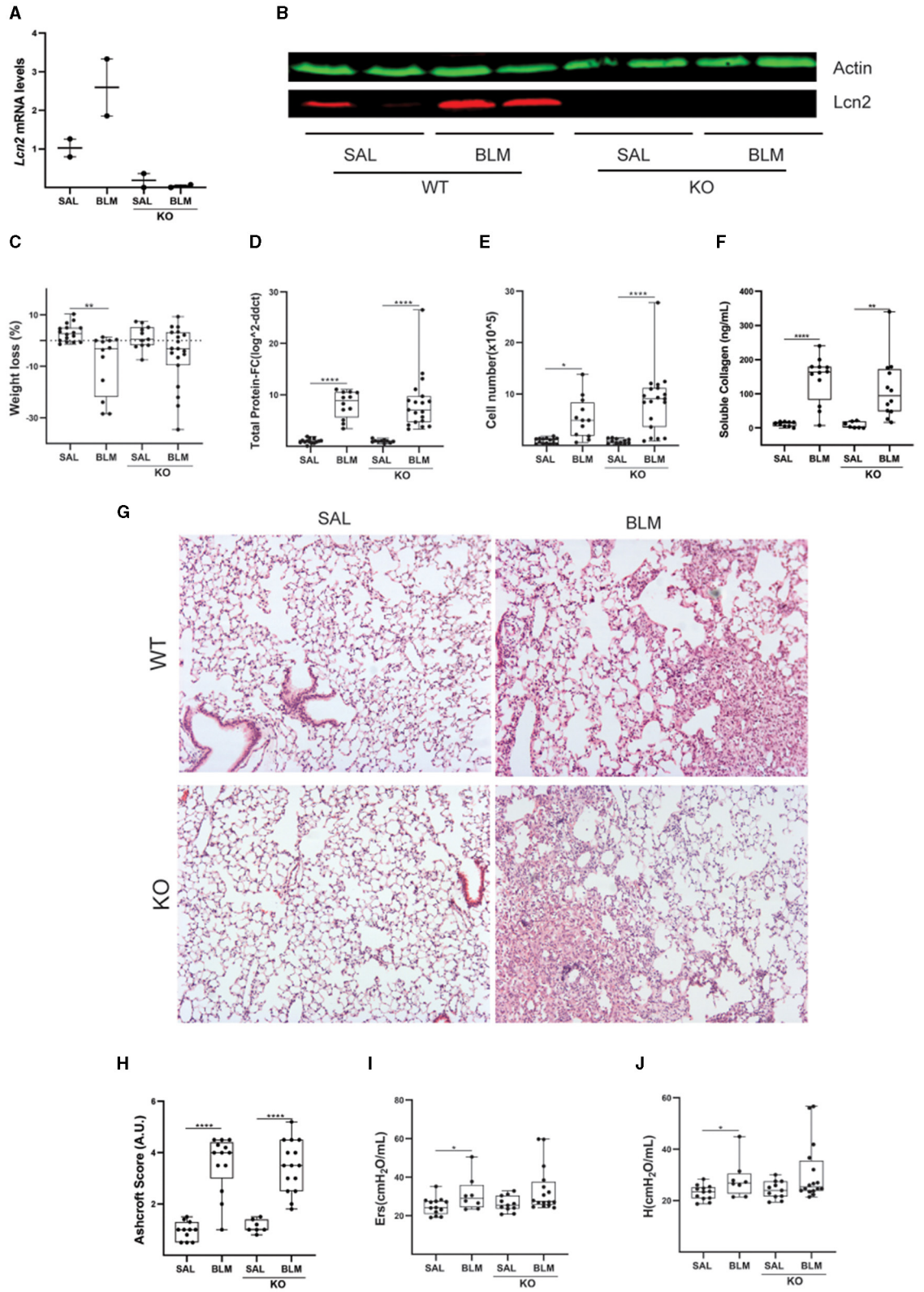

FIGURE 6
Lcn2 genetic deficiency has minor effects in bleomycin (BLM)-induced pulmonary inflammation and fibrosis. **(A)** *Lcn2* mRNA expression was interrogated with Q-RT-PCR; Values were normalized over the expression of the housekeeping gene *B2m* and presented as fold change over control; **(B)** Representative Western blot of Lcn2 expression (red) in lungs from WT and KO mice treated with BLM confirming the global depletion of *Lcn2* in KO mice. **(C)** Weight loss post-BLM administration. **(D)** Total protein concentration in BALFs, as determined with the Bradford assay. **(E)** Inflammatory cell numbers in BALFs, as counted with a hemacytometer. **(F)** Soluble collagen levels in the BALFs as detected with the Direct Red assay. **(G)** Representative H&E-stained lung sections (×10). **(H)** Ashcroft scoring of disease severity. **(I, J)** Indicated respiratory functions were measured with FlexiVent; statistical significance was assessed with one-way ANOVA; */**/**** denotes $p < 0.05/0.01/0.0001$.

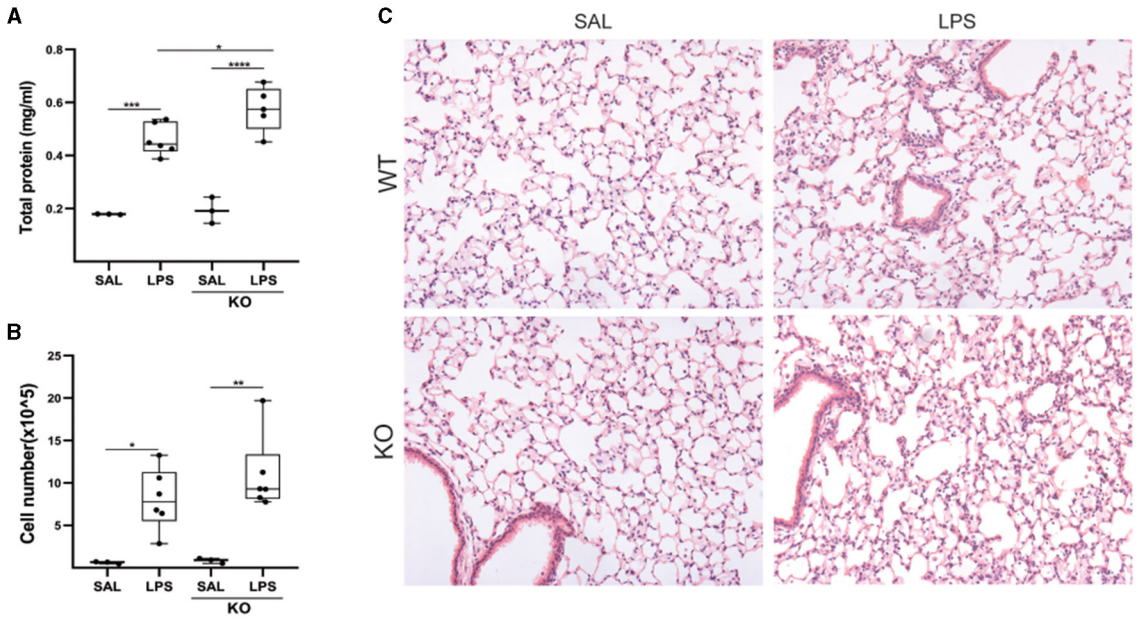

FIGURE 7
Lcn2 genetic deficiency has minor effects on LPS-induced pulmonary inflammation. **(A)** Total protein concentration in BALFs, as determined with the Bradford assay. **(B)** Inflammatory cell numbers in BALFs, as counted with a hemacytometer; **(C)** Representative H&E-stained sections of murine lungs of WT and Lcn2 KO mice (×10); statistical significance was assessed with one-way ANOVA; */**/***/**** denotes $p < 0.05/0.01/0.001/0.0001$.

Additionally, a high neutrophil to lymphocyte ratio (NLR) as measured from complete blood counts has also been associated with increased mortality in IPF (48). LCN2 has been shown to promote the formation of neutrophil extracellular traps (NETs) (49), which have been implicated in the pathogenesis of several diseases including IPF (50). In skin psoriasis, the amplification loop of LCN2 parallel to neutrophil-produced extracellular NETs was shown to participate in the enhancement and persistence of the local inflammatory response (51). The proinflammatory activity of NETs and LCN2 induction in psoriasis was suggested to be dependent on TLR4/IL-36R crosstalk and MyD88/nuclear factor-kappa B (NF-kB) downstream signaling (51).

Overall, although the possible role for LCN2 in IPF pathogenesis remains obscure, the acute increase in Lcn2 expression following both LPS-induced ALI and BLM-induced pulmonary inflammation and fibrosis suggests that Lcn2 is an acute phase protein of lung damage in mice, as previously suggested for acute kidney injury (9) and acute exacerbation of cystic fibrosis (52), correlating with epithelial damage and neutrophilic infiltration. Moreover, the increased LCN2 mRNA levels detected in IPF patients suggest that LCN2 levels can be used as surrogate biomarkers of pulmonary inflammation and a possible indicator of compromised pulmonary functions, urging for larger studies.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committees of the University Hospital of Heraklion (IRB numbers: 1045 and 17030). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. The animal study was approved by Institutional Animal Ethical Committee (IAEC) of Biomedical Sciences Research Center Alexander Fleming (#373/375), as well as the Veterinary Service and Fishery Department of the local governmental prefecture (#5508).

Author contributions

AG performed most presented experiments and analyzed the relative data, assisted by IB, PK, KT, TK, and AT. KN and SG performed FACS. ET performed human ELISAs. DF performed *in silico* data re-analysis and supervised all statistical analyses. KA led the relative clinical protocol and provided all human samples. AG, DF, ET, and VA wrote the article, which was critically read by all co-authors. All authors contributed to the article and approved the submitted version.

Funding

AG was supported by a fellowship from the Hellenic Foundation for Research and Innovation (#789). The research

was further partly supported through the Hellenic Foundation for Research and Innovation (HFRI) under the 2nd Call for HFRI Research Projects to support Faculty Members and Researchers (#3565 to VA). The funders had no role in the design of the study, collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1195501/full#supplementary-material

References

1. Martinez FJ, Collard HR, Pardo A, Raghu G, Richeldi L, Selman M, et al. Idiopathic pulmonary fibrosis. *Nat Rev Dis Primers.* (2017) 3:17074. doi: 10.1038/nrdp.2017.74

2. Raghu G, Remy-Jardin M, Myers JL, Richeldi L, Ryerson CJ, Lederer DJ, et al. Diagnosis of idiopathic pulmonary fibrosis. An official ats/ers/jrs/alat clinical practice guideline. *Am J Respir Crit Care Med.* (2018) 198:e44–68. doi: 10.1164/rccm.201807-1255ST

3. Vukmirovic M, Kaminski N. Impact of transcriptomics on our understanding of pulmonary fibrosis. *Front Med (Lausanne).* (2018) 5:87. doi: 10.3389/fmed.2018.00087

4. Mouratis MA, Aidinis V. Modeling pulmonary fibrosis with bleomycin. *Curr Opin Pulm Med.* (2011) 17:355–61. doi: 10.1097/MCP.0b013e328349ac2b

5. Fanidis D, Moulos P, Aidinis V. Fibromine is a multi-omics database and mining tool for target discovery in pulmonary fibrosis. *Sci Rep.* (2021) 11:21712. doi: 10.1038/s41598-021-01069-w

6. Fanidis D, Pezoulas VC, Fotiadis D, Aidinis V. An explainable machine learning-driven proposal of pulmonary fibrosis biomarkers. *Comput Struct Biotechnol J.* (2023) 21:2305–15. doi: 10.1016/j.csbj.2023.03.043

7. Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. Isolation primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. *J Biol Chem.* (1993) 268:10425–32. doi: 10.1016/S0021-9258(18)82217-7

8. Flower DR, North AC, Sansom EC. The lipocalin protein family: structural and sequence overview. *Biochim Biophys Acta.* (2000) 1482:9–24. doi: 10.1016/S0167-4838(00)00148-5

9. Devarajan P. Neutrophil gelatinase-associated lipocalin: a promising biomarker for human acute kidney injury. *Biomark Med.* (2010) 4:265–80. doi: 10.2217/bmm.10.12

10. Guardado S, Ojeda-Juárez D, Kaul M, Nordgren MT. Comprehensive review of lipocalin 2-mediated effects in lung inflammation. *Am J Physiol-Lung Cell Mol Physiol.* (2021) 321:L726–33. doi: 10.1152/ajplung.00080.2021

11. Betsuyaku T, Nishimura M, Takeyabu K, Tanino M, Venge P, Xu S, et al. Neutrophil granule proteins in bronchoalveolar lavage fluid from subjects with subclinical emphysema. *Am J Respir Crit Care Med.* (1999) 159:1985–91. doi: 10.1164/ajrccm.159.6.9809043

12. Eagan TM, Damås JK, Ueland T, Voll-Aanerud M, Mollnes TE, Hardie JA, et al. Neutrophil gelatinase-associated lipocalin: a biomarker in COPD. *Chest.* (2010) 138:888–95. doi: 10.1378/chest.09-2718

13. Treekitkarnmongkol W, Hassane M, Sinjab A, Chang K, Hara K, Rahal Z, et al. Augmented lipocalin-2 is associated with COPD and counteracts lung adenocarcinoma development. *Am J Respir Crit Care Med.* (2020). doi: 10.1164/rccm.202004-1079OC

14. Kangelaris KN, Prakash A, Liu KD, Aouizerat B, Woodruff PG, Erle DJ, et al. Increased expression of neutrophil-related genes in patients with early sepsis-induced ARDS. *Am J Physiol Lung Cell Mol Physiol.* (2015) 308:L1102–1113. doi: 10.1152/ajplung.00380.2014

15. Huang Z, Li H, Liu S, Jia J, Zheng Y, Cao B. Identification of neutrophil-related factor LCN2 for predicting severity of patients with influenza A virus and SARS-CoV-2 infection. *Front Microbiol.* (2022) 13:854172. doi: 10.3389/fmicb.2022.854172

16. Ntatsoulis K, Karampitsakos T, Tsitoura E, Stylianaki EA, Matralis AN, Tzouvelekis A, et al. Commonalities between ARDS. pulmonary fibrosis and COVID-19: the potential of autotaxin as a therapeutic target. *Front Immunol.* (2021) 12:687397. doi: 10.3389/fimmu.2021.687397

17. Ikezoe K, Handa T, Mori K, Watanabe K, Tanizawa K, Aihara K, et al. Neutrophil gelatinase-associated lipocalin in idiopathic pulmonary fibrosis. *Eur Respir J.* (2014) 43:1807–9. doi: 10.1183/09031936.00192613

18. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. *Nature.* (2004) 432:917–21. doi: 10.1038/nature03104

19. Ali MK, Kim RY, Brown AC, Donovan C, Vanka KS, Mayall JR, et al. Critical role for iron accumulation in the pathogenesis of fibrotic lung disease. *J Pathol.* (2020). doi: 10.1002/path.5401

20. Molyneaux PL, Cox MJ, Willis-Owen SA, Mallia P, Russell KE, Russell AM, et al. The role of bacteria in the pathogenesis and progression of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med.* (2014) 190:906–13. doi: 10.1164/rccm.201403-0541OC

21. Lynch DA, Sverzellati N, Travis WD, Brown KK, Colby TV, Galvin JR, et al. Diagnostic criteria for idiopathic pulmonary fibrosis: a Fleischner Society White Paper. *Lancet Resp Med.* (2018) 6:138–53. doi: 10.1016/S2213-2600(17)30433-2

22. Barbayianni I, Ninou I, Tzouvelekis A, Aidinis V. Bleomycin revisited: a direct comparison of the intratracheal micro-spraying and the oropharyngeal aspiration routes of bleomycin administration in mice. *Front Med (Lausanne).* (2018) 5:269. doi: 10.3389/fmed.2018.00269

23. Mouratis MA, Magkrioti C, Oikonomou N, Katsifa A, Prestwich GD, Kaffe E, et al. Autotaxin and Endotoxin-Induced Acute Lung Injury. *PLoS One.* (2015) 10:e0133619. doi: 10.1371/journal.pone.0133619

24. Adams TS, Schupp JC, Poli S, Ayaub EA, Neumark N, Ahangari F, et al. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci Adv.* (2020) 6:eaba1983. doi: 10.1126/sciadv.aba1983

25. Habermann AC, Gutierrez AJ, Bui LT, Yahn SL, Winters NI, Calvi CL, et al. Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *Sci Adv.* (2020) 6: aba1972. doi: 10.1126/sciadv.aba1972

26. Mayr CH, Simon LM, Leuschner G, Ansari M, Schniering J, Geyer PE, et al. Integrative analysis of cell state changes in lung fibrosis with peripheral protein biomarkers. *EMBO Mol Med.* (2021) 13:e12871. doi: 10.15252/emmm.202012871

27. Strunz M, Simon LM, Ansari M, Kathiriya JJ, Angelidis I, Mayr CH, et al. Alveolar regeneration through a Krt8+ transitional stem cell state that persists in human lung fibrosis. *Nat Commun.* (2020) 11:3559. doi: 10.1038/s41467-020-17358-3

28. Stuart T, Butler A, Hoffman P, Hafemeister C, Papalexi E, Mauck WM, et al. Comprehensive integration of single-cell data. *Cell.* (2019) 177:1888–902.e1821. doi: 10.1016/j.cell.2019.05.031

29. Hao Y, Hao S, Andersen-Nissen E, Mauck WM, Zheng S, Butler A, et al. Integrated analysis of multimodal single-cell data. *Cell.* (2021) 184:3573–87.e3529. doi: 10.1016/j.cell.2021.04.048

30. Zhuang H, Wang H, Ji Z. findPC: An R package to automatically select the number of principal components in single-cell analysis. *Bioinformatics.* (2022) 38:2949–51. doi: 10.1093/bioinformatics/btac235

31. McInnes L, John H, James M. UMAP: uniform manifold approximation and projection for dimension reduction. *arXiv*. (2020) 1802.03426. doi: 10.48550/arXiv.1802.03426

32. Becht E, McInnes L, Healy J, Dutertre C-A, Kwok IWH, Ng LG, et al. Dimensionality reduction for visualizing single-cell data using UMAP. *Nat Biotechnol.* (2019) 37:38–44. doi: 10.1038/nbt.4314

33. Hu C, Li T, Xu Y, Zhang X, Li F, Bai J, et al. CellMarker 2.0: an updated database of manually curated cell markers in human/mouse and web tools based on scRNA-seq data. *Nucleic Acids Res.* (2023) 51:D870–D876. doi: 10.1093/nar/gkac947

34. Tashiro J, Rubio GA, Limper AH, Williams K, Elliot SJ, Ninou I, et al. Exploring animal models that resemble idiopathic pulmonary fibrosis. *Front Med (Lausanne).* (2017) 4:118. doi: 10.3389/fmed.2017.00118

35. Chen H, Bai C, Wang X. The value of the lipopolysaccharide-induced acute lung injury model in respiratory medicine. *Expert Rev Respir Med.* (2010) 4:773–83. doi: 10.1586/ers.10.71

36. Jaberi SA, Cohen A, D'Souza C, Abdulrazzaq YM, Ojha S, Bastaki S, et al. Lipocalin-2: structure, function, distribution and role in metabolic disorders. *Biomed Pharmacother.* (2021) 142:112002. doi: 10.1016/j.biopha.2021.112002

37. Guo X, Sunil C, Qian G. Obesity the development of lung fibrosis. *Front Pharmacol.* (2021) 12:812166. doi: 10.3389/fphar.2021.812166

38. Galaris A, Fanidis D, Stylianaki EA, Harokopos V, Kalantzi AS, Moulos P, et al. Obesity reshapes the microbial population structure along the gut-liver-lung axis in mice. *Biomedicines*. (2022) 10:2. doi: 10.3390/biomedicines10020494

39. Oikonomou N, Harokopos V, Zalevsky J, Valavanis C, Kotanidou A, Szymkowski DE, et al. Soluble TNF mediates the transition from pulmonary inflammation to fibrosis. *PLoS ONE.* (2006) 1:e108. doi: 10.1371/journal.pone.0000108

40. Xiao X, Yeoh BS, Vijay-Kumar M. Lipocalin 2: an emerging player in iron homeostasis and inflammation. *Annu Rev Nutr.* (2017) 37:103–30. doi: 10.1146/annurev-nutr-071816-064559

41. Han MK, Zhou Y, Murray S, Tayob N, Noth I, Lama VN, et al. Lung microbiome and disease progression in idiopathic pulmonary fibrosis: an analysis of the COMET study. *Lancet Respir Med.* (2014) 2:548–56. doi: 10.1016/S2213-2600(14)70069-4

42. O'Dwyer DN, Ashley SL, Gurczynski SJ, Xia M, Wilke C, Falkowski NR, et al. Lung microbiota contribute to pulmonary inflammation and disease progression in pulmonary fibrosis. *Am J Respir Crit Care Med.* (2019) 199:1127–38. doi: 10.1164/rccm.201809-1650OC

43. An HS, Yoo JW, Jeong JH, Heo M, Hwang SH, Jang HM, et al. Lipocalin-2 promotes acute lung inflammation and oxidative stress by enhancing macrophage iron accumulation. *Int J Biol Sci.* (2023) 19:1163–77. doi: 10.7150/ijbs.79915

44. Kang SS, Ren Y, Liu CC, Kurti A, Baker KE, Bu G, et al. Lipocalin-2 protects the brain during inflammatory conditions. *Mol Psychiatry.* (2018) 23:344–50. doi: 10.1038/mp.2016.243

45. Jang E, Lee S, Kim JH, Kim JH, Seo JW, Lee WH, et al. Secreted protein lipocalin-2 promotes microglial M1 polarization. *FASEB J.* (2013) 27:1176–90. doi: 10.1096/fj.12-222257

46. Warszawska JM, Gawish R, Sharif O, Sigel S, Doninger B, Lakovits K, et al. Lipocalin 2 deactivates macrophages and worsens pneumococcal pneumonia outcomes. *J Clin Invest.* (2013) 123:3363–72. doi: 10.1172/JCI67911

47. Kinder BW, Brown KK, Schwarz MI, Ix JH, Kervitsky A, King TE. Baseline BAL neutrophilia predicts early mortality in idiopathic pulmonary fibrosis. *Chest.* (2008) 133:226–32. doi: 10.1378/chest.07-1948

48. Mikolasch TA, George PM, Sahota J, Nancarrow T, Barratt SL, Woodhead FA, et al. Multi-center evaluation of baseline neutrophil-to-lymphocyte (NLR) ratio as an independent predictor of mortality and clinical risk stratifier in idiopathic pulmonary fibrosis. *EClinicalMedicine.* (2023) 55:101758. doi: 10.1016/j.eclinm.2022.101758

49. Li H, Feng D, Cai Y, Liu Y, Xu M, Xiang X, et al. Hepatocytes and neutrophils cooperatively suppress bacterial infection by differentially regulating lipocalin-2 and neutrophil extracellular traps. *Hepatology.* (2018) 68:1604–20. doi: 10.1002/hep.29919

50. Khawaja AA, Chong DLW, Sahota J, Mikolasch TA, Pericleous C, Ripoll VM, et al. Identification of a novel HIF-1α-α(M)β(2) integrin-NET axis in fibrotic interstitial lung disease. *Front Immunol.* (2020) 11:2190. doi: 10.3389/fimmu.2020.02190

51. Shao S, Fang H, Dang E, Xue K, Zhang J, Li B, et al. Neutrophil extracellular traps promote inflammatory responses in psoriasis via activating epidermal TLR4/IL-36R crosstalk. *Front Immunol.* (2019) 10:746. doi: 10.3389/fimmu.2019.00746

52. Eichler I, Nilsson M, Rath R, Enander I, Venge P, Koller YD. Human neutrophil lipocalin, a highly specific marker for acute exacerbation in cystic fibrosis. *Eur Respir J.* (1999) 14:1145–9. doi: 10.1183/09031936.99.14511459

Frontiers in Medicine

Translating medical research and innovation into improved patient care

A multidisciplinary journal which advances our medical knowledge. It supports the translation of scientific advances into new therapies and diagnostic tools that will improve patient care.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact