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Acute exacerbations of interstitial lung disease: what is the best treatment?

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There is an increasing recognition that patients with various forms of chronic fibrosing interstitial lung diseases (CFILD) are at risk for an acute exacerbation (AE) of their disease that can rapidly lead to death (1). While acute exacerbations of Idiopathic pulmonary fibrosis (AE-IPF) appear to be more common than acute exacerbations for other forms of CFILD and a considerable number of manuscripts have been published on risk factors, pathogenesis and treatments, no randomized and adequately powered clinical trials of therapies for AE-CFILD have been published to date. But because only a relatively small number of patients at any single center develop an AE over time, such a study would be very difficult to perform. Furthermore, funding entities with adequate resources to support a large clinical trial of licensed drugs are reluctant to support an evaluation drugs that are off patent.

Treatment approaches to fibrotic lung diseases and, especially, IPF have evolved considerably over the past two decades. The first clinical practice guideline on the diagnosis and management of patients with IPF (2) suggested that cytotoxic drugs such as azathioprine or cyclophosphamide (CYC) could be useful to diminish or stop the progression of IPF. But these drugs had never been tested in randomized controlled trials that were adequately powered with robust endpoints. But over a decade later a key National Institutes of Health (NIH) sponsored study provided results that went against the assumption that the cytotoxic agent, azathioprine, was beneficial in treating patients with IPF (3). In contrast to the perception that azathioprine in combination with low-dose corticosteroids may be a useful therapy for IPF, treatment with azathioprine was shown by the PANTHER-IPF trial to actually be detrimental to a substantial number of patients and associated with a significantly increased risk of adverse events including hospitalization and death when compared to controls. Additionally, a prospective study of CYC for patients with progressive IPF did not find benefit, and drug-related adverse events occurred in two-thirds of this study cohort (4). Recently updated clinical practice guidelines on the management of patients with IPF do not support the use of cytotoxic drugs for the treatment of IPF (5).

The advent of anti-fibrotic therapy with pirfenidone or nintedanib has had a significant impact on disease progression in IPF, and new data support anti-fibrotic therapy using nintedanib for patients with CFILD (,7). Nonetheless, significant numbers of patients with IPF as well as those with CFILD other than IPF can suffer acute exacerbations of their fibrotic lung disease despite treatment with anti-fibrotic drugs. While many retrospective case series that examined different drug therapies for acute exacerbations of IPF have been published in the literature (8), no therapy other than lung transplantation has been shown to provide long-term survival in a randomized clinical trial. However, despite a lack of clinical trial evidence, current international consensus guidelines make a weak recommendation, based on very low quality evidence, that corticosteroids should be used to treat the majority of patients with

AE-IPF in addition to supportive care (9).

In this issue of the journal Innabi et al. (10) review published literature on the treatment of ILD associated with connective tissue disease (CTD) with CYC and note that significant although modest benefit has been found for patients with ILD associated with systemic sclerosis. They also examined published literature on using CYC to treat acute exacerbations of ILD (AE-ILD) and conclude that cumulative studies, which are predominantly single-center, retrospective, and inadequately powered, do not support significant benefit when using CYC to treat acute exacerbations of IPF or CTD-ILD. Additionally, they note that while consensus criteria have been established that define acute exacerbations of IPF (11), specific criteria for diagnosing acute exacerbations of other non-IPF forms of ILD have yet to be determined, and a standardized approach to treatment of AE-ILD has yet to be established.

Because toxicities associated with CYC therapy are substantial and such therapy must be carefully monitored for adverse events (12), adequately powered, randomized, controlled trials should be conducted to prove whether or not CYC or other pharmacologic therapies provide significant benefit for patients who develop an episode of AE-ILD. A number of registered trials (www.clinicaltrials.gov) are seeking a definitive answer to this question, and the Phase 3 multi-center, double-blind, randomized, placebo-controlled EXAFIP study (NCT02460588) is being conducted in France to evaluate the efficacy of methylprednisolone with or without CYC for treating AE-IPF (13). While a number of other registered Phase 2 or 3 trials are also evaluating therapies for AE-IPF, no trials have been registered for treating acute exacerbations of non-IPF ILD.

Acute exacerbations of IPF can even occur in patients with a limited extent of fibrosis and well-preserved lung function (12), and the widespread acute lung injury that characterizes AE-IPF is an important cause of accelerated disease progression and mortality. Randomized, controlled trials that evaluate novel therapies for acute exacerbations of IPF as well as non-IPF fibrosing ILDs are much needed. In this regard, a recent study by Donahoe et al. (14) that sought to reduce autoantibodies via a combination of plasma exchange, administration of rituximab, and intravenous immunoglobulin showed that some patients with AE-IPF had prolonged survival responses. A better understanding of the pathogenesis and triggers of acute exacerbations as well as optimal strategies for prevention and making an early diagnosis of AE-IPF and acute exacerbations of other forms of chronic fibrosing ILD will likely lead to improved survival for patients with these devastating forms of fibrotic ILD. Clearly, successful treatment of episodes of AE-CFILD remains elusive, and progress is likely to be slow and incremental. Treatment strategies that employ novel approaches and biologic response modifiers rather than cytotoxic drugs may prove to be of significant benefit to patients.

Table 1. Therapies for acute exacerbations of IPF

Pharmacologic	Corticosteroids (e.g. high-dose, pulsed intravenous)* Corticosteroids plus immunomodulatory/cytoxic agents** - Cyclophosphamide - Calcineurin inhibitors (Cyclosporine A, tacrolimus) Rituximab with plasma exchange** Human recombinant thrombomodulin** Antibiotics (e.g. macrolides, co-trimoxazole)** Anti-fibrotic drugs (e.g. continue if already using prior to AE)**
Non-pharmacologic	Supportive care - Supplemental oxygen - Symptom palliation (e.g. opioids for relief of dyspnea) - Assisted ventilation Hemoperfusion with polymyxin B-immobilized fibers** Lung transplantation

*Weak recommendation, very low quality evidence (Reference 9); **Unproven therapy; no guideline recommendation

References

1. Kolb M, Bondue B, Pesci A, Miyazaki Y, Song JW, Bhatt NY, et al. Acute exacerbations of progressive-fibrosing interstitial lung diseases. Eur Respir Rev. 2018 Dec 21;27(150):180071. doi: 10.1183/16000617.0071-2018. PMID: 30578331.
2. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000 Feb;161(2 Pt 1):646-64. doi: 10.1164/ajrccm.161.2.ats3-00. PMID: 10673212.
3. Idiopathic Pulmonary Fibrosis Clinical Research Network, Raghu G, Anstrom KJ, King TE Jr, Lasky JA, Martinez FJ. Prednisone, azathioprine, and N-acetylcysteine for pulmonary fibrosis. N Engl J Med. 2012 May 24;366(21):1968-77. doi: 10.1056/NEJMoa1113354. Epub 2012 May 20. PMID: 22607134; PMCID: PMC3422642.
4. Zisman DA, Lynch JP 3rd, Toews GB, Kazerooni EA, Flint A, Martinez FJ. Cyclophosphamide in the treatment of idiopathic pulmonary fibrosis: a prospective study in patients who failed to respond to corticosteroids. Chest. 2000 Jun;117(6):1619-26. doi: 10.1378/chest.117.6.1619. PMID: 10858393.
5. Raghu G, Rochwerg B, Zhang Y, Garcia CA, 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 Jul 15;192(2):e3-19. doi: 10.1164/rccm.201506-1063ST. Erratum in: Am J Respir Crit Care Med. 2015 Sep 1;192(5):644. Dosage error in article text. PMID: 26177183.
6. 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 Oct 31;381(18):1718-1727. doi: 10.1056/NEJMoa1908681. Epub 2019 Sep 29. PMID: 31566307.
7. Wells AU, Flaherty KR, Brown KK, Inoue Y, Devaraj A, Richeldi L, et al. Nintedanib in patients with progressive fibrosing interstitial lung diseases-subgroup analyses by interstitial lung disease diagnosis in the INBUILD trial: a randomised, double-blind, placebo-controlled, parallel-group trial. Lancet Respir Med. 2020 May;8(5):453-460. doi: 10.1016/S2213-2600(20)30036-9. Epub 2020 Mar 5. PMID: 32145830.
8. Biondini D, Balestro E, Sverzellati N, Cocconcelli E, Bernardinello N, Ryerson CJ, et al. Acute exacerbations of idiopathic pulmonary fibrosis (AE-IPF): an overview of current and future therapeutic strategies. Expert Rev Respir Med. 2020 Apr;14(4):405-414. doi: 10.1080/17476348.2020.1724096. Epub 2020 Feb 3. PMID: 31994940.
9. 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 Sep 1;198(5):e44-e68. doi: 10.1164/rccm.201807-1255ST. PMID: 30168753.
10. Innabi A, Gomez-Manjarres D, Alzghoul B, Chizinga M, Mehrad B, Patel D. Cyclophosphamide for the treatment of Acute Exacerbation of Interstitial Lung Disease: A Review of the Literature.
11. Collard HR, Ryerson CJ, Corte TJ, Jenkins G, Kondoh Y, Lederer DJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. Am J Respir Crit Care Med. 2016 Aug 1;194(3):265-75. doi: 10.1164/rccm.201604-0801CI. PMID: 27299520.
12. Baughman RP, Meyer KC, Nathanson I, Angel L, Bhorade SM, Chan KM, et al. Monitoring of nonsteroidal immunosuppressive drugs in patients with lung disease and lung transplant recipients: American College of Chest Physicians evidence-based clinical practice guidelines. Chest. 2012 Nov;142(5):e1S-e111S. doi: 10.1378/chest.12-1044. PMID: 23131960; PMCID: PMC3610695.
13. Naccache JM, Montil M, Cadranel J, Cachanado M, Cottin V, Crestani B, et al. Study protocol: exploring the efficacy of cyclophosphamide added to corticosteroids for treating acute exacerbation of idiopathic pulmonary fibrosis; a randomized double-blind, placebo-controlled, multi-center phase III trial (EXAFIP). BMC Pulm Med. 2019 Apr 11;19(1):75. doi: 10.1186/s12890-019-0830-x. PMID: 30971235; PMCID: PMC6458697.
14. Donahoe M, Valentine VG, Chien N, Gibson KF, Raval JS, Saul M, et al. Autoantibody-Targeted Treatments for Acute Exacerbations of Idiopathic Pulmonary Fibrosis. PLoS One. 2015 Jun 17;10(6):e0127771. doi: 10.1371/journal.pone.0127771. Erratum in: PLoS One. 2015;10(7):e0133684. PMID: 26083430; PMCID: PMC4470587.

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Cyclophosphamide for the treatment of Acute Exacerbation of Interstitial Lung Disease: A Review of the Literature

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Abstract. Acute exacerbation of interstitial lung disease is a serious and life-threatening event but little is known about its treatment. Cyclophosphamide has been proposed in randomized clinic trials as a treatment option in progressive cases of systemic sclerosis related interstitial lung disease. However, in acute exacerbation of interstitial lung disease, we found only small case series, and retrospective studies, mostly with no comparative groups which described the role of cyclophosphamide. Results of these studies showed mixed outcomes, with no robust evidence that cyclophosphamide adds any benefit in treating acute exacerbations of interstitial lung disease. More well-designed studies including randomized clinical trials are needed to better understand the role of cyclophosphamide during exacerbations of interstitial lung disease. In this review article, we summarize the current evidence on the use of cyclophosphamide in interstitial lung disease with a focus on the acute exacerbation events.

Key words: Cyclophosphamide, Interstitial Lung Disease, Acute Exacerbation

Abbreviations:
AE-ILD: acute exacerbation of interstitial lung disease
AE-IPF: acute exacerbation of idiopathic pulmonary fibrosis
AZA: azathioprine
CT: computed tomography
CTD-ILD: connective tissue disease-interstitial lung disease
CyA: cyclosporin A
CYC: cyclophosphamide
FAST: Fibrosing Alveolitis in Scleroderma Trial
FEV1: forced expiratory volume in one second
FVC: forced vital capacity
DLco: Diffusion capacity of carbon monoxide
HRCT: high resolution computed tomography
ILD: interstitial lung disease
IPF: idiopathic pulmonary fibrosis
MMF: mycophenolate mofetil
PaO_2/FIO_2: partial pressure of arterial oxygen to the fraction of inspired oxygen ratio
RA-ILD: rheumatoid arthritis interstitial lung disease
SLS I: Scleroderma Lung Study I
SLS II: Scleroderma Lung Study II
SSc-ILD: systemic sclerosis-interstitial lung disease

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Introduction

Acute exacerbation of interstitial lung disease (AE-ILD) is a serious life-threatening event, with a reported median post-acute exacerbation survival of only 3-4 months (1, 2). In patients with idiopathic

pulmonary fibrosis (IPF), acute exacerbations reduce survival and are the most common cause of death on autopsy. In large series of 461 patients with IPF, Song *et al*, reported that those who experienced acute exacerbations had in-hospital mortality rate of 50%, and the 1- and 5-yr survival rates from the initial diagnosis were 56% and 18%, respectively (3). In connective tissue disease-interstitial lung disease (CTD-ILD), a review of 155 patients found that the 10 patients who experienced acute exacerbation had a median survival of only 169 days after acute exacerbation (4).

Although the prognosis of AE-ILD disease is poor, treatment is unknown. Immunosuppressing agents such as cyclophosphamide (CYC) have been proposed as treatment option in the literature (5, 6). We sought to review the literature on the role of CYC in AE-ILD after reviewing how it is typically used in chronic ILD.

Methodology

An electronic search was implemented in PubMed, Google Scholar and Medline. Search terms included the term "cyclophosphamide", "exacerbation", "idiopathic pulmonary fibrosis", "lung fibrosis", "interstitial lung disease" and/or "connective tissue disease". Publications were only included in the review if they were written in English or the abstract was in English. No date limits were set. From the articles retrieved in the first search round, the search strategy was amplified by manual screening of the reference lists of identified studies. As the review is narrative and not systematic, the references were selected according to the relevance to the subject of the review.

Overview of Acute Interstitial Lung Disease Exacerbation

Currently, acute exacerbation is only defined in IPF, however similar criteria are often used to define acute exacerbation in other ILDs (1, 7). Definition and criteria for AE-IPF were published in 2007 and revised in 2016 by an international working group, which defined AE-IPF as an "acute, clinically significant, respiratory deterioration characterized by evidence of new widespread alveolar abnormality,". The 2016 International Working Group Report proposed the following diagnostic criteria for AE-IPF: (a) previous or concurrent diagnosis of IPF; (b) acute worsening or development of dyspnea typically less than one month in duration; (c) presence of new bilateral ground-glass opacities and/or consolidations superimposed on a background pattern of UIP on high resolution computed tomography (HRCT) scans; and (d) clinical deterioration not fully explained by heart failure or fluid overload (1, 8, 9). The 2007 criteria for diagnosis of AE-IPF were strict, requiring careful exclusion of pulmonary infection, left heart failure, pulmonary embolism, and identifiable causes of acute lung injury (9). However, some of these causes, including infection and drug toxicity, were allowed as triggers of acute exacerbation in the 2016 updated criteria for AE-IPF. Also, bronchoalveolar lavage is no longer necessarily required for the diagnosis of AE in the 2016 criteria compared with the 2007 criteria. The new criteria provide a broader inclusion compared to the original 2007 criteria and make it easier to diagnose acute exacerbation (10). Figure 1 demonstrates computed tomography (CT) of the chest from a 68-year-old male patient who was admitted to the hospital for acute exacerbation of ILD.

Acute exacerbation in IPF is common and has a poor prognosis. The incidence of AE-IPF ranges from 4 to 20% per year among IPF patients in reported studies (3, 11). The wide variation in incidence of AE-IPF could be due to different definitions and criteria of acute exacerbation used by different authors. In one large retrospective study, 1- year and 3-year incidences of acute exacerbation in IPF were 14.2% and 20.7%, respectively (3).

The incidence, clinical characteristics and prognosis of acute exacerbation patients in CTD-ILD and other less common causes of ILD have yet to be fully studied as compared to acute exacerbation in IPF. The incidence of CTD-ILD exacerbation is less frequent than AE-IPF, however the prognosis of acute exacerbation in CTD-ILD is poor and comparable to the poor prognosis in IPF exacerbation. In one study six patients out of 83 CTD-ILD developed acute exacerbation of CTD-ILD with an overall incidence of 7.2% and a 1-year incidence 1.25%. Five out of the six patients died and one survived for discharge (12).

There is no standardized accepted approach to the treatment of AE-ILD, however, in clinical practice, some patients are treated with high dose immunosuppression, typically with pulse corticosteroids

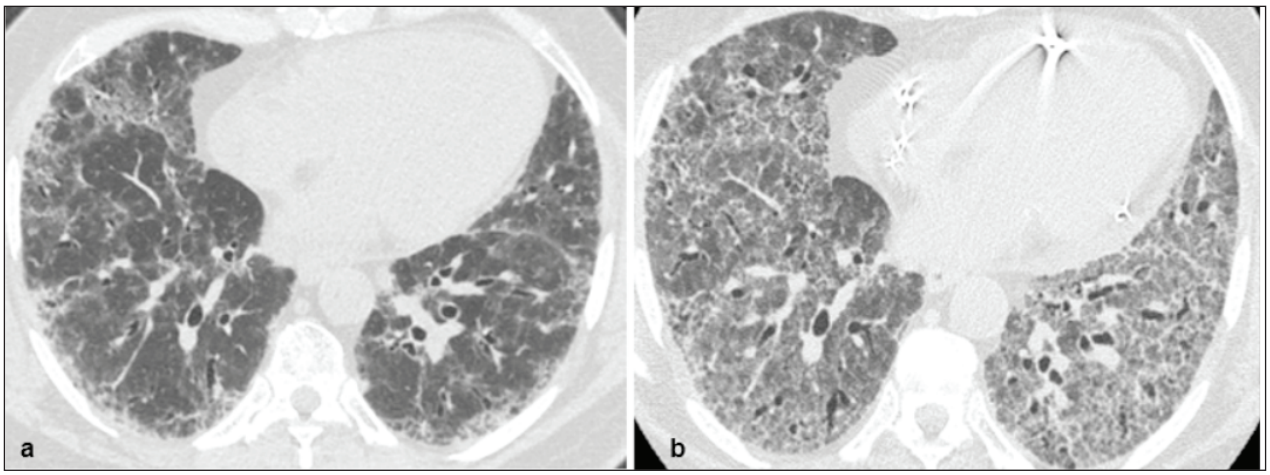

Figure 1. A: Axial CT of a patient with familial ILD. B: Axial CT of the same patient 6 months later at the time of an acute exacerbation and shows worsening bilateral ground-glass opacification. Abbreviation: CT, computed tomography, ILD: interstitial lung disease

of 500–1000 mg per day of methylprednisolone for three days along with antibiotics (13). The evidence for treating AE-ILD with corticosteroids is weak and some argue that it might be even harmful (14). It is reasonable to withhold treatment in patients with contraindications to therapy, as it is unclear how much this treatment affects outcomes (15). Other immunosuppressive agents, including cyclosporine A (CyA), tacrolimus, rituximab and intravenous CYC have also been used to treat AE-ILD. Newer therapies are being reported and investigated (6). Some patients also require mechanical ventilation support and mixed results have been reported from emergent lung transplantation (16).

Role of Cyclophosphamide in the Management of Chronic CTD-ILD

Cyclophosphamide is an alkylating agent and potent immunosuppressive medication that induces apoptotic cell death in rapidly proliferating cells, including clonally expanding lymphocytes (17). Cyclophosphamide can be administered orally or intravenously. In cases of rapidly progressive ILD, intravenous CYC has the advantage of a rapid onset of action, after which, maintenance therapy with an oral agent can be established (18, 19). Intravenous CYC has a favorable safety profile as compared to daily oral CYC. Daily oral CYC results in a higher cumulative dose, increasing the risk of side effects (17). The standard oral dosage for patients with normal renal function is 2 mg/kg/day and intravenous doses range between 500 and 1000 mg/m^2 body surface area administered every four to six weeks. Therapy generally is provided for six months to one year (20).

Cyclophosphamide is associated with significant toxicities and side effects. The most common side effects include nausea and hair thinning/alopecia. It is notorious for causing hemorrhagic cystitis and bladder cancer due to exposure of the bladder to acrolein, a metabolite of CYC. The risk of each is related to total cumulative dose, with a total dosage greater than 100 grams most strongly associated with bladder cancer. To reduce total dosage, duration of CYC usage is often limited to periods shorter than 12 months. Other side effects include bone marrow suppression with associated risks of bacterial and opportunistic infections, pulmonary toxicity, acute hepatic failure, cardiac toxicity, gonadal toxicity and an increase in hematological, solid and skin malignancies. CYC is teratogenic and should be avoided throughout pregnancy (20).

CYC has been widely studied in CTD-ILD, most commonly systemic sclerosis-interstitial lung disease (SSc-ILD) (21). In a treatment algorithm of SSc-ILD that was developed in 2016–2017, expert consensus was that intravenous CYC should be used as a second-line induction therapy, after mycophenolate mofetil (MMF) (22). To date, it has been studied in four randomized clinical trials in ILD (18, 23-25). The studies were mostly conducted in patients with SSc-ILD. It has also been suggested as a treatment option for severe, progressive disease or refractory

disease in rheumatoid arthritis-ILD (RA-ILD), idiopathic inflammatory myositis-ILD, and Sjögren's-associated ILD (26).

The first landmark randomized controlled trial that studied CYC in SSc-ILD was the Scleroderma Lung Study I (SLS I). It was a double-blind, randomized, placebo-controlled clinical trial that examined the efficacy of oral CYC for the treatment of SSc-ILD. One hundred fifty-eight subjects were randomized to oral CYC (up to 2 mg/kg/day) or placebo for 12 months. Of the 158 patients, 145 completed at least six months of treatment and were included in the analysis. The primary outcome was forced vital capacity (FVC). This study demonstrated a modest but statistically significant improvement in the mean absolute difference in FVC percent predicted between treatment and placebo group which was 2.53%, favoring CYC group (P<0.03). The mean absolute difference in total lung capacity (TLC) percent predicted was also significant (4.09%), favoring CYC group. There was no difference for DLCO and DLCO/VA. In addition, CYC had a modest beneficial effect on dyspnea, thickening of the skin, and the health-related quality of life. The SLS I study showed that the more extensive the lung fibrosis and/or skin involvement (FVC < 70% predicted, worse HRCT fibrosis score or worse skin thickening), the more likely the benefit from the medication (24). Sub-analysis of the SLS I trial revealed that CYC therapy was also associated with significant improvement in HRCT fibrosis score (27). Extension of the SLS I study published in 2007 showed that the favorable outcome of CYC on FVC continued to improve after cessation of CYC treatment reaching a maximum at 18 months (six months after stopping CYC therapy) with a mean 4.16% FVC difference versus placebo (p=0.01). The beneficial effects of CYC disappeared one year after CYC was terminated. In contrast, the positive effect on dyspnea persisted through 24 months (28). In the SLS I trial there was a higher frequency of adverse events (hematuria, leukopenia, neutropenia, anemia, and pneumonia) and higher withdrawal from treatment in the CYC arm compared with placebo, but there was no significant increase in serious adverse events (24). Based on this study, the European League Against Rheumatism (EULAR) has recommended that "cyclophosphamide should be considered for treatment of SSc-ILD, in particular for patients with scleroderma with progressive ILD (strength of recommendation: A)" (29).

Due to the need for safer long-term treatment option, the SLS investigators conducted the Scleroderma Lung Study II (SLS II) which provided further insight into the role of MMF and CYC in patients with SSc-ILD. This study randomized patients in the United States to either two years of MMF or one year of oral CYC followed by one year of placebo. Patients in both arms had significant improvements in lung function over the two-year course of the study neither arm was superior over the other. Leukopenia and thrombocytopenia occurred less often in patients administered MMF than in those who received CYC. Similar to what was seen in the SLS I study, the more severe the fibrosis, the larger the effect of treatment (23).

In the third placebo-controlled trial using intravenous CYC, the Fibrosing Alveolitis in Scleroderma Trial (FAST), 45 patients were randomized to either receive placebo or intravenous CYC (600 mg/m2 body surface area intravenously at monthly intervals for six months) and oral prednisolone (20 mg on alternate days) followed by azathioprine (AZA) maintenance therapy (2.5 mg/kg/day). There was a numerical trend towards benefit in the treatment group with a 4.2% difference in FVC percent predicted, though it did not reach statistical significance (18). The difference in FVC was actually more pronounced in FAST than in SLS I (+4.2% vs. +2.5% respectively), but the smaller number of subjects in FAST (n=45) compared with SLS I (n=158) impacted the ability to achieve statistical significance. The increase in FVC in SLS I, SLS II, and FAST trial and other uncontrolled studies is supportive of efficacy of CYC in CTD-ILD (30).

The fourth randomized controlled trial by Zhang *et al*, compared intravenous CYC for 12 months versus MMF (1.5 g daily) for 12 months in 60 patients with SSc-ILD. All participants in intervention and control groups received prednisolone, with the starting dose titrated according to disease severity (as deemed by trial doctors) and all participants weaned to 10 mg daily within four weeks. A total of 45 patients completed this trial. Patients in both groups with FVC ≤ 75% predicted and forced expiratory volume in one second (FEV1) ≤ 75% predicted had significant statistical improvement in FVC and FEV1. Interestingly, for the patients with diffusion capacity for carbon monoxide (DLco) ≤ 65%, there were significant increases in the CYC group, which

has not been observed in the other trials (25).

In a recent Cochrane review that included the prior four studies, it was found that a small benefit may be achieved from the use of CYC in patients with CTD-ILD in mean difference in percent predicted FVC when compared with placebo, but not in the difference in the percent predicted DLco or mortality. Modest clinical improvement in dyspnea may be noted with the use of CYC (20). A randomized clinical trial of rituximab versus CYC in progressive CTD-ILD (including scleroderma-ILD, IIM-ILD, and MCTD-ILD), with change in FVC as the primary outcome, is currently going in the United Kingdom (RECITAL study, NCT01862926) (31).

Cyclophosphamide and ILD exacerbation

International guidelines for diagnosis and treatment of IPF state that supportive care remains the mainstay in the management of acute exacerbation of idiopathic pulmonary fibrosis (AE-IPF). However, some authors suggest the use of high-dose corticosteroid including methylprednisolone and prednisolone in treating of AE-IPF. This practice is mainly based on weak recommendations by international guidelines and not based in randomized controlled trials (32, 33). The use of immunosuppressants, including CYC and CyA have been reported in few retrospective studies and small case series. The addition of intravenous CYC to corticosteroid in AE-IPF has been listed as potential treatment option in the American Thoracic Society international working group report for AE-IPF and per the French practical guidelines (5, 8). The rationale for using CYC as immunosuppressant agent in AE-IPF was driven mainly by experience in other connective tissue diseases, such as SSc-ILD, and vasculitis (34, 35).

Okamoto *et al*, Ambusoni *et al*, and Parambil *et al* initially reported a total of 35 patients, with different definitions of AE-IPF, who used CYC and corticosteroids in addition to various supportive care measures for AE-IPF. These case series reported mixed results, and no clinically significant improved outcomes could be concluded from the data reported (36-38). Similarly, a 2004 retrospective study by Al-hameed *et al*, reviewed 25 patients who were admitted for AE-IPF with no identifiable cause. All patients died within the first 90 days of admission. All patients received treatment with corticosteroids. A smaller number (8 patients) were treated with immunosuppressive agents, predominantly CYC. Using corticosteroid and CYC did not seem to alter the prognosis in the previous cohort (39).

Two more recent small retrospective studies described the efficacy of corticosteroids and intravenous CYC therapy for AE-IPF. First study by Morawiec *et al*, who used high-dose pulses of corticosteroid followed by CYC regimen in 10 patients (with 11 episodes) with AE-IPF and seven IPF patients with sub-acute exacerbation (with an onset of symptoms between 30-90 days prior to treatment). The median age of the cohort was 67 years, and the median partial pressure of arterial oxygen to the fraction of inspired oxygen ratio ($PaO_2/FIO_{2)}$ at the time of exacerbation was 262. Treatment regimen was methylprednisolone pulses (1000 mg) at days 1 to 3 followed on day 4 by escalating regimen of CYC (initial intravenous dose 500 mg, increased by 200 mg every 2 weeks, maximum dose administered 1500 mg). All patients were alive one month after treatment was initiated. At three months, 72% of the patients were alive: all patients with sub-acute exacerbation and 55% patients with acute exacerbation. Cause of death was respiratory failure in all cases (40). The second study was also a small retrospective case series conducted in Italy. In this case series the authors evaluated the outcome in terms of survival of intravenous pulse doses of high-dose corticosteroid (methylprednisolone 1000 mg per day for three consecutive days) followed by monthly CYC administration (maximum six doses) in a cohort of patients with AE-IPF. A total of 11 patients were assessed. The median age of the cohort was 66 years, and the median PaO_2/FIO_2 at the time of exacerbation was 208. A median of 5 monthly pulse doses of CYC were administered, with 4 patients receiving all 6 doses. Four patients died before completion, and 3 patients developed adverse events (hematuria, epistaxis without thrombocytopenia and Pseudomonas pneumonia) and interrupted the pulse administration. Overall survival at three months was 73%, at six months was 63%, at 12 months was 55%, at 18 months was 45% and at two years was 27%. In-hospital mortality was 9% (35). Both studies suggested the potential benefit of corticosteroids plus intravenous CYC therapy considering the low side effects and the safety profile. However, their cohorts had small number of patients and they did not include a comparison group which makes it unclear if

corticosteroids plus intravenous CYC provide any benefit over other treatments.

A more recent study by Hozumi *et al*, published the largest retrospective, multicenter study about the use of CYC in AE-IPF. It included 102 patients with first episode of AE-IPF. The mean age of the cohort was 74 years and only 8 patients required intubation and mechanical ventilation. Based on propensity scores, 26 matched patient pairs were made. Efficacy of corticosteroids plus intravenous CYC therapy for the first acute exacerbation was compared with that of corticosteroid monotherapy. The post-acute exacerbation 90-day survival rate of their entire cohort was 65%. No significant differences between matched-group were observed in post-acute exacerbation 90-day survival rates (85% vs 77%; p = 0.70), cumulative survival rates, or incidence of adverse events. The authors concluded that the routine use of intravenous CYC with corticosteroids in AE-IPF was not beneficial when compared to corticosteroids alone (41). Another 2019 study by Aso *et al*, a Japanese inpatient database was utilized to study and analyze outcomes of patients with AE-IPF who received CYC in addition systemic corticosteroids, versus systemic corticosteroids alone. The results of an instrumental variable analysis showed no difference between the two groups in respect to in-hospital mortality and ventilator-free days (42). Table 1 summarizes these studies.

Due to the unmet need for effective treatment for AE-IPF and due to the lack of randomized controlled trials in this field, a phase III trial is being conducted. The EXAFIP study (NCT02460588) is a French national multicenter double-blind placebo-controlled randomized trial that is enrolling patients to evaluate the efficacy of CYC compared to placebo on early survival in patients treated with corticosteroids. The primary outcome is all-cause mortality rate at 3 months with other secondary outcomes. This

Table 1. Summary of the main studies that reported the use CYC in IPF exacerbation.

Study	Design	Size	Intervention	Clinical Outcome
Ambrosini et al. (2003)(37)	Single-center, retrospective study	5 patients	Steroids followed by CYC	Four patients died within one month, 1 patient lived at least 1.5 years.
Al-Hameed et al. (2004)(39)	Single-center, retrospective study	25 patients	Steroids or combination of steroids + CYC	All patients died in-hospital. One patient was discharged and returned after 30 days and died.
Parambil et al. (38)	Single-center, retrospective study	2 patients	Steroids followed by CYC	Both patients treated after biopsy. Both patients died in-hospital
Okamoto et al. (2006)(36)	Single-center, retrospective study	28 patients	Combination of steroids + CYC or Steroids + CyA	Twenty-four patients died within 4 months. Report did not specify whether the survivors received cyclophosphamide or cyclosporin. Survival rate at 1-month was 14% and 3-month was 14%.
Morawiec et al. (2011)(40)	Single-center, retrospective study	17 patients	Steroids followed by CYC	Ten patients had AE-IPF; survival rate at 1-month was 100%, at 3-month was 55%, and at 6-month was 40%. Seven patients had SAE-IPF, survival rate at 1-month was 100%, at 3-month was 100 %, and at 6-month was 71%.
Novelli et al. (2016)(35)	Single-center retrospective study	11 patients	Steroids + CYC	Survival rate at 3-month was 73%, at 6-month was 63%, at 12-month was 55%, at 18-month was 45% and at 2-year 27%.
Hozumi et al. (2019)(41)	Retrospective, multicenter study	102 patients	Steroid versus steroids plus CYC	No significant differences in 90-day survival rate between matched groups.
Aso et al. (2019)(42)	Retrospective, nationwide data base study	1847 patients	Steroids versus steroids + CYC	No significant differences between the two groups with respect to in-hospital mortality.

AE-IPF: acute exacerbation of idiopathic pulmonary fibrosis, SAE-IPF: subacute exacerbation of idiopathic pulmonary fibrosis, CI: calcineurin inhibitor, CyA: cyclosporin A, CYC: cyclophosphamide, TAC: tacrolimus.

study should provide better insights on the efficacy and side effects of CYC in AE-IPF (43).

With regards the treatment approach for acute exacerbation in CTD-ILD, it has not yet been established but conventionally it is treated with corticosteroids (12). Other drugs have been additionally administered including CyA with inconsistent outcomes (44, 45). In a retrospective study by Suda *et al*, six patients with CTD-ILD were treated with high dose corticosteroids. Four of 6 patients were given IV 500–750 mg/m2 CYC and 2 received 2–3 mg/kg/day CyA. In the previous cohort, all patients required mechanical ventilation and 5 of 6 died. The patient who survived only received corticosteroids (12). Tomiyama *et al*, reported 13 case of SSc-ILD exacerbation. Ten patients were treated with CYC in combination with corticosteroids or other immunosuppressant. Mortality was high in the previous cohort as it was 46% (46).

Ota *et al*, retrospectively reviewed 17 patients with RA-ILD who required hospitalization at the University of Tokyo Hospital due to an acute exacerbation (12 patients) or subacute exacerbation (5 patients). Patients were classified into four groups: patients who received glucocorticoids only, glucocorticoids plus tacrolimus, glucocorticoids plus CyA, or CYC in combination with other drugs. Five patients received intravenous CYC with dose varying between 750 to 1200 mg. The total number of doses also varied between 2 to 9 doses. Interestingly, the patients who received CyA or CYC had more severe disease compared to other treatment group, and all five patients in the CYC group were alive for the average observation period of 474 days. It is difficult to discriminate whether the better prognosis in this group was due to CYC alone or combination of some drugs. The authors concluded that for severe cases with low respiratory function, intensive therapy, including CYC, has a potential to improve the prognosis the acute exacerbation of RA-ILD (47).

Schulze *et al*, identified 14 patients who were admitted to the intensive care unit with severe ILD and were treated with cyclophosphamide. Twelve patients were mechanically ventilated, and 7 patients were supported by extracorporeal membrane oxygenation. Corticosteroids and antibiotics were given to all patients. The absolute cyclophosphamide doses varied from 400 mg up to 1.000 mg (mean dose 807.1 mg) and the mean time between diagnosis and the initiation of cyclophosphamide was 11.3 days. Five patients received plasmapheresis. The authors observed a positive prognostic effect in patients with SSc and ANCA-associated-vasculitis and reduced overall survival was found for Goodpasture syndrome, dermatomyositis, cryptogenic organizing pneumonia and drug reaction with eosinophilia and systemic symptoms. They also concluded that additional plasmapheresis and initiation of cyclophosphamide within ten days following initial diagnosis of ILD were associated with improved prognosis (48). Table 2 summarizes the previous studies.

Conclusion and Consideration for Future Research

Cyclophosphamide has been shown to have a beneficial effect in the chronic management of scleroderma-ILD in landmark randomized controlled

Table 2: Summary of the main studies examing the use of CYC in CTD-ILD exacerbation

Study	Design	Size	Intervention	Clinical outcome
Suda et al. (2009)(12)	Retrospective, single center	6 patients	Steroids or combination of steroids + CYC or steroids + CyA	Five patients died. The patient who survived received only steroids.
Tomiyama et al. (2016)(46)	Single-center, Retrospective study	13 patients	Steroids or combination of steroids + CYC or steroids + CI	Twelve patients received combination therapy. Almost half of the patients died on follow up.
Ota el al. (2017)(47)	Single-center, Retrospective study	17 patients	Steroid or combination of steroids + TAC or steroids + CyA or steroids + CYC (or combination of more than 2 immunosuppressants)	The five patients who received CYC had more severe disease and were alive at 474 days follow up.
Schulze et al. (2019) (48)	Single-center, Retrospective study	14 patients	Steroid + CYC or steroid + CYC + plasmapheresis	Patients with Scc-ILD and ANCA-associated-vasculitis ILD had an improved survival.

CI: calcineurin inhibitor, CyA: cyclosporin A, CYC: cyclophosphamide, TAC: tacrolimus

trials (18, 23-25). Although this effect was beneficial for the groups studied, it was modest in terms of lung function improvement and was mainly confined to progressive cases of SSc-ILD. However, for AE-ILD, we found retrospective and small studies with a few cases and non-comparative groups. They showed mixed results and did not show definite benefit in AE-IPF or CTD-ILD (12, 35-42, 46-48).

Decision-making in the treatment of people with lung fibrosis in the setting of acute exacerbation is unclear, and there are still questions that need to be answered in this field. The clinician must balance a high level of need for therapy in a severely sick population against the potential for adverse effects as CYC is well known for wide range of toxicities. It is not clear whether evidence of efficacy of CYC in progressive CTD-ILD can be extrapolated to the acute exacerbation at this time.

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References

1. P. Spagnolo, W. Wuyts, Acute exacerbations of interstitial lung disease: lessons from idiopathic pulmonary fibrosis, Curr Opin Pulm Med 23(5) (2017) 411-417.
2. I.N. Park, D.S. Kim, T.S. Shim, C.M. Lim, S.D. Lee, Y. Koh, W.S. Kim, W.D. Kim, S.J. Jang, T.V. Colby, Acute exacerbation of interstitial pneumonia other than idiopathic pulmonary fibrosis, Chest 132(1) (2007) 214-20.
3. J.W. Song, S.B. Hong, C.M. Lim, Y. Koh, D.S. Kim, Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome, Eur Respir J 37(2) (2011) 356-63.
4. Y. Toyoda, M. Hanibuchi, J. Kishi, H. Kawano, S. Morizumi, S. Sato, M. Kondo, T. Takikura, T. Tezuka, H. Goto, Y. Nishioka, Clinical features and outcome of acute exacerbation of interstitial pneumonia associated with connective tissue disease, J Med Invest 63(3-4) (2016) 294-9.
5. V. Cottin, B. Crestani, D. Valeyre, B. Wallaert, J. Cadranel, J.C. Dalphin, P. Delaval, D. Israel-Biet, R. Kessler, M. Reynaud-Gaubert, B. Aguilaniu, B. Bouquillon, P. Carré, C. Danel, J.B. Faivre, G. Ferretti, N. Just, S. Kouzan, F. Lebargy, S. Marchand-Adam, B. Philippe, G. Prévot, B. Stach, F. Thivolet-Béjui, J.F. Cordier, F.N.R. Centre, N.o.C.C.f.R.L. Diseases, Diagnosis and management of idiopathic pulmonary fibrosis: French practical guidelines, Eur Respir Rev 23(132) (2014) 193-214.
6. W.H. Amundson, E. Racila, T. Allen, H.E. Dincer, R. Tomic, M. Bhargava, D.M. Perlman, H.J. Kim, Acute exacerbation of interstitial lung disease after procedures, Respir Med 150 (2019) 30-37.
7. W.D. Travis, U. Costabel, D.M. Hansell, T.E. King, D.A. Lynch, A.G. Nicholson, C.J. Ryerson, J.H. Ryu, M. Selman, A.U. Wells, J. Behr, D. Bouros, K.K. Brown, T.V. Colby, H.R. Collard, C.R. Cordeiro, V. Cottin, B. Crestani, M. Drent, R.F. Dudden, J. Egan, K. Flaherty, C. Hogaboam, Y. Inoue, T. Johkoh, D.S. Kim, M. Kitaichi, J. Loyd, F.J. Martinez, J. Myers, S. Protzko, G. Raghu, L. Richeldi, N. Sverzellati, J. Swigris, D. Valeyre, A.E.C.o.I.I. Pneumonias, 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 188(6) (2013) 733-48.
8. H.R. Collard, C.J. Ryerson, T.J. Corte, G. Jenkins, Y. Kondoh, D.J. Lederer, J.S. Lee, T.M. Maher, A.U. Wells, K.M. Antoniou, J. Behr, K.K. Brown, V. Cottin, K.R. Flaherty, J. Fukuoka, D.M. Hansell, T. Johkoh, N. Kaminski, D.S. Kim, M. Kolb, D.A. Lynch, J.L. Myers, G. Raghu, L. Richeldi, H. Taniguchi, F.J. Martinez, Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report, Am J Respir Crit Care Med 194(3) (2016) 265-75.
9. H.R. Collard, B.B. Moore, K.R. Flaherty, K.K. Brown, R.J. Kaner, T.E. King, J.A. Lasky, J.E. Loyd, I. Noth, M.A. Olman, G. Raghu, J. Roman, J.H. Ryu, D.A. Zisman, G.W. Hunninghake, T.V. Colby, J.J. Egan, D.M. Hansell, T. Johkoh, N. Kaminski, D.S. Kim, Y. Kondoh, D.A. Lynch, J. Müller-Quernheim, J.L. Myers, A.G. Nicholson, M. Selman, G.B. Toews, A.U. Wells, F.J. Martinez, I.P.F.C.R.N. Investigators, Acute exacerbations of idiopathic pulmonary fibrosis, Am J Respir Crit Care Med 176(7) (2007) 636-43.
10. N. Enomoto, Y. Oyama, Y. Enomoto, H. Yasui, M. Karayama, M. Kono, H. Hozumi, Y. Suzuki, K. Furuhashi, T. Fujisawa, N. Inui, Y. Nakamura, T. Suda, Differences in clinical features of acute exacerbation between connective tissue disease-associated interstitial pneumonia and idiopathic pulmonary fibrosis, Chron Respir Dis 16 (2019) 1479972318809476.
11. C.J. Ryerson, V. Cottin, K.K. Brown, H.R. Collard, Acute exacerbation of idiopathic pulmonary fibrosis: shifting the paradigm, Eur Respir J 46(2) (2015) 512-20.
12. T. Suda, Y. Kaida, Y. Nakamura, N. Enomoto, T. Fujisawa, S. Imokawa, H. Hashizume, T. Naito, D. Hashimoto, Y. Takehara, N. Inui, H. Nakamura, T.V. Colby, K. Chida, Acute exacerbation of interstitial pneumonia associated with collagen vascular diseases, Respir Med 103(6) (2009) 846-53.
13. G. Leuschner, J. Behr, Acute Exacerbation in Interstitial Lung Disease, Front Med (Lausanne) 4 (2017) 176.
14. S.A. Papiris, E.D. Manali, L. Kolilekas, C. Triantafillidou, I. Tsangaris, K. Kagouridis, Steroids in idiopathic pulmonary fibrosis acute exacerbation: defenders or killers?, Am J Respir Crit Care Med 185(5) (2012) 587-8.
15. S. Cuerpo, J. Moisés, F. Hernández-González, M. Benegas, J. Ramirez, M. Sánchez, À. Agustí, J. Sellares, Acute exacerbations of idiopathic pulmonary fibrosis: Does clinical stratification or steroid treatment matter?, Chron Respir Dis 16 (2019) 1479973119869334.
16. J.S. Lee, A. Fischer, Current and emerging treatment options for interstitial lung disease in patients with rheumatic disease, Expert Rev Clin Immunol 12(5) (2016) 509-20.
17. A.S. Jee, T.J. Corte, Current and Emerging Drug Therapies for Connective Tissue Disease-Interstitial Lung Disease (CTD-ILD), Drugs 79(14) (2019) 1511-1528.
18. R.K. Hoyles, R.W. Ellis, J. Wellsbury, B. Lees, P. Newlands, N.S. Goh, C. Roberts, S. Desai, A.L. Herrick, N.J. McHugh, N.M. Foley, S.B. Pearson, P. Emery, D.J. Veale, C.P. Denton, A.U. Wells, C.M. Black, R.M. du Bois, 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 54(12) (2006) 3962-70.
19. T.J. Corte, R. Ellis, E.A. Renzoni, D.M. Hansell, A.G. Nicholson, R.M. du Bois, A.U. Wells, Use of intravenous cyclophosphamide in known or suspected, advanced non-specific interstitial pneumonia,

Sarcoidosis Vasc Diffuse Lung Dis 26(2) (2009) 132-8.
20. H. Barnes, A.E. Holland, G.P. Westall, N.S. Goh, I.N. Glaspole, Cyclophosphamide for connective tissue disease-associated interstitial lung disease, Cochrane Database Syst Rev 1 (2018) CD010908.
21. A. Perelas, A.V. Arrossi, K.B. Highland, Pulmonary Manifestations of Systemic Sclerosis and Mixed Connective Tissue Disease, Clin Chest Med 40(3) (2019) 501-518.
22. A. Fernández-Codina, K.M. Walker, J.E. Pope, S.A. Group, Treatment Algorithms for Systemic Sclerosis According to Experts, Arthritis Rheumatol 70(11) (2018) 1820-1828.
23. D.P. Tashkin, M.D. Roth, P.J. Clements, D.E. Furst, D. Khanna, E.C. Kleerup, J. Goldin, E. Arriola, E.R. Volkmann, S. Kafaja, R. Silver, V. Steen, C. Strange, R. Wise, F. Wigley, M. Mayes, D.J. Riley, S. Hussain, S. Assassi, V.M. Hsu, B. Patel, K. Phillips, F. Martinez, J. Golden, M.K. Connolly, J. Varga, J. Dematte, M.E. Hinchcliff, A. Fischer, J. Swigris, R. Meehan, A. Theodore, R. Simms, S. Volkov, D.E. Schraufnagel, M.B. Scholand, T. Frech, J.A. Molitor, K. Highland, C.A. Read, M.J. Fritzler, G.H.J. Kim, C.H. Tseng, R.M. Elashoff, S.L.S.I. Investigators, Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomised controlled, double-blind, parallel group trial, Lancet Respir Med 4(9) (2016) 708-719.
24. D.P. Tashkin, R. Elashoff, P.J. Clements, J. Goldin, M.D. Roth, D.E. Furst, E. Arriola, R. Silver, C. Strange, M. Bolster, J.R. Seibold, D.J. Riley, V.M. Hsu, J. Varga, D.E. Schraufnagel, A. Theodore, R. Simms, R. Wise, F. Wigley, B. White, V. Steen, C. Read, M. Mayes, E. Parsley, K. Mubarak, M.K. Connolly, J. Golden, M. Olman, B. Fessler, N. Rothfield, M. Metersky, S.L.S.R. Group, Cyclophosphamide versus placebo in scleroderma lung disease, N Engl J Med 354(25) (2006) 2655-66.
25. G. Zhang, T. Xu, H. Zhang, S. Ye, Q. Wang, L. Zhang, Y. Lei, R. Luo, X. Zhang, [Randomized control multi-center clinical study of mycophenolate mofetil and cyclophosphamide in the treatment of connective tissue disease related interstitial lung disease], Zhonghua Yi Xue Za Zhi 95(45) (2015) 3641-5.
26. B. Wallace, D. Vummidi, D. Khanna, Management of connective tissue diseases associated interstitial lung disease: a review of the published literature, Curr Opin Rheumatol 28(3) (2016) 236-45.
27. J. Goldin, R. Elashoff, H.J. Kim, X. Yan, D. Lynch, D. Strollo, M.D. Roth, P. Clements, D.E. Furst, D. Khanna, S. Vasunilashorn, G. Li, D.P. Tashkin, Treatment of scleroderma-interstitial lung disease with cyclophosphamide is associated with less progressive fibrosis on serial thoracic high-resolution CT scan than placebo: findings from the scleroderma lung study, Chest 136(5) (2009) 1333-1340.
28. D.P. Tashkin, R. Elashoff, P.J. Clements, M.D. Roth, D.E. Furst, R.M. Silver, J. Goldin, E. Arriola, C. Strange, M.B. Bolster, J.R. Seibold, D.J. Riley, V.M. Hsu, J. Varga, D. Schraufnagel, A. Theodore, R. Simms, R. Wise, F. Wigley, B. White, V. Steen, C. Read, M. Mayes, E. Parsley, K. Mubarak, M.K. Connolly, J. Golden, M. Olman, B. Fessler, N. Rothfield, M. Metersky, D. Khanna, N. Li, G. Li, S.L.S.R. Group, Effects of 1-year treatment with cyclophosphamide on outcomes at 2 years in scleroderma lung disease, Am J Respir Crit Care Med 176(10) (2007) 1026-34.
29. O. Kowal-Bielecka, J. Fransen, J. Avouac, M. Becker, A. Kulak, Y. Allanore, O. Distler, P. Clements, M. Cutolo, L. Czirjak, N. Damjanov, F. Del Galdo, C.P. Denton, J.H.W. Distler, I. Foeldvari, K. Figelstone, M. Frerix, D.E. Furst, S. Guiducci, N. Hunzelmann, D. Khanna, M. Matucci-Cerinic, A.L. Herrick, F. van den Hoogen, J.M. van Laar, G. Riemekasten, R. Silver, V. Smith, A. Sulli, I. Tarner, A. Tyndall, J. Welling, F. Wigley, G. Valentini, U.A. Walker, F. Zulian, U. Müller-Ladner, E. Coauthors, Update of EULAR recommendations for the treatment of systemic sclerosis, Ann Rheum Dis 76(8) (2017) 1327-1339.
30. S.C. Mathai, S.K. Danoff, Management of interstitial lung disease associated with connective tissue disease, BMJ 352 (2016) h6819.
31. P. Saunders, V. Tsipouri, G.J. Keir, D. Ashby, M.D. Flather, H. Parfrey, D. Babalis, E.A. Renzoni, C.P. Denton, A.U. Wells, T.M. Maher, Rituximab versus cyclophosphamide for the treatment of connective tissue disease-associated interstitial lung disease (RECITAL): study protocol for a randomised controlled trial, Trials 18(1) (2017) 275.
32. G. Raghu, H.R. Collard, J.J. Egan, F.J. Martinez, J. Behr, K.K. Brown, T.V. Colby, J.F. Cordier, K.R. Flaherty, J.A. Lasky, D.A. Lynch, J.H. Ryu, J.J. Swigris, A.U. Wells, J. Ancochea, D. Bouros, C. Carvalho, U. Costabel, M. Ebina, D.M. Hansell, T. Johkoh, D.S. Kim, T.E. King, Y. Kondoh, J. Myers, N.L. Müller, A.G. Nicholson, L. Richeldi, M. Selman, R.F. Dudden, B.S. Griss, S.L. Protzko, H.J. Schünemann, A.E.J.A.C.o.I.P. Fibrosis, An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management, Am J Respir Crit Care Med 183(6) (2011) 788-824.
33. M.M. Juarez, A.L. Chan, A.G. Norris, B.M. Morrissey, T.E. Albertson, Acute exacerbation of idiopathic pulmonary fibrosis-a review of current and novel pharmacotherapies, J Thorac Dis 7(3) (2015) 499-519.
34. K. de Groot, L. Harper, D.R. Jayne, L.F. Flores Suarez, G. Gregorini, W.L. Gross, R. Luqmani, C.D. Pusey, N. Rasmussen, R.A. Sinico, V. Tesar, P. Vanhille, K. Westman, C.O. Savage, E.E.V.S. Group), Pulse versus daily oral cyclophosphamide for induction of remission in antineutrophil cytoplasmic antibody-associated vasculitis: a randomized trial, Ann Intern Med 150(10) (2009) 670-80.
35. L. Novelli, R. Ruggiero, F. De Giacomi, A. Biffi, P. Faverio, L. Bilucaglia, S. Gamberini, G. Messinesi, A. Pesci, Corticosteroid and cyclophosphamide in acute exacerbation of idiopathic pulmonary fibrosis: a single center experience and literature review, Sarcoidosis Vasc Diffuse Lung Dis 33(4) (2016) 385-391.
36. T. Okamoto, H. Ichiyasu, K. Ichikado, H. Muranaka, K. Sato, S. Okamoto, K. Iyonaga, M. Suga, H. Kohrogi, [Clinical analysis of the acute exacerbation in patients with idiopathic pulmonary fibrosis], Nihon Kokyuki Gakkai Zasshi 44(5) (2006) 359-67.
37. V. Ambrosini, A. Cancellieri, M. Chilosi, M. Zompatori, R. Trisolini, L. Saragoni, V. Poletti, Acute exacerbation of idiopathic pulmonary fibrosis: report of a series, Eur Respir J 22(5) (2003) 821-6.
38. J.G. Parambil, J.L. Myers, J.H. Ryu, Histopathologic features and outcome of patients with acute exacerbation of idiopathic pulmonary fibrosis undergoing surgical lung biopsy, Chest 128(5) (2005) 3310-5.
39. F.M. Al-Hameed, S. Sharma, Outcome of patients admitted to the intensive care unit for acute exacerbation of idiopathic pulmonary fibrosis, Can Respir J 11(2) (2004) 117-22.
40. E. Morawiec, I. Tillie-Leblond, V. Pansini, J. Salleron, M. Remy-Jardin, B. Wallaert, Exacerbations of idiopathic pulmonary fibrosis treated with corticosteroids and cyclophosphamide pulses, Eur Respir J 38(6) (2011) 1487-9.
41. H. Hozumi, H. Hasegawa, K. Miyashita, H. Yasui, Y. Suzuki, M. Kono, M. Karayama, K. Furuhashi, D. Hashimoto, N. Enomoto, T. Fujisawa, N. Inui, Y. Nakamura, K. Yokomura, H. Nakamura, T. Suda, Efficacy of corticosteroid and intravenous cyclophosphamide in acute exacerbation of idiopathic pulmonary fibrosis: A propensity score-matched analysis, Respirology 24(8) (2019) 792-798.
42. S. Aso, H. Matsui, K. Fushimi, H. Yasunaga, Systemic glucocorticoids plus cyclophosphamide for acute exacerbation of idiopathic pulmonary fibrosis: a retrospective nationwide study, Sarcoidosis Vasc Diffuse Lung Dis 36(2) (2019) 116-123.
43. J.M. Naccache, M. Montil, J. Cadranel, M. Cachanado, V. Cottin, B. Crestani, D. Valeyre, B. Wallaert, T. Simon, H. Nunes, Study protocol: exploring the efficacy of cyclophosphamide added to corticosteroids for treating acute exacerbation of idiopathic pulmonary fibrosis; a randomized double-blind, placebo-controlled, multi-center phase III trial (EXAFIP), BMC Pulm Med 19(1) (2019) 75.
44. B. Bradley, H.M. Branley, J.J. Egan, M.S. Greaves, D.M. Hansell, N.K. Harrison, N. Hirani, R. Hubbard, F. Lake, A.B. Millar, W.A. Wallace, A.U. Wells, M.K. Whyte, M.L. Wilsher, B.i.T.S.S.o.C.C.

British Thoracic Society Interstitial Lung Disease Guideline Group, T.S.o. Australia, N.Z.T. Society, I.T. Society, Interstitial lung disease guideline: the British Thoracic Society in collaboration with the Thoracic Society of Australia and New Zealand and the Irish Thoracic Society, Thorax 63 Suppl 5 (2008) v1-58.
45. S.M. Koo, S.Y. Kim, S.M. Choi, H.K. Lee, K.I.L.D.S. Group, Korean Guidelines for Diagnosis and Management of Interstitial Lung Diseases: Part 5. Connective Tissue Disease Associated Interstitial Lung Disease, Tuberc Respir Dis (Seoul) 82(4) (2019) 285-297.
46. F. Tomiyama, R. Watanabe, T. Ishii, Y. Kamogawa, Y. Fujita, Y. Shirota, K. Sugimura, H. Fujii, H. Harigae, High Prevalence of Acute Exacerbation of Interstitial Lung Disease in Japanese Patients with Systemic Sclerosis, Tohoku J Exp Med 239(4) (2016) 297-305.
47. M. Ota, Y. Iwasaki, H. Harada, O. Sasaki, Y. Nagafuchi, S. Nakachi, S. Sumitomo, H. Shoda, S. Tohma, K. Fujio, K. Yamamoto, Efficacy of intensive immunosuppression in exacerbated rheumatoid arthritis-associated interstitial lung disease, Mod Rheumatol 27(1) (2017) 22-28.
48. A.B. Schulze, G. Evers, A. Kümmel, F. Rosenow, J. Sackarnd, J.P. Hering, C. Schülke, J.A. Engelbertz, D. Görlich, P.J. Barth, G. Lenz, H. Becker, M. Mohr, L.H. Schmidt, Cyclophosphamide pulse therapy as treatment for severe interstitial lung diseases, Sarcoidosis Vasc Diffuse Lung Dis 36(2) (2019) 157-166.

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Bronchoscopic performance of bronchoalveolar lavage in germany – a call for standardization

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Abstract. Background: Bronchoalveolar lavage (BAL) is a widely used clinical tool in diagnosing interstitial lung diseases. Although there are recommendations and guidelines, the procedure is not completely standardized. Varying approaches likely influence the conclusiveness of BAL data and may be one reason for the divergent judgement of their value between different centers. **Objectives:** To evaluate how BAL is performed in Germany using an electronically based survey. **Methods:** We conducted a cross-sectional online survey among all members of the German Respiratory Society. **Results:** 608 members responded to the survey and of these 500 perform lavages. Most bronchoscopists (344/500) do not use a tube and have no anesthesiologist present during the procedure (405/500). Propofol is used by 76.8% and midazolam by 67.9% (n = 405), often in combination. A major difference was noted regarding the total volume of instillation. Many respondents use a predefined fixed amount of instilled volume (202/500), whereas an almost equal number use variable volumes based on the recovery (196/500). The minimum recovery volume predefined by 217/499 ranged from 3-150 ml (median 30 ml; mean 42.2 ± 55.1 ml). Most respondents did not transport their samples in special medium (61.5%) or on ice (72.8%). The average time between recovery and arrival at the lab was 115.6±267.0 min (n = 323). **Conclusion:** This study shows the broad spectrum of variations in the performance of BAL in Germany, which could have a negative effect on the method's clinical value. There is a need for training and standardization of BAL performance.

Key words: Bronchoscopy; bronchoalveolar lavage; interstitial lung disease; BAL survey

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Introduction

Bronchoalveolar lavages (BAL) were first performed in animal studies in 1961, aiming to harvest alveolar macrophages for research purposes (1,2). Three years later, this method was introduced in

humans (2,3). Apart from the diagnosis of infectious diseases, BAL is an important tool in the diagnosis of interstitial lung diseases (ILD)(4–6). However, its use for the diagnosis of ILD has varied over the decades. According to the ATS/ERS/JRS/ALAT statement on idiopathic pulmonary fibrosis (IPF) from 2011, BAL should not be performed for diagnostic evaluation of IPF in the majority of patients (7). This point of view changed, and now the current guideline on the diagnosis of IPF from 2018 recommends BAL again in the diagnostic algorithm of IPF (8). However, although a recommendation for BAL was made, there were strong and divergent opinions on the use of BAL in ILD within the guideline committee. Given the fact that members of this committee should mainly base their recommendation on published data, this heterogeneity is surprising and may be the result of different personal experience on the value of BAL. Although there are guidelines on the BAL procedure, the authors discovered that there is a huge divergence in the performance of the BAL procedure itself. It is likely that different approaches affect the results of BAL and, thus, influence and limit their clinical value. Such differences in real world experience may be one reason for the divergent judgement of the value of BAL between different centers. Therefore, we performed an electronically based survey, which evaluated the performance of BAL in Germany.

Materials and Methods

We conducted a cross-sectional survey (see supplementary material) among all members of the German Respiratory Society (DGP). Members were contacted by email. Their participation was voluntary and all collected data was pseudonymized. Members were sent a corresponding link. Using this link, participants answered questions online via a database that was specifically developed for this purpose. The survey questions were developed jointly by the authors and are shown in the Supplement. Since the methods could vary within a single medical institution, the questions applied to the specific survey participant and not to the method practiced at the institution. Members of the DGP who did not participate in the survey within 2 weeks were asked in a second email to take part. Members, who responded to the survey and do not perform BAL, stopped the survey after the first question.

All responses were evaluated in a pseudonymized form. The authors did not know which response corresponded to which survey participant. The present study is a survey of a bronchoscopy method (how do I perform a BAL). It was on a generalized technique on an individual physician level and not a patient-based data survey. Individual patient information or patient data were not collected. Thus, an ethics committee vote or patient consent was not necessary.

A descriptive data evaluation was performed by calculating absolute numbers, percentages, medians, and means. Whenever possible standard deviations are given. However in most cases the data are nominal and not continuous. In these cases median or absolute numbers were calculated. The participants entered the data directly into an electronic database. The data were transferred to an Excel file and finally imported into and calculated using JMP® 14.20, SAS Institute Inc.

Results

3070 members of the German Respiratory Society (DGP) were contacted to take part in the study. The survey was taken by 608 members, of these 500 performed BAL. BAL was done mainly for suspected sarcoidosis or ILD (63%; 315/500) followed by infections (22%; 110/500). The average number of BALs performed per year by the physicians who specified this was 162 ± 251 (95% CI: 139-185; n = 464).The primary outcome was transplant-free survival. Data was obtained from the Social Security death index and the electronic medical record. Date of last follow-up, death, or lung transplantation was recorded.

Technical requirements for BALs - flexible tube, rigid bronchoscope, or without tube

The vast majority of the bronchoscopists performed BAL without intubation (Figure 1). Only 79/500 (15.8%) respondents, routinely intubated with a flexible tube. Thirty bronchoscopists (6.0%) used a rigid bronchoscope for intubation. Since only 47 participants (9.4%) said that an anesthesiologist is present when performing the BAL, it can be concluded that the majority perform the procedure via a flexible tube without the support of an anesthesiologist.

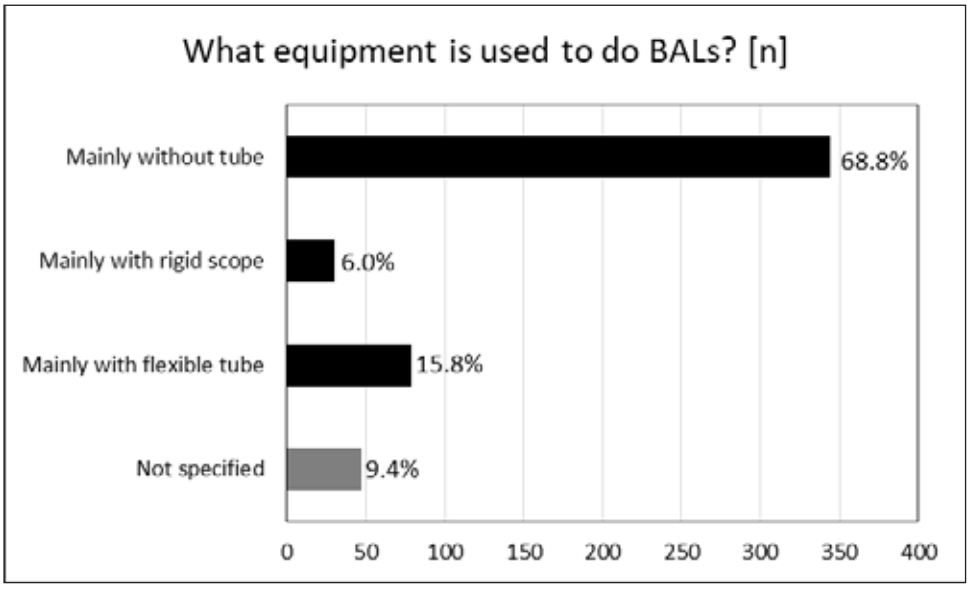

Figure 1. The equipment used for BAL is shown. Given are the number and percentage of participants (n = 500).

Analgosedation and local anesthesia

For the sedation, 76.8% (311/405) of the respondents used propofol and 67.9% (275/405) midazolam. Therefore, many respondents used both drugs, most probably as a combination, while opioids were used by 12.4% (50/405) of the participants.

Local anesthesia for bronchoscopy can be performed in several ways, either as a single mode of application or in combination. Inhalation of a local anesthetic was used by 32.8% (164/500) of participants, spray application of a local anesthetic to the throat by 73.6% (368/500), and instillation of a local anesthetic to the bronchial system by 76.4% (382/500). A total of 60.2% (301/500) reported that the instillation of a local anesthetic to the bronchial system is performed immediately before BAL. There is a marked difference in the general instillation of a local anesthetic to the bronchial system depending on whether an anesthesiologist is present during bronchoscopy (29.8%; 14/47) or not (90.6%; 367/405).

Procedures to instill and recover lavage fluid

More than half (64.4%; 322/500) of the physicians instilled the fluid directly through the working channel, while 22.2% (111/500) used a separate catheter, which was inserted into the working channel (Figure 2a). If the working channel was used directly for instillation and recovery of lavage fluid, 70.2% (226/322) flushed the working channel with saline before application of the first aliquot (Figure 2b).

A major technical variation between the responding physicians was the instilled volume of saline used for BAL. Figure 3 shows what the respondents used as a criterion for their instilled fluid volume. While 202 physicians (40.4 %) used a fixed total amount of instillation volume, 196 (39.2%) physicians reported that the total amount is based on the recovery. In the case where the total amount was based on the instilled volume, the median volume instilled was 100 ml (10% percentile: 100 ml, 90% percentile: 200 ml). If the recovery was used as a criterion for the total instilled amount, the median target recovery volume was 50 ml (10% percentile: 30 ml, 90% percentile: 100 ml). However, the aimed target recovery volume was very low in some cases (minimally 3ml). Independent of this, 43.5% of the physicians defined a minimum amount for the recovery (median = 30 ml). The aliquot volume was usually 20ml (median 20 ml, 10% percentile 20ml, 90% percentile 100ml). After instillation, 61.1% (305/499) of respondents recovered lavage fluid manually and 23.6% (118/499) used mechanical suction of which 5.6% (28/499) used support without reduction of

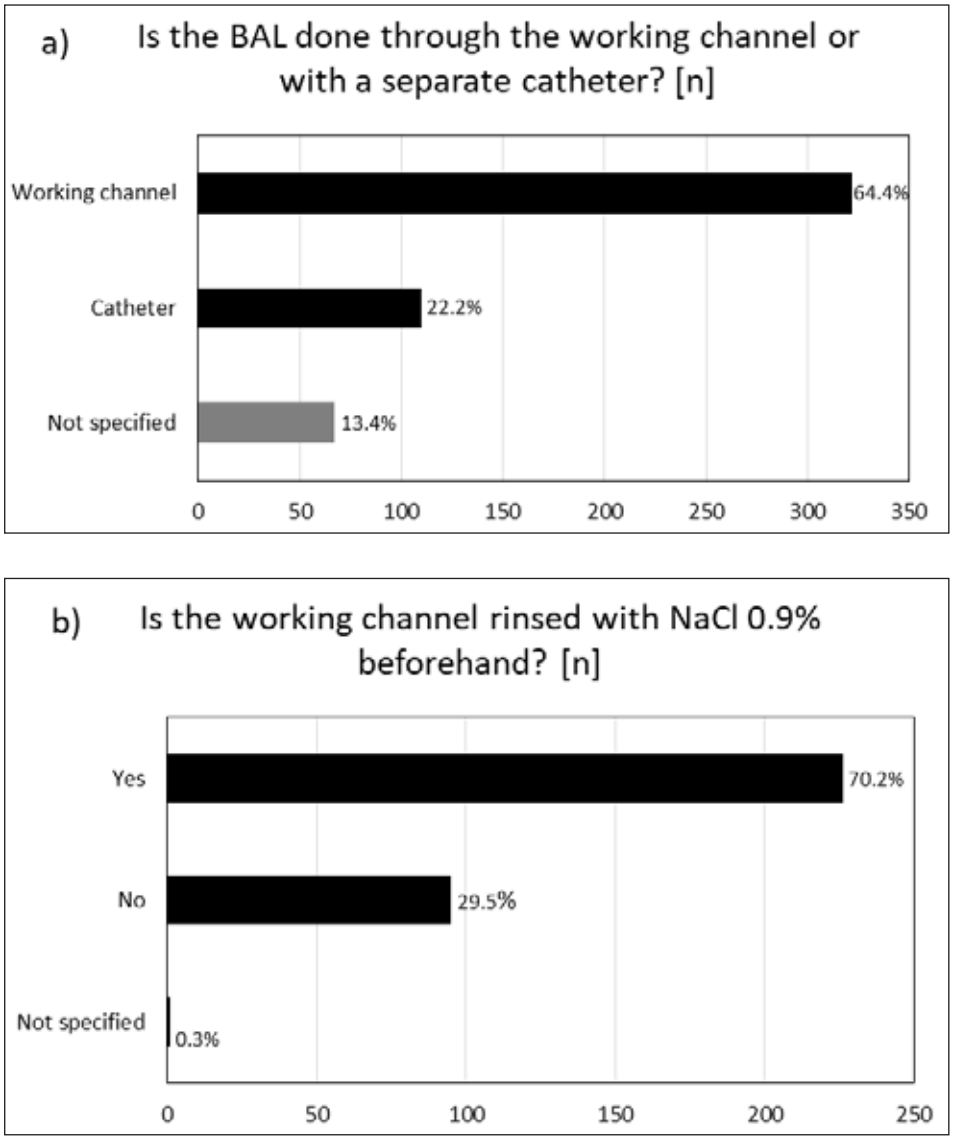

Figure 2. a) Participants were asked whether the BAL procedure is done directly through the working channel or by using a separate catheter. The results are given in number and percentage of all participants (n = 500). b) Of the participants that used the working channel directly, the number and percentage of participants are given that rinse the channel with saline before performing BAL (n=322).

applied negative pressure (Figure 4). The first portion of the lavage aspirate was routinely discarded by a median of 50.0% of bronchoscopists (n = 196), the median volume discarded was 20 ml (n = 164). Slightly less than half (43.5%; 217/499) predefined a minimal recovery volume to indicate a successful BAL (Figure 5). The median predefined minimal recovery was 30 ml.

Site of lavage

In case of diffuse ILD, the middle lobe or the lingula were used as standard sites for BAL by 56.1% (280/499) of respondents, while 26.1% (130/499) performed BAL in those segments with the most prominent interstitial lung abnormalities; 17.8% (89/499) did not respond to this question.

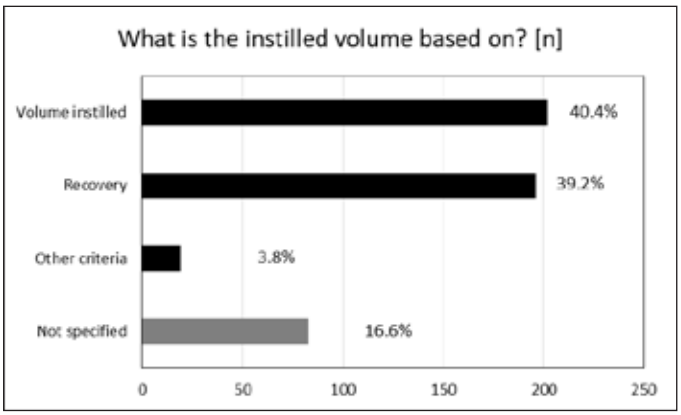

Figure 3. The participants were asked what they base their instilled volume for BAL on. The results are given in number and percentage of participants (n = 500).

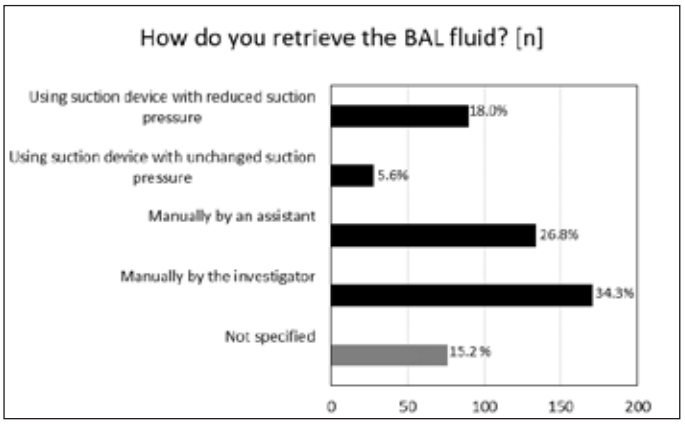

Figure 4. The participants were asked how they retrieve the BAL fluid. Given are the number and percentage of participants (n = 499).

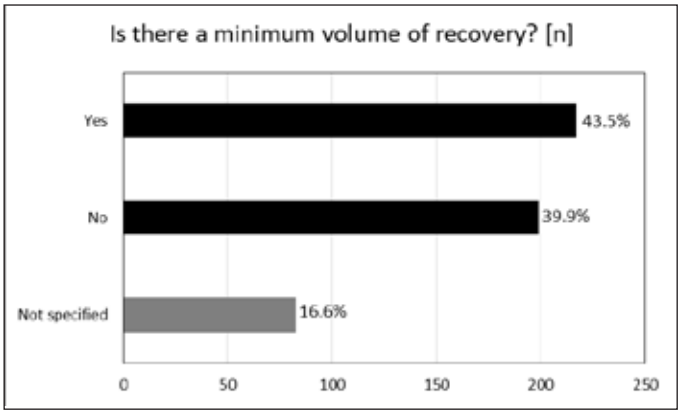

Figure 5. The participants were asked whether they define a minimum recovery volume. Given are the number and percentage of participants (n = 499).

In patients with localized lesions suspicious for organizing pneumonia, inflammatory infiltrates, malignant lesions, or similar, the majority (78.0%; 389/499) performed BAL in the area of greatest abnormality, while only 4.2% (21/499) did not; 17.8% (89/499) did not respond to this question.

Sample transport and cell analysis

In the majority of the cases, BAL was not transported in a special transport medium (no special medium: 61.5%; special medium: 20.6%; not specified: 17.8%). The containers used to collect BAL samples were usually made of plastic (71.5%). Few participants used glass (3.4%). The remaining did not specify (18.8%) or did not know (6.2%). When asked whether BAL was transported on ice, 72.8% said no, while 8.2% said yes.

About 50% of the pulmonology departments had their samples analyzed externally and around 30% in their own institution (see Table 1).

The average time between BAL recovery and the time the sample arrived at the lab was about two hours (mean 115.6 ± 267.0 min.; 95% CI: 86.4-144.9; n = 323). When asked how much time passes between BAL recovery and cytological or immunocytological analysis, 28.5% (data not shown) and 36.5% of the participants, respectively, did not know (Figure 6). The time to cytological or immunocytological analysis was more than 1 hour in 63.3% (data not shown) and 83.3% (n = 210) of the cases, respectively.

Discussion

In the past years, since the 2011 IPF guideline, the scientific interest in the diagnosis of ILDs has focused mainly on diagnostic algorithms, radiologic and histologic criteria, as well as transbronchial cryobiopsies. BAL was not the focus of scientific attention. However, the quality and thus how conclusive BAL data are is crucially dependent on how BAL is technically performed (9). Conferences over the years have repeatedly been held on the BAL procedure including timing, sources of cells, use of findings, and perturbation of BAL components (10). There have been calls for training and standardization. Nevertheless, the ATS guideline on clinical utility of BAL is vague and rarely gives clear instructions on how to implement the procedure (9). The present survey evaluated how BAL is currently performed in Germany. The study showed that in part there are great variations in the technical procedure, which could strongly affect the conclusiveness of the BAL results. We will discuss the different aspects of the study sequentially.

Table 1. Where is the cytological and immunocytological analysis done?

Site of analysis	Cytology [n]	Immunocytology [n]
Internally at hospital	171(34.3%)	134(26.9%)
Externally	229(45.9%)	251(50.3%)
Not done	1(0.2%)	14(2.8%)
Not specified	97(19.4%)	99(19.8%)
Unknown	1(0.2%)	1(0.2%)

Technical requirements for BAL

Guidelines only report on performing BAL in the setting of a flexible bronchoscopy (9,11). Studies analyzing the impact of doing BALs through a rigid bronchoscope or through a flexible tube are missing. However, as "rigid" usually means using a flexible bronchoscope through a rigid scope, an impact of a rigid conduit or a tube on the quality and results of the BAL is unlikely. Neither a rigid scope nor an intubation with a tube is necessary to perform a BAL, and the setting should be chosen based on other procedures planned during the bronchoscopy such as transbronchial cryobiopsy or endobronchial ultrasound (EBUS). Based on the survey, it cannot be concluded whether patients were intubated for the procedure, i.e. with a flexible tube, or if the procedure was performed in patients who were intubated for other procedures done during the same bronchoscopy.

Analgosedation and local anesthesia

According to the survey results, BAL is most frequently executed without an anesthesiologist in moderate sedation using a combination of propofol and midazolam. Performing BAL under general anesthesia yields similar results to local anesthesia (11). Although there are recommendations on sedation during bronchoscopy (12), no recommendation on the optimal drugs for sedation has been given

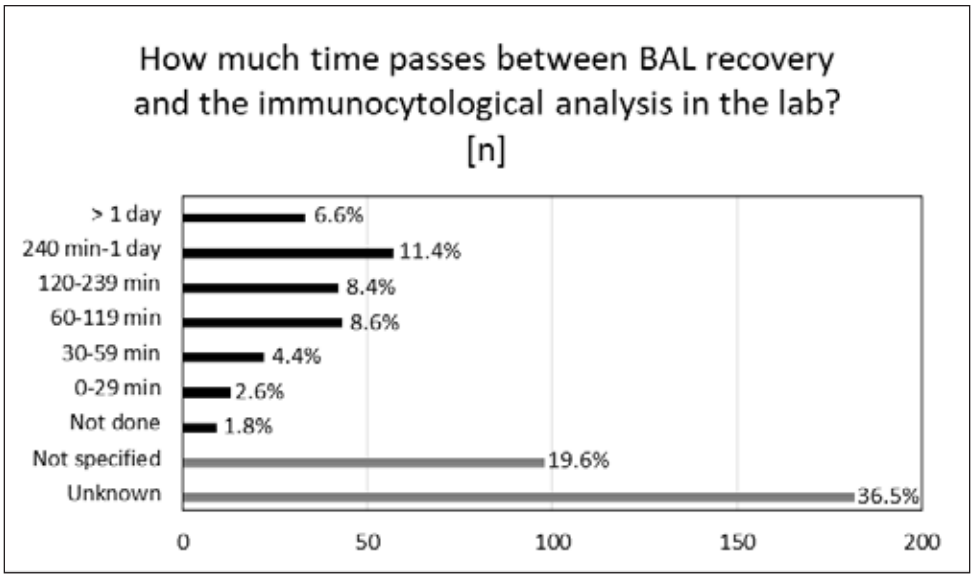

Figure 6. The participants were asked how much time passes between BAL recovery and immunocytological analysis. Given are the number and percentage of participants (n =499).

to date. More important than the choice of specific drugs is that sedation is deep enough to prevent coughing, which would have a negative effect on cell recovery and contamination with bronchial secretions and especially blood (13).

An interesting finding of the present study is that roughly 1 out of 5 physicians routinely perform the BAL in the presence of an anesthesiologist. Whether this has a direct impact on patient outcome (i.e. higher safety standards) has not been conclusively investigated so far. However, it can be assumed that the presence of an anesthesiologist is not primarily motivated by the BAL, but by other factors such as an additional cryobiopsy. We assume that performing BAL is only a minor burden for the patients. Therefore, with sufficient pulmonary resources, no special precautions (e.g. the presence of an anesthesiologist or intubation) have to be taken. At the same time, the consumption of health care resources rises markedly if an anesthesiologist is routinely involved in bronchoscopy – especially given the fact that under these circumstances additional personnel is involved on top of the specialist (e.g. specialized nurses).

This study highlights that in the vast majority of bronchoscopies propofol is used for sedation and can be considered the (informal) standard medication for sedation in Germany. Only a minority (about 12%) routinely apply analgesics (i.e. opioids) during bronchoscopy.

According to the present study, inhalation of local anesthetics is rarely performed. In contrast, the application of these agents to the throat and the bronchial system must be viewed as standard. Uncertainty remains whether there is a clinically meaningful impact of local anesthetic application with regard to BAL quality/interpretation (i.e. bacterial death/cell destruction). Duddridge et al. report that lidocaine at concentrations of 1.5% and 4% show negligible effects on BAL metabolic activity if the supernatant is not removed promptly from the harvested cells (14). Interestingly, the presence of an anesthesiologist results in markedly reduced application of local anesthetics to the bronchial system. This is most likely explained by the fact that in contrast to pulmonologist-guided bronchoscopies including analgosedation, patients receive muscle relaxants during (rigid) bronchoscopy if anesthesiologists are involved. However, these points are not addressed in the current guideline (9).

Procedures to instill and recover lavage fluid

Taking into account that the European Society of Pneumology (one of the two founding societies of the ERS) published its recommendations as early

as 1989, there is a surprising degree of heterogeneity in instillation and recovery procedures. While these guidelines recommend instillation through the working channel of the fiberoptic bronchoscope (11), 22% of respondents use a separate plastic catheter. The guidelines also address the containers in which the recovery fluid is collected. Ones without silicone coating and some plastic containers promote cell adherence to the container surface and thus may influence the BAL result (9). However, to date, to the authors' knowledge, no data are available on the adherence of recovered cell types to the plastic surface of these separate plastic catheters. The guideline for the performance of BALs does not clearly define the criterion, which should be used as an orientation for the instilled volume. Although an instilled volume of 100-300 ml is recommended, at the same time a minimal recovery of more than 30% is considered sufficient. With a recovery smaller than 10%, the BAL is considered inconclusive (9). In the present study, only 40% chose a predefined fixed amount of instillation volume. Thirty-nine percent used a variable amount based on the recovered volume. Interestingly, the predefined instilled volume was less than 100 ml in 3.9% and thus falls below the recommended lower limit. In 51%, the aimed instilled volume corresponded to the suggested minimal amount of 100 ml. It is possible that some physicians, who use the fixed amount of instillation volume, adapt this during bronchoscopy to higher volumes when they notice that the recovery would be too low.

Interestingly, a great technical variation is found in the predefined target recovery volume. Costabel et al. recommend recovering at least 25 ml of the instilled saline (15). The Clinical Practice Guideline of the ATS from 2012 recommends that the minimal total volume retrieved should be greater or equal to 5% of the instilled volume, i.e. 5-15 ml (optimal sampling retrieves >30%) (9). In the present survey, only 0.5% fall below the lower limit of 10 ml defined in the guideline. The median sought recovery volume was 50 ml. However, the described lower limit in the guideline should be evaluated critically. If 20 ml portions are used and the recovery is merely 10% per portion, it would mean that when performing BALs through a working channel (volume of the working channel about 2 ml) only fluid from the working channel would be aspirated. This fluid would not have had contact with the bronchial system let alone with the alveolar region. Accordingly, we consider the recommendation of a recovery of more than 50 ml (50-60 % of the instilled volume) given by Costabel as a good orientation (15).

Should we discard the first portion? According to Klech and Pohl (11) the first aspiration may be significantly different from the subsequent ones due to the high proportion of bronchial washing. This is especially relevant in cases with bronchial inflammation or mucus contamination. In the present survey 43.5% of the physicians reported that they discard the first aspirate. However, to the authors' knowledge, no data are available on this topic so far. The instilled aliquots are usually 20 ml (11). In the European recommendation from 1989, an aspirate after each instillation is suggested (11). However, there are no data that instillation of the first 60 or 80 ml as a single portion might be better because the large alveolar bed cannot be reached with the first 20 ml. It should be discussed whether the danger of repeated "bronchial washings" might be increased. Unfortunately, data on this topic concerning BAL are still lacking. Regarding recovery, several respondents use manual suction, which is also suggested in the European recommendations. However, 6% use mechanical support without reduction of negative pressure, which should be avoided.

Site of BAL

The preferred site of BAL by the majority of respondents reflects existing recommendations. The Report of the European Society of Pulmonology Task Group on BAL (11) recommends a standard site of sampling in diffuse interstitial lung disease, preferably the middle lobe or the lingula. The reason is that approximately 20% more fluid and cells can be recovered from these lobes than from the lower lobes (16). Based on the data available at the time of publication, the task forces summarized that, in general, lavage at one site would give sufficient clinical information and may be considered representative of the whole lung (11). Most respondents in the current survey follow this approach. Yet, in contrary, a more recent ATS clinical practice guideline (9) suggests that the lavage site should be chosen on the basis of the results of a high-resolution computed tomography performed before bronchoscopy. This is

Table 2. Recommendations for the transport of the recovered BAL fluid according to Meyer et al. (6)

(1)	The BAL recovery should be collected in containers that prevent cells from adhering to the vessel wall. Otherwise, cell loss could result (e.g. silicon coated glass containers or containers made of polypropylene or plastic containers especially developed for cell culture) (7).
(2)	BAL samples may be transported at room temperature if the analyzing laboratory is in the same hospital and there is no transport delay.
(3)	If the expected transport time is up to one hour, the material can be sent in its native form, but on ice (4° C). It is important to ensure that the transport fluid does not freeze.
(4)	If the expected time of transport is more than one hour, the material should be transferred to a nutrient solution. Media for cell culture are suitable (i.e.: MEM+25 mM HEPES). In case the transport is at room temperature, the addition of a bacteriostatic agent to the culture medium can be considered (i.e. 0.1ml Pen/Strep). This, for example, makes postal shipping over 24 h possible without cooling [12].
(5)	If the analyzing laboratory cannot work up the native sample immediately, it is recommended to transfer it to a culture medium. The sample should be cooled until it is worked up, which should not be more than 24 h.

preferable to a "traditional" BAL site (i.e. the middle lobe or lingula) based on low evidence data suggesting superior results if a lavage is performed in areas with more extensive parenchymal change. However, since then, no further higher-grade evidence has been generated reflecting this recommendation. With localized lesions, the majority of respondents chose the area with the most prominent changes for lavage, which reflects the recommendations by the European task group report (11).

Sample transport and cell analysis

Although over 70% of the hospitals transport BAL without cooling and the majority without special transport medium, about half the samples are processed externally. The average transport time to the analyzing laboratory is about 2 hours. Many participants did not know how long it takes until their samples are cytologically and immunocytologically analyzed. This means that the BAL fluid is often transported under poor conditions. Without cooling and special nutrients, the cells already begin to deteriorate and die after 60 minutes (17). The correct transport is essential to preserve the quality of the BAL cells. Therefore, it is important to ensure optimal transport so that the quality of the cells is as good as possible. The diagnostic evaluation is strongly dependent on the preservation of the original material. Table 2 summarizes the recommendations for the sample transport.

However, not only the transport is important, but also how BAL is processed and evaluated in the laboratory. This influences the quality of the analysis and thus the accuracy of the results.

Conclusions

The present study demonstrates the broad heterogeneity of how BAL is performed in Germany. Some variations, however, should clearly be considered questionable, because they have a negative effect on the conclusiveness of the BAL (e.g. recovery volume, type and duration of sample transport). Thus, even for the "simple examination" BAL there is a need for training. Other variations are the result of missing standardizations (e.g. a broad range of recommended total instilled volumes: 100-300 ml). Such a standardization, even though it is often based on expert opinion, is highly warranted and would establish a basis for future comparative studies to optimize the BAL method. Although it is almost certain that variations in performing BAL are also seen in other European countries, further studies beyond Germany would underline the urgency for standardization and training further.

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References

1. Myrvik Q, Leake ES, Fariss B. Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity. J Immunol. 1961 Feb;86:128–32.

2. Toetsch M, Guzman J, Theegarten D, Schmid K., Costabel U. [Bronchoalveolar lavage]. Pathologe. 2007;28:346–53.
3. Keimowitz RI. Immunglobulins in normal human tracheobronchial washings. J Lab Clin Med. 1964;63:54–9.
4. Gharsalli H, Mlika M, Sahnoun I, Maalej S, Douik El Gharbi L, Mezni F El. The utility of bronchoalveolar lavage in the evaluation of interstitial lung diseases: A clinicopathological perspective. Semin Diagn Pathol. 2018 Sep;35(5):280–7.
5. Kebbe J, Abdo T. Interstitial lung disease: the diagnostic role of bronchoscopy. J Thorac Dis . 2017 Sep;9(S10):S996–1010. A
6. Tanriverdi H, Uygur F, Örnek T, Erboy F. et al. Comparison of the diagnostic value of different lymphocyte subpopulations in bronchoalveolar lavage fluid in patients with biopsy proven sarcoidosis. Sarcoidosis Vasc Diffus Lung Dis. 2015;32:305–12.
7. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, 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 Mar 15;183(6):788–824.
8. 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(5):e44–68.
9. Meyer KC, Raghu G, Baughman RP, Brown KK, Costabel U, du Bois RM, et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med. 2012 May 1;185(9):1004–14.
10. Reynolds HY. Bronchoalveolar Lavage - Obtaining Biologic Specimens from the Respiratory Tract Surface. Sarcoidosis Vasc Diffus Lung Dis. 2008;25:5–9.
11. Klech H, Pohl W. Fiberbronchoscopy in Technical recommendations and guidelines for bronchioloalveolar Lavage (BAL). Report of the ERS Task Group. Eur Respir J. 1989;2:561–85.
12. Hautmann H, Eberhardt R, Heine R, Herth F, Hetzel J, Hetzel M, et al. Empfehlung zur Sedierung in der flexiblen Bronchoskopie. Pneumologie. 2011 Nov 14;65(11):647–52.
13. Bonella F, Ohshimo S, Bauer P, Guzman J, Costabel U. Bronchoalveolar lavage. In: Interventional Pulmonology . European Respiratory Society Journals Ltd; 2010. p. 59–72.
14. Duddridge M, Kelly CA, Ward C, Hendrick DJ, Walters EH. The reversible effect of lignocaine on the stimulated metabolic activity of bronchoalveolar lavage cells. Eur Respir J. 1990;3:1166–72.
15. Costabel U, Barth J, Behr J, Buhl R, Kanzow G, Klech H. [Recommendations for diagnostic bronchoalveolar lavages]. Pneumologie. 1993;47:607–19.
16. Pingleton SK, Harrison GF, Stechschulte DJ, Wesselius LJ, Kerby GR, Ruth WE. Effect of location, pH, and temperature of instillate in bronchoalveolar lavage in normal volunteers. Am Rev Respir Dis. 1983 Dec;128(6):1035–7.
17. Costabel U. [Atlas of the Bronchoalveolar Lavage]. Stuttgart: Thieme; 1994.

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Transbronchial cryobiopsy for diagnosing parenchymal lung diseases: real-life experience from a tertiary referral center

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Abstract. Background and Objectives: Transbronchial cryobiopsy (cryo-TBB) is increasingly being used in the diagnosis of diffuse parenchymal lung diseases (DPLD). Varying diagnostic success and complication rates have been reported. Herein we report our experience with cryo-TBB, focusing on diagnostic yield, factors affecting diagnosis, and safety. **Methods:** This retrospective study was conducted in a tertiary referral chest diseases hospital. Data regarding the patients, procedures, complication rates, diagnostic yield, and the final diagnosis made by a multidisciplinary committee at all diagnosis stages were evaluated. **Results:** We recruited 147 patients with suspected DPLD. The definitive diagnosis was made pathologically in 98 of 147 patients (66.6%) and using a multidisciplinary approach in 109 of 147 (74.1%) cases. The number of samples had a significant effect on diagnostic success. Histopathologic diagnostic yield and diagnostic yield with a multidisciplinary committee after a single biopsy were 50%, and histopathological diagnostic yield and diagnostic yield with multidisciplinary committee increased to 71.4% and 85.7%, respectively, with a second biopsy (p = 0.034). The incidence of mild-to-moderate hemorrhage was 31.9%; no severe hemorrhage occurred. Pneumothorax rate was 15.6%, and the mortality rate was 0.68%. **Conclusions:** Cryo-TBB has sufficient diagnostic yield in the context of a multidisciplinary diagnosis with acceptable complication rates. Performing at least 2 biopsies and from at least 2 segments increases diagnostic success.

Key words: Transbronchial cryobiopsy, bronchoscopy, parenchymal lung diseases, diagnostic yield

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Introduction

Diffuse parenchymal lung diseases (DPLDs) are a heterogeneous group of lung parenchymal disorders with varying treatment options and prognoses. Given this heterogeneity, arriving at a differential diagnosis is critical (1, 2). However, arriving at a differential diagnosis could be challenging given the similar and

overlapping features between diseases. In most cases, further invasive procedures are required for accurate diagnosis following appropriate radiologic and physiological evaluations (3). Surgical lung biopsy (SLB) continues to be the gold standard in identifying the possible causes of DPLDs and their histopathologic patterns despite the associated mortality and morbidity (4-6). The 60-day mortality rate after SLB has been reported to range from 2% to 4%, signifying the need for less-invasive diagnostic procedures (5, 7). Among these minimally invasive methods, conventional transbronchial forceps biopsy offers a less-invasive approach; however, its diagnostic yield is low owing to small biopsy samples and crush artefacts (8, 9). Transbronchial cryobiopsy (cryo-TBB), which seems to be safe procedure with lower complication and mortality rates compared than SLB in DPLD diagnosis (4). Comparing the results from published cryo-TBB case series is challenging given the differences in technical details of the procedure such as the use of bronchial blockers, non-use of fluoroscopy, and performing the procedure through an intubation tube. These different procedural approaches can also result in differences in diagnostic yield and complication rates (10).

This study aimed to report our experience with the diagnostic yield and complications of cryo-TBB applied at our tertiary referral chest diseases hospital.

Methods

This retrospective study was held in Yedikule Chest Diseases and Thoracic Surgery Hospital interventional pulmonology unit between January 2014 and December 2019. . The total number of patients eligible for cryobiopsy was 155. Among those, 4 patients developed desaturation immediately after the intubation with a rigid bronchoscope, the cryo probe could not be moved distally in 3 patients, and 1 patient developed arrhytmia. Excluding these 8 cases, the study included data from 147 patients. The diagnostic steps are detailed in the scheme (Figure 1) Inclusion criteria were DPLD patients who could not be differentially diagnosed with clinical, laboratory, immunological and high resolution computed tomography (HRCT) data and were indicated for surgical lung biopsy and evaluated with cryo-TBB. All patients had pulmonary function tests (PFTs), complete blood count, HRCT, and echocardiography. The procedure was not applied to patients who had bleeding tendency (INR> 1.5, Platelet count <50.000 / microL), estimated Pulmonary artery pressure > 40 mmHg, uncontrolled cardiac arrhythmia, unstable angina, severe hypoxemia (pO2 <55 mm Hg despite oxygen support), carbon monoxide diffusing capacity (DLCO) <35%, forced expiratory volume

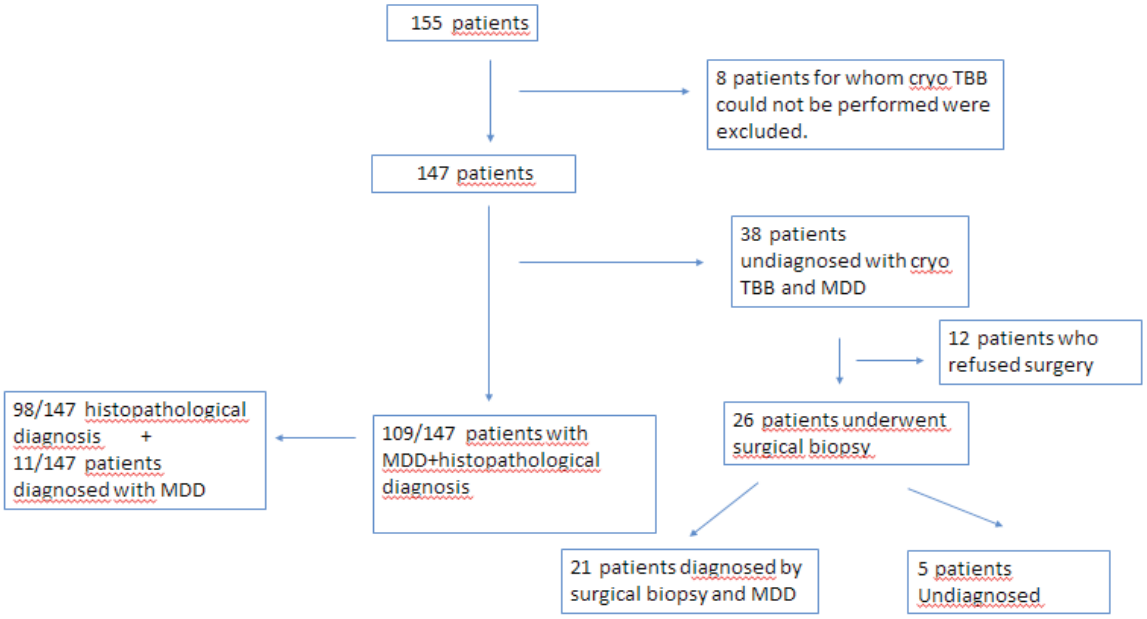

Figure 1. Diagnostic algorithm of the patients

(FVC)<50% and whose procedure was terminated without cryo-TBB due to cardiac and respiratory instability detected after intubation.

Procedure

Anticoagulant treatments were discontinued before the procedure basis the indications in a patient. HRCT were used to identify the biopsy location before the procedure. Basis the radiological pattern, samples were biopsied from ≥1 distinct lobes. The procedure was performed under anesthesia with intravenous propofol and remifentanil, and intubation was achieved with a rigid bronchoscope. Biopsies were performed under fluoroscopy guidance with a flexible bronchoscope passed through a rigid tube, and the bronchoscope was placed in the determined bronchus. A flexible cryoprobe, 90 cm in length and 1.9 mm in diameter, was used for biopsy (ERBE, Germany) (shown in Figure 2a). The probe was cooled using nitrous oxide (N_2O), which caused the probe tip temperature to reach –89°C in a few seconds.

The cryoprobe, which was directed through a flexible bronchoscope in the determined bronchus, was placed perpendicularly to the chest wall up to 10–20 mm from the thoracic wall under fluoroscopy guidance (shown in Figure 2b). When the cryoprobe was placed in the determined position, it was cooled for an average of 5–6 s and withdrawn using the flexible bronchoscope, with the frozen lung tissue attached to the probe tip. The frozen tissue sample was placed in a formalin solution for fixation without damage. The uninflated Fogarty balloon, which was placed in the lobar bronchus closest to the biopsy segment, was inflated after each biopsy and slowly deflated for bleeding control (shown in Figure 2c). Hemorrhages were classified basis the following criteria: grade 1 hemorrhage (mild) if endoscopic aspiration was required; grade 2 (moderate) hemorrhage if additional endoscopic procedures were required (bronchial occlusion and/or cold saline); and grade 3 (severe) hemorrhage if surgical interventions because of hemodynamic or respiratory instability, transfusions, and/or intensive care unit admission were required (4). Patients who could not undergo

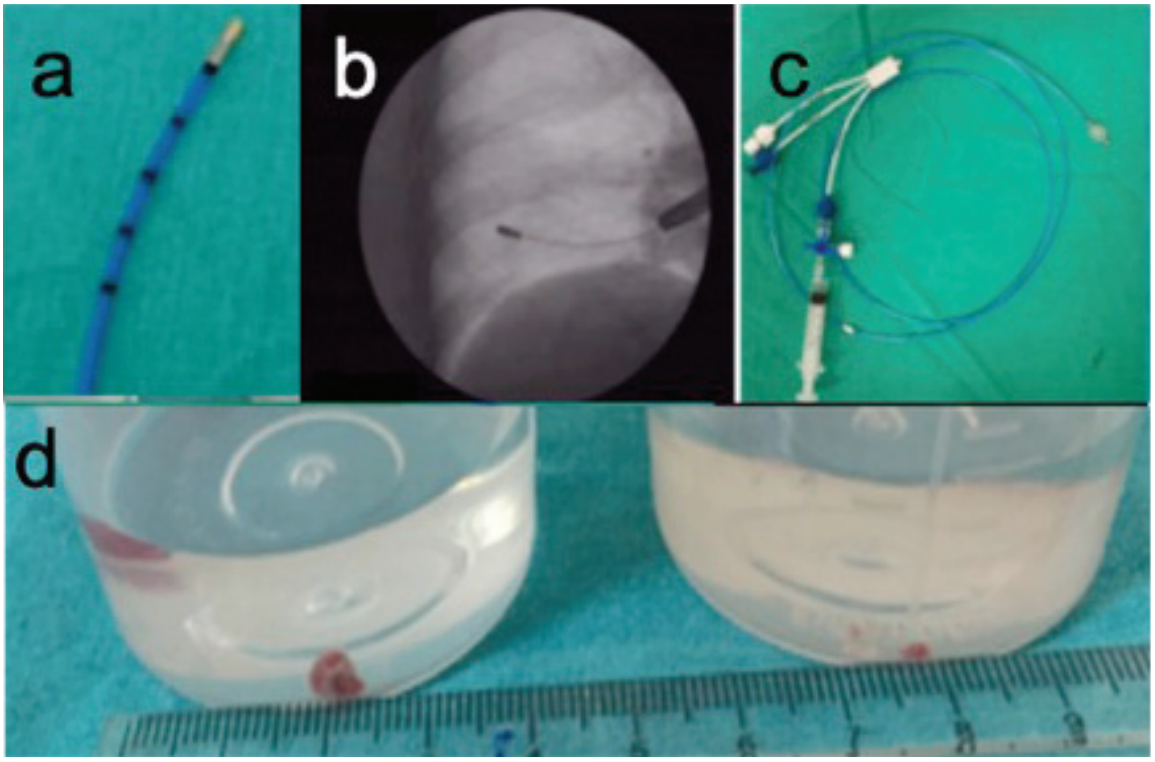

Figure 2. a) The flexible 1.9 mm diameter and 900 mm length cryoprobe, b) Marks on the distal area of the probe (1 cm between each mark) c) Fogarty baloon d) Cryo-transbronchial lung biopsy samples from one of the patients vs conventional biopsy sample comparison

cryobiopsy in the exclusion criteria were included in the bleeding assessment. Chest x-ray control was planned 2 hours after the procedure to check for an iatrogenic pneumothorax.

Clinical information, radiological features, and biopsy results were then evaluated by a multidisciplinary committee including clinicians, radiologists, and pathologists. This study was approved by the ethics committee of our institution.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Windows 22.0. Continuous variables were presented as means ± standard deviations and medians (min-max) and categorical variables as numbers and percentages. Parametric test assumptions (normality and homogeneity of variances) were checked before comparing the groups. Categorical values were analyzed using the Fisher exact test. The Mann–Whitney U test and paired samples t tests were used to compare continuous variables. A p value < 0.05 was considered statistically significant.

Results

147 patients were enrolled to the study. Among those, 4 patients developed desaturation immediately after the intubation with a rigid tracheoscopy, the cryo probe could not be moved distally in 3 patients, and 1 patient developed arrhytmia. Excluding these 8 cases, the study included data from 147 patients.

The average age of the patients analyzed in the study was 56.4 ± 13 and 82 of them were female (55.8%) and 65 were male (44.2%). The baseline characteristics of the patients are provided in Table 1.

Table 1. Patients' characteristics at baseline and procedural details

Patient number (n)		147
Mean age (years±SD)		56.4±13
Gender n (%) Female		82 (55.8)
Male		65 (44.2)
Smoking (Pack-years) (n=95, mean±SD)		12.5±16.8
CCI (n) 0-2		90
>3		57
FVC % predicted (Mean±SD)		82.4±18.5
DLCO% predicted (Mean)		59.01±18.1
Biopsy location	Right lung (n,%)	134 (91.2)
	Left lung (n,%)	13 (8.8)
	One segment	73
	Two different segments	69
	Three different segments	5
Mean biopsy number (n±SD)		3.3±1
Freezing duration (seconds, min-max)		4.8±1.2 s (4-10)
Small axis diameter (mm±SD)		2.8±0.6
Large axis diameter (mm±SD)		6.9±2.3

CCI Charlson Comorbidity index, FVC: Forced vital capacity, DLCO: Carbon monoxide diffusion capacity, cTBB: Transbronchial lung cryobiopsy, SD: standard deviation

The number of biopsies taken from the right and the left lung were 134 (91.2%) and 13 (8.8%), respectively. Biopsies were taken from one segment in 73 (49.6%) patients, from two different segments in 69 (46.9%) patients, and from three different segments in 5 (3.4%) patients. Cryo-TBB was performed on different segments of the same lobe in 61 cases and two different lobes and different segments in 15 cases . The mean biopsy number per patient was 3.3 ± 1 (range 1-6). Mean freezing time during the procedure was 4.8 ± 1.2 seconds (range: 4-10). The shortest and longest diameters of the biopsy materials were 2.8 ± 0.6 mm, 6.9 ± 2.3 mm, respectively (Figure 2d).

Histopathological diagnosis was provided in 98/147 cases (66.6%). The most common diagnosis was nonspecific interstitial pneumonia (NSIP) (n=46, 46.9%). Fourteen cases were diagnosed with granulomatous inflammation (n=14, 14.3%), 12 were malignancy (n=12, 12.2%), 6 with usual interstitial pneumonia (UIP) (n=6, 6.1%), 6 with organizing pneumonia (OP) (n=6, 6.1%), and 6 with hypersensitivity pneumonitis (HP) (n=6, 6.1%). The pathological diagnoses and diagnostic yield are showed in Table 2.

Table 2. Histopathological and multidisciplinary diagnoses and diagnostic yield

		Diagnosis	**n (%)**
DIAGNOSIS	HISTOPATHOLOGICAL DIAGNOSIS	Non-Specific Interstitial Pneumonia	46 (46.9)
		Hypersensitivity Pneumonia	6 (6.1)
		Usual Interstitial Pneumonia	6 (6.1)
		Organizing Pneumonia	6 (6.1)
		Granulomatous Inflamation	14 (14.3)
		Malignancy	12 (12.2)
		Adenocarcinoma	10
		Breast cancer metastas	1
		Lymphoma	1
		Others[a]	8 (8.2)
		Non-diagnostic	49
		Diagnosis	**n (%)**
	MULTIDISCIPLANARY COUNCIL DIAGNOSIS	Non-Specific Interstitial Pneumonia	38 (34.8)
		Hypersensitivity Pneumonitis	20 (18.3)
		Idiopathic pulmonary fibrosis	4 (3.7)
		Sarcoid	12 (11)
		Organizing Pneumonia	7 (6.4)
		Malignancy	12 (12.2)
		Adenocarsinoma	10
		Breast cancer metastas	1
		Lymphoma	1
		Others[b]	15 (13.8)
		Non-diagnostic	38

Others[a]: Alveolar microlithiasis (1), Non-spesific inflammation (1), Mosaic pattern (1), Histiocytosis X (1), Folicular bronchiolitis (1), Chronic eosinophilic pneumonia (1), Acute Lung Injury (1), Unclassifiable interstitial lung disease (1)
Others[b]: Rheumatoid lung disease (1), Tuberculosis (1), Drug induced lung disease (1), Alveolar microlithiasis (1), Nonresolving pneumonia (1), Mosaic attenuation (1), Histiocytosis X (1), Follicular bronchiolitis (1), Acute interstitial pneumonia (1), Unclassifiable interstitial lung disease (1)
SD: standard deviation

A final diagnosis was made in 109 (74.1%) of 147 cases evaluated by the multidisciplinary committee. The most common diagnosis was NSIP in 38 patients. Other diagnoses are shown in Table 2.

SLB was performed in 26 (17.6%) out of 38 patients who could not be diagnosed after multidisciplinary evaluation, 21 patients were diagnosed while pathological diagnosis could not be made in 5 patients (11 UIP, 5 HP, 2 Adenocarcinoma, 1 NSIP, 1 Emphysema, 1 Anthracosis). The remaining 12 patients were patients who were not suitable for SLB due to their general condition or who did not accept a further examination.

Overall histopathological yield and diagnostic yield with multidisciplinary approach were 66,6% and 74,1%, respectively. Histopathological diagnostic yield and diagnostic yield with multidisciplinary approach after a single biopsy was 50%, and histopathological diagnostic yield and diagnostic yield with multidisciplinary approach increased to 71.4% and 85.7% by a second biopsy (p = 0.034). No further increase in diagnostic yield was observed when more than two samples were taken (Table 3).

The histopathological diagnostic yield was 61.9% when multiple biopsies were taken from a single segment, and 69.8% with multidisciplinary approach, while the histopathological diagnostic yield was 72.9% when multiple biopsies were taken from different segments of the same lobe and 81.1% with multidisciplinary approach. There is no statistically significant difference in diagnostic yield when biopsy is taken from single or different lobes.

Complications occurred in 77 (49.6%) of 155 patients (patients who were excluded from cryo-TBB and patients who included in the study). 69 (46.9%) patients who developed complications due to the cryo-TBB procedure included in the study. Hemorrhage developed in 47 (31.9%) patients; grade 1 in 28 (19%) patients, grade 2 in 19 (12.9%) patients. Grade 3 hemorrhage was not observed in any patient. Pneumothorax was developed in 23 (15.6%) patients after the procedure. Of those with pneumothorax, 14 (60.9%) had a tube thoracostomy. Respiratory failure occurred in 3 patients (2%), 2 of these patients were discharged with a short-term noninvasive mechanical ventilation application in the pulmonology wards, 1 patient was admitted to an intensive care and administered invasive mechanical ventilation and died on the 15th day (Table 4).

When the factors affecting the development of pneumothorax and hemorrhage were evaluated, there was no significant difference in pneumothorax rates between patients who underwent 1 and 2 biopsies and those who had more than 3 biopsies (Table 3). While pneumothorax was detected in 13.7% of patients who underwent single-segment biopsy, and in 17.6% of patients who underwent 2 or more segment biopsies, but it was not statistically significant. There was no significant difference between pneumothorax rates in patients who underwent a biopsy from the same and different lobes. The pneumothorax rate was 6.3% when a biopsy was taken from the upper lobes, and 18.3% when a biopsy was taken from the lower lobes. No statistically significant difference was found in

Table 3. Diagnostic yield according to the number of samples

	Overall diagnostic yield (%)	**1-2 biopsy**	**(diagnostic yield)**	**(n=31)**	**3 biopsy (diagnostic yield) (n=116)**	**p value***
Pathological diagnosis	66.6	20 (64.51%)			78 (67.2%)	0.995
		1 biopsy (n=10) 5 (50%)	2 biopsy (n=21) 15 (71.4%)	p=0.244*		
Diagnosis with Multidisciplinary comittee	74.1	23 (74.1%)			86 (74.1%)	0.775
		1 biopsy (n=10) 5 (50%)	2 biopsy (n=21) 18 (85.7%)	p=0.034*		

**p value of the comparison between 1 and 2 biopsies*
***p value of the comparison between 1-2 and ≥3 biopsies.*

Table 4. Safety outcomes and length of hospital stay (n=147)

Complications	**n (%)**
Pneumothorax Oxygen therapy Tube Thoracostomy	23/147 (total) 9 (39.1) 14 (60.9)
Hemorrhage	Grade 1: 28/147 (19) Grade 2: 19/147 (12.9) Grade 3: 0/147
Respiratory failure	3/147 (2.0)
Mortality within 30 days	1/147 (0.7)
Length of Hospital stay Discharged same day Discharged next day 1-3 days >4 days Hospital stay (mean days±SD)	 114 (77.5) 33/147 (22.4) 15/33 (45.5) 18/33 (54.5) 4.3±2.8

terms of hemorrhage rates in biopsies taken from the same or different lobes and biopsies taken from the upper and lower lobes.

Discussion

Cryo-TBB is used for the diagnosis of DPLD as an alternative to SLB at many centers. Our study presents real-world data from patients who underwent cryo-TBB at our tertiary referral hospital with experience in interventional bronchoscopy. We found a sufficient histopathologic diagnostic yield of 66.6%, and when these histopathologic diagnoses were combined with clinical and radiographic information using a multidisciplinary approach, the diagnostic yield reached 74.1% for specific diagnoses in most cases. In addition, cryo-TBB was associated with a lower pneumothorax rate and controllable hemorrhagic complications.

Reports from various centers indicate the diagnostic yield in DPLD with cryo-TBB to range from 50% to 100% and the complication rates (e.g., pneumothorax) to range from 1% and 30% (4, 11, 12). These varying results are probably because of the lack of standardization in patient selection and cryo-TBB techniques. A 2019 review assessed the literature on cryo-TBB and furnished an evidence-based expert panel report (14). However, the cryo-TBB technique remains unstandardized. In our series of 147 cases, histopathologic diagnostic yield with cryo-TBB was 66.6%. When these histopathologic diagnoses were combined with a multidisciplinary approach, the diagnostic yield was 74.1%. The most frequently made histopathologic diagnoses and histopathologic diagnosis rates differ among the published reports by centers where the procedures were performed. Several studies have reported diagnostic yields between 44% and 91% (13, 15-17). This wide distribution in histopathologic diagnosis rates could be attributed to the different equipment used at the centers, differing experience, and the differing perspectives of pathologists.

The higher diagnostic yield with cryo-TBB over conventional TBB has been attributed to the larger biopsy size (18-20). The sample size may affect the diagnostic yield. Although the optimal sample size for cryo-TBB materials remains unestablished in the literature, some pathologists contend that samples of 5 mm diameter (equivalent to the size of the full area seen with a 4X microscope objective lens) are sufficient (21). In our study, the shortest mean diameter was 2.8 ± 0.6 mm and the longest was 6.9 ± 2.3 mm. Wälscher et al reported the average diameter of the biopsy sample in their study to be 5 mm (22). However, our diagnostic success rate is similar to that in the study by Wälscher et al (22). In the study by Ravaglia (10) that included 699 patients, the shortest diameter was 4.6 ± 1.2 mm, and the longest diameter was 6.3 ± 1.9 mm; the histopathologic diagnostic yield was 87.8%. In comparison, the short diameter in our study was smaller. We suppose that the differences in diagnostic yield could be because of the differences in the biopsy diameter. Considering the effect of the heterogeneity of the disease and the distribution of parenchymal pathology on the diagnosis, usually more than 1 biopsy samples (mean number of biopsies per patient, 3.3 ± 1) were collected in our study. The optimal number of biopsies for cryo-TBB remains undetermined in the literature. Similar to those in the 2 studies by Ravaglia, the diagnostic yield in our study significantly increased in patients with 2 biopsies instead of 1 (10, 23). No significant difference was observed in the diagnostic yield with ≥2 biopsies.

The "Transbronchial Cryobiopsy for the Diagnosis of Interstitial Lung Diseases: CHEST Guideline and Expert Panel Report" published in 2019 recommends collecting biopsy samples from at least

2 different regions (14). We performed cryo-TBB on different lobes in patients with radiographic interlobar heterogeneity. In patients with diffuse radiographic patterns in both the upper and lower lobes, cryo-TBB was generally performed on different segments of the same lobe. In our study, we found that collecting 2 biopsy samples from different parts of the same lobe or from different lobes significantly increased the diagnostic yield. In line with our observation, Ravaglia, in 2 distinct studies, reported that collecting biopsy samples from 2 different locations significantly increased diagnostic yield (8, 23).

Pneumothorax is one of the most common complications reported to be associated with cryo-TBB. However, the incidence of pneumothorax varies significantly across publications, ranging from 1% to 30% (4, 8, 11, 18-20, 24-26). Deep sedation and jet ventilation have been reported to increase the incidence of pneumothorax (4). In fact, in the study by Alvarez et al, the incidence of pneumothorax on performing cryo-TBB with local anesthesia and under conscious sedation was 4.7% (12). In our study, the incidence of pneumothorax was 15.6%, and all procedures were performed under deep sedation by intubation with a rigid bronchoscope. The incidence of pneumothorax could also be affected by the sample size and technical. In fact, in the study by Ravaglia et al. (10), the incidence of pneumothorax was higher in case of biopsy from >1 and/or upper lobes. In our study, while the incidence of pneumothorax due to cryo-TBB was unaffected by the number of biopsies, we observed that it increased in cases of biopsies performed on the lower lobes and on multiple regions.

Hemorrhage is another common complication associated with cryo-TBB, with reported incidence between 2.5% and 87% (8, 12, 18-20, 22, 24, 25, 27, 28). In our study, in accordance with the literature, grade 1 and grade 2 hemorrhages occurred in 31.9% of the patients. Hemorrhages were easily controlled with adrenaline and cold saline in addition to the use of the Fogarty balloon placed in the bronchus in each patient. Six patients needed absorbable hemostat use. None of the patients had a life-threatening bleeding requiring transfusion, intensive care unit follow-up, or surgical intervention. Although hemorrhage has been reported to be more common in biopsies on the lower lobes (10), the incidence of bleeding was not associated with the number of samples or sampling strategy (≥1 regions, lower or upper lobes) in our study.

While 77.5% of our patients were discharged on the same day, the average hospital stay among our hospitalized patients was 4.3 ± 2.8 days. In the study by Ravaglia et al (4), the average hospital staying among patients who underwent SLB was 6.1 days. In terms of hospital stay, cryo-TBB could be considered as a cost-effective method.

Studies have reported mortality rates associated with cryo-TBB to range between 0% and 4.1% (4, 24, 33, 34). The mortality rate among our patients was 0.7%: 3 patients needed non-invasive mechanical ventilation after the procedure, and 2 of these patients recovered in a short time (1 exitus). However, 1 patient was taken to the intensive care unit and invasive mechanical ventilation was needed on the 5th day; the patient subsequently died because of diffuse alveolar damage and respiratory failure. In a retrospective study by Ravaglia et al, patients who underwent cryo-TBB and SLB were compared (0.3% vs 2.7%), and the mortality rate in the SLB group was higher than that in our study (4). In the study by Hutchinson that included 12,000 patients who were examined using SLB, mortality rate was reported to be 1.7% among elective cases, respectively. This result is higher than the mortality rates in patients who underwent cryo-TBB in our study (35).

Our study has some limitations. First, it is a retrospective study. Second, we could not compare results with FOB or SBL without general anesthesia and jet ventilation. Furthermore, this study did not include a control group, and cryo-TBB was not compared with surgical lung biopsy in the same population.

Conclusion

Our results show that cryo-TBB has sufficient diagnostic success in most DPLD cases. The diagnostic yield increases with at least 2 biopsies and biopsies from at least 2 segments. Cryo-TBB has a higher diagnostic success, controllable complication and lower mortality rates; it can hence be considered as a first step in the diagnostic strategy for DPLD in patients requiring invasive diagnosis with a multidisciplinary committee at a center with expertise in these procedure.

Statement of Ethics: This research comply with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. All authors state that subjects (or their parents or guardians) have given their written informed consent and that the study protocol was approved by the Yedikule Chest Disease and Thoracic Surgery Education and Research Hospital ethical committee on human research.

Conflict of Interest: The authors have no conflicts of interest to declare.

Authors' Contributions: All authors participated in the study design and/or or implementation, analysis, and interpretation of the data. All authors had full access to the data, participated in manuscript development, and gave final approval before submission.

References

1. Mikolasch TA, Garthwaite HS, Porter JC. Up- date in diagnosis and management of intersti- tial lung disease. Clin Med (Lond). 2016 Dec; 16 Suppl 6:s71–8.
2. Poletti V, Ravaglia C, Gurioli C et al. Invasive diagnostic techniques in idiopathic interstitial pneumonias. Respirology. 2016 Jan;21(1):44–50.
3. Nead MA, Morris DG. InterstitialLungDisease: A Clinical Over view and General Approach. In: Fishman AP, Elias JA, Fishman JA, Grippi MA, Senior RM, Pack AI. Fishman's Pulmonary Diseases and Disorders. 4th edition McGrawHill 2008; 1105-24.
4. Ravaglia C, Bonifazi M, Wells AU et al. Safety and Diagnostic Yield of Transbronchial Lung Cryobiopsy in Diffuse Parenchymal Lung Diseases: A Comparative Study versus Video-Assisted Thoracoscopic Lung Biopsy and a Systematic Review of the Literature. Respiration. 2016;91(3):215-27. doi: 10.1159/000444089.
5. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013; 188: 733–748.
6. Raghu G, Collard HR, Egan JJ, 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.
7. Kreider ME, Hansen-Flaschen J, Ahmad NN et al. Complications of video-assisted thoraco- scopic lung biopsy in patients with interstitial lung disease. Ann Thorac Surg. 2007 Mar; 83(3):1140–4.
8. Pajares V, Puzo C, Castillo D, et al. Diagnostic yield of transbronchial cryobiopsy in interstitial lung disease: a randomized trial. Respirology. 2014; 19:900–6
9. Tomasetti S, Cavazza A, Colby TV et al. Transbronchial biopsy is useful in predicting UIP pattern. Raspir Res.2012 Oct 29;13:96. doi: 10.1186/1465-9921-13-96
10. Ravaglia C, Wells AU, Tomassetti S et al. Diagnostic yield and risk/benefit analysis of trans-bronchial lung cryobiopsy in diffuse parenchymal lung diseases: a large cohort of 699 patients. BMC Pulm Med. 2019; 19: 16. doi: 10.1186/s12890-019-0780-3
11. Iftikhar IH, Alghothani L, Sardi A, Berkowits D, Musani AI. Transbronchial lung cryobiopsy and video-assisted thoracoscopic lung biopsy in the diagnosis of diffuse parenchymal lung disease: a meta-analysis of diagnostic test accuracy. Ann Am Thorac Soc. 2017;14:1197–211.
12. Bango-Alvarez A, Ariza-Prota M, Torres-Rivas H, et al. Transbronchial cryobiopsy in interstitial lung disease: experience in 106 cases – how to do it. ERJ Open Res. 2017;3:00148–2016
13. Hetzel J, Maldonado F, Ravaglia C, et al. Transbronchial Cryobiopsies for the diagnosis of diffuse parenchymal lung diseases: expert statement from the Cryobiopsy working group on safety and utility and a call for standardization of the procedure. Respiration. 2018;95:188–200.
14. Maldonado F, Danoff SK, Wells AU et al. Transbronchial Cryobiopsy for the Diagnosis of Interstitial Lung Diseases: CHEST Guideline and Expert Panel Report CHEST (2019), doi: https://doi.org/10.1016/j.chest.2019.10.048.
15. DiBardino DM, Haas AR, Lanfranco AR, Litzky LA, Sterman D, Bessich JL. High complication rate after introduction of transbronchial cryobiopsy into clinical practice at an Academic Medical Center. Ann Thorac Soc. 2017;14:851–7.
16. Lentz RJ, Taylor TM, Kropski JA, et al. Utility of flexible bronchoscopic cryobiopsy for diagnosis of diffuse parenchymal lung diseases. J Bronchology Interv Pulmonol. 2018;25:88–96.
17. Ussavarungsi K, Kern RM, Roden AC, Ryu JH, Edeli ES. Transbronchial cryobiopsy in diffuse parenchymal lung disease: retrospective analysis of 74 cases. Chest. 2017;151: 400–408
18. Babiak A, Hetzel J, Krishna G et al. Transbronchial cryobiopsy: a new tool for lung biopsies. Respiration. 2009; 78(2):203–8
19. Yarmus L, Akulian J, Gilbert C, et al. Cryoprobe transbronchial lung biopsy in patients after lung transplanta- tion: a pilot safety study. Chest. 2013 Mar; 143(3):621–6.
20. Kropski JA, Pritchett JM, Mason WR et al. Bronchoscopic cryobiopsy for the diagnosis of diffuse parenchymal lung disease. PLoS One. 2013 Nov;8(11):e78674.
21. Colby TV, Tomassetti S, Cavazza A, Dubini A, Poletti V. Transbronchial cryobiopsy in diffuse lung disease: update for the pathologist. Arch Pathol Lab Med. 2017;141:891–900.
22. Wälscher J, Groß B, Eberhardt R et al. Transbronchial Cryobiopsies for Diagnosing Interstitial Lung Disease: Real-Life Experience from a Tertiary Referral Center for Interstitial Lung Disease. Respiration 2018 DOI: 10.1159/000493428
23. Ravaglia C, Wells AU, Tomassetti S, et al. Transbronchial lung cryobiopsy in diffuse parenchymal lung disease: comparison between biopsy from 1 segment and biopsy from 2 segments - diagnostic yield and complications. Respiration. 2017;93:285–92
24. Hagmeyer L, Theegarten D, Wohlschlager J, et al. The role of transbronchial cryobiopsy and surgical lung biopsy in the diagnostic algorithm of interstitial lung disease. Clin Respir J. 2016;10:589–95.
25. Sharp C, McCabe M, Adamali H, Medford AR. Use of transbronchial cryobiopsy in the diagnosis of interstitial lung disease-a systematic review and cost analysis. QJM. 2017;110:207–14.
26. Gershman E, Fruchter O, Benjamin F, et al. Safety of cryo-transbronchial biopsy in diffuse lung diseases: analysis of three hundred cases. Respiration. 2015;90:40–6.
27. Fruchter O, Fridel L, Rosengarten D, Raviv Y, Rosanov V, Kramer MR. Transbronchial cryo-biopsy in lung transplantation patients: First report. Respiralogy (2013)18, 669-673. Doi:10.1111/resp.12037.
28. Griff S, Ammenwerth W, Schonfeld N, et al. Morphometrical analysis of transbronchial cryobiopsies. Diagn Pathol. 2011;6:53.
29. Casoni GL, Tomassetti S, Cavazza A, et al. Transbronchial lung cryobiopsy in the diagnosis of fibrotic interstitial lung diseases. PLoS One. 2014;9:e86716.

30. Echevarria-Uraga JJ, Perez-Izquierdo J, Garcia-Garai N, et al. Usefulness of an angioplasty balloon as selective bronchial blockade device after transbronchial cryobiopsy. Respirology. 2016;21:1094–9.
31. Linhas R, Marcoa R, Oliveira A, Almedida J, Neves S, Campaninha S. Transbronchial lung cryobiopsy: associated complications. Rev Port Pneumol. 2017;23:331–7.
32. Tomic R, Cortes-Puentes GA, Murugan P, Joo Kim H, Amin K, Dincer HA. Acute exacerbation of interstitial lung disease after cryobiopsy. J Bronchology Interv Pulmonol. 2017;24:319–22
33. Cascante JA, Cebollero P, Herrero S, et al. Transbronchial Cryobiopsy in Interstitial Lung Disease: Are We on the Right Path? Journal of bronchology & interventional pulmonology. 2016;23(3):204-209
34. Sriprasart T, Aragaki A, Baughman R, et al. A Single US Center Experience of Transbronchial Lung Cryobiopsy for Diagnosing Interstitial Lung Disease With a 2-Scope Technique. Journal of bronchology & interventional pulmonology. 2017;24(2):131-135
35. Hutchinson JP, Fogarty AW, McKeever TM, Hubbard R. In-hospital mortality after surgical lung biopsy for interstitial lung disease in the United States. 2000–2011. Am J Respir Crit Care Med. 2016;193:1161–7

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Does 1-minute walk test predict results of 6-minute walk test in patients with idiopathic pulmonary fibrosis?

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Abstract. Background: The six-minute walk test (6MWT) is a readily available tool used to evaluate functional capacity in patients with idiopathic pulmonary fibrosis (IPF). However, it is often logistically challenging to perform in the context of a busy clinical practice. We sought to investigate if the 1MWT distance (1MWD) predicts the 6MWT distance (6MWD), and if an abbreviated walk could accurately predict outcomes in IPF patients. **Methods:** Baseline demographics and pulmonary function testing of IPF patients evaluated at a tertiary referral center between 2010 and 2017 were collected. 6MWT variables at baseline as well as 1 and 6 minutes were collected. Time to death, lung transplantation, or most recent follow-up was ascertained. **Results:** There were 177 patients, the majority of whom (80%) were male. The mean age was 67 ± 9 years and mean FVC was 64 ± 18% predicted. Forty eight (27%) patients used oxygen supplementation during the 6MWT. The median 6MWD was 366 meters (IQR: 268-471) while the median 1MWD was 65 meters (IQR: 46-81). Stratified by the median, 89 patients were "High Walkers" based on the 6MWD ≥ 366m (HW_6) and 88 patients were "Low Walkers" (LW_6). HW_6 had a higher FVC% (70 ± 15 vs 57 ± 18, p= 0.001), higher DLCO% (45 ± 12 vs 34 ± 14, p= 0.001) and higher 1MWD (83 ± 28 vs 47 ± 16, m p= 0.001). Median transplant-free survival was better in HW_6 vs LW_6 (27 ± 16 vs 22 ± 18 months, log rank p= 0.018). There was a strong correlation between the 1MWD and the 6MWD (r= 0.91, Spearman's correlation, p < 0.0001). Also, the transplant-free survival curves stratified by 1MWD were very similar to the curves for 6MWD, showing a lower survival in the LW_1 cohort (log rank p= 0.009). **Conclusion:** The 1MWD obtained during the first minute of a 6MWD shows a strong correlation to total 6MWD and retains its ability to predict transplant-free survival. 1MWT may serve as a practical substitute for the more cumbersome 6MWT. Our findings require further validation prospectively in larger cohorts of IPF patients.

Key words: idiopathic pulmonary fibrosis, 6-minute walk test, 1-minute walk test, prognostic

Background

The 6-minute walk test (6MWT) is a simple, objective, and reproducible measurement of functional capacity. It is a submaximal exercise test used in the clinical evaluation of patients with various forms of cardiovascular and pulmonary diseases, including idiopathic pulmonary fibrosis (IPF). IPF is a chronic, progressive, fibrotic interstitial lung disease of unknown etiology occurring predominantly in patients over sixty and is characterized by reduced lung volumes and impaired gas exchange (1). The natural history of IPF varies, but it is associated with a poor prognosis, with a median survival after diagnosis of 2 to 5 years (2). Among the clinical and physiologic predictors associated with survival in IPF, the 6MWT has been increasingly used over the

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past 5 years as a secondary endpoint in IPF clinical trials (3-5). The primary outcome measurement is the six-minute walk distance (6MWD), but other parameters have been investigated as predictors of outcomes, including the rest and nadir oxygen saturation (SpO_2), Borg Dyspnea Index Scale, and heart rate recovery. Validation of a shorter walk test may result in a logistically easier test, and therefore more universally applicable in routine clinical care.

The 6MWT initially evolved from a 12-minute walk and indeed high correlation coefficients between the two-minute, six-minute, and 12-minute tests have previously been demonstrated (6,7). This data suggests that the time chosen to assess exercise tolerance by walking tests may not be critical. Therefore, since the 6MWT is a reliable, valid, and responsive measure of disease status in IPF, we sought to investigate if the distance during first minute of a 6MWT (1MWD) predicts the 6MWD, and if an abbreviated walk is also predictive of outcomes in IPF patients.

Methods

We conducted a retrospective study of IPF patients presenting to the Inova Advanced Lung Disease Clinic from May 2010 to February 2017. Eligible patients had a confident IPF diagnosis according to the criteria of the *American Thoracic Society/European Respiratory Society* (8,9). Patients were excluded if they were deemed to be clinically unstable or acutely ill, requiring more than 6 Liters/min O_2 via nasal cannula, or had a major comorbidity (e.g., ischemic heart disease, malignancy) that could impact their performance on the walk. Local IRB committee approval was obtained (# 17-2673), the need for informed consent was waived.

Baseline demographics were collected including: age, gender, height, weight, body mass index (BMI), and date of initial consultation. Pulmonary function tests (PFT) including the forced vital capacity (FVC %), forced expiratory volume in first second (FEV_1 %), FEV_1/FVC ratio, single breath diffusing capacity for carbon monoxide (DLCO %), total lung capacity (TLC %) were recorded. PFTs were performed according to American Thoracic Society (ATS) standards (10,11) (*V6200 Autobox DL; SensorMedics, Yorba Linda, CA*) and expressed as percent predicted (12-14).

6MWT

Each patient performed the 6MWT according to the ATS guidelines with the instruction to walk as far they could in 6 minutes by an experienced operator using standard verbal prompts (15). Patients were instructed to walk without jogging or running. They were permitted to slow down or stop to rest, if needed, and were encouraged to resume walking as soon as they were able. Patients walked on room air or using their usual oxygen flow rate, which was established during a prior oxygen titration study to maintain SpO_2 at least 88%. Oxygen saturation, heart rate and Borg score were measured and recorded at the start of the 6MWT, at 1 minute intervals during the test, at the end of the study, and at one minute of recovery after conclusion of the walk test. Cutaneous pulse oximetry and heart rate (*Nellcor N-20PA; Puritan Bennett, Inc., Pleasanton, CA*) were obtained using a forehead probe. The 6MWT was stopped if the patient experienced chest pain, intolerable dyspnea, leg cramps, diaphoresis, or SpO_2 dropped below 80%. All analyses were performed from measurements taken at 1 and 6 minutes of the same test.

The primary outcome was transplant-free survival. Data was obtained from the Social Security death index and the electronic medical record. Date of last follow-up, death, or lung transplantation was recorded.

Statistical considerations and data analysis

All demographic and pulmonary function data are presented as the mean ± standard deviation (SD) or the median, depending on the distribution, and according with interquartile ranges if continuous, or as frequencies, if categorical. Group comparisons were performed using *Student's* t-test or *Wilcoxon's* rank sum test for continuous variables; or Pearson's chi-square test or *Fisher's* exact test, for categorical variables, where appropriate.

The Spearman correlation between the 1MWD and 6MWD was analyzed. Patients were divided into groups based on their 1MWD and 6MWD: high walker (≥ median distance); and low walker (< median distance). *Kaplan-Meier* survival analysis and the log-rank test were used to compare the one minute and six minute high vs low walker groups in terms of transplant-free survival times. All the

statistical analyses were performed using GraphPad (*GraphPad; ver. 7, La Jolla, CA*) or *SAS (ver. 9.4, Cary NC)*. p-values ≤ 0.05 were considered as statistically significant.

Results

There were 177 patients identified with data available for analysis. The majority (80%) was male (Table 1). The baseline characteristics included age: 67 ± 9 years, BMI: 28 ± 5 kg/m², FVC% predicted: 64 ± 17 (n= 153), FEV_1% predicted: 69 ± 16, (n= 153), DLCO% predicted: 40 ± 14 (n= 115). The median survival of the group from the time of the 6MWT was 24 (10 - 35, 95% IQR) months.

In terms of the 6MWT, 27% of the patients required supplemental O_2 during the walk (Table 1). The mean 6MWT distance was 363 (268 - 471, 95% IQR) meters, with a corresponding 1MWD of 65 (46 - 81, 95% IQR) meters. Patients were stratified according to the 6MWD median (366 meters) into high (HW_6) and low walkers (LW_6) (89 and 88 patients respectively).

Comparison between HW_6 and LW_6 revealed that HW_6 had a younger age (64 ± 9 vs 67 ± 8 years, p= 0.001), higher FVC% predicted (70 ± 15 vs 57 ± 18, p= 0.001), higher DLCO% predicted (45 ± 12 vs 34 ± 14, p= 0.001), and a higher 1MWD (83 ± 28 vs 47 ± 16, m p= 0.001). The 1-year mortality after the initial 6MWT was significantly lower in the HW_6 group compared to the LW_6 group (31 vs 16%, p= 0.019).

Patients were also stratified according 1MWD median into high walkers (HW_1) (≥ 65 m) and low

Table 1. Demographics and functional characteristics of patients, stratified by median 6MWD (≥ 366 meters (n= 89) versus < 366 meters (n= 88))

Variables	All patients n = 177	HW_6 (6MWD ≥ 366 meters) n = 89	LW_6 (6MWD < 366 meters) n = 88	p value
Age	67 ± 9	64 ± 9	67 ± 8	0.001*
Gender, male (%)	142 (80)	81 (91)	61 (69)	0.001*
BMI	28 ± 5	28 ± 5	29 ± 5	0.51
FVC %	64 ± 18	70 ± 15	57 ± 18	0.001*
FEV_1 %	69 ± 16	76 ± 15	62 ± 18	0.001*
DL_{CO} %	40 ± 14	45 ± 12	34 ± 14	0.001*
FEV_1/FVC %	81 ± 12	79 ± 15	84 ± 9	0.02*
1 MWT, meters	65 ± 29	83 ± 28	47 ± 16	0.001*
6 MWT, meters	363 ± 141	478 ± 68	246 ± 90	0.001*
Supplemental O_2 (%)	48 (27)	8 (9)	40 (45)	0.001*
GAP Index				
1 (%)	37 (21)	30 (34)	7 (8)	
2 (%)	60 (34)	33 (37)	27 (31)	
3 (%)	30 (17)	10 (11)	20 (23)	0.004*
Mortality, 1 year (%)	42 (24)	14 (16)	28 (32)	0.02*
Mortality, overall (%)	62 (35)	25 (28)	37 (42)	0.03*
Median Survival, months	24 ± 17	27 ± 16	22 ± 18	0.02*

Data reported as mean ± SD, or frequency with percent (%)

*Significance tests for comparisons between high and low walker patients (HW_6 and LW_6) based on paired t-test for continuous patient characteristics and Fisher's exact test for categorical patient characteristics

Abbreviations: 6MWT: 6-minute walk test; 6MWD: 6-minute walked distance; HW6: High Walkers patients stratified according to the 6MWD median (≥ 366 m); LW_6: Low Walkers patients stratified according to the 6MWD median (< 366 m); BMI: body mass index; FVC %: forced vital capacity, % predicted; FEV_1 %: forced expiratory volume in 1 second, % predicted; DL_{CO} %: monoxide carbon diffusion capacity, % predicted; 1MWD: walked distance during first minute of 6MWT; GAP index: Gender Age and Pulmonary function test index

walkers (LW_1) (< 65 m). For the most part, patients who were High Walkers during the 6MWT were also High Walkers in the 1 minute time interval, and vice versa, with the exception of about less than 10% of patients who had inconsistent results between time intervals, reflective of pacing variability or perhaps fatigue (8 HW_1 (9%) became LW_6, while 81 HW_1 (91%) stayed HW at 6 minutes).

There was a strong correlation between 1MWD and 6MWD (r= 0.91, Spearman's correlation, p <0.0001) (Figure 1a). However we did observe a less favorable, but still statistically significant correlation between SpO_2 at 1 and 6 minutes (Figure 1b, r=0.69, p<0.0001). With regards to qualifying for supplemental oxygen, 38 patients desaturated below 88% based on the 6MWT, while only 7 did so on the 1MWT (Figure 1b).

The transplant-free survival of LW_6 was significantly lower than the HW_6 group (Figure 2). Interestingly, when we stratified the patients based on HW_1 versus LW_1, the transplant-free survival curve was very similar, showing a lower survival in the LW_1 cohort (Figure 3).

Discussion

In this paper, we demonstrate that the 1MWD obtained from the 6MWT has a strong correlation with the 6MWD in IPF patients. In addition, the information gleaned at one minute during the course of a 6MWT imparts similar prognostic information. We postulate that this modification could render a walk test more feasible to obtain on a more frequent basis in patients with IPF during the course of their routine clinical follow-up.

The 6MWT has evolved over time. As a simple measure of physical fitness, Balke *et al.* developed the specific time of 12 minutes and was responsible for the introduction of the 12-minute walk test (6). In 1982, Butland and colleagues evaluated the possibility of reducing the time patients would be required to walk (7). The health status of patients under study was often so poor that patients would frequently lack the motivation for even 12 minutes of physical activity. Their validation study demonstrated that after a slight initial burst of speed, patients walked at a constant speed, suggesting that shorter tests may be as informative. Subjects showed a remarkable ability to pace themselves during this test. The high correlation coefficients between the 2-minute, 6-minute, and 12-minute tests indicated that they were all similar measures of exercise tolerance. This data suggests that the time chosen to assess exercise tolerance by walking tests may not be critical.

The association between 6MWD and survival in IPF patients has been well demonstrated by several authors. In 2006, *Lederer et al.* analyzed survival of 454 patients with IPF listed for lung transplant. The area under the receiver operator curve (ROC) showed that the 6MWD was better than the FVC

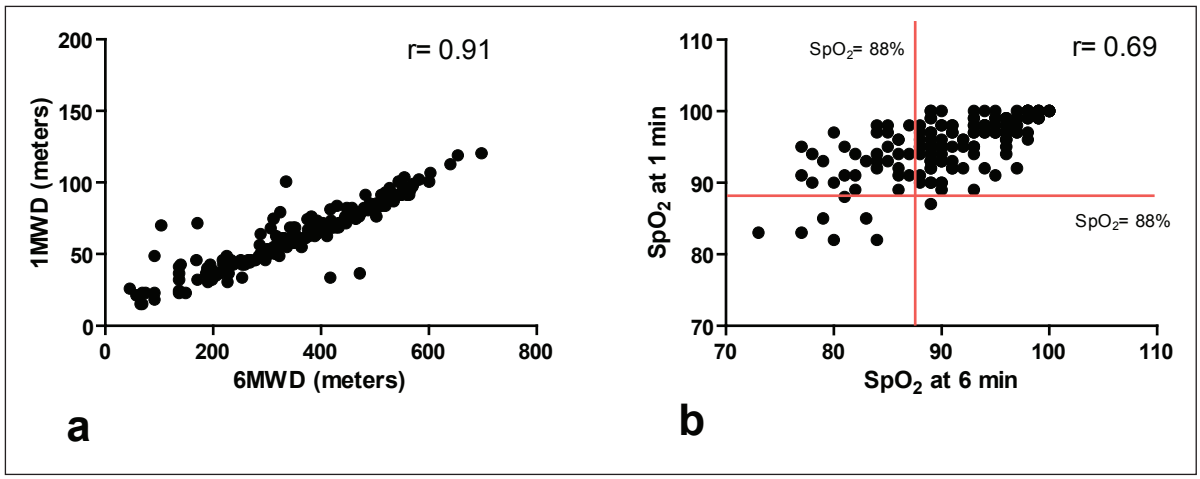

Figure 1. Correlation between a) 1-minute walked distance and 6-minute walked distance (1MWD and 6MWD) and b) 1-minute SpO_2 and 6-minute SpO_2

Abbreviations: r: Pearson's correlation coefficient; 6MWD: 6-minute walked distance; 1MWD: walked distance during first minute of 6MWT; 6MWT: 6-minute walk test; SpO_2: arterial oxygen saturation

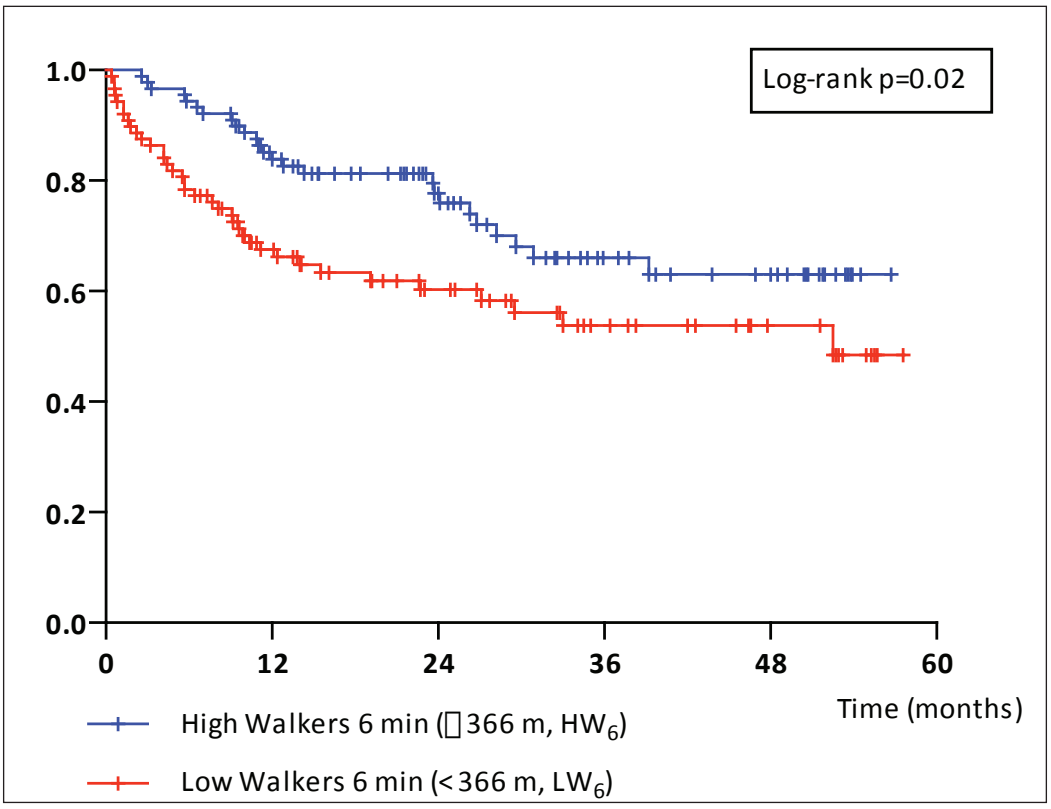

Figure 2. Transplant-Free Survival of IPF Patients, stratified by 6-minute walked distance (6MWD)
Abbreviation: 6MWD: six-minute walked distance

% predicted in discriminating survival at 6 months with a distance lower than 207m strongly and independently associated with an increased mortality rate for wait-listed IPF patients (16). In 2014, du Bois *et al.* provided additional evidence of the independent contribution of 6MWD to prediction of mortality in IPF. They found that a baseline 6MWD lower than 250 m was associated with increase in mortality risk (hazard ratio, 2.12; 95% CI, 1.15-3.92) and also a decline of 50 m or more at 24 weeks was associated with nearly 3-fold increase in mortality risk (hazard ratio, 2.73; 95% CI, 1.60-4.66) (17). In another study, change in 6MWD was also highly predictive of mortality; a 24-week decline of greater than 50 m was associated with a fourfold increase in risk of death at 1 year (hazard ratio, 4.27; 95% CI, 2.57-7.10; p<0.001) (18). Although we did not evaluate serial change in the 1MWT, we hypothesize that this will impart similar prognostic information. This hypothesis should ideally be validated in a future prospective study.

The reliability, validity, and responsiveness of the 6MWT has been demonstrated in reports from large cohorts of prospectively collected data in the context of independent clinical trials (18-20). From these studies, the 6MWD was weakly correlated with other physiologic measures, which attests to its providing complimentary physiologic information not reflected in standard physiologic pulmonary function testing. The estimated minimal clinically important difference (MCID) for the 6MWT in IPF has been estimated between 24-45 m (18). We speculate that the MCID for a 1MWT would be relatively less given that there is less time for other factors to confound distance to manifest during the course of the study performance. This could prove to be an additional advantage of a shorter test.

Although widely studied, there are ongoing issues which require further investigation to improve the performance characteristics of the 6-minute walk test. Weir *et al.* analyzed if changing the wording from "far" to "fast" might facilitate a better effort and

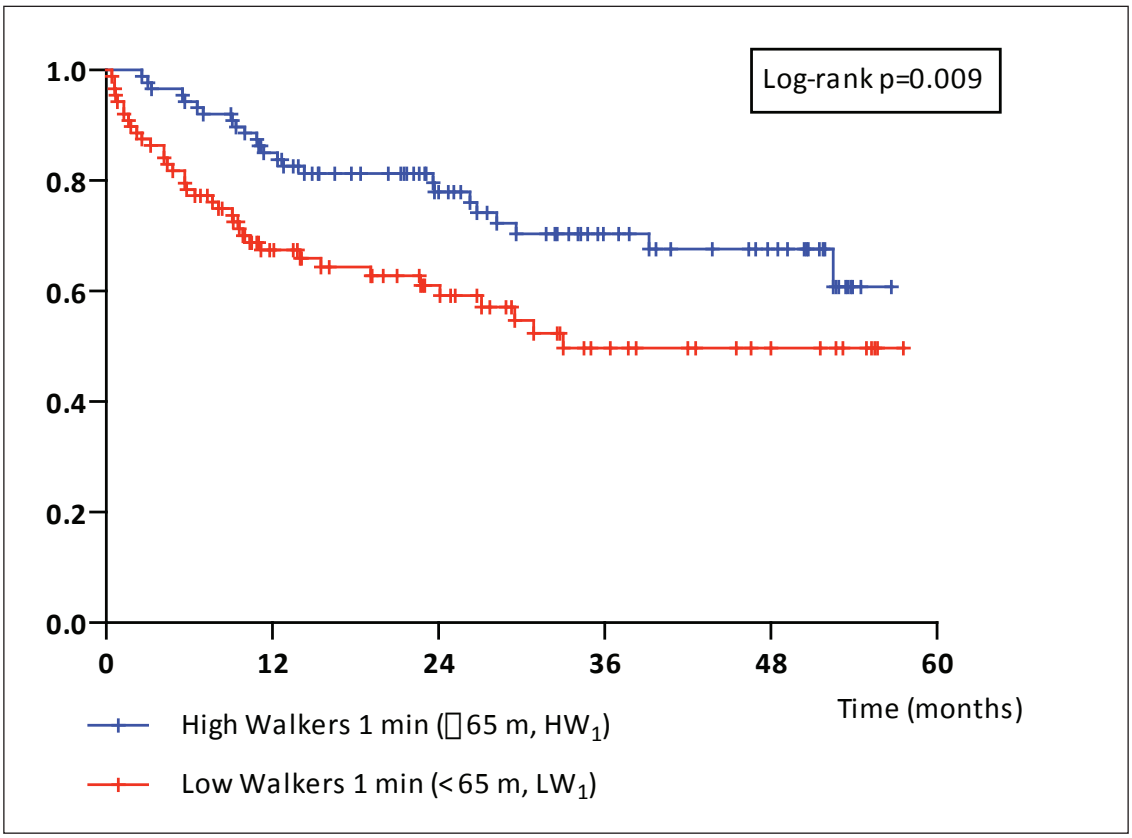

Figure 3. Transplant-Free Survival of IPF patients, stratified by 1-minute walked distance (1MWD)
Abbreviation: 1MWD: 1-minute walked distance

greater distance during the 6MWT (21). Twenty-four patients (ten with pulmonary hypertension, eight with IPF, six with non IPF-ILD) were enrolled and completed 6MWTs in random order with differing instructions. Interestingly, the greatest distance was obtained with the fast instruction, exceeding the standard ("far") instruction distance by a mean of 52.7 meters. This highlights that patients may not walk as far as they are able with the standard ATS instruction for 6MWT. This is likely due to feedforward subconscious pacing (or tele-anticipation) of having to walk 6 minutes. It is likely that patients, including those in our current cohort, would walk further if they know that they only have to complete one minute of walking. This would likely lead to less variability and greater reproducibility of the test. This is supported by prior data demonstrating that the variance of the 12-minute test was slightly greater than that of the six-minute test, which was slightly greater than that of the two-minute test; that is, the longer the patients walked, the greater the spread of results.

Our study has several limitations that are worth noting. First, it was a retrospective study and the 1MWT distance came from the 6MWT against which it was compared. Ideally, these should have been two separate tests. Since patients may be able to walk further if instructed to walk for only one minute, the correlation between two independent tests may not be as good as we have demonstrated. However, we speculate that since a 1MWT might be more conducive to a maximal effort with less time for variability, the reproducibility and precision of serial 1MWT may be better than 6MWT. These are issues that can only be verified in the context of a prospective study. In addition, the oxygen saturation observed at 1 minute often underestimated the degree of desaturation at 6 minutes and "missed"

17.5% (31/177) of patients who qualified for supplemental oxygen (based on desaturation < 88% at 6 minutes). However, the need for implementing supplemental oxygen in IPF patients whose SpO_2's breach the 88% threshold has never been validated.

We also didn't account for the influence of any comorbid conditions or antifibrotic therapy in our analysis, however, we feel that any such confounders would have affected both the 6MWT and 1MWT equivalently. It is noteworthy that our cohort had a relatively high mortality rate, possibly because the 6MWTs were not necessarily obtained from the time of the initial presentation, but during the course of follow-up with survival analyzed from the time of the walk.

Although there was a high correlation between the 1MWD and 6MWD, further studies are needed to validate the test performance characteristics and prognostic value of distance walked in a 1MWT compared to the standard 6MWT in an independent cohort of patients with IPF. Shortening the time is just one modification that could improve the walk test for future use. Additionally, the evaluation of alternate instruction, such as changing the wording from "far" to "fast" and the integration of other parameters (such as the oxygen saturation and Borg dyspnea score) may further improve the clinical value of the walk test.

Conclusions

The 6MWD is a valid and practical method for evaluating disease status in patients with IPF and provides objective information regarding the functional status and near-term prognosis of patients with IPF. One-minute distances ≥ 65 m and < 65 m were defined as high walkers and low walkers, respectively, and a strong correlation appeared between the 6-minute distance and 1-minute distance in terms of predicting survival. Shorter walk times could be easier for both patients and clinicians, but further studies are necessary to identify appropriate thresholds as well as the role of serial changes in the prediction of outcomes among IPF patients.

Although more research is needed to validate the findings, the results suggest that the 1-minute test may be a practical substitute for the 6-minute test by providing similar prognostic information more quickly and easily than the 6-minute test.

List of Abbreviations

1MWT: 1-minute walk test
1MWD: 1-minute walked distance
6MWT: 6-minute walk test
6MWD: 6-minute walked distance
IPF: idiopathic pulmonary fibrosis
SpO_2: arterial oxygen saturation
Borg: Borg Dyspnea Index Scale
BMI: body mass index
PFT: pulmonary function tests
FEV1: forced expiratory volume in first second
FVC: forced vital capacity
DL_{CO}: diffusion lung capacity of carbon monoxide
TLC: total lung capacity
ATS: American Thoracic Society

Declarations

Ethics approval and consent to participate

Local IRB committee (Inova Fairfax Hospital) approval was obtained (# 17-2673). The need for informed consent was waived because of the retrospective nature of the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during this study are available from the corresponding author on reasonable request

Competing interest

SDN is a consultant for Actelion, Bellerophon, Roche-Genentech, Boerhinger-Ingelheim, Pliant, Merck, United Therapeutics and Bayer Pharmaceuticals. He is also on the Speakers' Bureau for Roche-Genentech, Boerhinger-Ingelheim, and Bayer Pharmaceuticals.

AWB has served on an advisory board for Promedior, Theravance, and Genentech, and serves on the Speakers' Bureau for Genentech.

NAW has served on advisory board for Gilead Sciences.

OAS has served as a consultant and serves on the Speakers' Bureau for Lung Rx/United Therapeutics, Actelion and Bayer.

JP has served as a consultant for Boerhinger-Ingelheim

Other authors have no competing interest

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Authors' contributions: FSN, JP, SDN analyzed and interpreted the patient data. FSN, JP, NAW, AWB, OAS, CK, SDN were major contributor in writing the manuscript. All authors read and approved the final manuscript

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References

1. Richeldi L, Collard HR, Jones MG. Idiopathic pulmonary fibrosis. Lancet Lond Engl. 2017 May 13;389(10082):1941–52.
2. Hutchinson J, Fogarty A, Hubbard R, McKeever T. Global incidence and mortality of idiopathic pulmonary fibrosis: a systematic review. Eur Respir J. 2015 Sep;46(3):795–806.
3. King TE, Bradford WZ, Castro-Bernardini S, Fagan EA, Glaspole I, Glassberg MK, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. N Engl J Med. 2014 May 29;370(22):2083–92.
4. Noble PW, Albera C, Bradford WZ, Costabel U, Glassberg MK, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. Lancet Lond Engl. 2011 May 21;377(9779):1760–9.
5. Noble PW, Albera C, Bradford WZ, Costabel U, du Bois RM, Fagan EA, et al. Pirfenidone for idiopathic pulmonary fibrosis: analysis of pooled data from three multinational phase 3 trials. Eur Respir J. 2016 Jan;47(1):243–53.
6. Balke B. A SIMPLE FIELD TEST FOR THE ASSESSMENT OF PHYSICAL FITNESS. REP 63-6. Rep Civ Aeromed Res Inst US. 1963 Apr;1–8.
7. Butland RJ, Pang J, Gross ER, Woodcock AA, Geddes DM. Two-, six-, and 12-minute walking tests in respiratory disease. Br Med J Clin Res Ed. 1982 May 29;284(6329):1607–8.
8. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, 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 Mar 15;183(6):788–824.
9. Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, 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 Sep 15;188(6):733–48.
10. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995 Sep;152(3):1107–36.
11. American Thoracic Society. Single-breath carbon monoxide diffusing capacity (transfer factor). Recommendations for a standard technique--1995 update. Am J Respir Crit Care Med. 1995 Dec;152(6 Pt 1):2185–98.
12. Morris JF. Spirometry in the evaluation of pulmonary function. West J Med. 1976 Aug;125(2):110–8.
13. 13. Goldman HI, Becklake MR. Respiratory function tests; normal values at median altitudes and the prediction of normal results. Am Rev Tuberc. 1959 Apr;79(4):457–67.
14. Burrows B, Kasik JE, Niden AH, Barclay WR. Clinical usefulness of the single-breath pulmonucy diffusing capacity test. Am Rev Respir Dis. 1961 Dec;84:789–806.
15. Holland AE, Spruit MA, Troosters T, Puhan MA, Pepin V, Saey D, et al. An official European Respiratory Society/American Thoracic Society technical standard: field walking tests in chronic respiratory disease. Eur Respir J. 2014 Dec;44(6):1428–46.
16. Lederer DJ, Arcasoy SM, Wilt JS, D'Ovidio F, Sonett JR, Kawut SM. Six-minute-walk distance predicts waiting list survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006 Sep 15;174(6):659–64.
17. du Bois RM, Albera C, Bradford WZ, Costabel U, Leff JA, Noble PW, et al. 6-Minute walk distance is an independent predictor of mortality in patients with idiopathic pulmonary fibrosis. Eur Respir J. 2014 May;43(5):1421–9.
18. du Bois RM, Weycker D, Albera C, Bradford WZ, Costabel U, Kartashov A, et al. Six-minute-walk test in idiopathic pulmonary fibrosis: test validation and minimal clinically important difference. Am J Respir Crit Care Med. 2011 May 1;183(9):1231–7.
19. Nathan SD, du Bois RM, Albera C, Bradford WZ, Costabel U, Kartashov A, et al. Validation of test performance characteristics and minimal clinically important difference of the 6-minute walk test in patients with idiopathic pulmonary fibrosis. Respir Med. 2015 Jul;109(7):914–22.
20. Brown AW, Nathan SD. The Value and Application of the 6-Minute-Walk Test in Idiopathic Pulmonary Fibrosis. Ann Am Thorac Soc. 2018;15(1):3–10.
21. Weir NA, Brown AW, Shlobin OA, Smith MA, Reffett T, Battle E, et al. The influence of alternative instruction on 6-min walk test distance. Chest. 2013 Dec;144(6):1900–5.

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Telomere length assessment in blood leukocytes of patients with sarcoidosis

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Abstract. Background: Accelerated aging and telomere shortening have been studied in many chronic diseases such as interstitial pulmonary fibrosis and chronic obstructive pulmonary disease. Different studies have shown that patients with these diseases have shorter telomere lengths than controls; this can be a marker of the progression and outcome of the disease. So far, a few studies have been evaluated the telomere length in sarcoidosis. In this study we determine the telomere length in patients with sarcoidosis and compare it with control subjects. **Objective:** Our aim is to compare telomere length in patients with sarcoidosis and normal population. Methods: We select 58 patients with sarcoidosis who were visited in the sarcoidosis clinic of Masih Daneshvari Hospital. 58 sex and age-matched (with±2 years) healthy control subjects were selected. Telomere length was measured by quantitative real time PCR as described by Cawthon on peripheral blood sample. The telomere repeat copy number (T) to single-gene copy number(S) ratio was calculated using the comparative Ct method. Results: The mean and standard deviation of telomere length in the patient and control group was 0.65 ± 0.05 and 0.72 ± 0.07 respectively. There was a statistically significant difference between the two groups. (P = 0.031). Conclusion: Sarcoidosis is an inflammatory disease that can involve many organs. Like other chronic diseases, aging phenomenon occurs in that; which led to decrease cellular and tissue telomere length. This article demonstrates shorter telomere length in Iranian sarcoidosis patients compared to normal population.

Keywords: Telomere, sarcoidosis, aging

Introduction

Sarcoidosis is a multiorgans chronic granulomatous inflammatory disease with unknown etiology (1, 2). Variable incidence has been reported in different studies ranging from 0.73 per 100,000 to 71 per 100,000 in Japanese and American population, respectively (3, 4). Recently it has been declared that sarcoidosis incidence in Iranian population is almost 1-2 cases per 100,000 people (1). However, its etiology and pathogenesis is not completely understood.

Recently more attention has been paid to the association between the phenomenon of aging and cellular oxidative stress and the occurrence of chronic pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and interstitial pulmonary fibrosis (IPF). The process of "aging" or "senescence" is defined as a condition associated with the

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progressive reduction of homeostasis in the body, which begins after the fertility phase has been completed, leading to an increased number of diseases and also disorders of DNA repair (5-7).

Human telomeres consist of thousands of hexameric nucleotides, TTAGGGs, and related proteins, including the Shelterin complex, or the Telosome (8). They play a key role in establishing the integrity of the chromosomes and their stability (9, 10). In many cells with high proliferation rate, the length of the telomere is dynamic. The telomere length in human somatic cells decreases from 20 to 200 bp in each cell division. So this process gradually causes chromosomal shortening in any cell division. In long term this phenomenon causes telomere attrition which leads to apoptosis or irreversible ending of the cell cycle (11). Telomeres, as a replication index, determine the number of cell divisions throughout the life; eventually transmit senescence as a signal. Many causes such as the imbalance of oxidant / antioxidant factors lead to telomeres attrition, accelerated aging and shorter life span (12-14). The rate of telomeres length shortening is similar in different tissues. Therefore, measuring telomere length in cells such as peripheral blood leukocytes or oral mucosal cells that are readily accessible can reflect telomere length in other tissues in patients with chronic diseases and healthy individuals (11, 15).

In many chronic diseases such as IPF and COPD, accelerated aging and telomere shortening have been studied. Different studies have shown that these individuals have shorter telomere lengths than controls; which can be a marker of the progression and outcome of the disease (16-19).

There are some studies that have evaluated the telomere length in sarcoidosis (20, 21). In our best knowledge, this study is the first one in Iran, which aimed to determine the telomere length in patients with sarcoidosis and compare it with control subjects.

Methods

Fifty-eight sarcoidosis patients who were visited in the sarcoidosis clinic in Masih Daneshvari Hospital were enrolled in the study. Their diagnosis was confirmed with clinical, radiological and pathological data. The patients with any other chronic lung disease, active cardiovascular disease, malignancy, infection or other chronic inflammatory diseases like inflammatory bowel

disease, were excluded. A group of 58 sex and age-matched (±2 years) healthy control subjects, were selected. This study was approved by the Ethical Committee of the National Research Institute of Tuberculosis and Lung Disease (NRITLD) and written consent was obtained from all the participants.

The blood samples [Angiotensin converting enzyme (ACE), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)] are obtained at the first visit in the clinic. The blood sample for PCR was also drawn.

Blood samples were drawn from all patients and controls (5ml) and transferred into EDTA-containing tubes. All samples were centrifuged at 1000×g for 10 minutes at 4°C and stored at -70°C for DNA extraction. Genomic DNA was extracted from buffy coats with Qiagen (QIAamp DNA Blood Mini Kit, Germany) and quantified by spectrophotometer (Hitachi 1800, Japan). Telomere length was measured by quantitative Real Time-PCR as described by Cawthon (22) .All samples were run in triplicate, using the SYBR green method and 35 ng of DNA. The sequences of the telomere primers for tel-1 and tel-2 and 36B4 as single copy gene for normalized technique were as follows:

Tel-1F 5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTTT-3'

Tel-2R 5'-GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT-3'

Single-1F 36B4F, 5'-CAGCA AGTGGGAAGGTGTAATCC-3

Single-2R 36B4R, 5'-CCCATTCTATCATCAACGGGTACAA-3'

The telomere repeat copy number (T) to single gene copy number(S) ratio was calculated using the comparative Ct method. Positive controls (extracted from a normal healthy person) and negative controls (DDW+ master mix) were added for every PCR run. PCR was performed by a BioRad RT-PCR system (BioRad, USA).

Descriptive statistics in terms of mean, Standard deviations and percentages were used to describe characteristics of the studied patients. Comparison of categorical variables was conducted by Chi-Square test or Fisher's exact test accordingly. After

assessment of normality distribution of variables by Shapiro test, Student t-test and ANOVA test were used if data had normal distribution while Mann–Whitney and Kruskal–Wallis test were used in skewed data. A P-value less than 0.05 was considered a significant test. SPSS version 22 was used for all statistical analysis.

Results

In patients with sarcoidosis, there were 43% (n=25) male and 57% (n=33) female. Their mean age was 48 ± 9 years old. The mean and standard deviation of telomere length in the patient and control group was 0.65 ± 0.05 and 0.72 ± 0.07 respectively. There was a statistically significant difference between the two groups. (P = 0.031)(Figure1)

According to Scadding staging 23 patients (39.7%) were in stage one, 31 patients (53.4%) in stage two and 4 patients (6.9%) in stage 3 pulmonary involvement. No significant correlation between telomere length and stage of pulmonary involvement was seen based on ANOVA test. (P-value=0.488) (Figure 2)

Extrapulmonary organs (skin, eyes, liver and kidneys) were also involved in 27 patients (46.6%). No significant difference was seen with more organ involvements (Table 1).

The T/S ratio (indicative of telomere length) was 0.70 ± 0.05 in women and 0.68 ± 0.04 in men (P-value = 0.174). In both sexes, telomere length was studied in correlation of age separately with Spearman's rho. There was no statistically significant

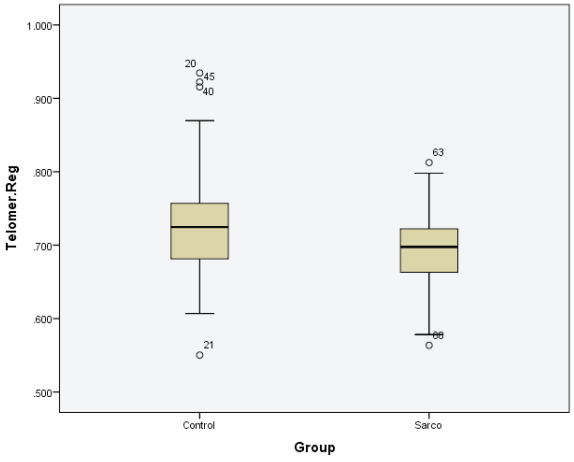

Figure 1. Comparison of telomere length in patients with sarcoidosis and controls.

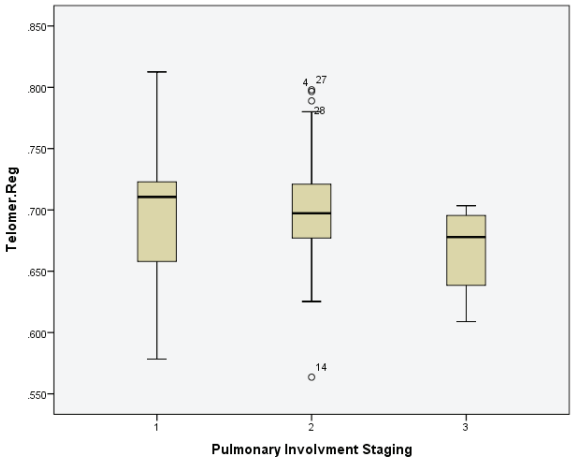

Figure 2. Comparison of telomere length among different stage of pulmonary involvement.

Table 1. Comparison of telomere length in patients with pulmonary involvement and in patients with pulmonary and extrapulmonary involvements.

Involvement	N	Mean	Standard Deviation	p-value
Pulmonary	31(53.4%)	0.69341	0.051745	0.678
Pulmonary + extra pulmonary	27(46.6%)	0.69922	0.054435	

Table 2. The relationship between telomere length and age in sarcoidosis subjects in each sex.

		Telomere	
Male	Age	Correlation Coefficient	0.249
		p-value	0.231
		Telomere	
Female	Age	Correlation Coefficient	-0.273
		p-value	0.124

correlation between age variation in subjects with sarcoidosis with different sex and telomere length. However, there was an inverse relationship between age and telomere length in female group, but it was not statistically significant (Table2).

There was no significant correlation between serum ACE level and telomere length in patients with sarcoidosis (P value = 0.84). According to the analysis performed by the spearman correlation coefficient, with increasing serum CRP, the telomere length decreased (P-value = 0.003) (Table 3).

No significant correlation between serum levels of ESR and telomere length in patients with sarcoidosis was seen (P-value = 0.86).

Discussion

Attrition of telomere length with aging and oxidative stress can be seen in many inflammatory situations and chronic disease. Our study demonstrated shortening of telomere length in Iranian sarcoidosis patients.

Telomere length is one of the cellular aging markers. With prolonged cell lifespan, the telomeres are shortened to reach the Hay-flick limit and the cell division is stopped, and thus the cell phenomenon may senescence and / or apoptosis (23,24). Cawthon has shown that telomere shortening will cause an increase in mortality rate specially in age-related diseases (25). Thus, length of telomere can be considered as a biomarker of cell destruction, oxidative stress and aging (13).

Interstitial lung disease (ILD) is a heterogeneous group of diseases which primarily involves the interstitium. Studies have shown shortening the telomere length in ILDs, especially in idiopathic pulmonary fibroses IPF (26). In 2015 Sadr and his colleagues showed that COPD patients have a shorter telomere (T/S ratio) in comparison to control group (19).

Table 3. The relationship between telomere length and CRP level in subject with sarcoidosis.

	CRP	N	Mean	Standard deviation	p-value
T/S regression	No	43	0.70804	0.050707	0.003
	Yes	15	0.66192	0.043266	

(0.61±0.08 in comparison to 0.69±0.09/P-value <0.001)

This is the first study to evaluate the telomere length in Iranian patients with sarcoidosis. This study showed that the telomere length is shorter in patients with sarcoidosis compared to the control group. Results of this study are compatible with Madea and Guan findings (20, 27). None of our patients were smokers; so oxidative stress was not an important or effective parameter in the telomere length analysis. Extrapulmonary organs (skin, eye, liver and kidney) were also involved in 27 patients (46.6%). It was expected that with increased organ involvement, the inflammation and stress levels in the body would increase; and therefore telomeres would have a greater reduction than single organ involvement. However the comparison of telomere length between these two groups didn't show any remarkable difference. Although Thompson showed that the length of telomere in patients with ocular sarcoidosis is decreased in comparison to control group, the researches done in field of telomere and Sarcoidosis didn't study this comparison comprehensively (28).

Average length of telomere was 0.7±0.05 and 0.68±0.04 in women and men with sarcoidosis (P-value=0.174) respectively. Also no meaningful correlation was found between aging in both sexes with sarcoidosis and length of telomere. Although a reverse correlation was seen between aging and length of telomere in women, but it was not statistically significant. In Madea et al study, it has been shown that aging associated change in telomere length in both control and Sarcoidosis groups, and in both men and women, is the same. The only difference was higher decreasing rate of telomere length in men (27). Guan et al, reached to the same results in 2007 (20).

ACE is secreted by epithelioid cells in granuloma. Studies show that there is no difference between ACE level, sex and age. Most of the studies showed that patients with higher ACE level have more severe disease and more inflammation (29). Also there is a reverse correlation between Sarcoidosis chronicity and ACE level (30-33). In our study, there was no significant correlation between ACE level and telomere length; which was in agreement with Guan et al study.

Telomere length is decreased with increasing oxidative stress and inflammation. Wrong et al, have shown that with each ng/ml increase in CRP,

telomere would be shortened by 2.6x10-2 bp (34). In 2015 a negative correlation between telomere length of leukocyte in blood and CRP level was shown in women with polycystic ovary syndrome (35). Here we report that with increase in CRP, telomere length will be decreased (P-value=0.03). Although there was no significant correlation between telomere length and ESR level (P-value=0.86).

Conclusion

Sarcoidosis is an inflammatory disease that can involve many organs. Like other chronic diseases, aging phenomenon occurs in sarcoidosis; which lead to decrease cellular and tissue telomere length. This study demonstrated that patients with Sarcoidosis have statistically significant shorter Telomere length compared to healthy individuals, in an Iranian population.

References

1. Kiani A, Abedini A, Adcock I,Mirenayat M S, Taghavi K, Mortaz E, et al. Association between vitamin D deficiencies in sarcoidosis with disease activity , course of disease and stages of lung involvements. J Med Biochem 2018; 37: 103–9.
2. Elizabeth V. Arkema and Yvette C. Cozier. Epidemiology of sarcoidosis: current findings and future directions. Ther Adv Chronic Dis 2018; 9(11): 227–240.
3. Cozier YC, Berman JS, Palmer JR, Boggs DA , Serlin DM, Rosenberg L. Sarcoidosis in black women in the United States: Data from the black women's health study. Chest. 2011; 139:144–150.
4. Morimoto T, Azuma A, Abe S, et al. Epidemiology of sarcoidosis in Japan. Eur Resp J. 2008; 31:372–379.
5. Harley CB, Vaziri H, Counter CM and Allsopp RC. The telomere hypothesis of cellular aging. Exp Gerontol 1992; 27: 375–82.
6. Blackburn EH. Structure and function of telomeres. Nature 1991; 350: 569–73.
7. Dillin A, Gottschling DE, Nyström T. "The good and the bad of being connected: the integrons of aging". Curr Opin Cell Biol. 2013; 26: 107–12.
8. Holohan B, Wright WE, Shay JW. Cell biology of disease: Telomeropathies: an emerging spectrum disorder. J Cell Biol 2014; 205 (3): 289–99.
9. Jang JS, Choi YY, Lee WK, Choi JE, Cha SI, Kim YJ, et al. Telomere length and the risk of lung cancer. Cancer Sci 2008; 99 (7): 1385–9.
10. Chan SW, Blackburn EH. New ways not to make ends meet: telomerase, DNA damage proteins and heterochromatin. Oncogene 2002; 21: 553–63.
11. Houben JM, Mercken EM, Ketelslegers HB, Bast A, Wouters EF, Hageman GJ, et al. Telomere shortening in chronic obstructive pulmonary disease. Respir Med 2009; 103 (2): 230–6.
12. Von Zglinicki T. Telomeres and replicative senescence: is it only length that counts? Cancer Lett 2001; 168:111–6.
13. Von Zglinicki T.Role of oxidative stress in telomere length regulation and replicative senescence. Ann N Y Acad Sci 2000; 908: 99–110.
14. Von Zglinicki T, Saretzki G, Docke W, Lotze C. Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? Exp Cell Res 1995; 220: 86-193.
15. Hemann M, Strong M, Hao L, Greider C. The Shortest Telomere, Not Average Telomere Length, Is Critical for Cell Viability and Chromosome Stability. Cell, 2001;107, 67–77.
16. Lee J, Sandford A, Man P, Sin D. Is the aging process accelerated in chronic obstructive pulmonary disease? Curr Opin Pulm Med. 2011; 17:90–97.
17. Amsellem V, Gary-Bobo G, Marcos E, Maitre B, Chaar V, Validire P and et al. Telomere Dysfunction Causes Sustained Inflammation in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med .2001; 1358–1366.
18. Kropski JA, Blackwell TS,Loyd JA.The genetic basis of idiopathic pulmonary fibrosis. ERJ Express. 2015.
19. Sadr M, Noori Mugahi1 S, Hassanzadeh G, Nadji S, Kiani A, Abedini A, et al. Telomere Shortening in Blood Leukocytes of Patients with Chronic Obstructive Pulmonary Disease. Tanaffos 2015; 14(1): 10-16.
20. Guan J, Maeda T, Sugano M, Oyama J, Higuchi J, Suzuki T and et al. An Analysis of Telomere Length in Sarcoidosis Journal of Gerontology: Biological Sciences Copyright 2007 by The Gerontological Society of America.2007; 1199–120.
21. Snetselaar R, van Moorsel CHM, Kazemier KM, van der Vis JJ, Zanen P, van Oosterhout MFM, Grutters JC. Telomere length in interstitial lung diseases. Chest. 2015 Oct;148(4):1011-1018.
22. Cawthon RM. Telomere measurement by quantitative PCR. Nucleic Acids Res 2002; 30: e47.
23. Ito K, Barnes PJ. COPD as a disease of accelerated lung aging. Chest 2009; 135 (1): 173- 80.
24. Liu JP. Molecular mechanisms of ageing and related diseases. Clin Exp Pharmacol Physiol 2014; 41 (7): 445- 58.
25. Cawthon RM, Smith KR, O'Brien E, Sivatchenko A, Kerber RA. Association between telomere length in blood and mortality in people aged 60 years of older. Lancet 2003; 361: 393-5.
26. Kropski JA, Blackwell TS,Loyd JA.The genetic basis of idiopathic pulmonary fibrosis. ERJ Express. 2015.
27. Maeda T, Guan J, Higuchi Y , Oyama J , Makino N. Aging-Related Alterations of Subtelomeric Methylation in Sarcoidosis Patients. Journal of Gerontology Journal of Gerontology 2009; 752–760.
28. Thompson I, Liu B, Sen H, Dumitriu B, Calado R, Hirani S,et al. Telomere Length of peripheral leukocytes is shortened in Ocular Sarcoidosis patients . ARVO Annual Meeting. 2013, Volume 54, Issue 15.
29. Yasar Z, Özgül MA, Cetinkaya E, et al. Angiotensin-converting enzyme as a predictor of Extrathoracic involvement of sarcoidosis. Sarcoidosis Vasculitis and Diffuse Lung Diseases 2015; 32; 318-324
30. Studdy P, Bird R, James DG et al: Serum ACE in sarcoidosis and other granulomatous disorders. Lancet, 1978; 1441-54.
31. Lieberman J: Elevation of serum angiotensin-converting-enzyme (ACE) level in sarcoidosis. Am J Med, 1975; 59: 365–72.
32. Silverstein E, Friedland, J Lyons HA et al: Evaluation of ACE in granulomatous lymph nodes and serum in sarcoidosis: Clinical and possible pathogenic significance. Ann NY Acad Sci, 1976; 278: 498–513.
33. Kahkouee S, Samadi K, Alai A,Abedini A, Rezaiian L. Serum ACE Level in Sarcoidosis Patients with Typical and Atypical HRCT Manifestation. Pol J Radiol, 2016; 81: 458-461.
34. Wong J , De Vivo I, Lin X, Fang S, Christiani D. The Relationship between Inflammatory Biomarkers and Telomere Length in an Occupational Prospective Cohort Study.Plos one. January 2014. Volume9. Issue 1.
35. Pedroso DC, Miranda-Furtado CL, Kogure GS, Meola J, Okuka M, Silva C, et al. Inflammatory biomarkers and telomere length in women with polycystic ovary syndrome. Fertil Steril. 2015 Feb;103(2):542-7.

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Sarcoidosis development during ulcerative colitis remission in a patient with a susceptible human leukocyte antigen serotype

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Abstract. The combination of sarcoidosis and ulcerative colitis (UC) is very rare, and its pathogenesis remains unknown. Hereditary factors as well as environmental factors have been speculated, including an association with the human leucocyte antigen (HLA) genotype. A 62-year-old Japanese woman with UC presented with complaint of a cough. Abnormal shadows were evident on the chest X-ray during mesalazine therapy. Multiple indolent subcutaneous nodules were also detected. Transbronchial lung and skin biopsies showed non-caseous epithelioid granulomas, which were pathologically compatible with sarcoidosis. After steroid therapy, she became asymptomatic and the abnormal shadows and subcutaneous nodules disappeared. HLA serological typing revealed that she harbored the sarcoidosis-related HLA-DR14 allele, as well as UC-related HLA-B52 and HLA-DR15 alleles. This case suggests that a susceptible HLA genotype may influence the onset of the combination of sarcoidosis and UC.

Key words: Human leukocyte antigen, Inflammatory bowel disease, Sarcoidosis, Ulcerative colitis

Introduction

Sarcoidosis is a systemic granulomatous disorder characterized by the infiltration of activated lymphocytes, mainly in the lungs, skin, and lymph nodes. Ulcerative colitis (UC) is a relapsing non-transmural inflammatory bowel disease that mainly affects the large intestine. Although both diseases are associated with specific HLA subtypes, environmental factors are also important in their onset (1, 2). To date, there have been 24 reported cases of the combination of both diseases in the same patient (3-18). However, the underlying mechanisms of comorbidity are almost completely unknown. Herein we report on a UC patient who developed sarcoidosis and whose HLA subtype was examined by serological typing.

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Case report

A 62-year-old Japanese woman with UC was referred to our hospital complaining of a dry cough and exhibited abnormal shadows in a chest X-ray. She had developed UC when she was 38 years old and had suffered from it for 17 years before going into remission for 7 years, after being treated with mesalazine at the age of 55 years. She had no history of smoking. Her prior medical history showed no evidence of pulmonary diseases.

On admission, there were indolent elastic hard subcutaneous nodules in her hip, forearms, and legs. The laboratory findings are shown in Table 1.

Table 1. Laboratory data on admission. Laboratory data showed that angiotensin converting enzyme, lysozyme, and soluble interleukin-2 receptor were elevated. These data indicated sarcoidosis. Human leukocyte antigen (HLA) typing by serology revealed that the patient had the sarcoidosis-related HLA allele DR14 as well as the ulcerative colitis-related HLA alleles B52 and DR15.

Hematology			Biochemistry			Serology		
WBC	5,62	x10³/μL	TP	6,7	g/dL	KL-6	898	U/mL
Neu.	62,6	%	ALb	3,9	g/dL	SP-D	107	ng/mL
Ly.	23,8	%	AST	27	IU	sIL-2R	3520	U/mL
Eo.	5,2	%	ALT	27	IU	ACE	110	U/L
Ba.	0,7	%	LDH	312	IU	Lysozyme	53,9	μg/mL
Mo.	7,7	%	ALP	248	IU	SA-A	13,9	μg/mL
RBC	499	x10³/μL	γ-GTP	22	IU			
Hb	15,5	g/dL	BUN	13,2	mg/dL			
Hct	41,8	%	Cr	0,74	mg/dL			
Plt	203	x10³/μL	Na	142	mEq/dL			
BS	101	mg/dL	K	3,7	mEq/dL			
HbA1c	6,9	%	Cl	106	mEq/dL			
			Ca	9,3	mg/dL			
HLA typing by serology								
haplotype1 (A24, B62, DR14)								
haplotype2 (A26, B52, DR15)								

Legends: KL-6, Krebs von den Lungen-6; SP-D, surfactant protein D; sIL-2R, soluble interleukin-2 receptor; ACE, angiotensin converting enzyme; SA-A, serum amyloid A.

The serum levels of angiotensin converting enzyme (ACE) and lysozyme were elevated to 110 U/L and 53.9 μg/mL, respectively, and serum soluble interleukin-2 receptor was also high (3,520 U/mL). HLA typing by serology revealed haplotype 1 antigens HLA-A24, HLA-B62, and HLA-DR14, and haplotype 2 antigens HLA-A26, HLA-B52, and HLA-DR 15. Interestingly, it has been reported that HLA-DR14 is associated with sarcoidosis, and HLA-B52 and HLA-DR15 are associated with UC.

As shown in Figure 1, chest X-ray showed multiple nodular shadows in bilateral lung fields and right hilar lymphadenopathy. Chest computed tomography (CT) revealed granular and nodular shadows in the entire bilateral fields of the lungs, thickened bronchial walls of bilateral lower lobes, and right hilar and mediastinal lymphadenopathy (Figure 2). Gallium-scintigraphy revealed accumulation in bilateral lungs, right hip, bilateral forearms, bilateral groin, hypogastric subcutaneous nodules, and the lymph nodes in the mediastinum.

Although the findings described above suggested that she had sarcoidosis, we did not completely exclude

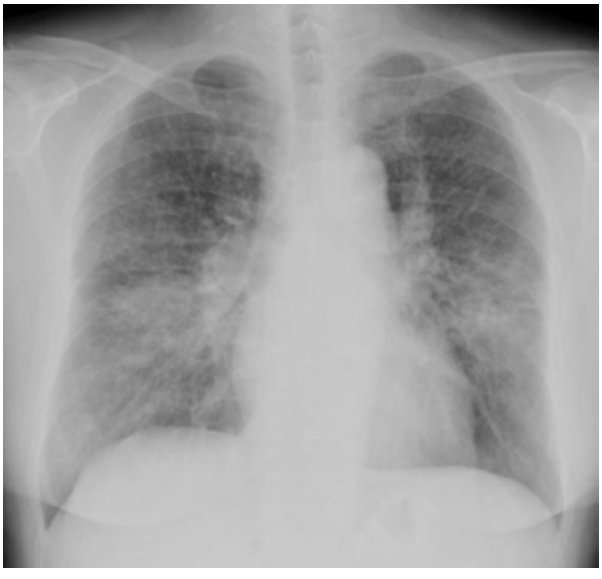

Figure 1. Chest X-ray showing multiple nodular shadows in bilateral lung fields and right hilar lymphadenopathy.

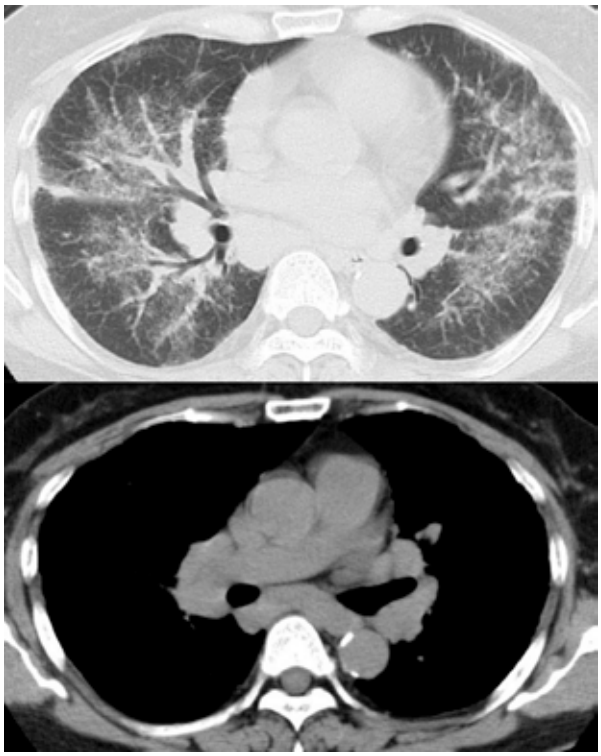

Figure 2. Chest CT showing ground glass opacities with small nodules, bronchial wall thickness in bilateral lung fields, and hilar and mediastinal lymph node swelling.

the possibility that she had pulmonary manifestations of UC or mesalazine-induced pneumonitis. Accordingly, we performed bronchoalveolar lavage (BAL) and examined the tissues of the lung and skin for granuloma to diagnose sarcoidosis. The total cell count in BAL fluid was elevated to 6.0 x 10^5 cells/mL (histiocytes 51%, lymphocytes 47.5%, neutrophils 1%, eosinophils 0.5%), and the CD4/CD8 ratio was as high as 7.91. Pathological examination of the lung and skin revealed non-caseating granulomas, as expected (Figure 3). We ultimately concluded that the patient had developed sarcoidosis and followed her carefully without steroid therapy.

Unfortunately, her cough gradually worsened. Fourteen months after the diagnosis of sarcoidosis, chest CT showed that a cystic lesion had appeared in the left upper lobe, and granular shadows in bilateral lungs increased steadily in size, resulting in partial consolidation (Figure 4). These findings led us to surmise that her health had deteriorated due to the progression of sarcoidosis, and we began to treat her with 30 mg/day of prednisolone. In response to the steroid therapy, her cough was cured, and the subcutaneous nodules and pulmonary consolidation both disappeared. However, the cystic lesion in the upper left lobe remained apparent on chest CT (Figure 4). We then reduced prednisolone carefully over a period of 6 months, and she has remained alive and well without steroids for over 1 year.

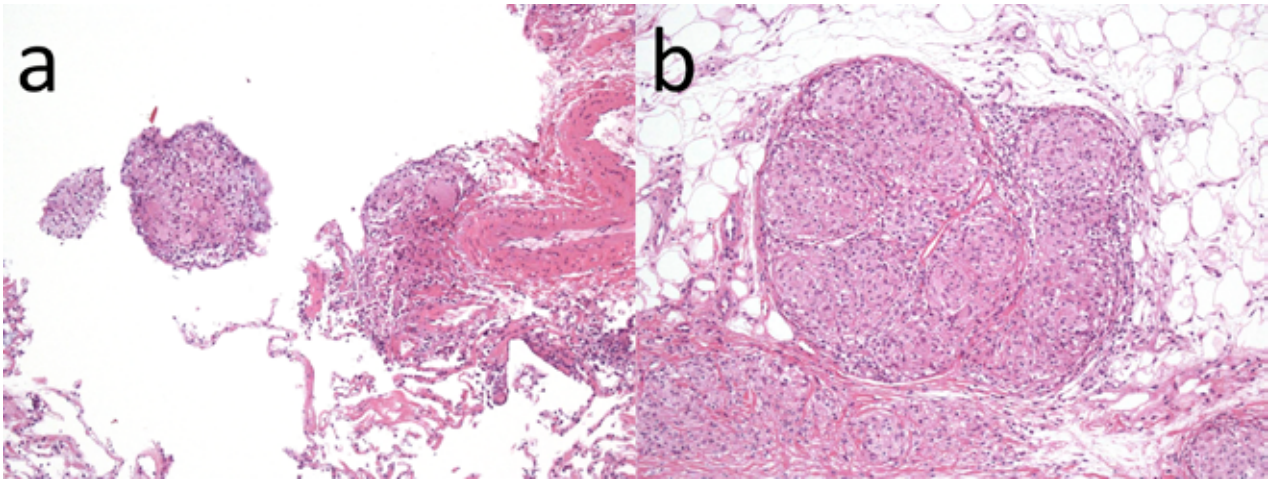

Figure 3. (a) A transbronchial lung biopsy specimen showing multiple non-caseous epithelioid granulomas including polynuclear giant cells around the pulmonary artery branches and bronchial epithelium. (b) Skin biopsy specimen showing multiple non-caseous epithelioid granulomas including polynuclear giant cells in subcutaneous adipose tissue just below the dermis. Magnification 10×, hematoxylin and eosin staining.

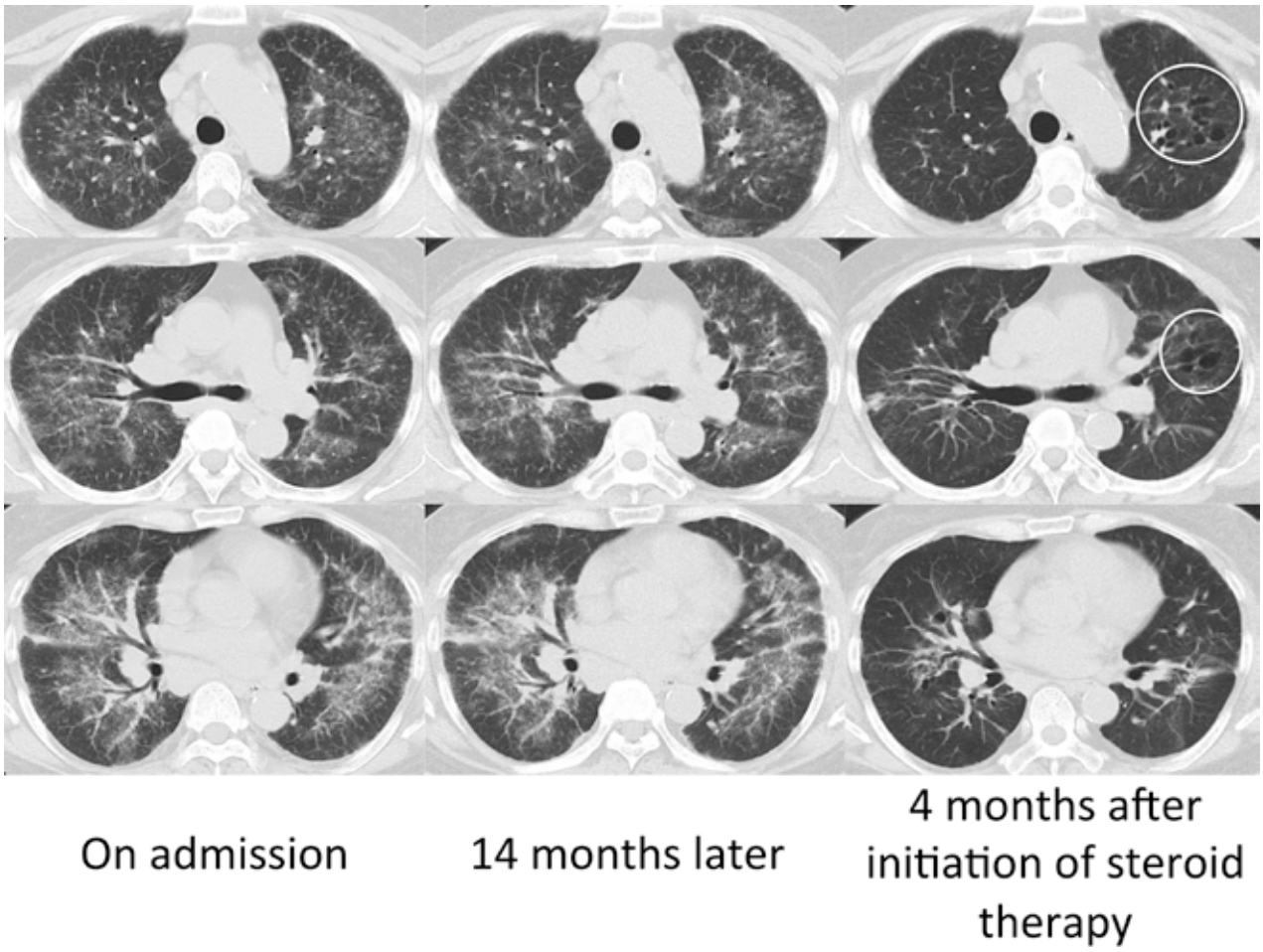

Figure 4. Clinical course of computed tomography (CT) findings. Compared with chest CT findings associated with sarcoidosis on admission, cystic lesions had appeared in the left upper lobe and multiple granular shadows in both lungs had increased 14 months later. Four months after the start of steroid therapy, the granular shadows had almost disappeared, but the left upper lobe cystic lesion (indicated by white circles) remained.

Discussion

To our knowledge, this is the first report of a patient with an HLA serotype associated with sarcoidosis (HLA-DR14) and UC (HLA-B52 and HLA-DR15) who was identified with both conditions. The allele frequencies of the respective antigenic types in the Japanese population are HLA-DR14: 10.01%, HLA-B52: 11.37%, and HLA-DR15: 19.62% (19). Table 2 summarizes 24 reported cases of the combination of sarcoidosis and UC, including the current case. Of the eight patients whose HLA serotype were analyzed (7, 8, 13, 14, 15, and the current report), six had an HLA serotype associated with sarcoidosis while two had an HLA serotype associated with UC. One report has demonstrated that sarcoidosis is clearly associated with HLA-DRB1*11, DRB1*12, DRB1*14, and DRB1*08 in Japan (20). The present case findings corroborate with this report in that she had HLA-DR14, which is a serotype of HLA-DRB1*14. It is also known that UC is significantly associated with specific HLA genotypes (21). HLA-DRB1*1502 (HLA-DR15) and HLA-B *52 are associated with UC development in Japan, and HLA-DRB1*0103 (HLA-B52) is associated with the disease in Europe and America (22). The present case findings were also consistent with these observations, in that she had HLA-B52 and HLA-DR15, which may have been related to the onset of UC.

Table 2. A summary of cases of sarcoidosis associated with ulcerative colitis (UC) reported from 1967 to 2015. There were 24 patients with both sarcoidosis and UC reported from 1967 to date, including the present case. Of the eight cases whose human leukocyte antigen (HLA) serotypes were analyzed (7, 8, 13, 14, 15, and the present case), six had an HLA allele associated with sarcoidosis, while two had an HLA allele associated with UC.

Case	Year	Age(yr)/ sex	Order of onset	Interval of onsets (yr)	HLA	Treatment of preceding diseases (sarcoidosis or UC)	Complications	Reference
1	1967	30/M	UC→SAR	2	N.D.	Steroid	Primary biliary cirrhosis	3
2	1969	26/M	UC→SAR	Autopsy	N.D.	Steroid, salazopyrin, total colectomy		4
3	1971	52/F	SAR→UC	13	N.D.	None		5
4	1981	64/F	Same time	0	N.D.	None		6
5	1986	44/F	UC→SAR	20	Three patients were HLA A1, B8 and DR3 positive.	Steroid	Toxic megacolon	7
6		31/M	Same time	0		None		
7		64/M	SAR→UC	12		Steroid		
8		38/F	SAR→UC	6		None		
9		33/M	SAR→UC	15		Steroid		
10		20/M	UC→SAR	16		Proctocolectomy	Ulinary stone	
11		37/F	SAR→UC	9		None		
12		47/F	UC→SAR	13		Proctocolectomy		
13	1987	30/M	UC→SAR	13	HLA B8	Steroid, sulfasalazine	Primary sclerosing cholangitis, bile duct carcinoma	8
14	1989	42/M	UC→SAR	13	N.D.	Symptomatic treatment	Sjogren's syndrome, sclerosing cholangitis	9
15	1995	38/M	UC→SAR	13	N.D.	Steroid, sulfasalazine, protocolectomy		10
16	1996	41/M	UC→SAR	6	N.D.	Tixocortol		11
17	1996	58/F	UC→SAR	36	N.D.	Steroid, proctocolectomy	Sjogren's syndrome	12
18	1997	22/M	UC→SAR	6	HLA A24, B52, B54, DR2, DR4	None	Insulin-dependent diabetes mellitus	13
19	1999	33/F	Same time	0	HLA DR52	None	Insulin-dependent diabetes mellitus	14
20	2001	38/F	UC→SAR	10	HLA A2, A28, B27, B44	Steroid, sulfasalazine		15
21	2003	53/M	UC→SAR	3	N.D.	Steroid, mesalamine	Appendiceal cancer	16
22	2005	33/F	UC→SAR	10	N.D.	Steroid	Dermatomyositis	17
23	2013	50/M	UC→SAR	8	N.D.	Steroid, salazopyrin		18
24	2015	62/F	UC→SAR	24	HLA A24, A26, B62, B52, DR14, DR15	Mesalazine		Our case

Legends: HLA, human leukocyte antigen; UC, ulcerative colitis; SAR, sarcoidosis; N.D., not described.

It has been reported that the prevalence of sarcoidosis is only 4.7–64.0 per 100,000. However, sarcoidosis seems to develop more frequently in UC patients (1) because it was observed in 8 of 680 UC patients in northern Europe (10). Although this suggests that sarcoidosis is not a rare disease in UC patients, sarcoidosis is not the only disease that affects the lung in UC patients. Extra-intestinal manifestations of UC and mesalazine-induced lung injury need to be differentiated. Pulmonary manifestations of UC include bronchiectasis, necrotizing bronchiolitis, bronchiolitis obliterans, and diffuse panbronchiolitis (23). We need to recognize that the extent of UC-related pulmonary manifestation is not always correlated with the activity of intestinal manifestation (24). On the other hand, mesalazine-induced lung injury has been reported to involve alveolar eosinophilic infiltrates, interstitial lymphocytic infiltrates, alveolar fibrosis, and non-necrotizing granulomas (25). If a patient with UC develops lung involvement during UC treatment, we need to distinguish carefully between lung involvements from extra-intestinal manifestations, mesalazine-induced lung injury, and sarcoidosis.

In conclusion, herein we reported the first case of a patient with an HLA serotype associated with susceptibility to both sarcoidosis and UC and who was identified with both conditions. However, cases of patients suffering from both conditions without susceptible HLA serotypes have also been reported (15). These cases suggest that there might be unknown common factors between sarcoidosis and UC. Further studies should be performed to better understand the associations between sarcoidosis and UC.

Conflicts of interest: None

References

1. Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Müller-Quernheim J. Sarcoidosis. Lancet 2014; 383: 1155-67.
2. Danese S, Fiocchi C. Ulcerative colitis. N Engl J Med 2011; 365: 1713-25.
3. Trujillo NP, Halstead LS, Ticktin HE. Chronic ulcerative colitis, xanthomatous biliary cirrhosis and sarcoidosis. Report of a case. Med Ann Dist Columbia 1967; 36: 170-4.
4. Jalan KN, MacLean N, Ross JM, Sircus W, Butterworth ST. Carcinoma of the terminal ileum and sarcoidosis in a case of ulcerative colitis. Gastroenterology 1969; 56: 583-8.
5. Watson DW, Friedman HM, Quigley A. Immunological studies in a patient with ulcerative colitis and sarcoidosis. Gut 1971; 12: 541-3.
6. Theodoropoulos G, Archimandritis A, Davaris P, Plataris J, Melissinos K. Ulcerative colitis and sarcoidosis: a curious association-report of a case. Dis Colon Rectum 1981; 24: 308-10.
7. Barr GD, Shale DJ, Jewell DP. Ulcerative colitis and sarcoidosis. Postgrad Med J 1986; 62: 341-45.
8. Van Steenbergen W, Fevery J, Vandenbrande P, et al. Ulcerative colitis, primary sclerosing cholangitis, bile duct carcinoma, and generalized sarcoidosis. Report of a unique association. J Clin Gastroenterol 1987; 9: 574-9.
9. Johnson CD. Obstructive jaundice in a patient with ulcerative colitis, Sjögren's syndrome and sarcoidosis. J R Soc Med 1989; 82: 362.
10. Fries W, Grassi SA, Leone L, et al. Association between inflammatory bowel disease and sarcoidosis. Report of two cases and review of the literature. Scand J Gastroenterol 1995; 30: 1221-3.
11. Le Gall F, Loeuillet L, Delaval P, Thoreux PH, Desrues B, Ramée MP. Necrotizing sarcoid granulomatosis with and without extrapulmonary involvement. Pathol Res Pract 1996; 192: 306-13.
12. Cox NH, McCrea JD. A case of Sjögren's syndrome, sarcoidosis, previous ulcerative colitis and gastric autoantibodies. Br J Dermatol 1996; 134: 1138-40.
13. Yoshioka K, Nishimura S, Kitai S, Kondo M. Association of sarcoidosis, insulin-dependent diabetes mellitus, and ulcerative colitis. Arch Intern Med. 1997; 157: 465-7.
14. Vial B, Chabot F, Antunes L, et al. Association of sarcoidosis and hemorrhagic rectocolitis in an insulin-dependent diabetic. [Article in French] Rev Mal Respir 1999; 16: 1151-4.
15. Nilubol N, Taub PJ, Venturero M, Lichtiger S, Bauer JJ. Ulcerative colitis and sarcoidosis. Mt Sinai J Med 2001; 68: 400-2.
16. Vaiphei K, Gupta N, Sinha SK, Nagi B, Singh K. Association of ulcerative colitis with pulmonary sarcoidosis, subcutaneous lipomatosis and appendiceal adenocarcinoma. Indian J Gastroenterol 2003; 22: 193-4.
17. Hayashi T, Nakamura T, Kurachi K, et al. Ulcerative colitis accompanied with sarcoidosis and dermatomyositis: report of a case. Dis Colon Rectum 2008; 51: 474-6.
18. Jarrot PA, Dury S, Rakotomalala A, et al. Association of sarcoidosis and ulcerative colitis: a review of 20 cases. Sarcoidosis Vasc Diffuse Lung Dis 2013; 30: 212-6.
19. Allele Frequencies in Worldwide Populations: http://www.allelefrequencies.net/hla6003a.asp
20. Ishihara M, Ohno S, Ishida T, et al. Molecular genetic studies of HLA class II alleles in sarcoidosis. Tissue Antigens 1994; 43: 238-41.
21. Satsangi J, Welsh KI, Bunce M, et al. Contribution of genes of the major histocompatibility complex to susceptibility and disease phenotype in inflammatory bowel disease. Lancet 1996; 347: 1212-7.
22. Asano K, Matsushita T, Umeno J, et al. A genome-wide association study identifies three new susceptibility loci for ulcerative colitis in the Japanese population. Nat Genet 2009; 41: 1325-9.
23. Camus P, Colby TV. The lung in inflammatory bowel disease. Eur Respir J 2000; 15: 5-10.
24. Black H, Mendoza M, Murin S. Thoracic manifestations of inflammatory bowel disease. Chest 2007; 131: 524-32.
25. Franco AI, Escobar L, García XA, et al. Mesalazine-induced eosinophilic pneumonia in a patient with ulcerative colitis disease: a case report and literature review. Int J Colorectal Dis 2016; 31: 927-9.

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Effectiveness of polymyxin B hemoperfusion in acute exacerbation of interstitial pneumonia: a retrospective analysis

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Abstract. Background; Acute exacerbation (AE) of interstitial pneumonia (IP) occurs commonly and has a poor prognosis. Polymyxin B hemoperfusion (PMX-DHP) has a beneficial effect on AE of some types of IPs, particularly idiopathic pulmonary fibrosis (IPF). However, little is known about the efficacy of PMX-DHP in the Korean population. The aim of this study was to examine the effectiveness of PMX-DHP in AE of IP. **Methods:** We conducted a retrospective study of 12 patients with AE of IP, including two patients with AE of IPF, who were treated with PMX-DHP at our center. Treatment with PMX-DHP was carried out once or twice. We collected and analyzed data on changes in oxygenation with PMX-DHP and survival after AE. **Results:** In patients with AE of IP, the ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen, or the P/F ratio, had significantly improved at the end of the treatment with PMX-DHP (87.0 [80.3 – 130.9] to 200.6 [105.0 – 245.5] mmHg, $p = 0.019$). The white blood cell (WBC) count had significantly reduced at the end of the treatment (12,400 [8,860 – 20,287] to 6,800 [3,950 – 15,775]/mm^3, $p = 0.050$). The 28-day and in-hospital mortality rates of patients after AE of IP were 41.7 % and 75.0 %, respectively. **Conclusion:** PMX-DHP improved oxygenation and reduced the WBC count in patients with AE, with either steroids alone or steroids and cyclophosphamide. Further studies are required to verify the potential benefits of PMX-DHP for patients with AE of IP.

Keywords: Lung Diseases; Interstitial; Disease Progression; Polymyxin B

Introduction

The clinical course of interstitial pneumonia (IP) is not clearly known and is highly variable (1-3). Idiopathic pulmonary fibrosis (IPF) is the most common type of idiopathic IP (IIP). Acute exacerbation (AE) of IPF is now well defined. It pathologically shares tissue damage patterns of acute respiratory distress syndrome (ARDS), such as diffuse alveolar damage (DAD) (4). AE of IPF has a high mortality rate during hospitalization (5, 6). AE also occurs in non-specific IP, IP associated with connective tissue disease, and chronic hypersensitivity pneumonitis (7-10).

Direct hemoperfusion with polymyxin B-immobilized fiber column (PMX-DHP) is effective for sepsis (11) and ARDS (12, 13). PMX-DHP might favorably affect the endotoxin levels, ratio of partial pressure of arterial oxygen (PaO_2) to the fraction of inspired oxygen (FiO_2), or the P/F ratio, and mortality in patients with sepsis. PMX-DHP also has favorable effects on patients with acute lung injury (ALI) or ARDS with pathological DAD (12, 13).

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Considering that the pathological findings of AE of IPF and ARDS are DAD, the use of PMX-DHP has been attempted in AE of IPF. In patients with AE of IPF, treatment with PMX-DHP significantly improves the P/F ratio and survival (14-17). Furthermore, in patients with other types of IPs, treatment with PMX-DHP improves the P/F ratio and survival (18-20). Since this has not been proven in the Korean population, the aim of this study was to investigate the safety and effectiveness of PMX-DHP in patients with IP.

Methods

Study population

We retrospectively examined the clinical records of consecutive patients with AE of IPF or other types of interstitial lung disease hospitalized and treated at the Chungnam National University Hospital from January 2018 to December 2019. AE of IPF was defined according to the criteria suggested by Collard et al (21). Patients who fulfilled the following criteria were diagnosed with AE of interstitial lung disease (ILD) (18, 19): (1) development or unexplained worsening of dyspnea within 30 days; (2) new bilateral ground-glass opacities and/or consolidation on high-resolution computed tomography; (3) stable P/F ratio < 300 mmHg; and (4) absence of apparent infection, pneumothorax, pulmonary thromboembolism, heart failure, and alternative causes of ALI, such as trauma, blood infusion, and toxic inhalation.

This study was approved by the Institutional Review Board of Chungnam National University (CNUH 2020-01-053), and the need for informed consent was waived because of the retrospective nature of the study.

PMX-DHP therapy

We administered PMX-DHP (PMX; Toray Medical Co., Ltd., Tokyo, Japan) to patients who were resistant to standard treatments, including corticosteroids alone or with cyclophosphamide. Treatment failure cases were defined as those where the oxygen demand did not decrease or increased after 24 hours after the clinician performed the standard treatment, and the PMX-DHP treatment was considered. A double-lumen catheter was inserted into the jugular or femoral vein. PMX-DHP was administered for 2 to 12 h (usually 6 h) at a flow rate of 100 mL/min and repeated once more within 24 h, if possible. Nafamostat mesilate was used as the anticoagulant.

Statistical analysis

Values are expressed as medians and interquartile ranges (IQRs) for continuous parameters. All statistical analyses were performed with the SPSS software, version 22.0 (IBM Corporation, Somers, NY, USA). We compared changes in the P/F ratio, vital signs, and other laboratory data between baseline and 24 or 48 h after the first PMX-DHP session using the Wilcoxon test. We performed comparisons between the two subgroups, IIP and non-IIP, using a general linear model for repeated measures. Cumulative survival was analyzed with the Kaplan–Meier method. Differences were considered significant at $p < 0.05$.

Results

Clinical features of patients

Table 1 shows the clinical characteristics of all patients. Twelve patients, including nine men and three women, with a median (IQR) age of 62.5 (56.0–77.5) years received a total of 20 cycles of PMX-DHP. Patients were classified into two subgroups: IIP (n = 7) and non-IIP (n = 5).

Most patients were diagnosed on the basis of radiologic findings, but one patient (No. 7) was diagnosed on the basis of findings of surgical biopsy. Two patients received corticosteroid therapy before onset, one of whom underwent immunosuppressive therapy with cyclophosphamide. One patient received pirfenidone before onset. Eight patients received mechanical ventilation with a median (IQR) duration of 12.0 (6.8–15.8) days.

Treatment and outcomes of patients

Table 2 shows the treatment and outcomes. Treatment with PMX-DHP was started after a median (IQR) duration of 48 (24.0 – 90.0) hours from the start of corticosteroid therapy. The median (IQR) number of cycles was two (one to two), and the median (IQR) duration was 6 (6 – 6) hours. Nine

Table 1. Clinical characteristics of patients

Patient Number	Sex	Age, years	Subgroup	Diagnosis	Duration of underlying disease, months	Previous therapy	Mechanical ventilation	Duration of ventilator
1	M	58	IIP	IPF	8	Pirfenidone	-	
2	M	66	IIP	IPF	0	-	+	9
3	M	71	IIP	Idiopathic AIP	0	-	-	
4	F	56	IIP	Idiopathic AIP	0	-	+	11
5	M	59	IIP	Unclassified IP	0	-	+	13
6	F	80	IIP	NSIP	0	-	-	
7	F	49	IIP	NSIP	0	-	+	16
8	M	56	Non-IIP	CPFE	18	-	+	15
9	M	84	Non-IIP	Drug-induced IP	0	-	+	6
10	M	78	Non-IIP	Drug-induced IP	0	-	-	
11	M	56	Non-IIP	DM-ILD	9	Steroid + Cyclophosphamide	+	4
12	M	76	Non-IIP	RA-ILD	15	Steroid	+	17

IIP: idiopathic interstitial pneumonia, IPF: idiopathic pulmonary fibrosis, AIP: acute interstitial pneumonia, NSIP: nonspecific interstitial pneumonia, CPFE: combined pulmonary fibrosis and emphysema, IP: interstitial pneumonia, DM: dermatomyositis, ILD: interstitial lung disease, RA: rheymatoid arthritis

Table 2. Treatment and outcomes of patients

Patient Number	PMX-DHP				Treatment		Outcome	Survival (from 1st PMX-DHP day)	Hospital stay	ICU stay
	Starting from steroid pulse therapy, days	Cycles	Duration, hours	Time delay between each cycle, days	Steroid	Others				
1	3	2	6	1	1,000		Dead	39	42	4
2	2	2	2	1	500		Dead	52	60	10
3	17	2	6-12	1	1,000		Alive		22	3
4	5	1	6		1,000		Dead	11	19	11
5	2	1	6		60		Alive		368	30
6	2	2	6	1	500		Alive		21	6
7	4	2	6	1	1,000		Dead	15	30	16
8	1	2	6	1	500		Dead	52	52	25
9	1	2	6	1	60		Dead	29	30	30
10	1	1	6		1,000		Dead	3	5	3
11	1	1	6		500	Cyclopho-sphamide	Dead	3	7	4
12	1	2	6	1	500		Dead	13	24	19

PMX-DHP: polymyxin B-immobilized fiber column, ICU: intensive care unit

Table 3. Clinical course of laboratory data based on the Wilcoxon test

Value	Baseline		24 hours			48 hours		
	Median, IQR	n	Median, IQR	n	p-value	Median, IQR	n	p-value
Lab								
pH	7.43 (7.36 – 7.49)	12	7.4 (7.33 – 7.47)	12	0.875	7.4 (7.35 – 7.45)	12	0.724
PaCO2, mmHg	41.0 (37.3 – 51.3)	12	41.5 (38.0 – 55.5)	12	0.694	44.0 (36.5 – 53.8)	12	0.965
PaO2, mmHg	74.0 (62.3 – 83.0)	12	95.5 (81.8 – 134.8)	12	0.013	108.0 (58.8 – 121.5)	12	0.071
P/F ratio, mmHg	87.0 (80.3 – 130.9)	12	201.6 (116.3 – 242.5)	12	0.007	200.6 (105.0 – 245.5)	12	0.019
WBC, /uL	12,400 (8,860 – 20,287)	12	8,180 (5,960 – 11,032)	12	0.003	6,800 (3,950 – 15,775)	12	0.050
Hb, g/dL	11.1 (9.1 – 12.7)	12	10.4 (9.5 – 12.1)	12	0.119	10.6 (9.4 – 11.7)	12	0.307
Platelet, x10^3/uL	230.0 (83.3 – 285.5)	12	169.0 (68.0 – 212.3)	12	0.034	155.5 (52.0 – 206.0)	12	0.034
CRP, mg/dL	12.4 (3.1 – 24.7)	12	13.3 (2.1 – 14.7)	11	0.541	3.8 (1.9 – 11.9)	7	0.018
IL-6, pg/mL	46.8 (9.7 – 414.1)	12	38.6 (7.9 – 228.5)	12	0.347	43.9 (2.1 – 204.8)	9	0.594
Vital sign								
Mean BP, mmHg	88 (82 – 97)	12	86 (80 – 98)	12	0.844	86 (80 – 92)	12	0.694
Heart rate, beats/min	111 (86 – 119)	12	102 (81 – 116)	12	0.182	108 (90 – 124)	12	0.969
Respiratory rate, beats/min	23 (22 – 25)	12	24 (21 – 26)	12	0.330	23 (18 – 25)	12	0.201
Body temperature,°C	37.1 (36.7 – 37.4)	12	37.0 (36.6 – 37.3)	12	0.366	36.7 (36.4 – 36.9)	12	0.071

IQR: interquartile range, pH: potential hydrogen, PaCO2: partial pressure or carbon dioxide, PaO2: partial pressure of oxygen, P/F ratio: ratio of arterial oxygen partial pressure to fractional inspired oxygen, WBC: white blood cell, Hb: hemoglobin, CRP: C–reactive protein, IL-6: interleukin–6, BP: blood pressure

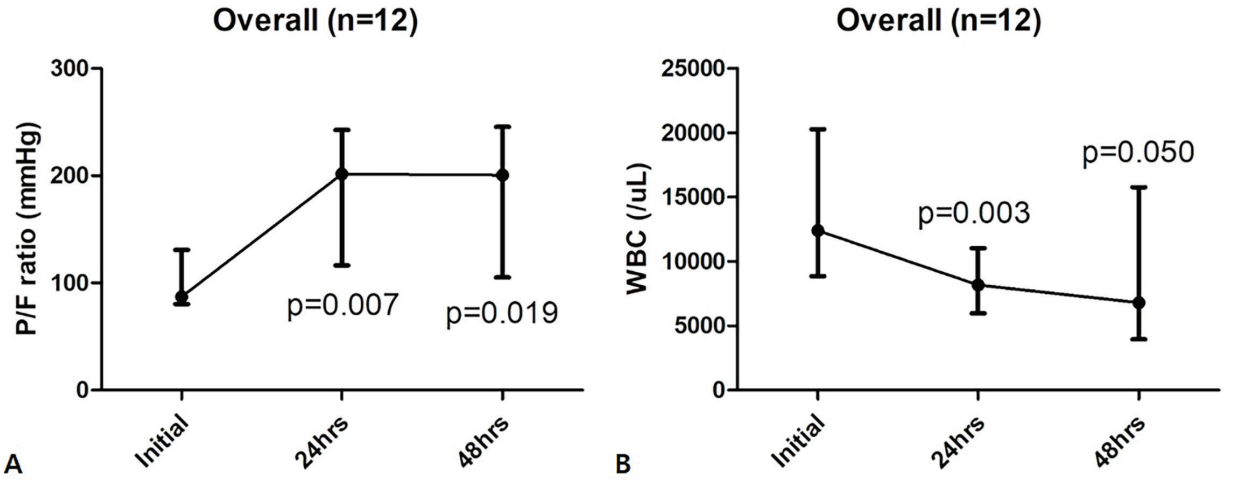

Figure 1. Change of P/F ratio and WBC count. The statistical analysis was performed with the Wilcoxon test. The p values indicate the comparisons with baseline values. Values are expressed as medians and IQRs (25 – 75%). A. P/F ratio, B. WBC
P/F ratio: ratio of arterial oxygen partial pressure to fractional inspired oxygen, WBC: white blood cell

Table 4. Change of laboratory data in subgroups

	Total	IIP (n = 7)	Non-IIP (n = 5)	p-value
Initial	12			
P/F ratio, mmHg	87.0 (80.3 – 130.9)	82.7 (76.3 – 191.7)	93.7 (82.0 – 125.4)	0.639
WBC, /uL	12,400 (8,860 – 20,287)	10,600 (6,840 – 14,400)	12,500 (12,210 – 23,275)	0.343
IL-6, pg/mL	46.8 (9.7 – 414.1)	14.0 (4.5 – 30.2)	277.4 (83.8 – 1748.5)	0.018
24 hours				
P/F ratio, mmHg	201.6 (116.3 – 242.5)	217.5 (197.1 – 250.0)	135.0 (82.0 – 233.9)	0.149
WBC, /uL	8,180 (5,960 – 11,032)	6,500 (4,700 – 11,110)	10,000 (8,180 – 15,950)	0.149
IL-6, pg/mL	38.6 (7.9 – 228.5)	13.0 (1.9 – 37.5)	288.0 (40.1 – 804.6)	0.010
48 hours				
P/F ratio, mmHg	200.6 (105.0 – 245.5)	197.8 (150.0 – 250.0)	203.3 (80.0 – 258.7)	0.755
WBC, /uL	6,800 (3,950 – 15,775)	5,180 (3,600 – 16,300)	8,900 (3,300 – 18,250)	0.530
IL-6, pg/mL	43.9 (2.1 – 204.8)	14.3 (1.9 – 66.0)	405.7 (324.9 – 405.7)	0.056

IIP: idiopathic interstitial pneumonia, P/F ratio: ratio of arterial oxygen partial pressure to fractional inspired oxygen, WBC: white blood cell, IL-6: interleukin-6

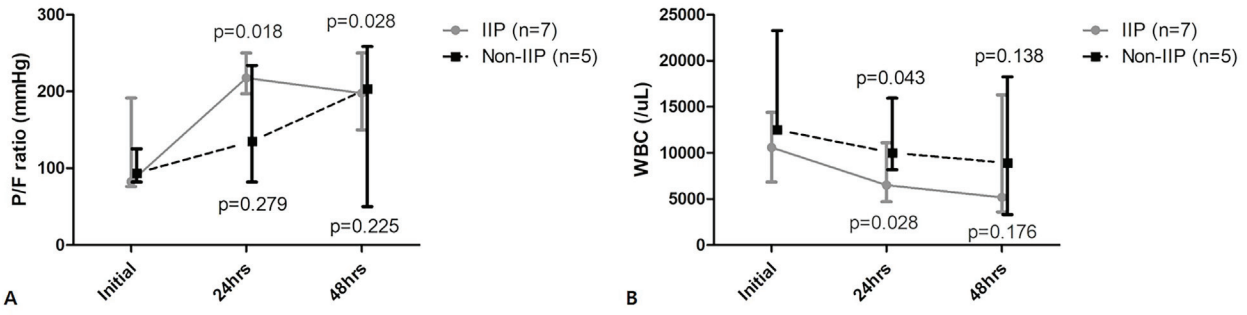

Figure 2. Change of P/F ratio and WBC count in the subgroup (IIP vs. non-IIP). The statistical analysis was performed with the Wilcoxon test. The p values indicate the comparisons with baseline values. Values are expressed as medians and IQRs (25 – 75%). A. P/F ratio, B. WBC
P/F ratio: ratio of arterial oxygen partial pressure to fractional inspired oxygen, WBC: white blood cell, IIP: idiopathic interstitial pneumonia

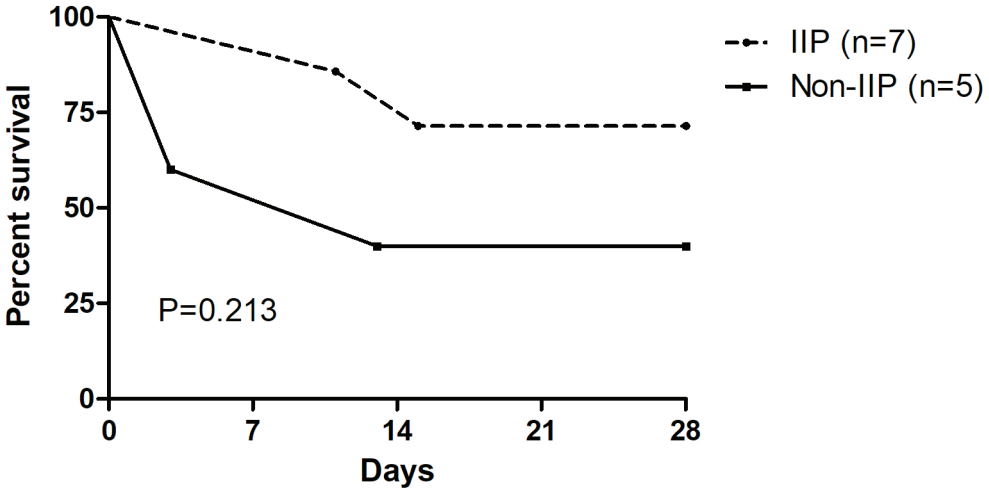

Figure 3. Comparison of the 28day-survival time of the patients in the subgroup. The median survival was longer and lower 28-day mortality in the IIP subgroup, but was not statistically significant by log-rank test (p = 0.213).
IIP: idiopathic interstitial pneumonia

of 12 patients died, and the median (IQR) survival after 1st PMX-DHP treatment among the patients who died was 15.0 (7.0 – 45.5) days. The median (IQR) duration of intensive care unit stay was 10.5 (4.0–23.5) days, and median (IQR) duration of hospital stay was 27.0 (19.5 – 49.5) days.

Clinical effects of PMX-DHP

The P/F ratio significantly improved in all patients from baseline to 24hours (median [IQR], 87.0 [80.3 – 130.9] mmHg vs. 201.6 [116.3 – 242.5] mmHg, p = 0.007) and 48hours (median [IQR], 87.0 [80.3 – 130.9] mmHg vs. 200.6 [105.0 – 245.5] mmHg, p = 0.019) after the 1st PMX-DHP treatment (Table 3, Figure 1A). Moreover, improvement in the P/F ratio after 24hours (p = 0.018) and 48hours (p = 0.028) was statistically significant in the IIP subgroup but not in the non-IIP group (24hours: p = 0.279; 48hours: p = 0.225) (Figure 2A). However, there was no statistically difference between the two subgroups (Table 4).

The WBC count significantly decreased in all patients from baseline to 24hours (median [IQR], 12,400 [8,860 – 20,287] vs. 8,180 [5,960 –11,032], p = 0.003) and 48hours (median [IQR], 12,400 [8,860

– 20,287) vs. 6,800 [3,950 – 15,775], p = 0.050) after the 1st PMX-DHP treatment (Table 3, Figure 1B). Moreover, decrement in the WBC count after 24 hours in both subgroups was statistically different (IIP: p = 0.028; non-IIP: p = 0.043) but not after 48 hours (IIP: p = 0.176; non-IIP: p = 0.138) (Figure 2B). However, there was no statistically difference between the two subgroups (Table 4).

The 28-day mortality was 41.7 % (five of 12 patients), and in-hospital mortality was 75.0 % (nine of 12 patients). The median (IQR) survival was 27.0 (19.5 – 49.5) days from admission and 15.0 (7.0 – 45.5) days from the 1st PMX-DHP treatment. In subgroup comparisons performed with the log-rank test, the 28-day and in-hospital mortalities were 28.6 % (two of seven patients) and 57.1 % (four of seven patients), respectively, in the IIP subgroup and 60.0 % (three of five patients) and 100.0 % (five of five patients), respectively, in the non-IIP subgroup (p = 0.213, p = 0.085) (Figure 3).

Side effects of PMX-DHP

To clarify the safety of PMX-DHP, we investigated the clinical course of vital signs and laboratory data (Table 3). Vital signs did not deteriorate during the PMX-DHP treatment, and no patient required additive vasopressors. None of the patients showed a tendency to bleed or required blood transfusion during the PMX-DHP treatment. There were no complications, such as pneumothorax or hematoma, associated with catheter insertion.

Discussion

This is the first study in Korea to retrospectively investigate the PMX-DHP treatment of patients with AE of IP. We found that PMX-DHP improved oxygenation and reduced the WBC count. Improved oxygenation and reduction in WBCs were found in both IIP and non-IIP subgroups. No improvement in survival was clearly identified. There were no complications during the PMX-DHP treatment.

Polymyxin B effectively reduces the level of endotoxins in blood during sepsis. The addition of PMX-DHP to conventional therapies improved survival of patients with sepsis and/or septic shock caused by abdominal gram-negative infections (11). The most common cause of ARDS is sepsis, a serious and widespread infection of the bloodstream. PMX-DHP improved the circulatory instability, oxygenation, and survival in patients with ARDS (12, 13). ARDS may be pathologically characterized by diffuse inflammatory findings in lung parenchyma, such as DAD, which is the most common surgical biopsy finding in AE with usual interstitial pneumonia (UIP) (4). Seo et al. first investigated the effect of the PMX-DHP treatment on AE of IPF. With the conventional corticosteroid treatment, four of six patients could be successfully weaned from mechanical ventilation and survived for over 30 days after the initial PMX treatment (14).

In this study, the P/F ratio improved in patients who received PMX-DHP, consistent with previous studies. Abe et al. reported that in patients with AE of IPF, the P/F ratio had significantly improved at the end of the 2nd treatment with PMX (mean ± standard error of mean [SEM] 173.9 ± 105.4 to 195.2 ± 106.8 Torr, p = 0.003) (15). Enomoto et al. reported that in patients with IPF, treatment with PMX-DHP elicited a significantly greater change in the P/F ratio (mean ± SEM, 58.2 ± 22.5 vs. 0.7 ± 13.3, p = 0.034) after 2 days compared to patients treated without PMX-DHP (17). Hara et al. reported that in patients with rapidly progressive IPs, the P/F ratio significantly improved 72 hours after PMX-DHP (median [IQR], 127.0 [91.1–150.9] vs. 152.8 [116.5–274.4], p = 0.02) (18). The mechanism through which PMX-DHP improves oxygenation in patients with AE of IP is unclear. However, Hara et al. found that the serum level of monocyte chemoattractant protein-1 (MCP-1) after PMX-DHP treatment had significantly reduced compared to the level before the PMX-DHP treatment (18). MCP-1 is produced by various cells, including monocytes. It belongs to the CC subgroup of chemokines and plays an important role in the recruitment and activation of monocytes during acute inflammation (22). MCP-1 is elevated in the bronchoalveolar lavage fluid and serum of patients with IPF or other types of IP (23, 24). Similarly, elevated CXC chemokines are associated with the pathological condition of IPF and other types of IP (25-27). Some inflammatory chemokines (e.g., neutrophil elastase, interleukin-8 (28), and interleukin-18 (29)) are immediately reduced in patients with ARDS after PMX-DHP. Seo et al. showed that reduction in interleukin-6 and interleukin-8 and plasminogen activator inhibitor-1 was found after

PMX-DHP (14). Noma et al. reported that MCP-1, interleukin-6, and interleukin-8 had reduced 72 h after PMX-DHP (30). These studies suggested that oxygenation improves because of the reduction in chemokines after PMX-DHP, but further studies are required.

In this study, the WBC count had decreased after the PMX-DHP treatment. Abe et al. showed that the WBC count had significantly reduced at the end of the 2nd treatment (13,330 ± 7,002 to 9,426 ± 5,188/mm^3, $p < 0.001$) (15). Enomoto et al. showed a smaller change in the WBC count (–630 ± 959 / μL vs. 4,500 ± 1190 /μL, $p = 0.002$) after 2 days of treatment (17). Enomoto et al. reported that three of the four patients with AE of IP who received 6- or 12-hours courses of PMX-DHP showed a decrease in serum interleukin-6 levels after PMX-DHP (20). Abe et al. showed PMX-DHP treatment in patients with acute exacerbation of interstitial pneumonia. After treatment, the cells absorbed by PMX were neutrophils and highly expressed HLA-DR, CD14, CD62L, and CD114. Additionally, serum MMP-9, which plays an important role in acute exacerbation of IP or acute respiratory distress syndrome, decreased after PMX (31). These studies showed reductions in WBC and chemokines, which may help improve the AE status through the reduction of inflammatory effects.

In this study, improvement in mortality was not confirmed in patients undergoing PMX-DHP, but there was a potential for improvement. Seo et al. reported that patients with AE of IPF survived more than 30 days after the PMX treatment (14). Takada et al. reported that six patients with rapidly progressive ILD who underwent PMX-DHP on the 1st day of steroid pulse therapy had significantly longer survival times than those who were treated with standard medication alone ($p < 0.01$) (19). Enomoto et al. reported that among patients with AE of IPF, the 12-month survival rate was significantly higher in patients treated with PMX-DHP (48.2 % vs. 5.9 %, $p = 0.041$). Treatment with PMX-DHP was an independent predictor of better prognosis (hazards ratio: 0.345; $p = 0.037$) (17). In our study, 28-day and in-hospital mortalities were 41.7 % and 75.0 %, respectively. AL-Hameed et al. described outcomes of AE of IPF in patients who were admitted to the intensive care unit. In their study, 24 of 25 patients died, resulting in overall mortality of 96% (6). Comparing these results, treatment with PMX-DHP might help improve survival.

Limitations

This study has some limitations. First, it was a small, retrospective, observational study at a single center. The pathological findings were unclear in most patients. In addition, the etiology, underlying disease and treatment, frequency and duration of the PMX-DHP treatment, time delay between every two PMX-DHP treatments, combination therapy, and adjustment of mechanical ventilation were diverse.

Conclusion

In conclusion, oxygenation improved stably without complications and the WBC count decreased when PMX-DHP was performed in patients with AE of IP. Improvements in survival were not statistically significant but may be of benefit for further studies. For patients with AE of IP, no particularly effective treatment could be established, and the prognosis was poor. Therefore, a large prospective trial is warranted for the future to confirm the improvement of the clinical course and survival of patients with AE of IP following the use of PMX-DHP.

Conflicts of Interest: No potential conflict of interest relevant to this article was reported.

References

1. Kim DS, Collard HR, King TE, Jr. Classification and natural history of the idiopathic interstitial pneumonias. Proceedings of the American Thoracic Society 2006; 3: 285-92.
2. Suzuki A, Kondoh Y, Brown KK, et al. Acute exacerbations of fibrotic interstitial lung diseases. Respirology (Carlton, Vic) 2019.
3. Travis WD, Costabel U, Hansell DM, et al. An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. American journal of respiratory and critical care medicine 2013; 188: 733-48.
4. Churg A, Muller NL, Silva CI, Wright JL. Acute exacerbation (acute lung injury of unknown cause) in UIP and other forms of fibrotic interstitial pneumonias. The American journal of surgical pathology 2007; 31: 277-84.
5. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. The European respiratory journal 2011; 37: 356-63.

6. Al-Hameed FM, Sharma S. Outcome of patients admitted to the intensive care unit for acute exacerbation of idiopathic pulmonary fibrosis. Canadian respiratory journal 2004; 11: 117-22.
7. Suda T, Kaida Y, Nakamura Y, et al. Acute exacerbation of interstitial pneumonia associated with collagen vascular diseases. Respiratory medicine 2009; 103: 846-53.
8. Kondoh Y, Taniguchi H, Kitaichi M, et al. Acute exacerbation of interstitial pneumonia following surgical lung biopsy. Respiratory medicine 2006; 100: 1753-9.
9. Inase N, Sakashita H, Ohtani Y, et al. Chronic bird fancier's lung presenting with acute exacerbation due to use of a feather duvet. Internal medicine (Tokyo, Japan) 2004; 43: 835-7.
10. Lee HK, Kim DS, Yoo B, et al. Histopathologic pattern and clinical features of rheumatoid arthritis-associated interstitial lung disease. Chest 2005; 127: 2019-27.
11. Cruz DN, Antonelli M, Fumagalli R, et al. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. Jama 2009; 301: 2445-52.
12. Tsushima K, Kubo K, Koizumi T, et al. Direct hemoperfusion using a polymyxin B immobilized column improves acute respiratory distress syndrome. Journal of clinical apheresis 2002; 17: 97-102.
13. Nakamura T, Kawagoe Y, Matsuda T, et al. Effect of polymyxin B-immobilized fiber on blood metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 levels in acute respiratory distress syndrome patients. Blood purification 2004; 22: 256-60.
14. Seo Y, Abe S, Kurahara M, et al. Beneficial effect of polymyxin B-immobilized fiber column (PMX) hemoperfusion treatment on acute exacerbation of idiopathic pulmonary fibrosis. Internal medicine (Tokyo, Japan) 2006; 45: 1033-8.
15. Abe S, Azuma A, Mukae H, et al. Polymyxin B-immobilized fiber column (PMX) treatment for idiopathic pulmonary fibrosis with acute exacerbation: a multicenter retrospective analysis. Internal medicine (Tokyo, Japan) 2012; 51: 1487-91.
16. Oishi K, Aoe K, Mimura Y, et al. Survival from an Acute Exacerbation of Idiopathic Pulmonary Fibrosis with or without Direct Hemoperfusion with a Polymyxin B-immobilized Fiber Column: A Retrospective Analysis. Internal medicine (Tokyo, Japan) 2016; 55: 3551-9.
17. Enomoto N, Mikamo M, Oyama Y, et al. Treatment of acute exacerbation of idiopathic pulmonary fibrosis with direct hemoperfusion using a polymyxin B-immobilized fiber column improves survival. BMC pulmonary medicine 2015; 15: 15.
18. Hara S, Ishimoto H, Sakamoto N, et al. Direct hemoperfusion using immobilized polymyxin B in patients with rapidly progressive interstitial pneumonias: a retrospective study. Respiration; international review of thoracic diseases 2011; 81: 107-17.
19. Takada T, Asakawa K, Sakagami T, et al. Effects of direct hemoperfusion with polymyxin B-immobilized fiber on rapidly progressive interstitial lung diseases. Internal medicine (Tokyo, Japan) 2014; 53: 1921-6.
20. Enomoto N, Suda T, Uto T, et al. Possible therapeutic effect of direct haemoperfusion with a polymyxin B immobilized fibre column (PMX-DHP) on pulmonary oxygenation in acute exacerbations of interstitial pneumonia. Respirology (Carlton, Vic) 2008; 13: 452-60.
21. Collard HR, Ryerson CJ, Corte TJ, et al. Acute Exacerbation of Idiopathic Pulmonary Fibrosis. An International Working Group Report. American journal of respiratory and critical care medicine 2016; 194: 265-75.
22. Melgarejo E, Medina MA, Sanchez-Jimenez F, Urdiales JL. Monocyte chemoattractant protein-1: a key mediator in inflammatory processes. The international journal of biochemistry & cell biology 2009; 41: 998-1001.
23. Iyonaga K, Takeya M, Saita N, et al. Monocyte chemoattractant protein-1 in idiopathic pulmonary fibrosis and other interstitial lung diseases. Human pathology 1994; 25: 455-63.
24. Suga M, Iyonaga K, Ichiyasu H, Saita N, Yamasaki H, Ando M. Clinical significance of MCP-1 levels in BALF and serum in patients with interstitial lung diseases. The European respiratory journal 1999; 14: 376-82.
25. Antoniou KM, Tzouvelekis A, Alexandrakis MG, et al. Different angiogenic activity in pulmonary sarcoidosis and idiopathic pulmonary fibrosis. Chest 2006; 130: 982-8.
26. Nakayama S, Mukae H, Ishii H, et al. Comparison of BALF concentrations of ENA-78 and IP10 in patients with idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia. Respiratory medicine 2005; 99: 1145-51.
27. Vasakova M, Sterclova M, Kolesar L, et al. Bronchoalveolar lavage fluid cellular characteristics, functional parameters and cytokine and chemokine levels in interstitial lung diseases. Scandinavian journal of immunology 2009; 69: 268-74.
28. Kushi H, Nakahara J, Miki T, Okamoto K, Saito T, Tanjo K. Hemoperfusion with an immobilized polymyxin B fiber column inhibits activation of vascular endothelial cells. Therapeutic apheresis and dialysis : official peer-reviewed journal of the International Society for Apheresis, the Japanese Society for Apheresis, the Japanese Society for Dialysis Therapy 2005; 9: 303-7.
29. Nakamura T, Kawagoe Y, Suzuki T, et al. Changes in plasma interleukin-18 by direct hemoperfusion with polymyxin B-immobilized fiber in patients with septic shock. Blood purification 2005; 23: 417-20.
30. Noma S, Matsuyama W, Mitsuyama H, et al. Two cases of acute exacerbation of interstitial pneumonia treated with polymyxin B-immobilized fiber column hemoperfusion treatment. Internal medicine (Tokyo, Japan) 2007; 46: 1447-54.
31. Abe S, Seo Y, Hayashi H, et al. Neutrophil adsorption by polymyxin B-immobilized fiber column for acute exacerbation in patients with interstitial pneumonia: a pilot study. Blood purification 2010; 29: 321-6.

SARCOIDOSIS VASCULITIS AND DIFFUSE LUNG DISEASES 2021; 38 (1); e2021013 DOI: 10.36141/SVDLD.V38I1.11277

Sarcoid vasculitis presenting with erythema nodosum-like lesions

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Dear Editor,

Although sarcoid vasculitis has been characterized as vasculitis associated with systemic sarcoidosis in nomenclature of vasculitides proposed by Chapel Hill Conference in 2012 (1), sarcoid vasculitis in skin lesions is rarely documented in cases with either cutaneous or systemic sarcoidosis and therefore not specifically mentioned in disorders with cutaneous vasculitis (2). We herein report a case of systemic sarcoidosis, in which granulomatous vasculitis was observed in a biopsied specimen taken from erythema nodosum-like lesion on the thigh.

A 39-year-old man was diagnosed with lung sarcoidosis based on the findings of bilateral hilar lymphadenopathy on plain chest X-ray, small nodular shadows and mediastinal lymphadenopathy by computed tomography, and histopathological features of epithelioid cell granuloma on lymph node biopsy by bronchoscopy two years previously. Neither ophthalmologic nor cardiac involvement was observed. Serum levels of angiotensin-converting enzyme (ACE) began to increase one year previously. He noticed asymptomatic skin lesions on the lower extremities two months previously, and was referred to our department. Physical examination showed a number of infiltrated erythematous plaques with induration on the bilateral lower extremities (Fig. 1a). Histopathological examination revealed multiple non-caseating epithelioid granulomas in the mid-dermis and subcutis (Fig. 1b).

In the mid-dermis, findings of fibrinoid necrosis and destruction of vascular wall with infiltration of histiocytes were observed (Fig. 2a). A small vein at the dermal-subcutaneous junction was infiltrated by a number of histiocytes with fibrinoid necrosis (Fig. 2b). Higher magnification revealed vasculitis with vessel wall fibrinoid necrosis and angiocentric infiltrates of sarcoidal granulomas characterized by collections of CD68-positive histiocytes surrounding and infiltrating into the affected vascular wall (Fig. 2c,d), and Elastica van Gieson staining showed absence of internal elastic lamina of the affected small vein (Fig. 2e). By contrast, the adjacent counterpart

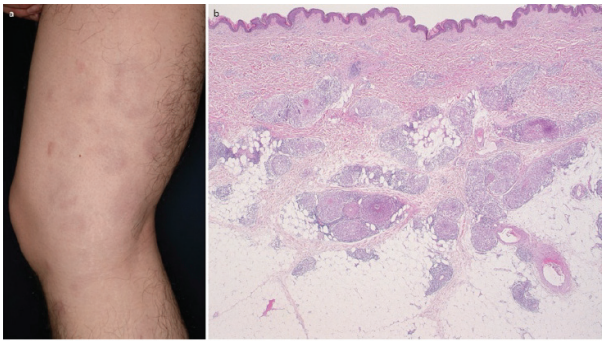

Figure 1. a) Multiple erythematous plaques with induration on the lower leg. b) Histological features showing non-caseating epithelioid cell granulomas with lymphocyte infiltration in the dermis and subcutis (×40).

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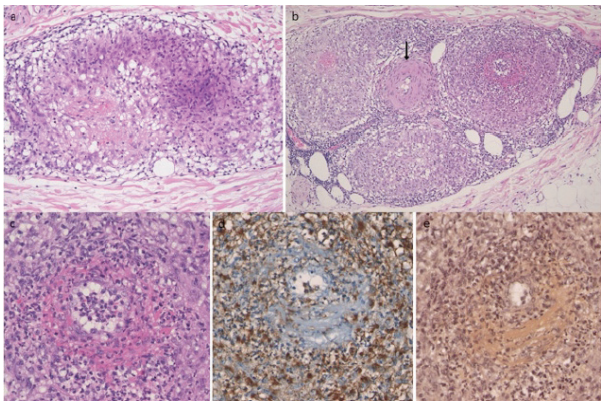

Figure 2. a) Sarcoid granuloma with venulitis showing destruction of vascular wall and fibrinoid necrosis in the mid-dermis (×200). Granulomatous vasculitis at the dermal-subcutaneous junction is characterized by an angiocentric infiltrate of histiocytes and multi-nucleated giant cells in and around the affected venous vessel wall (×100). The adjacent counterpart small artery (arrow) remained intact without involvement of the sarcoidal granulomas infiltration. Higher magnification showed fibrinoid necrosis with a predominant infiltrate of mononuclear cells in and around the vessel wall (×400). CD68 staining revealed an angiocentric infiltrate of CD68-positive histiocytes in and around the affected vessels (×400). b) Elastica van Gieson staining revealed absence of internal elastic lamina and loss of the elastic lamina of the involved vessel (×400).

small artery (arrow in Fig. 2b) remained intact without involvement of the sarcoidal granulomas infiltration. Serum levels of ACE (36.9 U/L, normal: 8.3-21.4) and soluble IL-2 receptor (2210 U/mL, normal: 121-613) were elevated, and neither PR3-ANCA nor MPO-ANCA was detected.

The present case developed erythema nodosum-like lesions on the lower legs. Erythema nodosum is a non-specific skin manifestation associated with sarcoidosis, whereas erythema nodosum-like lesion is a rare specific form of cutaneous sarcoidosis, in which skin biopsies reveal the presence of non-caseating epithelioid granulomas in the mid-dermis and subcutaneous tissues. The involved sites are the lower legs in almost all cases, but the symptoms such as tenderness and subcutaneous induration tend to be milder than those of erythema nodosum. Patients with erythema nodosum-like lesions experience no pain, or slight pain if any, in contrast to erythema nodosum. This type of skin lesion usually regresses spontaneously.

Granulomatous vasculitis is sometimes observed in cutaneous sarcoidosis, but rarely documented in the literatures. In a previous report, granulomatous vasculitis was observed in nearly 30% of patients (12/42), among whom venous involvement was observed in 11 patients (3). In a review by Yazdani Abyaneh et al. (4), granulomatous vasculitis in sarcoidosis is characterized by its association with chronic sarcoidosis, and clinical presentation with ulcers and livedo; while subcutaneous veins and arteries can be involved (4), and dermal venules are affected more often (3). Histopathology of sarcoid vasculitis in dermal or subcutaneous vessels were characterized by dense infiltration of sarcoid granulomas cuffing around and in the affected vessel walls leading to disruption of vessels. Clinical features of sarcoid vasculitis have been reported presenting with ulcerative sarcoidosis (5), plaque-type sarcoidosis (6), and annular form sarcoidosis (7). This is the first report of sarcoid vasculitis presenting with erythema nodosum-like lesion.

References

1. Jennette JC, Falk RJ, Bacon PA, et al. 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides. Arthritis Rheum 2013; 65:1–11.
2. Sunderkötter CH, Zelger B, Chen K-R, et al. Nomenclature of cutaneous vasculitis: dermatologic addendum to the 2012 revised international Chapel Hill consensus conference nomenclature of vasculitides. Arthritis Rheumatol 2018; 70: 171–184.
3. Takemura T, Shishiba K, Akiyama O, Oritsu M, Matsui Y, Eishi Y. Vascular involvement in cutaneous sarcoidosis. Pathol Int 1997; 47: 84–89.
4. Yazdani Abyaneh MA, Raghu P, Kircher K, Kutzner H, Kortz A, Carlson JA. Circumscribed cicatricial alopecia due to localized sarcoidal granulomas and single organ granulomas arteritis: a case report and systematic review of sarcoidal vasculitis. J Cutan Pathol 2015; 42: 746–756.
5. Poonawalla T, Colome-Grimmer MI, Kelly B. Ulcerative sarcoidosis in the legs with granulomatous vasculitis. Clin Exp Dermatol 2008; 33: 282–286.
6. Yamamoto T, Chen K-R. Perforating plaque-type pretibial sarcoidosis with granulomatous phlebitis. Am J Dermatopathol 2020; 42: 225–226.
7. Mizuno K, Nguyen CTH, Ueda-Hayakawa I, Okamoto H. Annular lesions of cutaneous sarcoidosis with granulomatous vasculitis. J Cutan Pathol 2017; 44: 494–496.